Obstetrics & Gynecology Science

Korean Society of Obstetrics and Gynecology
Korean Society of Maternal Fetal Medicine
Korean Society of Gynecologic Endocrinology
Korean Society of Gynecologic Endoscopy and Minimally Invasive Surgery
Korean Society of Ultrasound in Obstetrics and Gynecology
Korean Society of Contraception and Reproductive Health
Korean Urogynecologic Society
Korean Society of Endometriosis

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2020 Congress Events

2020.07.26 **The 13th Good Clinical Practice for Breast and Thyroid Disease for Gynecologists**

AI Hall, 12F, Daeyang AI Center, Sejong University

2020.08.23 **The 20th Annual Meeting of Korean Society of Gynecologic Endoscopy and Minimally Invasive Surgery**

Grand Auditorium, 6F, East Building, Asan Medical Center, Seoul, Korea

2020.09.05 **The 13th Symposium of Korean Society of Ultrasound in Obstetrics and Gynecology**

Grand Auditorium, B1F, The Catholic University of Korea Seoul St. Mary's Hospital, Seoul, Korea

2020.09.13 **The 25th Annual Congress of Korean Society of Gynecologic Endocrinology**

Convention Center, SETEC, Seoul, Korea

2020.09.25-26 **The 106th Annual Congress of Korean Society of Obstetrics and Gynecology**

Lotte Hotel Seoul, Seoul, Korea

2020.10.16-17 **The 63rd Annual Spring Meeting of Korean Society of Obstetrics and Gynecology**

Changwon Exhibition Convention Center, Changwon, Korea

2020.11.29 **The 30th Annual Congress of Korean Society of Gynecologic Endoscopy and Minimally Invasive Surgery**

Grand Hall and Theater, Grand Walkerhill, Seoul, Korea

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Mirena® is approved for contraception, treatment of heavy menstrual bleeding, dysmenorrhea and local progestogen treatment during estrogen replacement therapy.

Reference

1. 미레나® 국내 제품설명서 (최신개정일: 2018-11-26)
2. NICE 2018 Guideline [NG88]. Heavy menstrual bleeding: assessment and management (published: 2018-03-14)

Mirena®

[제품명] 미레나20마이크로그람/일(레보노르게스트렐) **[주성분]** 1세트(약 355mg) 중 레보노르게스트렐 52mg **[효능·효과]** 피임, 월경과다증, 월경곤란증, 에스트로겐 대체 요법시 프로게스틴의 국소적용 **[용법·용량]** 이 약은 자궁강에 삽입되며 삽입 후 5년간 유효하다. 이 약은 오직 자궁내시스템 삽입 경험이 있거나 이 약의 삽입 과정에 대한 훈련을 받은 의료 전문가에 의해서만 삽입되는 것이 권장된다. 이 약은 월경시작 7일 이내에 자궁 내에 삽입되며 월경 주기 중 어느 때라도 새로운 것으로 교체될 수 있다. 임신 초기 3개월 이내 유산된 경우에 이 약을 즉시 삽입할 수 있다. 산후에 삽입할 경우 자궁이 원상태로 회복될 때까지 기다려야 하며 분만 후 6주 이후에 실시해야 한다. 자궁의 회복이 상당히 지연된다면 분만 후 12주까지 기다리는 것을 고려해야 한다. 삽입이 어려운 경우 및/또는 삽입 중 혹은 삽입 후에 예외적인 통증이나 출혈이 나타날 경우에는 천공의 가능성이 고려되어야 하고 신체적 검사나 초음파 검사와 같은 적당한 조치를 취해야 한다. 이 시스템은 5년이 지나기 전에 제거되어야 한다. 만약 여성이 같은 방법을 사용해 피임을 계속 하기를 원한다면 원래의 시스템을 제거한 후 즉시 새로운 시스템을 삽입할 수 있다. 이 약을 제거한 후에 시스템이 손상되지 않았는지 확인해야 한다. **[사용상의 주의사항]** 1. 다음 환자에는 투여하지 말 것 의사가 이 레보노르게스트렐 자궁내시스템을 사용하는데 문제가 없는지 파악하기 위해 병력을 조사하거나 건강진단을 하게 된다. 임신을 했거나 의심되는 경우에는 이 자궁내시스템을 삽입해서는 안 된다. 또한 다음 경우일 때에도 삽입하여서는 안 된다. 생식기계(질, 자궁, 난소)에 감염증이 있는 경우, 생식기계에 암이 있거나 의심되는 경우(예: 자궁암, 자궁경부암) 또는 파파니콜로 도말검사 결과 비정상인 경우, 프로게스토겐-의존성 종양, 자궁에 진단되지 않는 비정상적인 출혈을 경험한 경우, 자궁강을 변형시키는 섬유종을 포함하는 선천적 또는 후천적으로 자궁이 비정상적인 구조를 하고 있는 경우, 자궁목 형성이상, 골반내 감염의 병력이 있거나 재발된 경우, 급성 또는 활동성 간 질환이 있거나 간종양(양성, 악성)이 있는 경우, 치료가 요구되는 혈전증이나 혈전색전증이 있는 경우, 출산 후 자궁내막염이나 또는 지난 3개월 내에 감염성 유산을 경험한 경우, 기타 하부 생식기계 감염이 있거나 세균성 질증을 포함하는 치료되지 않은 급성 자궁경부염이나 질염이 있는 경우, 환자나 상대자가 다수의 성관계를 가진 경우, 백혈병, 에이즈, 마약중독과 같은 감염에 이환되기 쉬운 경우, 생식기계 방선균증을 가진 경우, 예전에 삽입된 자궁내장치(IUD)가 있는 경우, 이 약물의 성분에 과민반응이 있는 경우, 유방암이 있거나 의심되는 경우, 자궁 외 임신의 병력이 있거나 발생할 가능성이 많은 경우, 세균성 심내막염의 병력이 있는 경우, 심장의 해부학적인 병변을 가진 여성이나 인공판막 치환술을 받은 후 심한 골반내 감염의 병력이 있는 경우, 뇌졸중이나 심근경색 같은 심한 동맥질환을 경험하였거나 가지고 있는 경우, hCG 수치가 상승한 최근의 영양막병을 가진 경우 2. 주요 이상반응 1) 아주 흔한(≥ 1/10) 이상반응: 두통, 복통, 생리 중 출혈 증가 및 감소를 포함한 출혈변화, 점상출혈, 희발월경 및 무월경, 음문질염, 생식기 분비물(예, 질분비물), 골반통 2) 천공: 자궁내장치 사용자들에 대한 대규모의 전향적 비교 비-중재 코호트 연구(N=61,448 명의 여성)에서 1년의 관찰기간 동안, 천공의 발생율은 전체 코호트에서 1000회 삽입 당 1.3건(95% 신뢰구간:1.1-1.6)이었다; 이 자궁내시스템의 코호트에서는 1000회 삽입 당 1.4건(95% 신뢰구간: 1.1-1.8)이었고, 구리 자궁내장치의 코호트에서는 1000회 삽입 당 1.1건(95% 신뢰구간: 0.7-1.6)이었다. 이 연구의 하위집단(미레나 또는 구리 자궁내장치를 사용한 N=39,009명의 여성)에서 관찰 기간을 5년까지로 연장했을 때, 전체 5년의 기간 동안 언제라도 감지된 천공의 발생률은 1000회 삽입 당 2.0건(95% 신뢰구간: 1.6-2.5)이었다. 이 연구는 장치 삽입 당시 수유 중인 경우와 출산 후 36주까지에 삽입했던 경우에 모두 천공 위험 증가와 관련이 있었음을 나타냈다. 이러한 위험인자들은 5년 동안 추적된 하위집단에서도 확인되었다. 이 두 위험인자들 모두 삽입한 자궁내장치의 종류와는 무관했다. **[전문의약품] [수입 및 판매자]** 바이엘코리아㈜ **[개정년월일]** 2018.11.26 **보다 자세한 사항은 제품설명서 전문 또는 바이엘 웹사이트, http://www.bayer.co.kr 를 참고하시기 바랍니다.**

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Aims and Scope

Obstetrics & Gynecology Science (NLM title: Obstet Gynecol Sci) is an international peer-review journal that published basic, translational, clinical research, and clinical practice guideline to promote women's health and prevent obstetric and gynecologic disorders. The journal has an international editorial board and is published in English on the 15th day of every other month. Submitted manuscripts should not contain previously published material and should not be under consideration for publication elsewhere.

The journal has been publishing articles since 1958. The aim of the journal is to publish original articles, reviews, case reports, short communications, letters to the editor, and video articles that have the potential to change the practices in women's health care.

The journal's main focus is the diagnosis, treatment, prediction, and prevention of obstetric and gynecologic disorders. Because the life expectancy of Korean and Asian women is increasing, the journal's editors are particularly interested in the health of elderly women in these population groups. The journal also publishes articles about reproductive biology, stem cell research, and artificial intelligence research for women; additionally, it provides insights into the physiology and mechanisms of obstetric and gynecologic diseases.

Obstetrics & Gynecology Science is the official journal of the following academic societies in Korea:
- Korean Society of Obstetrics and Gynecology
- Korean Society of Maternal Fetal Medicine
- Korean Society of Gynecologic Endocrinology
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- Korean Society of Ultrasound in Obstetrics and Gynecology
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- Korean Society of Endometriosis

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Background

Obstetrics & Gynecology Science continues in 2013 Korean Journal of Obstetrics & Gynecology (pISSN:2233-5188, eISSN: 2233-5196), which was first published in 1958.

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Review Article

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Obstetrics & Gynecology Science

The effect of antenatal magnesium sulfate on intraventricular hemorrhage in premature infants: a systematic review and meta-analysis

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Objective

The aim of this systematic review and meta-analysis study was to determine the pooled estimate of the effect of antenatal magnesium sulfate ($MgSO_4$) on intraventricular hemorrhage (IVH) in premature infants.

Methods

Two review authors independently searched all randomized clinical trials from international databases, including Medline (PubMed), Web of Sciences, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), and Research Registers of ongoing trials (ClinicalTrials.gov), from January 1989 to August 2017. Two independent review authors were responsible for data collection. After extracting the necessary information from the evaluated articles, meta-analysis of the data was performed using Stata version 14. Also, sources of heterogeneity among studies were determined by Meta regression.

Results

In this study, among 126 articles that were extracted from primary studies, 7 papers that evaluated the effect of $MgSO_4$ on IVH were eligible for inclusion in the meta-analysis. The results of the meta-analysis showed that pooled relative risk (95% confidence interval [CI]) was 0.80 (95% CI, 0.63 to 1.03) for the effect of $MgSO_4$ on IVH.

Conclusion

Results of this study showed that although $MgSO_4$ had a protective effect on IVH in premature infants, this effect was not statistically significant. Further studies are needed to determine the best dosage, timing, and gestational age to achieve the optimum effect of $MgSO_4$ on IVH.

Systematic Review Registration

International Prospective Register of Systematic Reviews (PROSPERO) Identifier: CRD42019119610

Keywords: Magnesium Sulfate; IVH; Premature infants; Systematic review; Meta-analysis

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Introduction

Intraventricular hemorrhage (IVH) consists of bleeding inside or around the ventricles, which are the areas in the brain that contain the cerebral spinal fluid. IVH is a common problem in premature infants, especially in very low birth weight infants (<1,500 g) [1]. Ancel et al. [2] reported that 56 children with normal ultrasound findings accounted for 35% of children with cerebral palsy. In the same study, children with isolated IVH, and those with white matter disease, accounted for 14% and 52% of cerebral palsy cases, respectively [2].

Many factors are involved in the incidence of IVH, the most important of which include respiratory distress, hypoxia-induced damage, ischemia, high or low blood pressure, increased venous blood pressure, pneumothorax, and hypovolemia [3]. Symptoms of IVH are nonspecific and differ according to the severity of the disease [4]. In severe and acute IVH cases, symptoms such as pale skin, acute anemia, respiratory dysfunction, and fontanel bulge can occur. Currently, cerebral ultrasound or magnetic resonance imaging is performed in the first 3 days of life and is repeated two or three times in suspected cases of IVH to assess its severity [5,6]. Bleeding from the germinal matrix around the brain ventricle based on IVH extension in brain ultrasonography is divided into four categories: grade 1 for limited bleeding to germinal matrix; grade 2 for IVH; grade 3 for bleeding with ventricular extension; and grade 4 for extension of bleeding to brain parenchyma. Grades 1 and 2 are automatically removed without any consequence; however, grades 3 and 4 are associated with severe consequences [7]. Given that preterm birth is a major cause of IVH, various methods have been proposed for its prevention [8]. Tocolytic treatments are among the conventional methods; however, there is disagreement about the best treatment method [9]. Magnesium sulfate, prostaglandin inhibitors, calcium channel blockers, and nitric oxide releasing drugs are some of the therapeutic methods in which positive effects have been reported [10,11]. Currently, magnesium sulfate is one of the most common methods used to prevent preterm delivery. Various studies have suggested that magnesium sulfate reduces the risk of brain injury in preterm infants [12-14]. In contrast, there are some studies that do not confirm the effect of magnesium sulphate on risk reduction of IVH, cerebral palsy, and perinatal mortality [15-17]. Therefore, given the controversy in the results of various studies in this field, the aim of this systematic review and meta-analysis study was to determine the effect of antenatal magnesium sulfate ($MgSO_4$) on IVH in premature infants.

Materials and methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [18]. The protocol of this study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42019119610).

1. Search strategy

Two review authors (YM and KM) independently searched all randomized clinical trials from international databases, including Medline (PubMed), Web of Sciences, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), and Research Registers of ongoing trials (ClinicalTrials.gov), from January 1989 to August 2017. The search was performed based on 11 English phrases and keywords, including "Magnesium Sulfate ($MgSO_4$)", "Heptahydrate Magnesium Sulfate", "Tocolysis Preterm", "Newborn", "Newborn Infant", "Newborn Infants", "Fetal", "Neonatal", "Preterm Prelabor Rupture of Membranes (PPROM)", "Prelabor Rupture of Membranes (PROM)", "Preterm Birth", "Premature Births", "Intraventricular hemorrhage (IVH)", "Cerebral Intraventricular Hemorrhages", "Neuroprotection", "Neuronal Protection", "Dystonic-Rigid Cerebral Palsy", "Mixed Cerebral Palsy", "Rolandic Type Cerebral Palsy", "Congenital Cerebral Palsy", "Spastic Diplegia", "Monoplegic Cerebral Palsy", "Athetoid Cerebral Palsy", "Dyskinetic Cerebral Palsy", "Atonic Cerebral Palsy", "Hypotonic Cerebral Palsy", "Diplegic Infantile Cerebral Palsy", "Spastic Cerebral Palsy", and "Cerebral Palsy (CP)". We exported the search results to the End-Note software version 9. The duplicated primary studies were deleted. The primary search results were reviewed based on the inclusion and exclusion criteria, and some of the articles were eliminated after reviewing their title and abstract. Subsequently, we investigated the search results and excluded some studies after full text review (Fig. 1).

2. Inclusion and exclusion criteria

We included all randomized controlled trials (RCTs) which assessed or reported the effect of antenatal magnesium sulfate

on IVH in premature infants. Therefore, only articles in which the primary outcome was IVH in premature infants, and gestational age between 24 to 37 weeks, were included in this review. Also, we considered primary studies in which IVH was diagnosed after birth by cranial ultrasound. We excluded duplicate citations, non- peer reviewed, cross-sectional studies, case control studies, review papers, book chapters, conference proceedings, and studies with other primary outcomes.

3. Data extraction and quality assessment

After assessment of the titles, abstracts, and texts, the full text of each selected article was retrieved for detailed analysis. Data were extracted using a data collection form with the name of the first author, date of publication, study title, study design, geographical setting, sample size, type of comorbidities (IVH-related), and main outcome. The entire process, from systematic search to final data extraction, was performed independently by two research experts (Kappa statistic for agreement for quality assessment; 0.75). Two reviewers (KM and ZN) independently evaluated the articles. Any disagreement was assessed by both reviewers, and if a consensus was not reached, a third author (YM) would evaluate the study. Moreover, quality assessment (using CONSORT) was determined by the same data extractor for each study. Risk of bias in the included studies was assessed using the Cochrane Risk of Bias Tool [19]. The bias domains that were assessed included sequence generation, allocation concealment, blinding, outcome data, and outcome reporting. Trials were rated as high risk of bias when the methodological flaw was likely to have affected the true outcome, low risk of bias if the flaw was deemed inconsequential to the true outcome, and unclear risk of bias when insufficient information was provided to permit judgment.

4. Statistical analysis

In this meta-analysis, we used two measures of association measurement: odds ratio (OR) and relative risk (RR). When the frequency of outcome (IVH in premature infants) is relatively low, OR and risk ratio provide similar estimates of RR [20]. We used logarithm and standard error logarithm RR for meta-analysis. The pooled RR with 95% confidence interval (CI) was derived through the DerSimonian and Laird method using random and fixed models [21]. Finally, for the estimated RR, we used the random effects model, since the test for heterogeneity was statistically significant in some analyses. In the present study, we used Cochran's Q test and I^2 statistic, with a significance level set at *P*-value <0.10 for evaluating statistical heterogeneity between the studies [22].

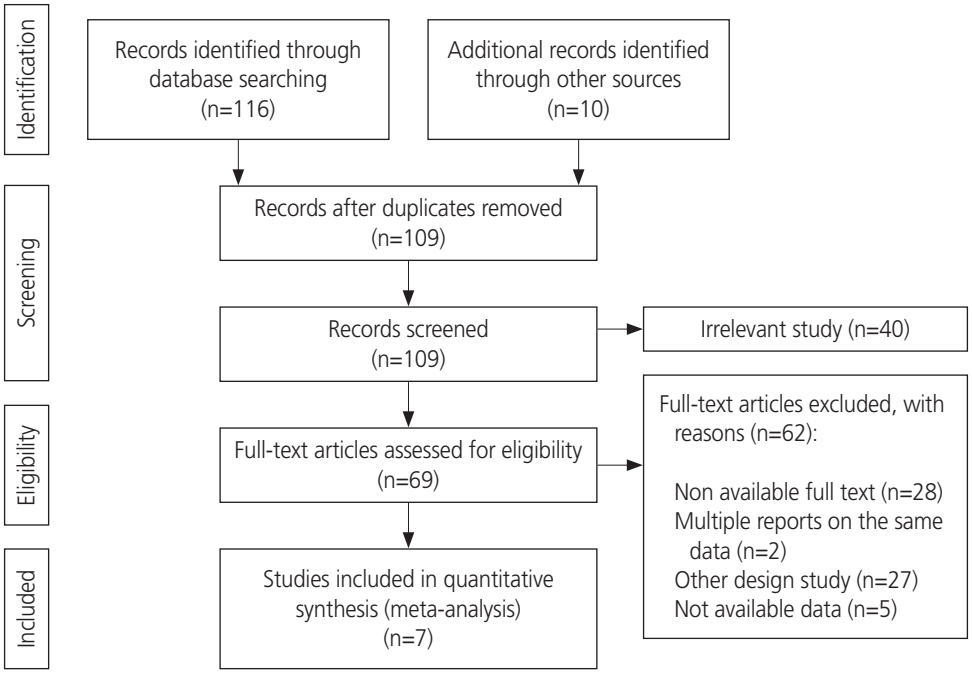

Fig. 1. Flow diagram of the literature search and study selection.

Table 1. Characteristics of studies included by principal outcome evaluated

Authors	Date of publication	Study name	Study design	Geographical setting	Sample size	Comorbidities (IVH)	Age of pregnancy (weeks)	Cases	Doses of intervention	RR (95% CI)	OR (95% CI)
Hirtz et al. [24]	2015	Antenatal magnesium and cerebral palsy in preterm infants	RCT	US	Intervention group: 953 Placebo group: 1,026	Cranial ultrasounds	24–37	PPROM/PTL	6 g loading and 2 g/hr infusion	-	0.57 (0.37–0.87)
Mirzamoradi et al. [27]	2014	Does magnesium sulfate delay the active phase of labor in women with premature rupture of membranes	RCT	Iran	Intervention group: 46 Placebo group: 46	Cranial ultrasounds	<34	PPROM	4 g loading and 2 g/hr infusion	-	3.2 (0.4–4.8)
Crowther et al. [10]	2003	Effect of magnesium sulfate given for neuroprotection before preterm birth	RCT	Australia	Intervention group: 535 Placebo group: 527	Cranial ultrasounds	<30	Planned and expected within 24 hr	4 g loading and 2 g/hr infusion	1.10 (0.90–1.33)	-
Mittendorf et al. [30]	2002	Association between maternal serum ionized magnesium levels at delivery and neonatal intraventricular hemorrhage	RCT	US	Intervention group: 72 Placebo group: 72	Cranial ultrasounds	<34	PTL	Before active phase (4 g loading and 2 g/hr infusion) After active phase (4 g single dose)	1.11 (0.53–2.34)	-
Horton et al. [25]	2015	The effect of magnesium sulfate administration for neuroprotection on latency in women with preterm premature rupture of membranes	RCT	US	Intervention group: 621 Placebo group: 638	Cranial ultrasounds	24–32	PPROM	6 g loading and 2 g/hr infusion	-	0.31 (0.10–0.96)
Rouse et al. [29]	2008	Magnesium sulfate for the prevention of cerebral palsy	RCT	US	Intervention group: 1,096 Placebo group: 1,145	Cranial ultrasounds	24–31	PPROM/PTL	6 g loading and 2 g/hr infusion	0.91 (0.78–1.08)	-
Marret et al. [26]	2006	Effect of magnesium sulphate on mortality and neurologic morbidity of the very-preterm newborn (of less than 33 weeks) with two-year neurological outcome: results of the prospective PREMAG trial	RCT	French	Intervention group: 286 Placebo group: 278	Cranial ultrasounds	<33	Planned and expected under 30 hr	4 g single dose	0.83 (0.62–1.09)	-

IVH, intraventricular hemorrhage; RR, relative risk; CI, confidence interval; OR, odds ratio; RCT, randomized controlled trial; PPROM, Preterm Prelabor Rupture of Membranes; PTL, preterm labor.

Table 2. Quality assessment of included studies according to the CONSORT checklist

Item No.	Hirtz et al. [24]	Mirzamoradi et al. [27]	Crowther et al. [10]	Mittendorf et al. [30]	Horton et al. [25]	Rouse et al. [29]	Marret et al. [26]
1a	No	Yes	Yes	No	No	Yes	Yes
1b	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2a	Yes	Yes	Yes	No	Yes	Yes	No
2b	Yes	Yes	No	No	Yes	No	Yes
3a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3b	Yes	Yes	Yes	No	Yes	Yes	Yes
4a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4b	Yes	Yes	No	Yes	Yes	Yes	Yes
5	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6a	Yes	Yes	Yes	Yes	Yes	No	Yes
6b	No	No	No	No	No	No	No
7a	No	Yes	No	Yes	No	Yes	Yes
7b	No	No	Yes	No	No	Yes	Yes
8a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8b	No	No	Yes	No	No	No	Yes
9	No	No	Yes	No	No	No	Yes
10	No	No	Yes	No	No	No	Yes
11a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11b	No	No	No	No	No	Yes	Yes
12a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12b	No	No	No	No	No	Yes	No
13a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13b	Yes	Yes	No	No	No	No	No
14a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14b	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	No	Yes	Yes	Yes	Yes	Yes	Yes
16	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17a	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17b	No	No	No	No	No	No	No
18	Yes	Yes	No	Yes	No	No	No
19	No	No	Yes	No	No	No	No
20	Yes	No	Yes	Yes	Yes	Yes	No
21	No	No	No	No	No	No	No
22	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	Yes	No	No	No	No	No	Yes
24	No	No	No	No	No	Yes	No
25	No	No	No	No	No	No	No
Total	22	23	24	19	20	24	26

An I^2 <50%, I^2 ≥50%, and I^2 ≥75% were considered to be evidence of "moderate", "substantial", and "considerable" heterogeneity, respectively [19]. In addition, to assess the source of heterogeneity between the studies, the authors conducted a meta regression and subgroup analysis. Publication bias was assessed by funnel plot, Egger and Begg's test, with a significance level set at *P*-value<0.10 [23]. The statistical analysis was performed using Stata 14.0 (Stata Corp, College Station, TX, USA) and Review Manager (RevMan), version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark).

Results

At the end of the database search, we obtained 126 articles. Among these articles, 17 were removed due to duplication. After reviewing the titles and abstracts of the articles, 40 were excluded due to non-relevance, resulting in 69 articles. After reviewing the full text of these 69 articles, only 7 were eligible for entry in the meta-analysis (Fig. 1).

The total sample size of the included studies in meta-analysis was 8,578 cases [10,24-29], in which 4 studies reported RR with a total sample size of 4,135 [10,26,28,29], and 3 studies reported OR with a total sample size of 4,443 [24,25,27] (Table 1). Table 1 shows the characteristics and results of the studies included in the meta-analysis.

As previously described in the methodology section, when the frequency of outcome (IVH in premature infants) is relatively low, OR and risk ratio provide similar estimates of RR [20]. Therefore, the OR and RR were combined, and the pooled RR was extracted with 95% CI.

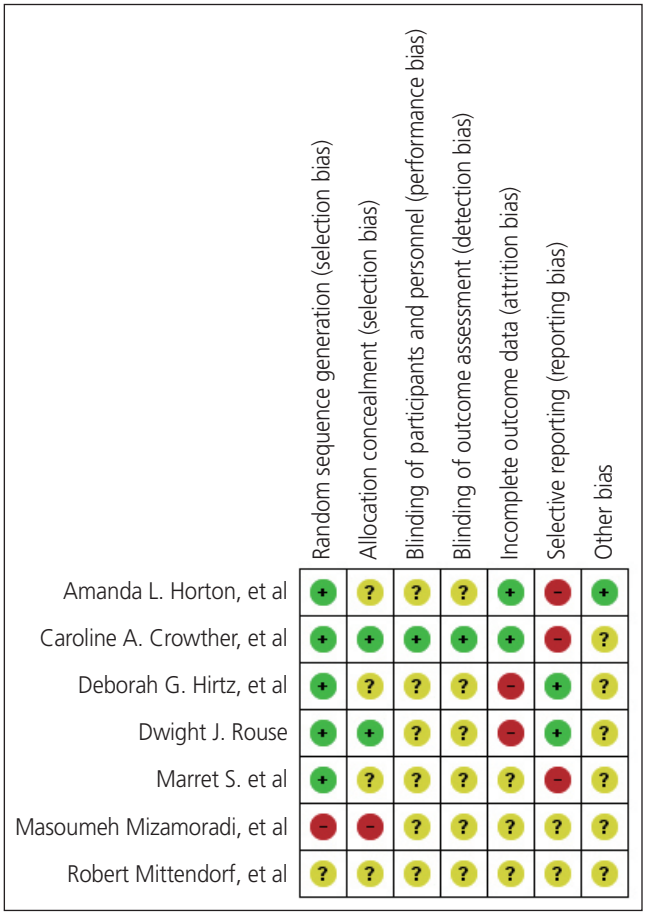

Fig. 3. Risk of bias summary (review authors' judgments about each risk of bias item for each included study).

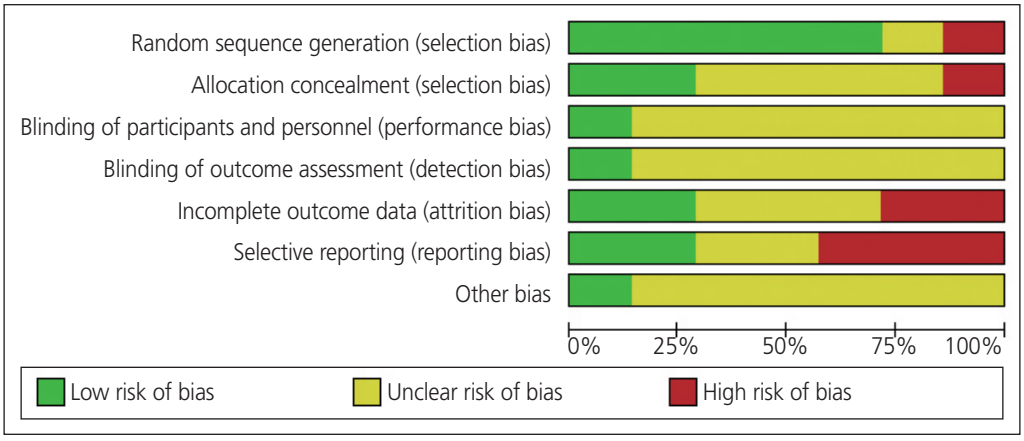

Fig. 2. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

1. Quality assessment and risk of bias

The quality assessment of the studies was performed by CONSORT checklist. Table 2 shows quality assessment of included studies according to the CONSORT checklist. As can be seen, the quality of all studies was high based on this checklist, except the study by Mittendorf et al. [30] (Table 2). The risk of bias was also performed for the articles included in the analysis. We considered the probability of the risk of bias according to sequence generation, allocation sequence concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other potential sources of bias. The results showed that most studies were low risk in terms of random sequence generation (selection bias) and allocation sequence concealment (selection bias). Additional information is provided in Fig. 2. Also, the study by Crowther et al. [10] presented the lowest risk among the studies Fig. 3. The studies did not provide adequate description of their methods, including randomization and blinding, which made it difficult for the researchers to make judgments about the risk of bias among the included studies. Also, outcome data was incomplete in all the included studies, so the risk of selective reporting was high or unclear (Fig. 2).

2. Meta-analysis

The results showed that pooled RR was 0.80 (95% CI, 0.63 to 1.03; I^2=63.0%; *P*=0.013), although $MgSO_4$ had a protective effect on IVH; however, this effect was not statistically significant (Fig. 4). The results also indicated the heterogeneity of the studies; however, bias in the publication of the results was not statistically significant (χ^2=3.25; *P*=0.352; I^2=8.2%); however, the CI of the test includes zero (Begg's test: Z=0.34; *P*=0.978) (Egger's test: t=0.10; *P*=0.930 95% CI, –0.743 to 0.779).

3. Subgroup analysis

Table 3 shows subgroup analysis by gestational age and $MgSO_4$. The RR for 6 g loading and 2 g/hr regimen of $MgSO_4$ on IVH in premature infants was 0.84 (95% CI, 0.72 to 0.98; I^2=42.5%; *P*=0.345). Also, RR for 4 g loading and 2 g/hr infusion $MgSO_4$ was 1.13 (95% CI, 0.94 to 1.36; I^2=27.8%; *P*=0.250); however, 4-g single dose $MgSO_4$ had a protective effect on IVH in premature infants (RR, 0.86; 95% CI, 0.66 to 1.12; I^2=0.0%; *P*=0.473) (Table 3).

The results of the subgroup analysis based on gestational age showed that the effect of $MgSO_4$ on IVH in premature infants between 24–37 weeks and <34 weeks were 0.93

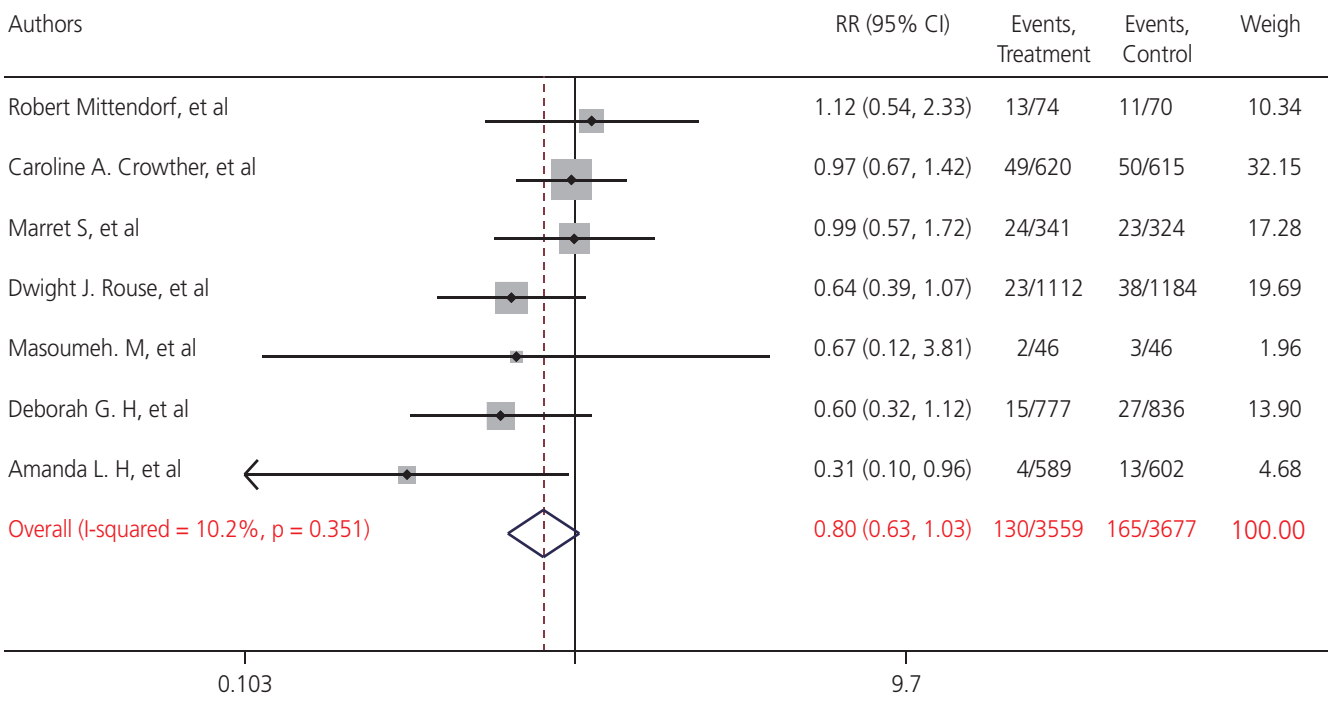

Fig. 4. Frost plot for relative risk (RR) and 95% confidence interval (CI) of magnesium sulfate ($MgSO_4$) on intraventricular hemorrhage (IVH).

Table 3. Summary relative risk (RR) Estimates (95% confidence intervals [CIs]) for randomized controlled trial studies conducted on the effect of antenatal magnesium sulfate ($MgSO_4$) on intraventricular hemorrhage in premature infants by gestational age, and $MgSO_4$ regimen

Subgroup	Number of studies (Sample size)	Summery RR (95% CI)	Between studies			Between subgroups	
			I^2	$P_{heterogeneity}$	Q	Q	$P_{heterogeneity}$
$MgSO_4$ regimen							
6 g loading and 2 g/h infusion	3 (6,592)[a]	0.84 (0.72–0.98)	42.5%	0.345	7.07	6.07	0.048
4 g loading and 2 g/h infusion	3 (1,298)[b]	1.13 (0.94–1.36)	27.8%	0.250	2.77		
4 g single dose	2 (832)[c]	0.86 (0.66–1.12)	0.0%	0.473	0.51		
Gestational age (wk)							
24 to 37	4 (7,654)	0.93 (0.83–1.05)	74.1%	0.009	11.57	16.21	0.013
<34	3 (1,842)	0.91 (0.70–1.18)	56.7%	0.099	4.62		

[a]Hirtz et al. [24], Horton et al. [25], and Rouse et al. [29]; [b]Mirzamoradi et al. [27], Crowther et al. [10], and Mittendorf et al. [30]; [c]Marret et al. [26] and Mittendorf et al. [30].

(95% CI, 0.83 to 1.05; I^2=74.1%; *P*=0.009) and 0.92 (95% CI, 0.70 to 1.18; I^2=56.7%; *P*=0.099), respectively (Table 3).

4. Meta regression

The results of the meta regression analysis, used to explore the sources of interstudy heterogeneity according to gestational age, indicated that the effect of $MgSO_4$ on risk of IVH is unrelated to gestational age (Q test=2.43, df=2, *P*-value=0.467).

Discussion

IVH is one of the most common complications in premature infants, and can cause long-term disability, cerebral palsy, mental retardation, seizures, behavioral and cognitive impairment, and death [31,32]. Studies have shown that the immature antioxidant system of the preterm infant can cause damage to the endothelial cells and alter brain hemostasis, can increase the susceptibility to reactive oxygen species, and, finally, increase the risk for IVH [33-35], Furthermore, studies have shown that approximately one third of cerebral palsy cases and IVH occur in premature infants [36]. Therefore, the present systematic review and meta-analysis study was designed to investigate the effect of antenatal $MgSO_4$ on IVH in premature infants.

The results of our study indicate that although $MgSO_4$ had a protective effect on IVH, this effect is not statistically significant (pooled RR, 0.80; 95% CI, 0.63 to 1.03). Although studies have shown $MgSO_4$ being used for the first time in obstetric practice, there is a controversy about its effect on the outcomes of premature infants [37,38]. Indeed, some studies have concluded that $MgSO_4$ is harmful due to increased risk of death and neurological problems for neonates [39-41]. In contrast, other studies have shown that $MgSO_4$ has a protective and beneficial effect on low birth weight infants [42-44]. For example, a study by Nelson et al. showed that the use of $MgSO_4$ can reduce the IVH incidence (OR, 0.14; 95% CI, 0.05 to 0.51) [42]. In a systematic review study conducted by Doyle et al. [45] with the aim of studying the effect of antenatal $MgSO_4$ on neurologic outcomes in preterm infants, the results showed that use of $MgSO_4$ dramatically reduced the risk of cerebral palsy in the children of women at risk of preterm birth (RR, 0.69; 95% CI, 0.54 to 0.87); also, a significant decrease was observed in the rate of substantial gross motor dysfunction (RR, 0.61; 95% CI, 0.44 to 0.85) [45]. In general, there are many reports that show that $MgSO_4$ increases the antioxidant properties of the brain, protects the brain cells against hypoxia and apoptosis, and normalizes platelet aggregation [46-50]. In other words, the $MgSO_4$ is a tocolytic method for preventing preterm labor. Epidemiological studies have shown that $MgSO_4$ in mothers leads to myocardial stability and blood supply in placenta and fetal brain, as well as reduction of the ischemic region and antioxidant effects, with decreased platelet adhesion in the fetus [51-53].

In contrast, some studies concluded that use of $MgSO_4$ had no effect, or had a minor and insignificant effect, on premature infants [16,17].The large RCTs that were conducted using different doses of $MgSO_4$ have shown an insignificant

reduction in the combined death, cerebral palsy, or gross motor dysfunction among premature infants [10,26,29]. For example, the results of a clinical trial study by Marret et al. [26] showed that although $MgSO_4$ has a protective effect on IVH, this effect was not statistically significant (OR, 0.83; 95% CI, 0.55 to 1.32). Meanwhile, the study by Crowther et al. [10] showed that magnesium sulfate had a protective effect on the risk of IVH; however, this effect was not statistically significant (OR, 0.83; 95% CI, 0.64 to 1.32). Also, a systematic review by Doyle et al. [45] that studied the effect of antenatal magnesium sulfate on neurologic outcome in preterm infants indicated that antenatal magnesium sulfate therapy has no statistically significant effect on pediatric mortality, or on other neurologic impairments or disabilities, in the early years of life of children (RR, 1.01; 95% CI, 0.82 to 1.23). A study by Petrova and Mehta [54] in 2012 revealed that there was no significant association between the use of magnesium sulfate, IVH, and parenchyma injury. Evidently, the results of these studies are consistent with our results. However, the results among various studies in this field are inconsistent, which may be due to differences in study design, study population or sample size, gestational age, follow-up patterns, and/or different doses of the $MgSO_4$.

There several limitations of this study that should be considered when interpreting the results. First, potential publication bias may exist in the observed results, since only some established electronic literature databases were searched. Second, language bias may threaten the results, given that only published articles in English were reviewed. Third, due to lack of detailed information, the quality assessment of the eligible studies may have been influenced by personal judgment. Finally, the last limitation is heterogeneity among the studies. Indeed, the included studies were not directly comparable with each other due to different methods for outcome assessment and experimental variation.

In conclusion, the results of this review showed that although $MgSO_4$ had a protective effect on IVH in neonates, this effect is not statistically significant. However, based on the heterogeneity in study population, sample sizes, gestational age, magnesium sulfate dosage, and follow-up patterns among the included studies, further investigation is needed to evaluate the best dosage, timing and gestational age for the optimum effect of magnesium sulfate on IVH.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Ethical approval

The study is applicable to Institutional Review Board (IRB: IR.IUMS.REC.1397.830).

Patient consent

There is no need for patient consent in this review article.

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Role of interleukin-6 (IL-6) in predicting gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is the most common pregnancy-associated metabolic disorder that is steadily increasing worldwide. Early diagnosis of pregnant women susceptible to GDM is the first step for deploying effective preventive treatment to reduce maternal, fetal, and neonatal complications. The diagnostic process of GDM is still controversial and interleukin-6 (IL-6) is one of the most recent markers used for the diagnosis of GDM. In this study, we aimed to systematically review the role of IL-6 in the diagnosis of GDM. In this systematic review, Google Scholar, Scopus, PubMed, ISI Web of Science, ProQuest, and MEDLINE databases were searched using the following keywords: GDM, screening, and IL-6, with the time interval 2009–2020. The quality of articles was assessed using the Strengthening the Reporting of Observational Studies in Epidemiology checklist. Twenty-four articles with desired quality that met the inclusion criteria were selected and reviewed further. Sixteen studies showed a statistically significant association, while 8 studies did not report any relationship between IL-6 levels and GDM. Based on the results of these studies, assessing the serum IL-6 levels can be investigated a newly established diagnostic biomarker for GDM. Therefore, through early diagnosis of susceptible women, effective measures can be implemented to reduce its complications.

Keywords: Diabetes, gestational; Screening; IL-6

Introduction

Diabetes is a metabolic disorder with steadily increasing prevalence. By 2014, 422 million adults were reported to have diabetes and at least 629 million people will be affected by 2045 if appropriate measures are not taken to reduce it. It has also been reported that high blood sugar causes 4 million deaths each year. The effects of diabetes go beyond the individual level, as it also affects the family and society and have wide-ranging socio-economic consequences [1]. Gestational diabetes mellitus (GDM) is a type of Diabetes, which is defined as diabetes diagnosed during the second or third trimester of pregnancy without prior detection [2]. GDM is a heterogeneous disorder resulting from the interactions between environmental and genetic factors [3]. Obesity and advanced maternal age are associated with the increasing prevalence of GDM worldwide. GDM heightens the potential risk of type 2 diabetes onset in the mother and her offspring [4]. The prevalence of GDM worldwide is estimated to be 17%, varying across different regions, with an estimate of 10% in North America and 25% in Southeast Asia [5].

GDM is associated with adverse pregnancy outcomes, including preeclampsia, polyhydramnios, fetal macrosomia, stillbirth, and neonatal complications such as hypoglycemia, hyperbilirubinemia, hypocalcemia, polycythemia, and respiratory distress [5]. Consequences of GDM extend beyond infancy and pregnancy, increasing the risks of metabolic

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syndrome, impaired glucose tolerance, and obesity in the offspring of affected mothers; it is a robust marker for the diagnosis of type 2 diabetes and diabetes-associated vascular diseases for the mother [6]. Various studies have shown that the lifestyle changes during pregnancy, especially in the early stages of pregnancy, can help in reducing the risk of GDM and also improve the adverse consequences associated with it [7].

Pregnancy represents a complex metabolic and physiological state in women. Insulin resistance plays a crucial role in the pathophysiology of GDM; in normal pregnancy, it can occur due to the increased secretion of diabetogenic placental hormones [8]. Despite over five decades of research, a common consensus on an internationally accepted screening method for GDM is yet to be achieved. Disagreements include the optimal time for screening, appropriate screening test, and general or selective screening methods [9]. According to World Health Organization (WHO), GDM can now be diagnosed with gestational glucose tolerance test using 75 grams of glucose at 24–28 gestational weeks [10]. Oral glucose tolerance test (OGTT) is an unpleasant test requiring consumption of 75 grams of glucose and delaying gastric discharge, which can cause nausea and vomiting. It is also a time-consuming method that requires overnight fasting before collection of 3 blood samples [11]. Moreover, an increase in the maternal blood glucose and fetal growth pathway occurs before 24 weeks of gestation, which has not been diagnosed in GDM. Early diagnosis of pregnant women with GDM and timely treatment can reduce the short- and long-term complications associated with it [12]. Researchers are currently investigating various markers to diagnose GDM, including interleukin-6 (IL-6) [13]. IL-6 is a cytokine produced by immune, adipose, and endothelial cells, and can have significant effects on glucose metabolism. IL-6 also affects pancreatic islet beta cells and enhances insulin secretion [14]. Additionally, inflammatory markers such as IL-6 have also been implicated in the pathogenesis of type 2 diabetes [15].

Numerous studies have been conducted on the association of IL-6 with GDM. Some studies have shown a statistically significant association between elevated IL-6 levels and GDM [4,13,16], while others did not report such relationships [17,18]. To this end, considering the contradictory results regarding the association between IL-6 and GDM, and based on the literature, there is no systematic review discussing the association between IL-6 and GDM. Therefore, in this systematic review, we aimed to investigate the association between IL-6 and GDM.

Methods

1. Search strategy

This study was conducted based on the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses. In order to collect data in a systematic manner, reliable databases such as MEDLINE, ISI Web of Science, PubMed, Scopus, Google Scholar, and ProQuest were used (Table 1).

2. Inclusion and exclusion criteria

Inclusion criteria included all the observational articles published in English and Persian from January, 2009 to February, 2020, in which the healthy pregnant women were in the age group of 18–40 years and screened for gestational diabetes during 24–28 gestational weeks.

Lack of access to the full text of articles, protocol studies, case studies, brief reports, all non-Persian and non-English articles, as well as studies on high-risk pregnant women (over 40 years of age, with body mass index (BMI) over 30, family history of type 2 diabetes, history of polycystic ovary syndrome, thyroid problems, hypertension, diabetes, and tobacco use, and any other disorders affecting the maternal and neonatal health) were excluded.

3. Study selection

In the initial search, 2,578 articles were fetched. Two differ-

Table 1. Search strategy

No.	Search term
#1	'Gestational diabetes' [tiab], OR 'GD' [tiab], OR 'Gestational Diabetes Mellitus' [tiab], OR 'GDM' [tiab], OR 'Diabetes, Pregnancy-Induced' [tiab], OR 'Pregnancy-Induced Diabetes ' [tiab]
#2	'Screening' [tiab], OR 'Predicting'[tiab], OR 'Diagnosis'[tiab]
#3	' Interleukin-6' [tiab], OR 'IL-6' [tiab]
#1 AND #2	
#1 AND #3	
#1 AND #2 AND #3	

ent researchers reviewed these articles and disagreements were resolved by a third one. Subsequently, 1,478 duplicated articles were removed. After reviewing the titles and abstracts, 900 more articles were excluded. After reviewing the full text of the articles in the next step, 100 articles were removed due to incompetence. Finally, 24 articles were considered sufficiently qualified and eligible for further reviewing (Fig. 1).

4. Quality assessment

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statements were used to assess the quality of the studies. The STROBE statement as a valid tool consists of a checklist of 22 items to assess the quality of different parts of the observational studies [19,20].

5. Data extraction

Initially, the selection and evaluation of studies were performed independently by the 2 researchers, and the disagreements were resolved by a third one. Information on the first author's name, year of publication, geographic region, study design, participants, BMI, sample, test time, test analysis method, diagnostic criteria of GDM, and levels of IL-6 were extracted and considered for the analysis.

Results

According to the process of search for articles, flowchart of which is presented in Fig. 1, 24 relevant high quality articles were selected and considered in this study after thoroughly reviewing the selected articles. Quality of the selected articles was assessed on the basis of the STROBE checklist (Fig. 2).

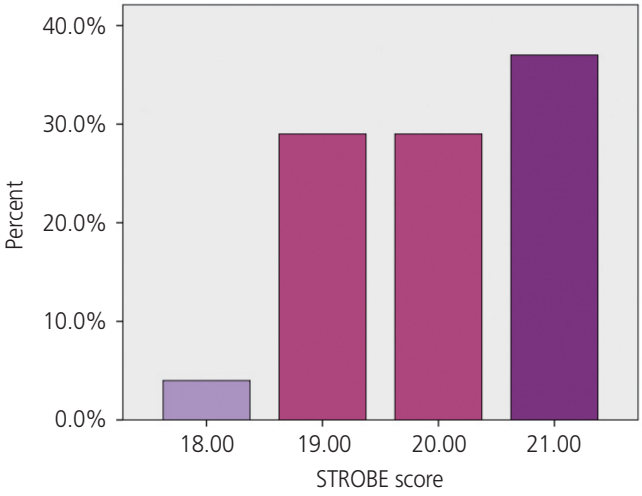

Fig. 2. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) score of different studies.

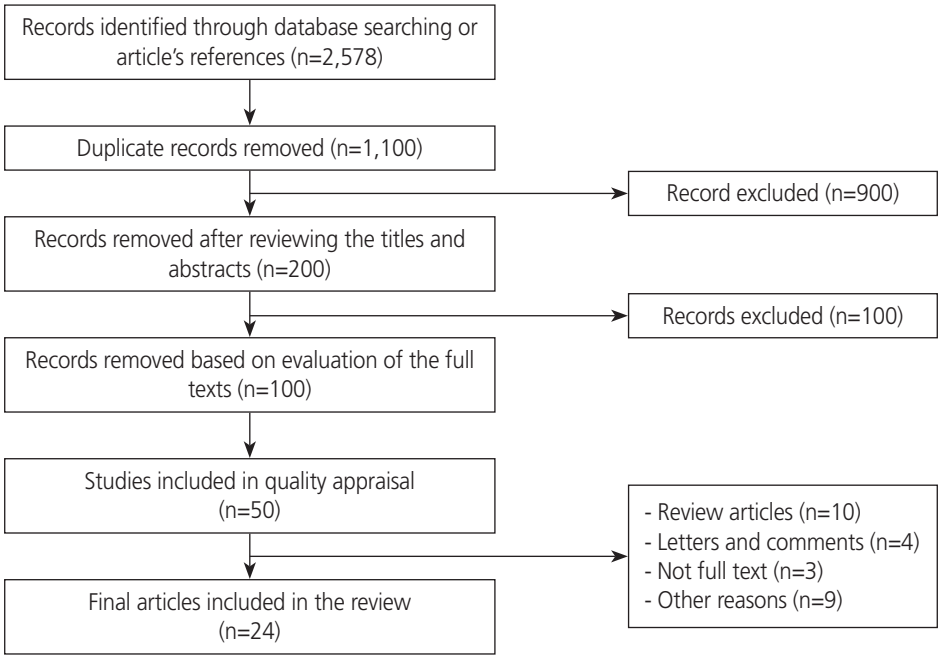

Fig. 1. Flow diagram for searching the articles.

These studies included articles published from 2009 to 2020, which were mainly case-control (n=17), cross-sectional (n=2), observational (n=2), cohort (n=2), and longitudinal (n=1) studies. A total of 2,806 pregnant women participated in these studies and blood samples were collected for measuring IL-6 levels in 3 studies during the first trimester (12%), in 18 studies during the second trimester (75%), and in 3 studies during the third trimester (12%) (Fig. 3).

The characteristics of the selected studies are listed in Table 2. These studies were conducted in different countries, including China (6), Turkey (4), US (2), Poland (2), India (2), Australia (1), Brazil (1), Prague (1), Tunisia (1), Saudi Arabia (1), Canada (1), Finland (1), and Greece (1). Moreover, for the diagnosis of GDM, 5 studies used the guidelines of Carpenter and Coustan, 1 study used the Indian criteria, 2 studies used the guidelines of International Association of Diabetes and Pregnancy Study Groups (IADPSG), 5 studies used the American Diabetes Association (ADA) guidelines, 4 studies used the National Diabetes Data Group guidelines, 2 studies used the China Diabetes Association Diabetes Branch guidelines, 1 study used the Australasian Diabetes in Pregnancy Society guidelines, 1 study used the Polish Diabetes Association guidelines, 1 study used the American College of Obstetricians and Gynecologists (ACOG) guidelines, 1 study used the Canadian Diabetes Association guidelines, 1 study used the WHO guidelines, 1 study used the Endocrine Society Clinical Practice Guideline/ACOG guidelines, 1 study used the ACOG/ADA guidelines, and 1 study used the Carpenter and Coustan/ADA guidelines. The majority of the studies measured IL-6 levels using the enzyme-linked immunosorbent assay (18), while the others employed multiplex immunoassay (4) or chemiluminescent immunoassay (2).

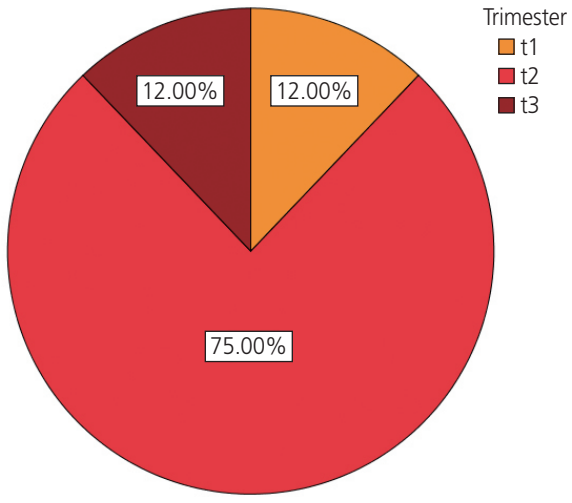

Fig. 3. Trimester in which the interleukin-6 test was performed.

Fig. 4 shows the significance level of the selected studies. As shown in this diagram, 16 studies exhibited a significant relationship between the serum level of IL-6 and GDM. Therefore, we can conclude that the serum IL-6 level may act as a suitable diagnostic marker for GDM.

Discussion

In the present systematic review, 24 articles were reviewed, most of which demonstrated a significant relationship between IL-6 levels and GDM. Thus, we concluded that IL-6 can be used as a marker to predict the occurrence of gestational diabetes.

Accurate and early diagnosis of women at a high risk of developing GDM provides an opportunity to manage the prenatal care models and apply future interventions to reduce the progression of gestational diabetes, and thereby its associated health care expenses and side effects [40]. However, the diagnostic criteria for GDM is still debatable. A previous study reported a linear relationship between maternal blood glucose levels and adverse perinatal outcomes [41]. The IADPSG recommends that the studies aimed to diagnose gestational diabetes must develop simpler, more cost-effective methods that do not require OGTT [42]. In recent years, there has been a great interest in identifying the role of inflammation during the development of GDM. Inflammatory factors can act as insulin antagonists and cause insulin resistance [43]. IL-6, as a cytokine, plays a crucial role in the pathophysiology of glucose intolerance and serves as a potential serum marker for early screening of glucose intolerance [43].

In type 2 diabetes, inflammatory cytokines may induce insulin resistance by suppressing multiple pathways in target tissues that are responsible for proper insulin signaling [44]. Insulin resistance is associated with abnormal secretion of proinflammatory cytokines, such as IL-6 [4]. In non-pregnant women, BMI and high body fat mass have been found to be associated with elevated levels of serum IL-6 [13]. IL-6 is also secreted by the placenta during pregnancy, which can lead to a chronic inflammatory process in adipose tissue and further assist the development of pregnancy-induced insulin

Table 2. Results from a systematic review of studies

Author	Geo-graphic region	Study design	GDM group		Control group		Sample	Time of collect test (wk)	Method of analysis test	Diagnos-tic criteria of GDM	GDM group	Control group	Results (*P*-value)
			Partici-pants	BMI	Partici-pants	BMI					IL-6	IL-6	
Wang et al. [21]	China	Case-control	60	NR	50	NR	Serum	24–28	ELISA	ADA	96.66 (88.33–106.66)[a]	83.32 (78.34–88.33)[a]	Significant (*P*<0.001)
Braga et al. [17]	Brazil	Observational	78	27.8 (23.6–32.1)[a]	98	22.8 (20.9–27.3)[a]	Serum	24–28	Multiplex immunoassay	Carpenter and Coustan	0.175 (0.120–0.298)[a]	0.155 (0.100–0.325)[a]	Not significant (*P*=0.77)
Sudharshana Murthy et al. [4]	India	Observational	30	25.7[b]	30	25[b]	Serum	24–28	ELISA	India criteria	2.96±1.37[c]	2.88±1.21[c]	Significant (NR)
Siddiqui et al. [13]	India	Case-control	53	25.3±4.08[c]	50	23.28±2.95[c]	Serum	24–31	ELISA (disclose)	ADA	0.72±0.67[d]	0.39±0.70[d]	Significant (*P*=0.001)
Šimják et al. [18]	Prague	Case-control	12	V1: 26.04±3.75[d] V2: 27.59±3.54[d]	12	V1: 27.63±4.59[d] V2: 28.74±4.63[d]	Serum	V1: 28–32 V2: 36–38	Multiplex immunoassay	IADPSG	V1 :4.33±0.81[d] V2: 5.02±1.13[d]	V1: 2.78±0.37[d] V2: 3.69±0.29[d]	Not significant (NR)
Yang et al. [16]	China	Cohort	21	31.60±3.70[c]	34	31.70±3.10[c]	Serum	<13	ELISA	Carpenter and Coustan	17/5 (12–23)[a]	11 (5–7)[a]	Significant (*P*=0.001)
Yu et al. [22]	China	Case-control	80	NR	60	NR	Serum	24–29	ELISA	CDADB	59.44±3.95	22.81±1.54	Significant (*P*=0.021)
Zhao et al. [23]	China	Cross sectional	29	28.10±3.20[c]	32	24.10±3.00[c]	Serum	24–28	ELISA	Carpenter and Coustan	5.10±1.20[c]	2.96±0.70[c]	Significant (*P*<0.001)
Abell et al. [24]	Australia	Cohort	25	28.0 (26.0–31.0)[a]	78	29.5 (25.5–34.0)[a]	Serum	12–15	ELISA	ADIPS	2.03 (1.67–2.90)[a]	1.92 (1.57–2.56)[a]	Not significant (*P*=0.428)
Zhang et al. [25]	China	Case-control	60	38.68±9.50[c]	60	28.74±7.26[c]	Serum	24–28	Multiplex immunoassay	ESCPG/ ACOG	5.85±1.41[c]	3.91±1.66[c]	Significant (*P*=0.002)
Bossick et al. [26]	USA	Case-control	18	37.10±7.70[c]	167	28.40±7.10[c]	Serum	13–28	Multiplex immunoassay	ACOG/ ADA	5.40±2.00[c]	3.90±2.10[c]	Significant (*P*=0.002)
Oztop et al. [27]	Turkey	Case-control	30	26.4 (17.3–44.1)[a]	20	24 (21–26)[a]	Serum	24–26	ELISA	ADA	3.10±0.90[c]	2.70±0.70[c]	Significant (*P*=0.04)
Qu et al. [28]	China	Case-control	53	32.17±4.36	57	23.62±3.51	Serum	22–30	ELISA	Carpenter and Coustan	4.43±0.45[c]	2.57±0.19[c]	Significant (*P*=0.0004)
Gümüş et al. [29]	Turkey	Case-control	101	27.00±5.00[c]	66	22.00±3.00[c]	Serum	24–28	ELISA	ADA	19.21±29.00[c]	15.49±19.13[c]	Not significant (*P*=0.933)

Table 2. Continued

Author	Geographic region	Study design	GDM group		Control group		Sample	Time of collect test (wk)	Method of analysis test	Diagnostic criteria of GDM	GDM group	Control group	Results (*P*-value)
			Participants	BMI	Participants	BMI					IL-6	IL-6	
Hassiakos et al. [30]	Greece	Case-control	40	25.36[e]	94	23.16[e]	Serum	11–14	Chemiluminescent immunoassay	IADPSG	2.0 (1.60)[f]	1.5 (1.01)[f]	Significant (*P*=0.001)
Kim et al. [31]	USA	Case-control	32	24.1±0.9[d]	227	24.9±0.6[d]	Serum	24–28	ELISA	Carpenter and Coustan	2.6±0.2[d]	2.9±0.9[d]	Not significant (NR)
Kuźmicki et al. [32]	Poland	Case-control	46	V1: 25.0 (24.2–28.0)[a] V2: 25.0 (24.2–28.0)[a]	45	V1: 23.0 (20.4–26.2)[a] V2: 23.0 (20.4–26.2)[a]	Serum	V1: 24–28 V2: 29–32	ELISA	PDA	V1: 0.99 (0.86–1.27)[a] V2: 1.13 (0.98–1.57)[a]	V1: 0.89 (0.75–1.07)[a] V2: 1.02 (0.83–1.22)[a]	Significant (*P*<0.05)
Nergiz et al. [33]	Turkey	Case-control	30	30.20±5.60[c]	30	29.10±30.00[c]	Serum	>24	ELISA	ACOG	32.5 (24.8–48)[a]	7.1 (4.2–9.8)[a]	Significant (*P*=0.001)
Özyer et al. [34]	Turkey	Case-control	35	30.40±4.30[c]	70	25.30±3.80[c]	Serum	24–28	Chemiluminescent sequential immunometric assay	Carpenter and Coustan/ ADA	2.0 (2.0–2.00)[a]	2.0 (2.0–2.51)[a]	Not significant (*P*=0.08)
Mrizak et al. [35]	Tunisia	Case-control	28	28.50±1.80[c]	24	29.7±2.23[c]	Serum	24–26	ELISA	NDDG	77.84±10.98[c]	53.17±1.45[c]	Significant (*P*=0.002)
Abdel Gader et al. [36]	Saudi Arabia	Cross-sectional	150	34.40±5.90[c]	100	30.60±4.60[c]	Serum	35–40	ELISA	NDDG	13.70+2.50[c]	13.90+15.30[c]	Not significant (NR)
Morisset et al. [37]	Canada	Longitudinal	20	28.20±7.50	27	24.20±4.30	Serum	26.1±3.7	ELISA	CDA	1.47±0.72	0.90±0.32	Significant (*P*≤0.05)
Pöyhönen-Alho et al. [38]	Finland	Case-control	38	30.6	21	26.9	Plasma	24–28	ELISA	ADA	4.9±2.21[c]	4.91±2.82[c]	Not significant (*P*=0.58)
Kuzmicki et al. [39]	Poland	Case-control	81	22.1 (20.5–24.9)[a]	82	23.1 (20.3–25.0)[a]	Serum	24–31	ELISA	WHO	1.0 (0.7–1.5)[a]	0.8 (0.5–1.1)[a]	Significant (*P*=0.006)

GDM, gestational diabetes mellitus; BMI, body mass index; IL, interleukin; V1, visit 1; V2, visit 2; ELISA, enzyme-linked immunosorbent assay; ADA, American Diabetes Association; IADPSG, International Association of Diabetes and Pregnancy Study Groups; CDADB, China Diabetes Association Diabetes Branch; ADIPS, Australasian Diabetes in Pregnancy Society; ESCPG, Endocrine Society Clinical Practice Guideline; ACOG, American College of Obstetricians and Gynecologists; PDA, Polish Diabetes Association; NDDG, National Diabetes Data Group; CDA, Canadian Diabetes Association; WHO, World Health Organization; NR, not reported; SD, standard deviation.

[a]Median (interquartile range); [b]Mean; [c]Mean±SD; [d]Means±standard error of the mean; [e]Median; [f]Median (SD).

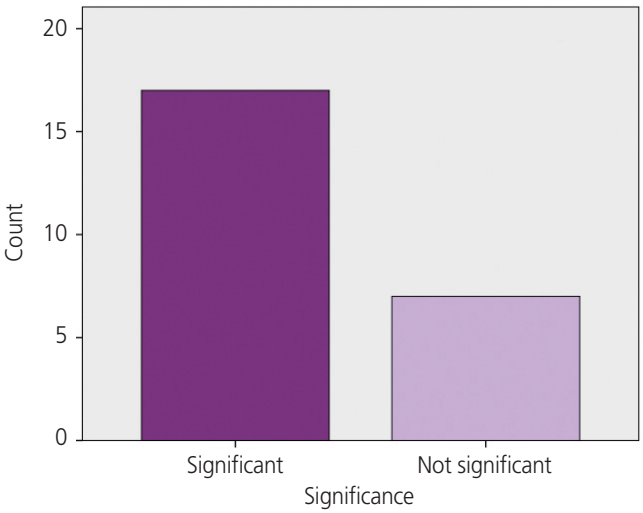

Fig. 4. Assessing the significance level of different studies.

resistance [45]. In GDM, similar to type 2 diabetes, insulin resistance is implicated in the pathophysiology [46]. Type 2 diabetes is considered a chronic inflammatory disease and IL-6 is a risk factor for the development of type 2 diabetes. Therefore, due to similar mechanisms underlying the onset of GDM and type 2 diabetes, IL-6 might act as an effective marker in predicting GDM.

In this systematic review, quantitative analysis of available data on the relationship between serum IL-6 levels and GDM was performed based on 24 selected articles, of which 16 articles showed an association between elevated serum IL-6 levels in women and GDM. For example, the results of a case-control study conducted by Siddiqui et al. [13] that aimed to investigate the association of IL-6 and C-reactive protein with GDM in Indian women demonstrated that the serum levels of IL-6 in women with GDM was significantly higher than in control women. Moreover, IL-6 levels were also associated with pre-pregnancy BMI, fasting blood sugar, and postprandial blood sugar . A study conducted by Yu et al. [22], which aimed at investigating the changes in gut flora and various inflammatory factors in patients with GDM, showed that the serum levels of inflammatory factors, including IL-6, were significantly higher in the case group than that in the control group; moreover, patients with GDM were highly susceptible to intestinal flora imbalances with elevated inflammatory factors, which affected the immune function in these patients and may play an important role in the development of diabetes. Furthermore, the findings of the study conducted by Zhao et al. [23], which aimed to examine the possible association of inflammatory markers with glucose intolerance and GDM in Chinese women, indicated that IL-6 levels were significantly higher in pregnant women with GDM or glucose intolerance compared to those in the healthy control group, and that there was a positive relationship between inflammatory cytokines, BMI, and HbA1c . In the study conducted by Zhang et al. [25], aimed at investigating the association between inflammatory and metabolic biomarkers in women with GDM in Mongolia, showed that the levels of inflammatory and placental biomarkers, including IL-6, in both serum and placenta showed a significant difference between women with GDM and those with healthy pregnancies.

The present review also included studies that did not show a correlation between the serum levels of IL-6 and GDM, such as the study conducted by Abell et al. [24], which aimed at evaluating the relationship between GDM risk using fasting glucose and serum biomarkers at the early pregnancy, and indicated that the serum IL-6 levels did not improve the ability to predict the risk of GDM. The results of another study conducted by Gümüş et al. [29] aimed to evaluate the possible association between clinical and biochemical parameters with GDM and gingivitis; this study showed that there was no association between the serum IL-6 levels and GDM. Similar results were obtained in an another study conducted by Özyer et al. [34] that aimed at investigating the association of inflammatory mediators, including IL-6, with glycemic status in pregnancy; the results of this study indicated that the maternal serum levels of inflammatory mediators are not relevant for assessing GDM during the late second or early trimester. The reason for such differences in the results of these studies can be attributed to the use of different methods and kits for measuring IL-6 levels and dissimilar diagnostic criteria for GDM. Also, the effects of confounding variables on serum IL-6 levels and GDM were not considered in all of the selected articles.

Increased IL-6 secretion during pregnancy has been associated with GDM in several studies. Conversely, OGTT testing with 75 grams of oral glucose, which is currently the gold standard test, is performed almost late in pregnancy and requires overnight fasting. Moreover, consumption of glucose is not very pleasant for a pregnant woman and it requires the collection of blood samples 3 times during the process. However, assessing IL-6 levels does not have the above-

mentioned challenges and it is easy, affordable, and tolerable for a pregnant woman. Therefore, IL-6 can be used as a marker for assessing the risk of GDM in pregnant women. Considering the increasing prevalence of gestational diabetes worldwide and the need for timely diagnosis and treatment of GDM to reduce adverse maternal and fetal complications, an acceptable marker such as IL-6 is urgently needed.

Conclusion

The results of this study indicate that serum IL-6 levels are significantly higher in pregnant women with GDM than in healthy pregnant women. Therefore, the evaluation of this marker as an acceptable, inexpensive, and readily available diagnostic criterion for assessing the risk of GDM can be investigated.

One of the limitations of this systematic review is the heterogeneity of the diagnostic criteria for GDM. Different commercial kits and assays have been employed to measure the serum IL-6 levels. Conversely, based on the extensive research and use of multiple studies with different ethnicities, it is difficult to control the effect of this variable. Additionally, all the previous studies that were considered did not offer adequate clinical information for performing a robust meta-analysis.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study has a code of ethics No. IR.ABZUMS.REC.1398.218 approved by Alborz University of Medical Sciences.

Patient consent

There is no need for patient consent in this review article.

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Fertility-sparing treatment in early endometrial cancer: current state and future strategies

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Endometrial cancer (EC) is the fifth most common cancer in women worldwide. Global estimates show rising incidence rates in both developed and developing countries. Most women are diagnosed postmenopausal, but 14–25% of patients are premenopausal and 5% are under 40 years of age. Established risk factors include age and hyperestrogenic status associated with nulliparity, obesity, and metabolic syndrome. Standard treatment for EC, which involves total hysterectomy and bilateral salpingo-oophorectomy, has excellent survival outcomes, particularly for low-grade endometrioid tumors. However, it leads to permanent loss of fertility among women who wish to preserve their reproductive potential. With current trends of reproductive-age women delaying childbearing, rising EC incidence rates, and a growing epidemic of obesity, particularly in developed countries, research on conservative non-surgical treatment approaches remains a top priority. Fertility-sparing treatment predominantly involves the use of oral progestins and levonorgestrel-releasing intrauterine devices, which have been shown to be feasible and safe in women with early stage EC and minimal or no myometrial invasion. However, data on the efficacy and safety of conservative management strategies are primarily based on retrospective studies. Randomized clinical trials in younger women and high-risk obese patients are currently underway. Here, we have presented a comprehensive review of the current literature on conservative, fertility-sparing approaches, defining the optimal candidates and evaluating tumor characteristics, reproductive and oncologic outcomes, and ongoing clinical trials. We have also summarized current guidelines and recommendations based on the published literature.

Keywords: Endometrial cancer; Fertility preservation; Conservative treatment; Progestin

Introduction

Endometrial cancer (EC) is the fifth most common gynecological cancer in developed countries, with an estimated worldwide incidence of 382,069 new cases per year [1,2]. The highest age-standardized incidence rates in 2018 were estimated to be 19.1 and 22.2 per 100,000 women in North America and Europe, respectively [2,3], attributable to the greater overall prevalence of obesity and metabolic syndromes [4]. Although the age-standardized rates were

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the lowest in middle-income countries, such as South Africa, Japan, and Brazil, these countries had the most rapid rates of increase in the past 10 years, at 11.3%, 5.9%, and 5% annually, respectively. Similar trends were observed for premenopausal women and an increased risk of EC over successive generations was more pronounced in some Asian countries, Costa Rica, and New Zealand [5].

Although EC is typically considered a postmenopausal cancer, 14%–25% of patients are premenopausal and 5% are younger than 40 years; majority of the patients tend to present with low-grade early stage tumors of the endometrioid subtype that are confined to the endometrium [6-8]. Historically, EC has been categorized into 2 clinicopathological types—type 1 EC, the estrogen-dependent endometrioid type associated with obesity that accounts for up to 85% of ECs, and type 2, the non-endometrioid subtypes that include serous, clear-cell, undifferentiated carcinomas and malignant-mixed Mullerian tumors and are typically not associated with obesity [9,10]. Tumor grade and myometrial invasion increase with age, accounting in part for the considerably worse prognosis of older patients [11,12].

Established risk factors for EC include exposure to exogenous estrogens or endogenous hyperestrogenic status associated with nulliparity, early age at menarche, late-onset menopause, age, and obesity [13]. Women with greater weight gain after 20 years of age had a 3-fold increased risk of EC of any subtype and a 6-fold increased risk of the endometrioid subtype [14]. A higher fasting insulin level has been linked to EC risk, regardless of the menopausal status [15]. It has also been causally linked to EC risk independently of body mass index (BMI) [16].

The survival outcomes following current standard surgical treatment for EC are good, ranging from 74% to 91%, particularly for women diagnosed with low-grade endometrioid tumors without lymph node involvement [17]. A Danish nationwide survey of EC patients with stage I tumors reported a 5- and 10-year disease-specific survival of 99% and 98%, respectively [18]. Younger women of reproductive age have significantly better disease-specific survival than older women independent of other prognostic indicators [19]. However, medical comorbidities can negatively impact survival outcomes. Diabetes and obesity are negative predictors of all-cause and disease-specific mortality, regardless of the tumor type, and the presence of more than 2 major comorbidities other than diabetes significantly predicts cancer-specific mortality [20]. Cardiovascular disease is also a frequent cause of death among women who survive EC beyond 5 years [21].

The standard treatment for EC involving total hysterectomy and bilateral salpingo-oophorectomy (THBSO) with or without lymphadenectomy is effective [22]. Fertility-sparing treatment approaches for patients who wish to preserve childbearing involve endocrine treatments with oral progestins, medroxyprogesterone acetate (MPA) or megestrol acetate (MA), or gonadotropin-releasing hormone (GnRH). More recently, levonorgestrel-releasing intrauterine devices (LNG-IUDs) have been used. However, there are inherent risks involved in conservative approaches, including the risk of the treatment being ineffective, the risk of relapse, and missing a diagnosis of ovarian or lymph node involvement (advanced stage disease) or synchronous ovarian cancer.

From this point forward, the term "fertility preservation" will refer to the preservation of the uterus. The role of ovarian preservation as part of the surgical management of EC is outside the scope of this review.

Given the current trends of women of reproductive-age delaying child-bearing and the increasing incidence of EC amongst nulliparous women [23,24], high-level evidence on alternatives to THBSO are urgently required. Generating evidence on conservative treatments for low-risk EC has become a top priority [25].

Defining candidates for fertility-sparing treatment

1. Young women desiring childbearing capacity

Women of child-bearing age with an EC diagnosis frequently have early-stage, low-grade disease [7,19,26] and although the standard treatment of THBSO is effective, they should be considered for non-surgical fertility-sparing treatment. The risks include missing occult lymph nodes or ovarian metastases. One possible solution could be a surgical exploration (including surgical node assessment) prior to fertility-sparing treatment. Rigorous follow-up is imperative. Estimates of age at first birth in the US show that 1 of 12 first births in 2006 were to women aged 35 and older, compared to 1 of 100 in 1970 [27].

2. Women with significant medical co-morbidities

For elderly women, substituting major surgery with effective

conservative alternatives may prevent major surgical complications. Patients with multiple medical comorbidities have a significantly higher risk of intra- and post-operative complications, including a prolonged hospital stay [28,29]. THBSO performed by laparoscopy in patients with early-stage EC was associated with less blood loss and pain, faster recovery, and a shorter hospital stay [30]. A recent meta-analysis of both the LACE [31] and the LAP2 [32] trials reported comparable survival outcomes for patients who had minimally invasive and those who had open surgery for EC [33]. Our group found that older patients with multiple pre-existing comorbidities, increased complexity of surgical procedures, higher American Society of Anesthesiologists (ASA) scores ≥2 (the ASA scoring system classifies patients' preoperative physiological status and operative risk), and high BMI were independent predictors of any adverse event [34]. A prediction model that quantifies the risks of adverse events in patients undergoing surgical procedures for EC has also been outlined [35].

3. Obese women in whom surgery is technically infeasible

Perioperative risks and length of hospital stay are higher in obese women [35]. The rate of conversion from laparoscopy to laparotomy increases with higher BMI, with 57% of patients with BMI ≥40 kg/m^2 requiring conversion [32]. Adverse events significantly increase healthcare costs [36]. The efficacy of treatment alternatives that are safe and less invasive needs to be addressed in this growing population of patients with obesity and EC, and the feMMe trial currently underway aims to evaluate fertility-sparing non-surgical treatment options in obese patients [37].

4. Tumor characteristics

Traditionally, the most suitable candidates for fertility-sparing treatment of EC have been patients with grade 1 tumors of the endometrioid subtype, with or without superficial invasion into the myometrium. These patients are more likely to present with estrogen- and progesterone-receptor positive tumors and progestin treatment results in encouraging outcomes. Patients with poorly differentiated tumors and deeply invasive disease (>50%) are considered ineligible for fertility-sparing treatment by most investigators. Some guidelines even suggest that any myometrial invasion should be considered a contraindication to fertility preservation [38]. Leitao et al. [39] compared grade 1 tumors diagnosed preoperatively with dilatation and curettage (D&C) or pipelle biopsy and found that significantly fewer tumors diagnosed by D&C were upgraded in the final hysterectomy specimen than those diagnosed by pipelle biopsy (8.7% vs. 17.4%; *P*=0.007). Given reported disparities between pathologists in differentiating between complex atypical endometrial hyperplasia and grade 1 EC [40,41], the accuracy of the final histological diagnosis for fertility-sparing treatment may be improved by a pathological review.

Patients with low-risk pathological features can expect a 5-year overall survival rate ranging from 80% to 90%. The accuracy of various methods to assess myometrial invasion have been widely reported in the literature. A meta-analysis of 9 studies found that both dynamic contrast-enhanced and diffusion-weighted magnetic resonance imaging (MRI) had high diagnostic accuracy in detecting deep myometrial invasion (86% pooled sensitivity for both methods) in women undergoing preoperative staging [42]. An earlier study suggested a high negative predictive value for deep myometrial invasion [43]. The implementation of both MRI and transvaginal ultrasonography (TVUS) appears to be highly effective in assessing myometrial invasion [44,45]. Similarly, comparisons of computed tomography, TVUS and MRI showed similar performance overall, but significantly better assessment of myometrial invasion with contrast-enhanced MRI [46]. TVUS performed by experts specializing in gynecological oncology had diagnostic accuracy comparable to MRI and greater inter-observer reproducibility for evaluating deep myometrial and cervical stromal invasion than that performed by general gynecologists [45,47].

5. Synchronous or metastatic ovarian cancer

The reported rate of synchronous ovarian cancer in women with EC varies from 5% to 29% [26,48,49]. Although EC patients aged ≤45 years are 5 times more likely to have synchronous ovarian tumors than women aged >45 years, nulliparity and not age is an independent risk factor for synchronous ovarian cancer [26]. A large multi-center analysis by the Korean Society of Gynecologic Oncology found that while the incidence of synchronous ovarian cancer in young women with EC was quite low (4.5%), synchronous ovarian cancer was not identified in women with low-risk EC [50]. A retrospective chart review of women aged 24–45 years found that 88% of coexisting ovarian tumors identified at

hysterectomy were synchronous primaries and 69% occurred in women with grade 1 EC [51]. There is evidence from the Surveillance, Epidemiology, and End Results database that the overall rate of synchronous ovarian cancer in EC patients has declined in the past 2 decades, but that rates peak between 29 and 47 years of age, followed by a significant decline [52].

Synchronous endometrial and ovarian carcinomas are seemingly independent co-occurrences of tumors at both the ovary and endometrium and are seen in 5% of EC patients and 10% of ovarian cancer patients.Two groups have demonstrated a clonal relationship between each site [53,54], disproving the concurrent theory of independent development of both tumors and suggesting that these cancers arise at one anatomic location and undergo restricted metastasis ("pseudometastases") to the second site. Failure to recognize a synchronous endometrial and ovarian carcinoma could allow the ovarian mass to progress and further metastasize, thereby changing a surgically curable disease into one requiring adjuvant chemotherapy with or without radiation, with an increased risk of recurrence and death from disease [55,56].

6. Family cancer history and genetic syndromes

Patients presenting with EC at a young age may have a genetic etiology. The risks associated with family history of EC have been well documented in meta-analyses and population-based studies [57,58] and may encompass a range of factors including shared environmental and lifestyle choices that may be influenced by genetic predisposition. The lifetime risk of EC in women with Lynch syndrome is estimated to be 40% to 60% and is primarily associated with germline mutations in one of the mismatch repair genes (MMR), *MLH1*, *MSH2*, *MSH6*, or *PMS2* [59]. Analysis of a large series of Lynch families showed that the cumulative risk of EC by 70 years of age was substantially higher among *MLH1* mutation carriers (54%), but the risks did not exceed 2% in women under age 40 [60]. Population-based studies show that MMR defects underlie <5% of EC at the population level, and the risk depends on which MMR gene is disrupted [61]. Current National Comprehensive Cancer Network (USA) guidelines for hereditary cancers recommend that all women with EC undergo genetic testing for MMR mutations, particularly women under 50 years of age and those from Lynch families [62]. Germline loss-of-function variants in the tumor suppressor gene *PTEN* have also been associated with increased risk of EC [63]. A comprehensive review of the evidence for genes implicated in hereditary EC and the available commercial testing panels have been presented in detail elsewhere [64].

At an increasing number of gynecological cancer centers, all patients diagnosed with EC are evaluated for Lynch syndrome by immunohistochemical staining of tumor histopathology specimens for MLH1, MSH2, PMS2, and MSH6 proteins, which are surrogate markers for Lynch syndrome. A diagnosis of Lynch syndrome and the associated risks for EC and ovarian cancer might impact a patient's decision regarding conservative, fertility-sparing treatment for EC.

Fertility-sparing management

1. Hormonal therapy

Fertility-sparing management of EC typically involves the use of the oral progestins MPA or MA, or more recently the LNG-IUD. The effectiveness of oral progestins remains unproven through randomized controlled clinical trials and the optimal dose and duration of progestin use have not been established. Current recommendations are MPA at a dose of 400–600 mg/day or MA at a dose of 160–320 mg/day for a minimum of 6 months, with follow-up assessment of treatment response using D&C and imaging [38,65]. The LNG-IUD releases 52 mg of intrauterine progestin at a consistent rate for up to 5 years but declines thereafter.

Progestin use in women undergoing fertility-sparing treatment for atypical hyperplasia and EC have consistently shown good overall response rates (76–81%) [66-68]. MA has been linked to higher remission probabilities compared to MPA and other hormonal treatments [66], which may be due to the relatively higher bioavailability of MA compared to MPA following oral administration [69]. A study of Korean women aged ≤40 years with grade 1 endometrioid EC treated with daily oral MPA or MA had similar rates of complete response (77.7%) after a median follow-up of 66 months; however, MPA was associated with a lower risk of recurrence compared to MA and BMI ≥25 kg/m^2 was a significant factor in failure to achieve a complete response [70]. Weight changes during treatment with progestin therapy were also shown to have a minimal effect on response rates, and pre-treatment and post-treatment BMI ≥25 kg/m^2 were significantly associ-

ated with a higher rate of recurrence, highlighting the importance of maintaining normal BMI during progestin treatment [71]. Obese patients are hyperestrogenic and suppressing the production of endogenous estrogen may be an effective alternative. A recent pilot study of a GnRH agonist combined with an aromatase inhibitor in fertility-sparing treatment of 6 obese women with grade 1 EC reported a complete response in all women at 3–6 months, and a pregnancy and live birth rate of 50% and 75% respectively [72]. A prospective observation study of LNG-IUD for 1 year plus a GnRH analogue for 6 months in patients aged <40 years with stage 1A EC showed results comparable to studies using MPA and MA, with a complete remission rate of 57% and a recurrence rate of 25% [73]. Although most studies report no significant toxicity among patients treated with high-dose oral progestins, adverse effects include thrombophlebitis, pulmonary emboli, weight gain, hypertension, and headaches, which may lead to low patient compliance [40,74,75].

The LNG-IUD circumvents the issues with patient noncompliance that accompany oral medication as well as the possible side-effects associated with high-dose oral progestins. A recent meta-analysis of 5 randomized controlled trials comparing the LNG-IUD to oral cyclic MPA treatment found that although regression rates for both were similar overall, regression rates were higher for non-atypical endometrial hyperplasia and mixed (atypical and non-atypical) endometrial hyperplasia therapy among non-obese women [76]. A prospective multi-center study to determine the efficacy of the LNG-IUD in combination with oral MPA at 500 mg/day in early-stage EC patients aged <40 years has been undertaken by the Korean Gynecologic Oncologic Group [77]. Several other randomized controlled trials are currently underway to evaluate the efficacy of LNG-IUDs with or without oral progestins or metformin in younger women with low-grade EC (Table 1).

2. Hysteroscopic tumor resection

Hysteroscopy as part of conservative management is controversial. Proponents claim it allows a more targeted removal of the primary site of disease plus adjacent margins and myometrium and permits a more accurate assessment of tumor characteristics through adequate biopsy sampling. Others find it challenging to distinguish the cancer tissue from surrounding hyperplasia. Studies reporting outcomes related to the use of hysteroscopy in conservative management are limited to case studies and case series. Alonso et al. [23] reviewed studies published between 1975 and 2014 of EC patients aged <40 years treated with initial hysteroscopic resection followed by hormone therapy for fertility-sparing and found that the complete response rate for patients with stage 1A grade 1 EC was 88.9%. Pregnancy rates in these studies among those who attempted conception, including through assisted reproductive technology (ART), ranged from 25% to 66%, suggesting the potential for superior outcomes compared to hormone treatment alone. Recent studies have confirmed these findings, reporting complete response rates in women with stage 1A EC of 89% to 97% following hysteroscopic fertility-sparing treatment, and pregnancy rates of over 45% among women who attempted conception [78,79]. Concerns have been raised about possible negative consequences of hysteroscopy due to EC cells spreading into the peritoneal cavity and the likelihood of adverse pregnancy outcomes due to mechanical damage to the basal layer of the endometrium causing Asherman's syndrome and a risk of placenta accreta [80].

3. Follow-up examinations

It is critically important that patients who are considered for fertility-sparing treatment understand and accept the need for close follow-up and endometrial sampling to evaluate the response to treatment. The optimal duration of progestin treatment that would achieve a complete response has been established, but there is little evidence to suggest added benefit beyond 6 months. A meta-analysis of 24 studies involving 370 patients undergoing fertility-sparing treatment showed that the remission probability after 6 months of treatment was 72% compared to 78% after 12 months of treatment, suggesting marginal benefit beyond 6 months [66]. There is variation across gynecologic oncology societies regarding the frequency of follow-up and method of endometrial sampling, primarily because of differences in treatment protocols, but current recommendations are for histological evaluation at 3–6 months by endometrial biopsy through D&C rather than aspiration biopsy [65,81]. Establishment of a complete response, defined as no evidence of progressive disease, is a good indicator that pregnancy may be pursued, with ongoing close follow-up for early detection of successful pregnancy or signs of recurrence. Patients who partially respond to treatment at 6 months may be advised to continue progestin treatment for an additional 3–6 months, and non-responders

Table 1. Current or ongoing clinical trials of conservative treatment for endometrial cancer (EC)

ClinicalTrials.gov ID	Start date	Study title	Aims (primary objective)	Design/intervention	Institution	Participants
NCT02035787	March 2014	Metformin with the levonorgestrel-releasing intrauterine device for the treatment of complex atypical hyperplasia (CAH) and endometrial cancer (EC) in non-surgical patients	To compare the rate of CR at 6 months in non-surgical grade 1 EC and CAH patients receiving metformin + LNG-IUS to 50%	Open label/metformin 850 mg twice daily added to standard non-surgical treatment with LNG-IUS	Chapel Hill, NC, USA	15 women 18 years or older with biopsy-proven complex atypical hyperplasia (n=15) or grade 1 EC
NCT01594879	January 2012	Treatment with medroxyprogesterone acetate plus LNG-IUS in young women with early stage endometrial cancer	To investigate the treatment efficacy of LNG-IUS plus MPA in young women with early stage EC	Phase 2 open label trial/ Mirena® (LNG-IUD), oral MPA oral progestin + LNG-IUS	Korean Gynecologic Oncology Group Study	39 patients <40 years of age with histologically confirmed as EC grade 1 that is confined to the endometrium based on the MRI evaluation
NCT00788671	November 2008	Levonorgestrel intrauterine device (IUD) to treat complex atypical hyperplasia (CAH) and grade 1 endometrioid endometrial carcinoma (G1EEC)	To learn the level of effectiveness of the Mirena® LNG-IUD in treating hyperplasia of the lining of the uterus and/or early-stage cancer of the lining of the uterus	Phase 2 open label trial/ levonorgestrel IUD	M.D. Anderson Cancer Center, TX USA	50 patients ≥18 with a diagnosis of complex atypical hyperplasia or endometrial biopsy within 3 months of study enrolment OR patients with a diagnosis of grade 1 endometrioid endometrial carcinoma
NCT03241914	July 2017	Megestrol acetate plus LNG-IUS in young women with early endometrial cancer	To see if MA plus LNG-IUS will not be inferior to returning the endometrial tissue to a normal state than MA alone in patients with early EC	Phase 2 & 3 randomized trial/oral MA 160 mg/day vs. oral MA 160 mg/day + LNG IUS for at least 3 months	Fudan University, Shanghai	40 patients 18-45 years of age; confirmed diagnosis of endometrioid EC based upon hysteroscopy; no myometrial invasion confirmed, extension beyond corpus, or enlarged lymph nodes confirmed by MRI
NCT02990728	March 2016	Mirena® ± metformin as fertility-preserving treatment for young Asian women with early endometrial cancer	To evaluate the efficacies of LNG-IUS, Mirena®, with or without metformin over ~6 month	Phase 2 randomized open label trial/Mirena® LNG-IUD vs. Mirena® LNG-IUD + Metformin	Chang Gung Memorial Hospital, Taiwan	120 patients ≤40 years old with histologic confirmed grade 1 endometrioid EC; tumor confined to the endometrial cavity with no evidence of metastasis on MRI and/or TVUS
NCT02397083	September 2015	Levonorgestrel-releasing intrauterine system with or without everolimus in treating patients with atypical hyperplasia or stage IA grade 1 endometrial cancer	To estimate the efficacy of the LNG-IUS device alone and in combination with everolimus to treat complex atypical hyperplasia or stage Ia grade 1 endometrioid EC with response rate	Phase 2 randomized open label trial/LNG IUD vs. LNG IUS + everolimus	M.D. Anderson Cancer Center, TX USA	270 patients ≥ 18 years of age with a diagnosis of CAH or grade1 endometrioid or focal grade 2 adenocarcinoma with predominantly grade 1 EC

Table 1. Continued

ClinicalTrials.gov ID	Start date	Study title	Aims (primary objective)	Design/intervention	Institution	Participants
NCT03463252	April 2018	Value of LNG-IUS as fertility-preserving treatment of EAH and EC	To analyze the effectiveness of LNG-IUS (Mirena®) in the fertility-sparing treatment of atypical endometrial hyperplasia and early EC, including pathology response and pregnancy outcome	Phase 2 & 3 randomized open label trial/ MPA 250–500 mg/day vs. MPA+LNG-IUS vs. LNG-IUS only; patients with contraindication to oral MPA is allocated to GnRH-agonist	West China Second University Hospital	224 patients ≤40 years old with a grade 1 endometrioid EC limited to the endometrium on MRI; and a strong desire for fertility preservation
NCT03077698	June 2017	An endometrial cancer study for women with recurrent or persistent endometrial cancer	To investigate the efficacy of sodium cridanimod in conjunction with progestin therapy in patients with recurrent or persistent EC, who have failed progestin monotherapy or who have been identified as PrR negative	Phase 2 open label multi-center trial/ sodium cridanimod in conjunction with MA therapy	Xenetic Biosciences, Inc	72 patients ≥18 years of age with histologically confirmed serous carcinoma or endometrioid type of EC; recurrent or persistent progressive disease that is refractory to established treatments
NCT03538704	January 2015	Effect of fertility-sparing therapy of early endometrial cancer	The purpose of this study is to investigate the effect of Fertility-sparing Therapy of Early Endometrial Cancer	Randomized open label trial/BMI ≥25 kg/m^2 given MPA 250 mg/ day plus Metformin 1–1.5 g/day vs. MPA 250 mg/day only	Peking University People's Hospital	80 patients 18 to 40 years old with FIGO stage 1a grade 1 with lesion confined to the endometrium; strong desire to preserve fertility
NCT01968317	October 2013	Megestrol acetate plus metformin to megestrol acetate in patients with endometrial atypical hyperplasia or early stage endometrial adenocarcinoma	To evaluate efficacy of MA plus metformin in returning the endometrial tissue to a normal state than MA alone in patients with endometrial atypical hyperplasia or early stage EC	Phase 2 randomized open label/oral metformin 500 mg third daily + MA 160 mg/ day vs. MA 160 mg/ day for 3 months	Fudan University, Shanghai	150 patients 18–45 years old with a confirmed diagnosis of endometrial atypical hyperplasia or low grade EC; desire for reproductive function or uterus
NCT04008563	August 2019	Bariatric surgery for fertility-sparing treatment of atypical hyperplasia and grade 1 cancer of the endometrium (B-FiERCE)	To determine the proportion of eligible women who agree to participate in the study (recruitment rate). The primary outcome will be met and a full scale randomized controlled trial to assess efficacy will be conducted if ≥40% recruitment rate is achieved patients' reasons for participation or non-participation will be recorded	A pilot randomized controlled trial to assess the feasibility of a full-scale randomized trial. participants will be randomized 1:1 to bariatric surgery plus pIUD or standard pIUD alone	University Health Network, Toronto	36 patients 18–41 years old with grade 1 endometrioid EC or complex atypical hyperplasia, and BMI ≥35 kg/m^2, no evidence of metastatic disease, a desire for fertility preservation and no contraindications to pIUD

BMI, body mass index; CR, complete response; EC, endometrial cancer; EAH, atypical endometrial hyperplasia; FIGO, International Federation of Gynecology and Obstetrics; CAH, complex atypical hyperplasia; GnRH, gonadotropin-releasing hormone; PrR, progesterone receptor; LNG-IUS, levonorgestrel-releasing intrauterine system; LNG-IUD, levonorgestrel-releasing intrauterine device; MA, megestrol acetate; MPA, medroxyprogesterone acetate; MRI, magnetic resonance imaging; IUD, intrauterine device; pIUD, progestin intrauterine device.

at the 6-month follow-up with persistent disease confirmed by D&C should be counselled to undergo hysterectomy.

Reproductive and oncologic outcomes

Reproductive outcomes in EC patients who undergo fertility-sparing treatment are promising, although estimates vary according to patient characteristics and whether ART is used. A multi-center study of 141 Korean women aged <40 years who were diagnosed with grade 1 endometrioid EC and treated with oral progestins showed that 73% of women who attempted conception, including a subset receiving fertility drugs, were successful, and that 66% had at least 1 live birth. Although the incidence of infertility in this cohort was higher than in the general population, those who received fertility treatments had similar 5-year disease-free survival rates compared to those who did not, and women who had at least 1 pregnancy had better disease-free survival rates compared to those who did not [82]. A more recent analysis of 118 Korean women with stage 1A grade 1-2 endometrioid EC treated with MPA and the LNG-IUD reported a live birth rate of 67% among those who tried to become pregnant and had full-term pregnancies. In this cohort, the median disease-free survival was 26 months among those who became pregnant, compared to 12 months among those who did not, suggesting that successful pregnancy may improve disease-free survival [83]. The benefits of pregnancy on recurrence were also documented by Ichinose et al. [84], who found that all women in their study receiving ART had a live birth and a lower risk of recurrence compared to those who did not achieve pregnancy. However, obesity negatively impacts pregnancy rates and lowers the probability of long-term treatment success among women receiving fertility-sparing treatment [85,86]. This remains an ongoing challenge to conservative management of EC, and high-quality data from randomized controlled trials are needed to successfully address this issue [87].

Future strategies and recommendations

The incidence of EC is rising, particularly in countries with rapid socioeconomic growth. Increases in obesity in these countries and a global decline in reproductive rates will increase the need for fertility-sparing approaches to treatment [5]. EC is among the 10 most frequent cancers in Latin American and Caribbean countries (7.7 per 100,000 women) and may reflect changes in reproductive patterns in conjunction with rising rates of obesity in these countries [88]. In high-income countries, the proportion of women with high BMI (≥25 kg/m^2) rose at a faster rate than the global average between 1980 and 2013, with the exception of Japan, where despite lower rates of obesity, EC rates continued to rise, highlighting the involvement of other risk factors [5]. The global prevalence of type 2 diabetes, which is known to be associated with EC and correlates with high BMI, has risen from 4.7% in 1980 to 9.0% in 2014 in adults aged >18 years [89]. Further discussion is warranted on the optimal approach to follow-up, including the frequency of endometrial re-evaluation in patients desiring fertility-sparing treatment, without compromising a patient's quality of life or increasing the risks associated with recurrence or survival.

Overall, mortality associated with conservative treatment of EC is low, and women aged <45 years with low-grade EC and minimal myometrial invasion who are treated with progestins seem to have good clinical outcomes comparable to those receiving primary hysterectomy [90]. However, there are reports in the published literature of fatalities following pregnancy in patients treated conservatively for well differentiated and non-invasive EC [91,92]. The importance of proper informed consent and strict follow-up procedures for patients opting for fertility-sparing treatment of EC cannot be overemphasized. Additional factors such as synchronous or metastatic ovarian cancer, or cancers associated with genetic predisposition need to be factored into current mortality estimates. Patients requesting fertility-sparing treatment should undergo testing for Lynch syndrome and be counselled prior to undertaking treatment. Additionally, patients with prior conditions that predispose to infertility, including obesity, polycystic ovarian syndrome, and anovulation, should be encouraged to seek ART for conception as soon as a complete response is achieved.

Feasibility studies for weight loss approaches with a view to improving EC treatment outcomes are critical to reducing the burden of this disease on healthcare resources. Significant reductions in excess body weight and improvements in biomarkers for insulin resistance and reproductive hormones among obese women undergoing bariatric surgery have been reported [93]. Bariatric surgery was shown to signifi-

cantly reduce the risk of EC among obese women [94]. These studies show promise for improving treatment in obese women, but further studies are necessary to determine the range of benefits on general health and the chance of fertility subsequent to fertility-sparing treatment for EC.

The importance of progesterone receptor status has been discussed with regard to predicting treatment response. While the Gynecologic Oncology Branch of the Chinese Medical Association requires progesterone receptor status in patients undergoing fertility-sparing treatment, the European Society for Medical Oncology (ESMO) does not currently recommend routine testing because patients who test negative for progesterone status still respond to treatment, although their chance of a response is significantly lower [65]. The inclusion of immunohistochemical markers [95] and serum levels of cancer antigen CA125 [96] to predict response to treatment will require further studies. Risk classification of tumors in a fertility-sparing setting using molecular markers for p53 and MMR abnormalities has been trialed with promising results [97]. Endometrial biomarkers to predict resistance to therapy would prove beneficial and are currently being evaluated (NCT00788671). The feMME trial (ANZGOG NCT01686126) is a randomized clinical trial that investigates the effectiveness of LNG-IUD in women with early stage EC and a BMI >30 kg/m^2. It is a 3-arm randomized multicenter phase 2 study of LNG-IUD ± metformin ± weight loss intervention in 165 women [37]. Metformin is the most widely used anti-diabetic drug, and there is evidence from *in vitro* studies to suggest that it also has anti-cancer properties [98]. Results are expected to become available in 2020. Additionally, 4 Chinese studies are currently evaluating fertility-sparing treatments for early-stage EC: MPA 250 mg/day ± metformin 1–1.5 g/day (NCT03538704); MPA 250–500 mg/day vs MPA + LNG-IUD vs LNG-IUD (NCT03463252); oral MA 160 mg/day ± LNG-IUD (NCT03241914); and MA 160 mg/day ± metformin (NCT01968317). A Taiwanese study is also currently evaluating LNG-IUD ± metformin in young Asian women under the age of 40 years with early stage EC (NCT02990728), and the Korean Gynecologic Oncology Group is evaluating LNG-IUD + MPA in young women with grade 1 EC confined to the endometrium (NCT01594879). With the exception of a Korean study, which recently published a 6-month update [99], these and other trials outlined in Table 1 have not been published at the time of writing this review.

Guidelines and recommendations

Comprehensive evidence-based guidelines and recommendations for EC management were developed by the ESMO, the European Society of Gynaecological Oncology, and the European Society for Radiotherapy & Oncology and published in 2015 [38]. The following summarizes their current recommendations for fertility-sparing treatment, including an update published in 2017 [100]:

- Conservative management should be considered in selected patients with grade 1 EC or premalignant disease; patients must be referred to specialized centers, undergo histological diagnosis using D&C with or without hysteroscopy, and have their diagnosis confirmed by a specialist gynecopathologist (Table 2). Overt myometrial invasion and adnexal involvement should be excluded

Table 2. Optimal candidates for non-surgical fertility-sparing treatment

Terms of candidates
1. Young women of child-bearing age (preferably women under age 40) diagnosed with EC
2. Definitive diagnosis of grade 1 tumor of the endometrioid subytpe with or without superficial invasion into the myometrium
3. Well differentiated tumors with ≤50% myometrial invasion assessesd by TVUS or MRI where TVUS is insufficient to assess
4. No evidence of synchronous or metachronous ovarian tumors evaluated by MRI or TVUS
5. No family history or hereditary cancer syndromes as evidenced by mutation testing primarily for Lynch Syndrome by immunohistochemical staining of tumor specimens for MMR proteins
6. No contraindications for hormonal treatment
7. Patients with comorbidities that preclude them from surgical treatment
8. Full acceptance and understanding that fertility-sparing treatment is not standard, carries a risk of recurrence, and requires close follow-up

EC, endometrial cancer; TVUS, transvaginal ultrasonography; MRI, magnetic resonance imaging; MMR, mismatch repair genes.

using pelvic MRI or expert TVUS.

- Current recommendations for fertility-sparing therapy involve the use of oral progestins, MPA (400–600 mg/day), MA (160–320 mg/day), or the LNG-IUD and assessment of patient responses by D&C, hysteroscopy, and imaging at 6 months. Minor variations of fertility-sparing treatment by other gynecologic oncology societies are primarily in regards to follow-up biopsy; the Japan Society of Gynecologic Oncology recommends endometrial biopsy at 3 months following progestin treatment [101], and the Korean Society of Gynecologic Oncology recommends endometrial biopsy at 3–6 month for fertility-sparing treatment of grade 1 endometrioid tumors confined to the endometrium [102]. In addition, the Gynecologic Oncology Branch of the Chinese Medical Association requires patients to be under the age of 40 and progesterone-receptor positive to be considered for fertility-sparing therapy [103].
- Patients who wish to conceive following a complete response should be advised to pursue pregnancy earlier and consider ART to improve success rates. Those not ready to conceive immediately should be offered low-dose progestin or the LNG-IUD. After completion of childbearing, THBSO should be considered, although preservation of the ovaries would depend on age and genetic risk factors.
- Patients should be apprised of the pros and cons of fertility-sparing treatment and that there are inherent risks associated with this approach, including the likelihood of future hysterectomy if there is disease recurrence. They should also be willing to accept close follow-up during and after treatment at least bi-annually, which may include D&C and imaging to evaluate recurrence.

Conclusion

There is consensus across various leading gynecologic oncology societies that fertility-sparing treatment of EC must be considered a research priority. Research to estimate the effectiveness of fertility-sparing treatment of EC is underway. Research is also required to understand the information needs of patients considering fertility-sparing treatment of EC. Finally, further research is urgently required to determine the predictors of response to hormonal treatment.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study is not applicable to Institutional Review Board.

Patient consent

There is no need for patient consent in this review article.

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Can vitamin C and interleukin 6 levels predict preterm premature rupture of membranes: evaluating possibilities in North Indian population

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Objective

We aimed to evaluate the relation between plasma ascorbic acid levels and the occurrence of preterm premature rupture of membranes (PPROM) and whether patients with ascorbic acid deficiency have predisposition to microbial invasion, as revealed by serum interleukin-6 (IL-6) levels and confirmed by placental culture sensitivity evaluation.

Methods

This controlled study involved 50 women with PPROM at gestational age between 28 and 36.6 weeks. Fifty patients with uncomplicated pregnancy and intact membranes were observed as control group. Serum ascorbic acid and IL-6 levels were evaluated for PPROM group subjects and controls, matched by their gestational age and body mass index. Repeat samples for IL-6 were taken after 48 hours of admission in PROM group subjects and at the onset of labor in controls. Placental membranes were sent for culture sensitivity evaluation after delivery.

Results

The mean plasma level of ascorbic acid was 0.60±0.35 mg/dL and 1.18±0.43 mg/dL in the PPROM and control groups, respectively, showing significant difference. Plasma IL-6 level rose from 18.18±5.94 pg/mL to 34.32±7.31 pg/mL after 48 hours of admission in the PPROM group (P=0.000). Plasma IL-6 level in controls rose from 5.59±4.36 pg/mL at admission to 7.36±3.67 pg/mL at the onset of labor. Membrane culture test results were positive in a total of 40 samples, of which 37 samples were from the PPROM group subjects.

Conclusion

Vitamin C deficiency, when complemented with elevated serum IL-6 level, can predict susceptibility to PPROM in the third trimester; the risk increases in women with prior history of similar pathology.

Keywords: Preterm premature rupture of fetal membranes; Ascorbic acid; Interleukin-6; Complications; Pregnancy

Introduction

Preterm premature rupture of membranes (PPROM) is the rupture of the chorion-amniotic membrane and leakage of amniotic fluid before onset of labor pains, prior to 37th week of pregnancy. Although PPROM involves 1–5% of pregnancies, it is responsible for 20–55% preterm deliveries [1]. PPROM is an important contributor to maternal and perinatal morbidity and mortality. The major cause of perinatal morbidity and mortality associated with PPROM is prematurity. Maternal sepsis is a rare but life-threatening complication reported in nearly 1% of cases [2].

It is postulated that mediators, released either from stretch-

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ing of membranes or infection, lead to activation of destructive enzymes in the matrix, thus causing rupture of amniotic membranes. Reactive oxygen species (ROS) is one of the factors involved in membrane destruction. Micronutrients stabilize the ROS, and hence, its deficiency leads to abnormal collagen structure synthesis that has been associated with increased risk of PPROM. The micronutrient Ascorbic acid (vitamin C) is an effective water-soluble antioxidant that scavenges several ROS, thus reducing oxidative stress [3]. It participates in the equilibrium between synthesis and degradation of collagen and this may be critical in reducing the occurrence of PPROM as well. Interleukin-6 (IL-6) is one of the inflammatory cytokines produced by macrophages, amnion, chorion, and decidual cells that is elevated in maternal serum and amniotic fluid in patients with PPROM with microbial invasion, even before the development of signs and symptoms of chorioamnionitis. A meta-analysis showed that IL-6 might be a sensitive and specific diagnostic marker for the early diagnosis of neonatal sepsis with premature rupture of membranes (PROM) [4]. Hence measuring IL-6 cytokine in maternal serum is a simpler and less invasive method of predicting impending infection in PPROM [5].

The present case-control study was conducted to investigate whether there is a relation of plasma ascorbic acid levels to the occurrence of PPROM and if those with deficiency have a significant predisposition to microbial invasion of amniotic membranes, as revealed by elevated IL-6 levels. Our aim was also to determine fetal morbidity and mortality in patients with PPROM and co-relate the outcome with serum vitamin C and IL-6 levels.

Materials and methods

This was a case control study conducted in a tertiary level hospital in Northern India for a period of 18 months. The study group subjects were hospitalized patients with PPROM. Control group subjects were patients from pre-natal clinics, identified during routine checkup.

Osaikhuwuomwan et al. [6] reported that 33 subjects in a study group and 21 in a control group had deficient serum vitamin C levels. Taking these as reference values and assuming 80% power and 5% type 1 error with equal proportion, sample size was calculated to be 38. Assuming 10% loss to follow-up, sample size was rounded off to 50 subjects.

The first 50 cases with PPROM between 28 and 36.6 weeks gestation, who fulfilled eligibility criteria, formed the study group. Control group was comprised of 50 patients with matching gestational age and body mass index, who were having uncomplicated pregnancies and intact membranes.

1. Inclusion criteria

Women, from 18 to 35 years of age, with singleton pregnancy between 28 and 36.6 weeks' gestational age, were included in the study.

2. Exclusion criteria

Women with anemia, diabetes mellitus, urinary tract infection, respiratory tract infection, vaginitis, polyhydramnios, history of smoking, and intake of micronutrients were excluded from the study.

Blood samples were collected initially from subjects in both groups, to establish the serum ascorbic acid and IL-6 levels, before the administration of any medication. Repeat samples for IL-6 were taken after 48 hours of admission in the study group and at the onset of labor in the control group. Speculum examination was done in cases with PPROM to confirm the presence of amniotic fluid and inspect its color and odor. Fetal well-being was evaluated by ultrasound. After routine workup the study group subjects were managed as per hospital protocol.

1) Serum IL-6

Maternal 2 mL venous blood was drawn into sterile silicone-coated tubes, refrigerated at 5°C, and centrifuged. Serum IL-6 was measured in batches by a single investigator, using an enzyme-linked immune-sorbent assay kit (R&D Systems, Minneapolis, MN, USA). This assay did not cross-react with other cytokines and had an inter- and intra-assay variation less than 5%.

2) Plasma ascorbic acid

Maternal 2 mL venous blood samples were collected in heparinized vial to establish ascorbic acid baseline. Serum ascorbic acid levels were evaluated using spectrophotometry.

Placental membranes of both groups were sent after delivery for aerobic culture sensitivity testing. Samples were collected in a universal sterile container without any preservative or additive and sent immediately after collection. They were refrigerated at 4–8°C, if there was a delay in sending

them to the microbiology laboratory. Samples were immediately processed in the microbiology laboratory. The placental membrane was well homogenized on a sterile Petri dish, using a bard-parker blade. The homogenized tissue was inoculated into 5% sheep blood agar (McConkey agar plates and brain heart infusion [BHI] enrichment media). Gram stain was also done from the tissue. Inoculated plates were incubated for 48 hours at 37°C and inspected for zones of growth. If no growth was detected then subculture was prepared, using BHI media. The report was considered negative only if there is no growth after 72 hours. Any growth in any of the culture plates were further evaluated for identification and sensitivity testing. Manual identification was done by biochemical tests and supported in a few cases by Vitek 2.0. Sensitivity testing of the isolates was done by disk diffusion test method following CLSI 2018.

Categorical variables are presented in number and percentage (%) and continuous variables as mean±standard deviation and median. Comparison of quantitative variables was performed by unpaired *t*-test/Mann-Whitney test. Qualitative variables were compared using χ^2 test /Fisher's exact test. *P*-value of <0.05 was considered statistically significant. The data was entered in MS Excel spreadsheet and analysis was done using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The accuracy of the multivariate predictors of gene mutations was measured using the area under the receiver operating characteristic (ROC) curve.

Table 1. Descriptive characteristics for preterm premature rupture of membranes (PPROM) and control groups

Parameters	PPROM group	Control group	*P*-value	Adjusted OR (95% CI)	*P*-value
Maternal age (yr)	24.36±3.45	24.08±3.33	0.639[a]	-	-
Gravidity (number)	2.72±1.01	2.24±1.04	0.021[a]	Gravidity≥3, 1.79 (0.73–4.37)	0.190
Presence of preterm birth history	18 (36)	5 (10)	0.002[b]	4.36 (1.39–13.71)	0.010
Low socio-economic status	34 (68)	17 (34)	0.003[b]	3.56 (1.48–8.59)	0.050
Smoking	3 (6)	2 (4)	0.646[b]	-	-
Caesarean section	14 (28)	10 (20)	0.349[b]	-	-
Gestational age at birth (wk)	34.00±1.54	39.30±1.76	0.000[a]	-	-
Birth weight (kg)	1.70±0.20	3.10±0.41	0.000[a]	-	-
Neonatal morbidity and mortality	37 (74)	12 (24)	0.000[b]	-	-

Data are shown as mean±standard deviation or number (%).
OR, odds ratio; CI, confidence interval.
[a]Independent *t*-test; [b]Pearson χ^2 test.

Table 2. Plasma ascorbic acid and interleukin (IL)-6 levels between preterm premature rupture of membranes (PPROM) group and control group

Parameters	PPROM group (n=50)	Control group (n=50)	*P*-value
Plasma ascorbic acid levels (mg/dL) at the time of enrolment	0.60±0.35	1.18±0.43	0.000[a]
Plasma IL-6 levels (pg/mL) at the time of enrolment	18.18±5.94	5.59±4.36	0.000[a]
Repeat plasma IL-6 levels (pg/mL)	34.32±7.31 (48 hr after admission)	7.36±3.67 (at the onset of labour)	-
No. of patients with low plasma ascorbic acid[b]	33 (66)	6 (12)	0.000[c]
No. of patients with high IL-6[b]	39 (78)	3 (6)	0.000[c]
No. of placental membrane culture (+)	37 (74)	3 (0.06)	0.000[c]

Data are shown as mean±standard deviation or number (%).
[a]Independent *t*-test; [b]Cut off levels for ascorbic acid was taken as ≤0.6 mg/dL [7] while cut off levels of IL-6 was taken as ≥15 pg/mL since this value predicted microbial invasion (confirmed on membrane culture sensitivity) of amniotic membranes in PPROM with high sensitivity and specificity; [c]Pearson χ^2 test.

Results

Descriptive characteristics of PPROM and control group are summarized in Table 1.

Univariate analysis showed patients, belonging to lower socioeconomic status and of higher gravida, were more prone to develop PPROM. Patients with a history of preterm birth were having five times higher risk of PPROM (odds ratio [OR], 5.06; 95% confidence interval [CI], 1.70–15.05) in the present pregnancy. The mean value of plasma ascorbic acid was 0.60±0.35 mg/dL in PPROM group and 1.18±0.43 mg/dL in the control group at the time of enrollment at the same gestational age. Patients with low ascorbic acid levels were having 14 times higher risk of PPROM (OR, 14.23; 95% CI, 5.06–40.04). Mean levels of plasma IL-6 at the time of enrollment in patients of PPROM group was higher (18.18±5.94 pg/mL) than control group (5.59±4.36 pg/mL). The difference was statistically significant (*P*-value=0.000) (Table 2). Its levels were directly proportional to the duration of leaking at admission; however, there was weak correlation (r^2 value=0.16).

In our study, the cutoff level for ascorbic acid was ≤0.6 mg/dL [7]; hence, predicting the chances of PPROM with sensitivity of 66% and specificity of 88% and ROC analysis showed an area under the curve (AUC) of 0.841 (95% CI, 0.78–0.93). The cutoff level of IL-6 was ≥15 pg/mL, and this value was found to predict microbial invasion (confirmed on membrane culture sensitivity tests) of amniotic membranes in PPROM, with a sensitivity of 85% and specificity of 86.67%, and ROC analysis showed an AUC of 0.915 (95% CI, 0.85–0.97) (Table 3 and Fig. 1).

After 48 hours of admission, repeat plasma IL-6 level rose

Table 3. The relationship between microbial invasion and interleukin (IL)-6 level

Laboratory test	Microbial invasion (+) (n=40)		Microbial invasion (–) (n=60)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IL-6[a]	34[b]	6[c]	8[d]	52[e]	85	86.67	80.95	89.65

n, number of subjects; PPV, positive predictive value; NPV, negative predictive value.
[a]IL-6: No. of patients with high IL-6 level (≥15 pg/mL) (n=42); [b]True positive; [c]False negative; [d]False positive; [e]True negative.

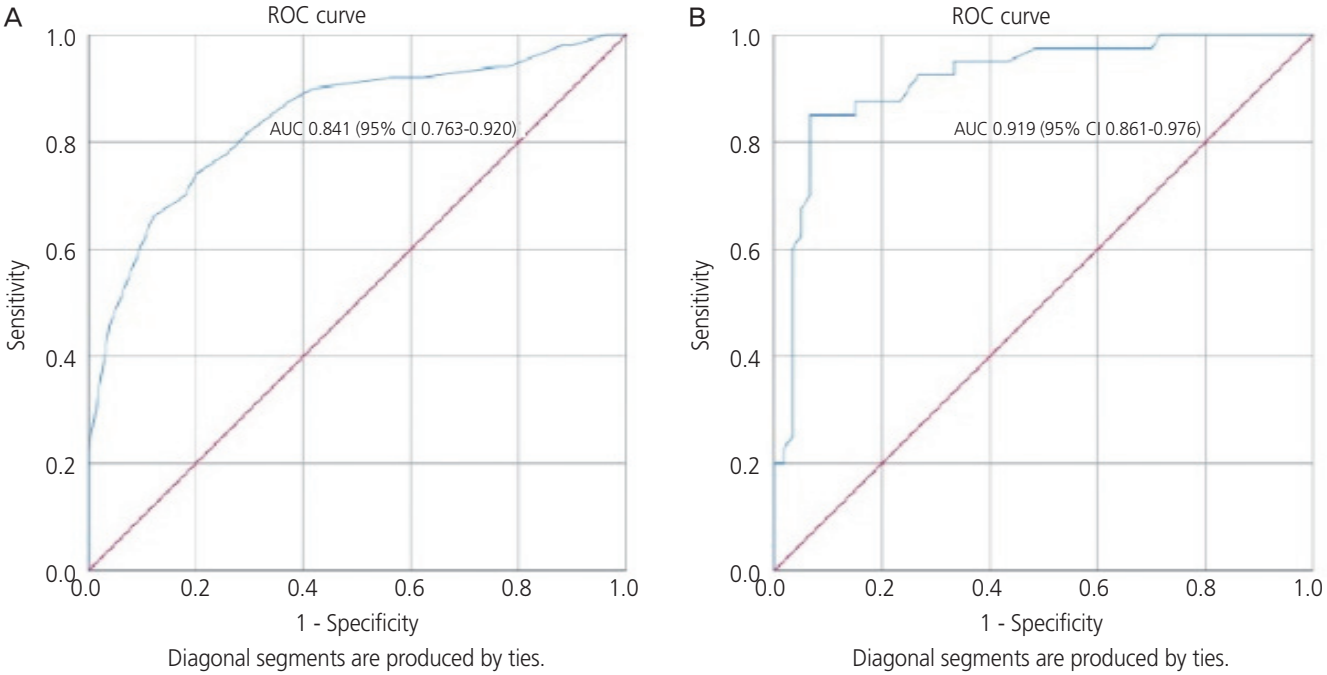

Fig. 1. (A) Receiver operating characteristic (ROC) curve for serum vitamin C as predictor of preterm premature rupture of membranes. (B) ROC curve for serum interleukin-6 as predictor of microbial invasion of placental membranes. AUC, area under the curve; CI, confidence interval.

from 18.18±5.94 to 34.32±7.31 pg/mL, the difference being statistically significant (*P*<0.000). However, the mean plasma level of IL-6 in controls rose from 5.59±4.36 at admission to 7.36±3.67 at the onset of labor. Membrane culture tested positive, suggesting microbial invasion in a total of 40 cases, of which 37 cases presented PPROM. Only 3 cases without PPROM had evidence of microbial invasion.

There was no difference in the mode of delivery in both the groups but statistically significant difference in birthweight of newborns existed. Neonatal morbidity developed in higher proportion in the PPROM group. Septicemia and conjunctivitis developed in 28% and 6% of babies in the PPROM group, while conjunctivitis developed in 4% in the control group. Respiratory distress syndrome was present in 24% of patients in the PPROM group, while it was present in only 10% of patients in the control group. Jaundice contributed equally (10%) to fetal morbidity in both groups. This proportional difference in the neonatal morbidity showed high statistical significance (*P*-value=0.001). Neonatal mortality was present in 6% of the patients from the PPROM group while none in the control group. Among women with low plasma vitamin C levels, 90.9% of neonates had one or other morbidity.

Discussion

Primary outcome (Table 2) revealed mean plasma level of ascorbic acid was significantly low while IL-6 level was high at the time of enrollment in the PPROM group. There was significant difference in the number of women with low serum ascorbic acid and high IL-6 level at the time of enrollment among PPROM and control group. IL-6 levels rose significantly high after 48 hours of admission among cases while the rise was moderately high among controls, comparing values during enrollment and at onset of labor. Secondary analysis revealed there was a significant difference between PROM group subjects and controls in terms of socioeconomic status, gravidity, history of preterm birth, birthweight, and perinatal morbidity (Table 1). However, multi-logistic analysis (Table 4) revealed that women having a history of preterm delivery and those with high serum IL-6 level at the time of enrollment were only at a significant high-risk of PPROM.

Vitamin C is a micronutrient that directly stimulates collagen synthesis by activating multiple genes. It is considered that it maintains the equilibrium between synthesis, stability, and degradation of collagen, which in turn may reduce the incidence of PPROM [8]. It also acts as an antioxidant and this role of vitamin C is most evident during periods of elevated oxidative stress. Phagocytes are activated because of many infections and they release ROS, which play a role in elimination of virus and bacteria [9]. These ROS are, however, not without side effects and may be harmful to the host cells, thus, playing a probable role in the pathogenesis of microbial invasion [10]. Ascorbic acid is assumed to play a role in protecting host cells against the detrimental effects of ROS, released by phagocytes, and hence, its levels are decreased during infections [11]. Therefore, ascorbic acid levels may be decreased in most infections, occurring after PPROM, thus acting as a cause as well as effect. This was evident in our study too. Ascorbic acid levels were significantly lower in the PPROM group, which might have triggered the occurrence of PPROM. Otherwise primary microbial invasion of placental membranes, as evident from the high IL-6 levels at the time of enrollment, was the cause for the decreasing level of ascorbic acid in PPROM group. Hence, IL-6 and ascorbic acid both can be utilized as marker for PPROM and

Table 4. Significant predictive factors for preterm premature rupture of membranes based on logistic regression test results

Factors	*P*-value	Adjusted OR (95% CI)
Low socioeconomic status	0.87	1.12 (0.25–4.84)
Presence of history of preterm birth	0.01[a]	7.93 (1.51–41.72)
Plasma ascorbic acid at the time of enrolment	0.53	2.13 (0.19–23.48)
Plasma IL-6 at the time of enrolment	0.00[a]	1.41 (1.11–1.80)
No. of patients with low serum ascorbic acid at the time of enrolment	0.50	0.38 (0.03–6.68)
No. of patients with high IL-6 at the time of enrolment	0.59	2.45 (0.07–50.63)

IL, interleukin; OR, odds ratio; CI, confidence interval.
[a]Significant *P*-value.

microbial invasion. Siega-Riz et al. [12] found a relationship between low vitamin C intake and an increased risk of PPROM (adjusted OR, 1.03; 95% CI, 0.90–1.17). Sharma and Mehta [3] observed low vitamin C concentration in patients with PPROM and an inverse relationship between the duration of the rupture of membranes and vitamin C levels with a significant increase in maternal morbidity, neonatal morbidity, and mortality. This inverse relationship evidenced in study by Sharma and Mehta [3] reinforces our findings though they did not measure any inflammatory marker in serum. However, the results of studies, defining the therapeutic role of the micronutrient are not encouraging. Steyn et al. [13] observed no significant difference in the occurrence of preterm labor with ascorbic acid supplementation (250 mg/day) and concluded that the therapeutic effects of vitamin C may be evident only in patients with deficiency of the micronutrient. Spinnato et al. [14] observed that high-dose vitamin C (1,000 mg/day) caused an increase in the incidence of PROM in a randomized double-blind placebo-control trial and warned against the empirical use of the micronutrient. Mercer et al. [15] observed that at high doses, vitamin C may cause collagen degradation and apoptosis, thus questioning the beneficial role of vitamin C in the prevention of PPROM. They concluded that vitamin C has no role in the remodeling process of normal fetal membrane, which may lead to weakening and rupture at term. However, a randomized double-blind placebo-controlled trial proved the effectiveness of vitamin C at a dose of 100 mg/day in preventing PPROM [16]. Hence, the therapeutic role of the micronutrient needs to be elucidated in deficient and sufficient subjects to evaluate bona fide effects. The dose has to be optimized after adjusting to plasma levels and duration of gestation, so that the inadvertent harmful effects, if any, are avoided.

Our body cannot synthesize vitamin C and diet is the main source of this micronutrient [13]. Inadequate intake is hence the possible cause for the prevalence of deficiency of vitamin C found in our study population. An imprudent attitude and casual approach in terms of clinical visits, drug intake, physical labor and diet among Indian women towards higher order pregnancies, could be a result from the limitations of social framework, prevalent in the country. This may also explain the observation made by us and in other studies [17,18] of higher association of PPROM with low socioeconomic status. A history of prior preterm delivery or PPROM was seen in a greater proportion in the study group, which is consistent with results of other studies [19,20]. Negara et al. [21] observed that there was stronger p53 expression and stronger apoptotic index in the PROM group than in the PROM-free group. This genetic link may explain the occurrence of repeated events of rupture of membranes in a woman. Our study found that there was no difference in the mode of delivery in the study and the control group, like Sharma and Mehta [3] did, while other studies [22,23] reported higher rate of caesarean deliveries among women with PPROM. Poor perinatal outcomes were observed in our study group, consistent with results of another study [24].

In conclusion our study demonstrates that low serum vitamin C levels alone can poorly predict the occurrence of PPROM. However, when this is complemented with elevated IL-6 level after 28 weeks, susceptibility to PPROM increases, given the subject has had prior history of such complication.

Measuring serum levels of IL-6 and vitamin C will assist in counselling and prognosticating patients with anticipated rupture of membranes. Optimizing the serum levels of ascorbic acid before conception and during pregnancy, particularly in those with micronutrient deficiency or a history of PPROM, can be a rewarding therapeutic intervention for the prevention of this obstetric complication.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institute Ethics Committee (S.No.IEC/VMMC/SJH/Thesis/October-2015). All tests were done free of cost in our hospital and were provided by the Government of India.

Patient consent

Informed written consent was taken from all subjects before enrolling them in the study.

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Secular trends in cesarean sections and risk factors in South Korea (2006–2015)

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Objective
This study aimed to investigate trends in the rate of cesarean sections (CSs) in South Korea from 2006 to 2015 and identify the risk factors associated with these changes.

Methods
Using the National Health Insurance Corporation dataset, all women who gave birth between 2006 and 2015 were included in the study. We investigated 1) the mode of delivery, 2) the complication rates during pregnancy (i.e., preeclampsia and placenta previa), and 3) pre-pregnancy factors (body mass index, hypertension [HTN], diabetes mellitus [DM], and other pre-existing medical conditions), and their trends during the study period.

Results
Over 10 years, the rate of CS increased from 36.3% in 2006 to 40.6% in 2015 (P<0.01). The rate of CS increased in primiparous women, women with multiple pregnancy, and women with preeclampsia. Maternal age and the incidence of placenta previa also increased. In contrast, the rate of vacuum deliveries and vaginal birth after CS decreased during the study period. The rate of women with pre-pregnancy obesity and DM increased, but the rate of women with pre-pregnancy HTN decreased.

Conclusion
The rate of CS in South Korea increased from 2006 to 2015. This trend may reflect changes in the rate of different risk factors. Identifying the causes of the increasing CS trend observed in this study will allow clinicians to monitor these factors and possibly reduce the rate of CS.

Keywords: Cesarean section; Trends; Risk factors

Introduction

Cesarean sections (CSs) are the most common major surgical procedure for women. There is no doubt that CS is a life-saving procedure for mothers and babies. This procedure can prevent adverse pregnancy outcomes such as perinatal asphyxia, stillbirth, uterine rupture, or obstetric fistula [1]. However, an increase in the use of CS is worrisome in many ways. The World Health Organization (WHO) reported that the rate of CS is increasing and nearly doubled between 2000 (12.1%) and 2015 (21.1%) [2,3]. CSs are associated with not only higher economic burden than those by vaginal births but also with health burden such as the risk of uterine rupture, abnormal placentation, ectopic pregnancy, stillbirth,

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preterm birth, and altered immune systems and obesity in children born through a CS [4].

The CS rate in South Korea ranks fifth among 24 countries in the Organization for Economic Cooperation and Development [5]. The rate of CS in other East Asian countries is less by 10%, although women in these countries share similar pelvic cavity characteristics. There is no definite consensus on the optimal rate of CS. However, the WHO recommends a rate of CS of approximately 10–15% because the maternal and neonatal health risks outweigh the benefits beyond 15% [6].

Understanding the trends in the factors affecting cesarean deliveries may benefit from assessing the effects of both maternal and healthcare provider characteristics on the change in CS rates and assist in designing effective interventions to optimize the rate of CS. Therefore, we examined recent trends in CS and the related factors that influence its rate.

Materials and methods

1. Study data

Study data were collected by merging the Korea National Health Insurance (KNHI) claims database with the National Health Screening Examination (NHSE) data.

In South Korea, 97% of the population is required to enroll in the KNHI program. The remaining 3% of the population is covered by the medical aid program. Thus, using the KNHI claims dataset, which contains information on all claims, nearly all information about diseases and their treatment can be identified from this centralized database, with the exception of procedures not covered by insurance.

As part of the KNHI healthcare system, all insurance subscribers and dependents are invited to participate in a biannual NHSE free of charge. The NHSE consists of 2 components: a health interview and a health examination. Therefore, pre-pregnancy information contained in the NHSE was also used in this study.

2. Study population

Based on the KNHI claims dataset, we identified all women who delivered between January 1, 2006 and December 31, 2015 (dataset 1). Using dataset 1, the delivery mode, pregnancy outcomes, and pre-pregnancy diseases were identified based on the International Classification of Diseases, 10th Revision (ICD-10) codes.

Women were included in the analysis if they had an NHSE 1 to 2 years before their delivery (dataset 2). Pre-pregnancy characteristics were evaluated in dataset 2.

3. Measurements of outcomes and pre-pregnancy and pregnancy characteristics

The mode of delivery was identified using electronic data interchange (EDI) codes in the KNHI claims database using dataset 1.

Pregnancy characteristics and pre-pregnancy diseases were evaluated using dataset 1. Information on pregnancy characteristics including primiparity, multiple pregnancy, instrumental delivery, vaginal birth after CS (VBAC), placenta previa, and preeclampsia was collected using EDI codes and ICD-10 codes. Information on pre-pregnancy diseases including diabetes mellitus (DM; E10–E14), hypertension (HTN; I10–I15), renal diseases (N10–N08, N10–N16), and cardiovascular–pulmonary diseases (I20–I28, I60–I69), hypothyroidism (E02–E03), hyperthyroidism (E05), and systemic diseases (M30–M36) was retrieved based on the ICD-10 codes.

Information on pre-pregnancy characteristics including smoking status, obesity, fasting glucose, liver function tests (LFTs), and total cholesterol (TC) levels was collected using dataset 2. Smoking status was categorized into current smokers, past smokers, and never smokers. Health examinations included the measurement of body mass index (BMI) in kilograms per square meter. Obesity was defined as BMI ≥25 kg/m^2 [7]. All blood samples were obtained after a minimum fasting period of 8 hours. The levels of fasting glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cholesterol were measured using enzymatic methods. A high fasting glucose level was defined as glucose ≥126 mg/L [8]. High LFTs were defined as AST and ALT levels >31 U/L [9]. High cholesterol was defined as cholesterol ≥200 mg/L [10].

4. Statistical analyses

The data are expressed as mean±standard deviation for continuous variables and as percentage for categorical variables.

Clinical and biochemical characteristics by delivery years were compared using analysis of variance (ANOVA) for differences in continuous variables and using the χ^2 test for categorical variables. Secular trends in the continuous and categorical variables were determined and compared across the years using ANOVA polynomial regression tests and chi-

square Cochran-Armitage tests, respectively. All tests were 2-sided, and $P<0.05$ was considered to indicate statistical significance. Statistical analyses were performed using SAS for Windows, version 9.4 (SAS Inc., Cary, NC, USA).

Results

During the study period, 4,190,949 deliveries were recorded.

1. Trend in cesarean section from 2006 to 2015

The rate of CS increased from 36.3% in 2006 to 40.6% in 2015, but unchanged from 2006 to 2011, and with a continuous increase each year after 2011 ($P<0.01$) (Fig. 1).

2. Trend in pregnancy factors related to cesarean section

The secular changes in maternal characteristics and pregnancy factors related to CS are summarized in Table 1. The mean maternal age at delivery increased from 29.92 years in 2006 to 31.85 in 2015. Likewise, the proportion of mothers who had advanced age (≥35 years and ≥40 years) increased. There were no changes in the rate of underage (<20 years) mothers during the study period. The rate of primiparity and multiple pregnancy increased, and the rate of CS increased in these high-risk groups. The number of vacuum deliveries and VBACs decreased during the study period. The prevalence of placenta previa increased during the study period.

3. Trend in pre-pregnancy diseases

Table 2 shows the secular changes in the prevalence of

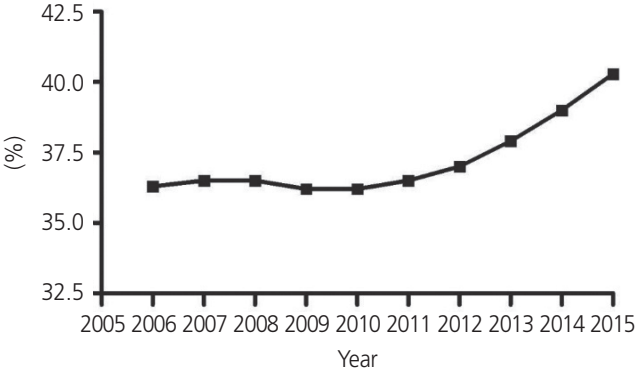

Fig. 1. Secular trends in the cesarean section rate between 2006 and 2015.

Table 1. Secular trends in maternal characteristics and pregnancy outcomes between 2006 and 2015

Characteristics	2006 (n=412,388)	2007 (n=454,582)	2008 (n=427,441)	2009 (n=407,106)	2010 (n=431,949)	2011 (n=431,570)	2012 (n=440,725)	2013 (n=395,706)	2014 (n=394,190)	2015 (n=395,292)	*P*-value
Age (yr)	29.92±3.79	30.12±3.80	30.39±3.92	30.59±3.91	30.87±3.83	31.04±3.89	31.25±3.88	31.49±3.93	31.67±3.96	31.85±3.99	<0.01
<20	0.66	0.41	0.40	0.37	0.36	0.42	0.39	0.42	0.39	0.35	<0.01
≥35	11.04	12.32	13.82	15.01	16.68	17.68	18.48	20.16	21.54	23.82	<0.01
≥40	1.06	1.11	1.30	1.54	1.75	2.03	2.21	2.43	2.64	2.79	<0.01
Primiparity	50.59	51.83	51.74	51.53	49.94	50.59	51.17	51.25	51.71	52.36	<0.01
CS in primiparity	35.42	35.83	36.04	36.09	36.41	36.85	37.79	39.27	40.77	42.55	<0.01
Multiple pregnancy	1.16	1.40	1.37	1.37	1.36	1.39	1.56	1.61	1.73	1.82	<0.01
V/D in multiple pregnancy	7.85	7.98	6.02	6.22	8.04	7.40	7.38	6.58	5.79	5.49	<0.01
Vacuum delivery	6.29	6.02	6.07	5.76	5.80	5.97	5.97	6.02	5.84	5.71	<0.01
VBAC	0.76	0.68	0.68	0.58	0.62	0.59	0.55	0.50	0.41	0.38	<0.01
Preeclampsia	2.04	2.06	1.93	1.93	2.01	1.90	2.04	1.86	1.86	1.96	<0.01
CS in preeclampsia	60.51	61.27	61.77	61.36	60.83	61.82	63.80	65.49	66.60	69.11	<0.01
Placenta previa	0.81	0.87	0.88	0.94	0.98	0.99	1.04	1.05	1.10	1.11	<0.01

Values are presented as mean±standard deviation or percentage.
CS, cesarean section; V/D, vaginal delivery; VBAC, vaginal birth after cesarean section.

Table 2. Secular trends in the prevalence of maternal pre-pregnancy comorbidities between 2006 and 2015

Characteristics	2006 (n=412,388)	2007 (n=454,582)	2008 (n=427,441)	2009 (n=407,106)	2010 (n=431,949)	2011 (n=431,570)	2012 (n=440,725)	2013 (n=395,706)	2014 (n=394,190)	2015 (n=395,292)	*P*-value
DM	1.12	1.05	0.99	0.94	0.98	1.03	1.06	1.09	1.09	1.11	<0.01
HTN	0.79	0.80	0.81	0.80	0.83	0.75	0.56	0.53	0.51	0.48	<0.01
Renal disease	1.98	1.92	1.91	1.83	1.88	1.81	1.76	1.77	1.57	1.60	<0.01
Cardio-vascular and pulmonary disease	0.53	0.56	0.57	0.54	0.58	0.58	0.56	0.55	0.53	0.52	<0.01
Hypothyroidism	2.28	2.48	2.76	2.91	3.07	3.02	3.13	3.15	3.04	3.22	<0.01
Hyperthyroidism	1.62	1.69	1.69	1.64	1.73	1.71	1.70	1.55	1.52	1.46	<0.01
Systemic disease	0.81	0.87	0.88	0.94	0.98	0.99	1.04	1.05	1.10	1.11	<0.01

Values are presented as percentage. Women were classified as having pre-pregnancy diseases if they were diagnosed with DM (E10–E14), HTN (I10–I15), renal diseases (N10–N08, N10–N16), cardiovascular and pulmonary diseases (I20–I28, I60–I69), and systemic diseases (M30–M36), based on the ICD-10 codes.
DM, diabetes mellitus; HTN, hypertension; ICD-10, International Classification of Diseases, 10th Revision.

maternal pre-pregnancy diseases. The prevalence of pre-pregnancy DM, cardiovascular-pulmonary disease, hyperthyroidism, and systemic disease was unchanged, but that of HTN and renal disease decreased during the study period. The prevalence of hypothyroidism increased.

4. Trend in pre-pregnancy factors related to cesarean section

The secular changes in pre-pregnancy risk factors related to CS were identified, as summarized in Table 3. The prevalence of pre-pregnancy obesity increased from 7.13% in 2006 to 10.67% in 2015. The prevalence of pre-pregnancy abnormal LFTs, high fasting glucose, and high TC also increased during the study period. The prevalence of pre-pregnancy smokers also increased.

Discussion

In this study, we evaluated secular trends in the rates of CS deliveries from 2006 to 2015 and their increase from 36.5% in 2007 to 40.3% in 2015. A previous study on changes in CS rate South Korea from 1982 to 2012 reported that the rate remained at 36% for the most recent 6 years of the study [11]. Our study evaluated 3 later years than that study and found that the rate increased significantly up to 40%. Increased maternal age, primiparity, pre-pregnancy obesity, multiple pregnancy, and placenta previa were contributing factors to the increase, while the rate of vacuum deliveries and VBACs decreased during the study period.

The rise in the rate of CS is a worldwide phenomenon. A multinational survey of the CS rates by the WHO reported an overall mean of 26.4% [12], and a recent global analysis of 150 countries reported a 12.4% increase in the rate of CS, with an average annual rate of increase of 4.4% over the past decades [13]. The trend in CS in Hong Kong, whose population shares similar pelvic sizes as that in our population, demonstrated a 10% increase during a 20-year study period, resulting in CS rates up to 24–25% [14]. European countries including Norway and England also showed moderate increases of 15.4% and 24.1%, respectively [15]. Our study found a significantly increasing rate over the study period, almost 3 times higher than that in 2015 when compared to the WHO-recommended CS rate. Herein, we explored the factors contributing to the increase in the rates of CS.

Table 3. Secular trends in maternal pre-pregnancy factors between 2006 and 2015

Characteristics	2006 (n=57,690)	2007 (n=73,822)	2008 (n=72,884)	2009 (n=78,468)	2010 (n=90,100)	2011 (n=92,802)	2012 (n=99,639)	2013 (n=89,093)	2014 (n=90,094)	2015 (n=93,315)	*P*-value
Obesity	7.13	7.03	7.34	7.74	8.42	8.76	9.09	9.29	9.87	10.67	<0.01
High AST	0.80	0.68	0.66	0.74	0.87	0.88	0.93	0.97	1.03	1.07	<0.01
High ALT	1.30	0.97	1.00	1.22	1.37	1.45	1.47	1.52	1.69	1.71	<0.01
High fasting glucose	0.13	0.12	0.12	0.14	0.17	0.17	0.18	0.20	0.25	0.23	<0.01
High TC	2.33	2.26	2.36	2.50	2.86	3.10	3.15	3.23	3.62	3.84	<0.01
Current smoking	3.77	4.20	4.47	4.31	4.88	5.38	5.80	5.77	5.93	5.44	<0.01

Values are presented as percentage. Obesity was defined as BMI ≥25 kg/m^2. High LFTs were defined as AST and ALT levels of >31 U/L. High fasting glucose levels were defined as a glucose level of ≥126 mg/L. High cholesterol was defined as a cholesterol level of ≥200 mg/L.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; BMI, body mass index; LFT, liver function test.

One important finding to consider is the increase in the rate of primary CS. This can lead to another CS in the future because VBACs have been decreasing as shown in our results. The risk of uterine rupture increases with VBAC attempts, and this catastrophic event may lead to litigation problems. In addition, the incidence of placenta previa was increased in our population. Placenta previa might be the cause for the rise in rates of CS, or the increased the rates of CS might be the cause of placenta previa. The risk of placenta previa in subsequent pregnancies increases with the number of previous cesarean deliveries [16,17]. Increased maternal age, *in vitro* fertilization, and previous myomectomy are also directly linked to the risk of placenta previa [18,19].

One cause of increased primary CS is maternal request without indication. A previous survey conducted in Korea showed that 6–7% of CS were preformed upon maternal request without indication [11,20]. The increase in women's dissatisfaction with long labor and vaginal delivery and fear of emergency CS in the middle of labor due to arrest of labor or failure to progress have resulted in lower obstetrician thresholds for CS deliveries. The decrease in operative deliveries using forceps or vacuum was another consequence observed in our population. As operative delivery is associated with pelvic floor injuries and acute perinatal injuries, obstetricians tend to perform CS instead of using forceps or vacuum. The society has generally become far less tolerant of poor outcomes, and a trend has been toward placing blame either on the individual or on the system involved. This condition, and the fear of litigation, makes clinicians reluctant to resist maternal requests.

The most pressing social issue in South Korea is the low birth rate, which was only 0.98 in 2018 [21]. The main reasons contributing to this situation are women balancing careers with having children, the high cost of raising children, lifestyle choices regarding marriage, gender equity values, and higher education levels of women [22]; people avoid getting married and having many babies. This social phenomenon results in increased nulliparity and maternal aging. Consistent with previous findings, maternal aging, multiple pregnancy, and pre-pregnancy obesity were associated with increased rates of CS in this study [23].

There has been a gradual increase in the mean age of pregnant women in Korea. Older maternal age leads to a further rise in the CS rate. Maternal aging is a consequence of women's higher education level, followed by marriage at an older

age. Increased maternal age poses higher obstetric complications because these women have more chronic diseases such as diabetes and HTN before and during pregnancy. The rate of preeclampsia was slightly decreased during 10 years, but a higher rate of CS in preeclamptic patients was observed. One possible explanation for the decrease in preeclampsia is the introduction of voucher programs for maternal health care covered by KNHI since 2008, and this program has allowed easier access to facility antenatal care and reduced the financial burden of low-income pregnant women [24]. Maternal aging also results in an increased failure to progress due to a contracted pelvis [25]. Women with contracted pelvis are likely to pass their pelvic dimensions to their own offspring. This phenomenon implies that the application of life-saving CS can evolutionarily increase the rates of contracted pelvises [26]. These factors related to aging lead to increased rates of CS. Several previous statistics in many countries showed the link between older maternal age and the increased rates of CS [27].

Our results showed a 50% increase in the frequency in multiple pregnancy and decreased numbers of vaginal deliveries in multiple pregnancy. The evolution of infertility therapy is one of the main reasons for the increase in multiple pregnancy. In Korea, the Ministry of Health and Welfare has established regulations on the number of embryo transfers regarding maternal age and status of embryos such that no more than 3 cleavage-stage embryos or 2 blastocysts can be transferred even after the age of 35. This has had a direct influence on adverse obstetrical outcomes including increase in the rate of CS deliveries. There are more chances of malpresentation in multiple pregnancy and even without malpresentation, and doctors tend to perform CS instead of vaginal delivery.

Several pre-pregnancy factors were associated with increased rates of CS deliveries. Pre-pregnancy obesity was one important factor that increased the rate of CS. There has been a rapid rise in maternal pregnancy BMIs in the past decades, and the prevalence of adult obesity in South Korea increased from 29.7% in 2009 to 32.4% in 2015 due to increase in childhood obesity and human microbiome issues, in agreement with our results [28,29]. Obesity is a well-known factor that contributes to adverse maternal and neonatal outcomes [30]. Associated factors that lead to CS are chronic diseases of the mother, macrosomia due to uncontrolled blood glucose levels, excess intra-abdominal adipose tissue causing obstructed labor, and induction agent intolerance due to large body volumes [31-33].

Despite the decreasing trend in smoking among people worldwide, our results showed that smoking prevalence among Korean women increased [34]. Changes in gender role, higher education, and social norms are contributing factors for the increase in smoking trend among women. Other than this factor, abnormal liver function and higher fasting glucose levels appear to be associated with the risk of CS. These factors may increase the rate of CS because they are strongly associated with comorbidities. However, the relationship between these factors and CS should be investigated in the future.

Several interventions have been implemented to reduce unnecessary CS, but their effectiveness was limited. However, counseling low-risk primipara mothers who favor CS on the risk of CS and educating them on optimal gestational weight gain and the importance of controlling body weight before pregnancy may help to prevent unnecessary CS. In low-risk pregnancies, women who want a primary CS should be counseled on the increased risks of perinatal mortality and morbidity resulting from CS. They include higher blood loss, infection rates, thromboembolism, and adjacent organ injuries in mothers and respiratory problems and mortality in babies [35,36]. One previous study conducted in China reported that attendance in a prenatal education course significantly reduced the rate of maternal CS requests [37]. Education on labor and normal delivery, the benefit of babies born vaginally, the perinatal risk of CS, pain management during labor, and the supporting roles of the husband or parents by skilled obstetricians, and maternal unit nurses or midwives may help to lower the number of CS as noted in this study.

This population-based cohort study reports trends in the rate of CS deliveries in South Korea over 10 recent years. However, the study had several limitations. First, social factors such as educational background, socioeconomic status, labor force participation, and publicity of the hospital, which are known to be associated with CS rates, were not considered. Second, the results of our study are not completely generalizable because our health insurance system is different from those in other countries and the population enrolled was East Asian. Third, the indications for primary CS were not described and may not be accurate because insurance does not cover the CS on maternal request.

We observed increasing trends in the rates of CS over the

recent decade. Maternal aging, increased primiparity, increased pre-pregnancy obesity, increased placenta previa, increased multiple pregnancy, and decreased vacuum deliveries and VBACs were major factors associated with the increased rates of CS. CS should be undertaken when medically necessary, and informed consent should include short-term and long-term effects of CS for the sake of the mother and the baby. Efforts should focus on providing CS to the appropriate women at the right time. In addition, public health programs to lower the rate of CS should be established, and multidisciplinary discussion should be performed in the future, whether national health insurance will cover "CS upon maternal request."

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board (IRB) of the Korea University Medical Center (IRB No. 2019GR0256).

Patient consent

All the information that was provided for the study had been de-identified; therefore, we were unable to obtain informed consents from the participants.

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Evaluation of maternal rhesus blood type as a risk factor in adverse pregnancy outcomes in Korea: a nationwide health insurance database study

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Objectives
The current study aimed to investigate whether pregnancy outcomes are affected by maternal rhesus (Rh) status by comparing the primigravida pregnancy outcomes of Rh-negative women with those of Rh-positive women.

Methods
The study data were collected from the Korea National Health Insurance Claims Database and the National Health Screening Program for Infants and Children. In total, 1,664,882 primigravida women who gave birth between January 1, 2007 and December 31, 2014, were enrolled in this study. As the risk and severity of sensitization response increases with each subsequent pregnancy, only primigravida women were enrolled. The patients were divided into 2 groups according to Rh status, and the pregnancy outcomes were compared.

Results
In total, 1,661,320 women in the Rh-positive group and 3,290 in the Rh-negative group were assessed. With regard to adverse pregnancy outcomes, there was no statistically significant difference between the 2 groups in terms of the prevalence of preeclampsia, postpartum hemorrhage, abruptio placenta, placenta previa, and uterine artery embolization. A univariate analysis revealed that none of the adverse pregnancy outcomes were significantly correlated to Rh status (preeclampsia: odds ratio [OR], 1.00, 95% confidence interval [CI], 0.81–1.23; postpartum hemorrhage: OR, 1.10, 95% CI, 0.98–1.24; abruptio placenta: OR, 0.80, 95% CI, 0.46–1.37; and placenta previa: OR, 1.08, 95% CI, 0.78–1.42). The adjusted ORs of postpartum hemorrhage and preterm birth did not significantly differ.

Conclusion
Maternal Rh status is not associated with adverse outcomes in primigravida women.

Keywords: Rhesus blood-group system; Pregnancy outcomes; Gravidity; National health programs

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Introduction

A person's blood type is determined according to the specific antigen types on the erythrocyte membranes of red blood cells (RBCs). The 2 main factors that determine the blood type are ABO (A, B, AB, and O) and rhesus (Rh) (positive or negative). Karl Landsteiner first discovered the ABO blood group system in 1900 when he was investigating the causes of some fatal transfusions [1]. The ABO blood group system was named according to the different agglutinins, or blood group antigens, including A, B, and H (or O) antigens, on the surface of human RBCs [1]. Based on the presence or absence of the Rh factor, the blood group system is called the Rh blood group system. Rh represents the first 2 letters of the name Macacus Rhesus. In 1940, during animal experiments, Landsteiner and other scientists discovered that rhesus monkeys and most human RBCs have antigenic Rh blood types, and this was used in naming the system [2]. With continuous studies of the Rh blood groups, the Rh blood group system was found to be the most complex system in the RBCs [3]. The discovery of the Rh blood type has played an important role not only in guiding blood transfusions more scientifically but also in improving experimental diagnoses and clinical immunotherapy.

The blood type may affect human health and diseases with a wide range of expression in human cells and tissues, including platelets, epithelium, and vascular endothelium [4,5]. Therefore, several studies showed the clinical significance of the biological characteristics of the ABO blood system, particularly with regard to cancer, cardiovascular disease, and pregnancy-related disease [6-8]. Numerous reports revealed that the ABO blood group may be associated with some risk factors for unfavorable pregnancy outcomes [9].

However, data about whether maternal Rh blood type alone, without consideration of alloimmune sensitization, is associated with the development of pregnancy-related diseases are limited. Moreover, the Rh-negative population is extremely small. Thus, a large population must be evaluated to assess the role of Rh blood type in identifying women at risk of developing pregnancy-related complications.

Thus, this study aimed to investigate whether pregnancy outcomes are affected by maternal Rh status by comparing the primigravida pregnancy outcomes of Rh-negative women with those of Rh-positive women in a nationwide population study.

Materials and methods

1. Health care in Korea

Approximately 97% of the Korean population is enrolled in the Korea National Health Insurance (KNHI) program. All claims data are stored in the KNHI claims database. As part of the KNHI system, a National Health Screening Program for Infants and Children (NHSP-IC) was started in 2007, and it includes information about physical examination findings, anthropometric measurements, and developmental screening results after birth. This study used information from the KNHI claims database to identify all women who gave birth between January 1, 2007 and December 31, 2014. Moreover, whether these women were Rh-positive or Rh-negative based on the applicable codes from the International Classification of Disease, 10th Revision was assessed. The data of women who met the inclusion criteria were linked to those of their offspring in the NHSP-IC database.

2. Dataset and outcomes

Fig. 1 shows the flowchart of participant enrollment. Using the KNHI claims data, we identified all women who gave birth between January 1, 2007 and December 31, 2014 (n=3,383,282). The maternal datasets were merged with those in the NHSP-IC database. Multigravida women (n=1,650,500), those whose offspring had not undergone

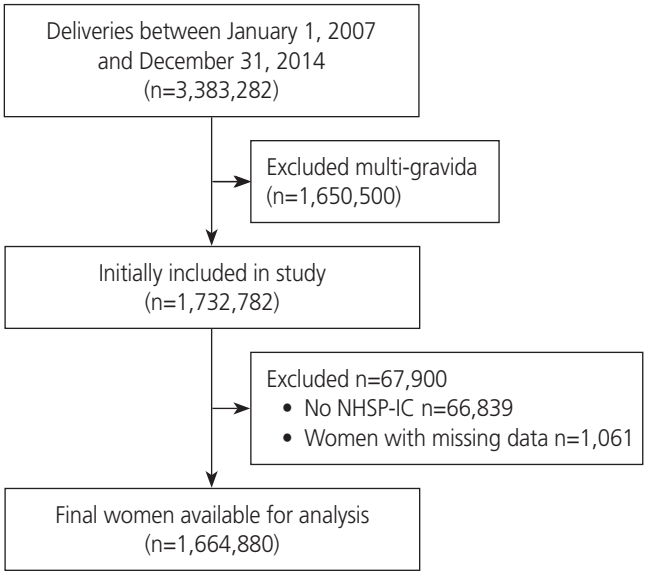

Fig. 1. Flowchart of participant enrollment. NHSP-IC, National Health Screening Program for Infants and Children.

NHSP-IC health examinations (n=66,839), and those with missing data (n=1,061) were excluded from the study. Data regarding maternal and offspring outcomes were extracted. The information included pregnancy outcomes, such as parity, type of delivery, pulmonary embolism, postpartum hemorrhage, abruptio placenta, placenta previa, and uterine artery embolization. Furthermore, the characteristics of the neonates, such as sex, whether delivered preterm, and birth weight, were evaluated. Preterm birth was defined as birth at a gestational age <37 weeks, low birth weight (LBW) as birth weight <2.5 kg, and large for gestational age (LGA) as birthweight >4.0 kg.

3. Statistical analysis

Continuous and categorical variables were expressed as mean±standard deviation and number (percentage), respectively. The clinical characteristics of the participants were compared using *t*-test for continuous variables and the χ^2 test for categorical variables. The risks of Rh-negative blood type were evaluated via multiple regression analyses. The risk of postpartum hemorrhage and preterm birth was adjusted for each related risk factor (postpartum hemorrhage: age, multiple pregnancy, cesarean section, preterm birth, LBW, LGA, preeclampsia, abruptio placenta, and placenta previa; preterm birth: age, multiple pregnancy, and preeclampsia). All tests were 2-sided, and a *P*-value <0.05 was considered statistically significant. Statistical analyses were performed using SAS for Windows (version 9.4; SAS, Cary, NC, USA).

Results

1. Baseline characteristics of the study population

The clinical characteristics of the participants are shown in Table 1. There were no significant differences in terms of age at delivery and multiple pregnancy and cesarean sec-

Table 1. Clinical characteristics of the participants

Characteristics	Rh-positive (n=1,661,320)	Rh-negative (n=3,290)	*P*-value
Age (yr)	29.8±3.7	29.7±3.5	0.444
Age >35 yr at delivery	161,506 (9.7)	317 (8.8)	0.058
Multiple gestation	33,479 (2.0)	82 (2.3)	0.270
Cesarean section	617,044 (37.1)	1,311 (36.4)	0.323
Preeclampsia	40,998 (2.5)	89 (2.5)	0.998
Postpartum hemorrhage	130,422 (7.9)	308 (8.5)	0.124
Placenta abruption	7,528 (0.5)	13 (0.4)	0.407
Placenta previa	18,899 (1.1)	43 (1.2)	0.757
Uterine artery embolization	2,101 (0.1)	6 (0.2)	0.501

Data were represented as mean±standard deviation and number (%).
Rh, rhesus.

Table 2. Neonatal weight differences between the rhesus (Rh)-positive and Rh-negative groups

Characteristics	Rh-positive (n=1,661,320)	Rh-negative (n=3,290)	*P*-value
Male sex	853,655 (51.38)	1,883 (52.20)	0.325
Preterm birth	57,406 (3.46)	129 (3.58)	0.691
Neonatal weight (g)	3.18±0.49	3.17±0.52	0.301
Normal weight	1,520,122 (91.50)	3,316 (91.93)	0.273
Low birth weight	82,309 (4.95)	181 (5.02)	0.221
Large birth weight	58,889 (3.54)	110 (3.05)	0.313

Data were presented as mean±standard deviation and number (%). Low birth weight: <2,500 g, large birth weight: >4,000 g, and preterm delivery: <37 gestational weeks.

tion rates between the Rh-positive group (n=1,661,320) and Rh-negative group (n=3,290). Moreover, adverse delivery outcomes did not significantly differ between the 2 groups. Moreover, preterm birth rates and birth weight (corrected for gestational age) did not significantly differ between the Rh-positive and Rh-negative groups (Table 2).

2. Univariate regression analysis of Rh blood type as a risk factor for pregnancy outcomes

Univariate regression analyses were performed to evaluate the associations between Rh blood type and various clinical outcomes (Table 3). The Rh blood type was not a risk factor for preterm birth (odds ratio [OR], 1.13, 95% confidence interval [CI], 0.91–1.41), LBW (OR, 1.01, 95% CI, 0.87–1.18), and LGA (OR, 0.86, 95% CI, 0.71–1.04). Rh blood type was not a risk factor for adverse pregnancy outcomes (preeclampsia: OR, 1.00, 95% CI, 0.81–1.23; postpartum hemorrhage: OR, 1.10, 95% CI, 0.98–1.24; abruptio placenta: OR, 0.80, 95% CI, 0.46–1.37; and placenta previa: OR, 1.08, 95% CI, 0.78–1.42). Moreover, age-adjusted univariate regression analyses revealed no significant relationships between the Rh blood type and these factors.

3. Multiple regression analysis of rhesus blood type as a risk factor for postpartum hemorrhage and preterm birth

Based on the univariate analysis, the adjusted ORs for postpartum hemorrhage and preterm birth were analyzed because there was no significant relationship between Rh blood type and clinical outcomes (Table 4). The risk factors for postpartum hemorrhage were age, multiple pregnancy, cesarean section, preterm birth, LBW, LGA, preeclampsia, abruptio placenta, and placenta previa. Preterm birth was adjusted for age, multiple pregnancy, and preeclampsia. After adjusting for the factors, no relationships were found between Rh blood type and postpartum hemorrhage (OR, 1.10, 95% CI, 0.98–1.23) and preterm birth (OR, 0.02, 95% CI, 0.85–1.23).

Table 3. Univariate regression analysis of rhesus blood type as a risk factor of pregnancy outcomes

Characteristics	OR (95% CI)	Age-adjusted OR (95% CI)
Multiple pregnancy	1.13 (0.91–1.41)	1.15 (0.92–1.43)
Cesarean section	0.97 (0.90–1.03)	0.97 (0.91–1.04)
Preterm birth	1.04 (0.87–1.24)	1.04 (0.87–1.24)
LBW	1.01 (0.87–1.18)	1.02 (0.88–1.18)
LGA	0.86 (0.71–1.04)	0.86 (0.71–1.04)
Preeclampsia	1.00 (0.81–1.23)	1.00 (0.81–1.24)
Postpartum hemorrhage	1.10 (0.98–1.24)	1.10 (0.98–1.23)
Placental abruption	0.80 (0.46–1.37)	0.80 (0.46–1.38)
Placental previa	1.08 (0.78–1.42)	1.07 (0.79–1.44)

OR, odds ratio; CI, confidence interval; LBW, low birth weight; LGA, large for gestational age.

Discussion

The proportions of blood type differ according to race, region, and ethnicity. In general, Africa, the Middle East, Europe, India, and Central Asia have higher Rh-negative blood rates than other regions. In Korea, Rh-negative is considered a rare blood type according to the Korean Red Cross standards, with an incidence of 0.4%; however, pregnancy can lead to several complications among Rh-negative women [10]. Currently, a non-O blood group may affect hemostatic balance disturbances and lead to an increased risk of embolization compared to an O blood group [11]. By contrast, another study showed a weak relationship between ABO blood type and gestational hypertension [12,13]. However, whether Rh blood type (negative or positive) is correlated to pregnancy outcomes is not known. Therefore, this study focused on the association between Rh-positive and Rh-negative blood groups and maternal pregnancy outcomes in 1,664,882 primigravida women in a large population study. The clinical characteristics did not differ significantly between Rh-positive (n=1,661,320) and Rh-negative women (n=3,290).

Several studies showed that the Rh blood type plays an important role in neonatal alloimmune disorders. The immune

Table 4. Multiple regression analysis of rhesus blood type as a risk factor of postpartum hemorrhage and preterm birth

Characteristics	Risk factor: adjusted OR (95% CI)
Postpartum hemorrhage[a]	1.10 (0.98–1.23)
Preterm birth[b]	0.02 (0.85–1.23)

OR, odds ratio; CI, confidence interval.
[a]Postpartum hemorrhage: adjusted for age, multiple pregnancy, cesarean section, preterm birth, low birth weight, large birth weight, preeclampsia, abruptio placenta, and placenta previa; [b]Preterm birth: adjusted for age, multiple pregnancy, and preeclampsia.

system of a Rh-negative mother considers Rh-positive fetal cells as foreign substances. Then, the mother's body produces antibodies against fetal blood cells. Antibodies, such as IgG1 and IgG3, cross back through the placenta to the baby [14,15]. Then, they destroy the baby's circulating RBCs, and this eventually develops into complications, such as preterm delivery, LBW, hydrops fetalis, hyperbilirubinemia, anemia, and bilirubin-induced neurological dysfunction [16-20].

In this study, there was no significant association between Rh blood type and neonatal outcomes, including preterm birth, LBW, and LGA. As previously mentioned, as the risk and severity of sensitization response increases with each subsequent pregnancy, we only included primigravida women in this analysis to reduce selection bias. During the first pregnancy, the initial exposure to fetal RBCs results in the formation of IgM antibodies, and these do not cross the placental barrier. Thus, no differences were observed in the first pregnancies of Rh-negative women [21]. Based on our results, the relationship between fetal gross and Rh blood type without an alloimmune effect is weak.

The World Health Organization reports that the major contributors to maternal death or maternal mortality are postpartum hemorrhage and hypertensive disorders [22]. Various studies have investigated whether there is an association between maternal ABO blood type and maternal adverse pregnancy outcomes [8,12,23-25]. In brief, maternal ABO blood type was correlated to the risk of preeclampsia. However, its association with GDM, preterm delivery, LBW, and SGA remains controversial.

Compared to the ABO types, studies about Rh blood type are limited. Thus, this study evaluated the differences in the rate of cesarean section, preeclampsia, postpartum hemorrhage, abruptio placenta, placenta previa, and uterine artery embolization among pregnant women with different Rh blood types. However, there was no significant association between Rh blood type and pregnancy outcomes, even preeclampsia, in primiparous women. Lee et al. [24] showed that Rh D-positive mothers had a slightly increased risk of preeclampsia in a large cohort study in Sweden (OR, 1.07, 95% CI, 1.03–1.10). However, in this study, multiparous women were enrolled, and only the Rh D antigen was evaluated. In a previous research, more than 50 Rh antigens have been identified at the serological level encoding 2 genes (RHD and RHCE). Among these antigens, Rh D, C, c, E, and e were considered the most clinically significant, and Rh D has the strongest antigenicity [26]. In our study, different findings were obtained, which might be attributed to the fact that we did not assess the differences in these antigens.

However, we did not find any associations between Rh blood type and adverse pregnancy outcomes. This result might be explained partly by the limitations of our research. First, this study only included Rh blood type classifications. However, the differences between the specific Rh blood type or the ABO classification were not assessed because the ABO blood group is not considered a disease category in the KNHI program. Second, as described above, the exclusion of multigravida pregnant women might have influenced the relatively varying results of the rates of preeclampsia compared to other previous studies. Third, with regard to neonatal outcomes, attention is now shifting towards short- and long-term morbidity [27]; however, in this study, only neonatal weight was included, and more detailed data are required to evaluate neonatal outcomes. To the best of our knowledge, this is the first nationwide retrospective research about the association between Rh blood type and adverse pregnancy outcomes in Korea. Hence, further studies must be conducted for a more detailed evaluation of the Rh blood type among pregnant women.

In conclusion, there were no significant differences in the risk of adverse pregnancy outcomes between Rh-positive and Rh-negative primigravida women in Korea.

Acknowledgments

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study protocol was approved by the institutional review board of Korea University Medical Center (IRB number: 2019GR0256).

Patient consent

This paper is a retrospective study and does not require written informed consent.

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Cervicovaginal fluid cytokines as predictive markers of preterm birth in symptomatic women

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Objective
Here, we investigated whether cytokines in the cervicovaginal fluid (CVF) can be predictive markers of preterm birth (PTB).

Methods
A multi-center prospective cohort study was conducted on 59 singleton pregnant women hospitalized for preterm labor (PTL) and/or preterm premature rupture of membranes (pPROM) between 22 weeks and 36 weeks 6 days of gestation from 2014 to 2015. The levels of 13 inflammatory cytokines (macrophage inflammatory protein [MIP]-1α, MIP-1β, tumor necrosis factor [TNF]-α, interleukin [IL]-1β, IL-6, IL-8, IL-17α, granulocyte colony stimulating factor [G-CSF], IL-7, IL-4, IL-5, IL-10, and IL-13) were measured using a multiplex bead-based immunoassay and that of fetal fibronectin (fFN) was measured using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using Student's *t*-test, Mann-Whitney *U* test, Pearson's correlation, and receiver operating characteristic (ROC) curve analysis in SPSS version 20.0.

Results
Among the 13 cytokines assessed, the levels of 3 cytokines (MIP-1α, IL-6, and IL-7) were negatively correlated with gestational age at delivery (P=0.028, P=0.002, and P=0.018, respectively). Sensitivities of MIP-1α, IL-6, and IL-17α were 70%, 80%, and 75%, respectively, and their specificities were 57%, 65%, and 69%, respectively. The sensitivity and specificity of fFN were 33% and 95%, respectively.

Conclusion
In symptomatic women diagnosed with PTL and/or pPROM, cytokines from cervicovaginal fluid, especially IL-6 and IL-17α, could be better predictive markers of PTB than fFN.

Keywords: Cytokines; Interleukin-6; Interleukin-17A; Biomarkers; Preterm birth

Introduction

Preterm birth (PTB), defined as birth before 37 weeks of gestation, is a major cause of neonatal morbidity and mortality [1,2]. Over the past decade, the prevalence of preterm births has steadily increased worldwide. Morbidity and mortality of infants born before 34 weeks of gestation are more than those of infants born after 34 weeks. Therefore, antenatal steroid administration is recommended for patients with preterm labor (PTL) and preterm premature rupture of membranes (pPROM). However, prediction methods and therapies

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for the same are not well studied.

PTB can be classified into two categories: spontaneous and iatrogenic. Spontaneous PTB accounts for up to 75% of all PTBs [3]. About 45% of PTBs are caused by PTL with intact membranes, while 30% are caused by the pre-labor rupture of fetal membranes. Causes of spontaneous PTB include activation of inflammatory reactions in the gestational tissues and secretion of inflammatory cytokines as an immune response to ascending infection of the genital tract and abnormal vaginal microorganisms [4-7].

Many studies aimed at predicting PTB have been conducted. Commonly used screening tests include risk scoring, cervical length measurement, and detection of biochemical markers. Risk scoring involves quantification of risk factors for spontaneous PTB and/or pPROM, including PTB history, cervical conization history, low socioeconomic status, low body mass index, smoking, drug use, anxiety, multifetal gestation, gestational diabetes, and gestational hypertension [6-9]. Tests for fetal fibronectin (fFN) and phosphorylated insulin-like growth factor binding protein-1 (IGFBP1) are now commercially available [10,11]. The level of fFN in cervicovaginal fluid (CVF), leaked due to the disruption of maternal choriodecidual tissues and through matrix remodeling, was used as a diagnostic marker for predicting PTB using a non-invasive method. Lockwood et al. described the clinical utility of fFN to predict spontaneous PTB in symptomatic women with high sensitivity (81.7%) and specificity (82.5%), based on a fFN threshold concentration of ≥50 ng/mL [3,8]. However, its specificity and sensitivity were confounded by unprotected vaginal intercourse, digital examination, bleeding, or contamination with amniotic fluid [1,12]. Cervical length measurement using transvaginal sonograms in patients with PTL and pPROM have also been used to predict PTB; however, it requires a skilled ultrasonographer and shows limited correlation with CVF [13-15].

Increased levels of inflammatory cytokines in CVF signify intra-amniotic infection that causes PTB [16]. Several inflammatory cytokines, including interleukin (IL)-1, -2, -4, -6, -8, -10, -12 and -17, tumor necrosis factor-alpha (TNF-α), interferon gamma (INF-γ), regulated on activation, normal T cell expressed and secreted (RANTES), and C-reactive protein (CRP), have been detected in the CVF, amniotic fluid, and blood of asymptomatic and symptomatic pregnant women [3,4,17,18]. IL-6 and matrix metalloproteinase-8 (MMP-8) levels in the amniotic fluid were significantly correlated with pPROM [19,20]. We also found that the levels of cytokines such as IL-1b, IL-6, IL-7, IL-7a, and TNF-α were significantly increased in the amniotic fluid of patients with cervical insufficiency [16]. Thereafter, the objective of our study was to determine whether inflammatory cytokines could be measured using non-invasive methods. Recent studies have shown that IL-6, IL-8, macrophage inflammatory protein (MIP)-1α, and MIP-1β levels in the CVF of patients with pPROM were correlated with those in the amniotic fluid [21]. IL-1β, IL-6, IL-8, and TNF-α levels were also elevated in patients with intact membranes in PTL [22]. There have also been studies in which IL-6 was shown to be a useful marker for predicting preterm delivery.

In the preset study, we expected that the levels of inflammatory cytokines in the CVF would be elevated in patients with PTL and pPROM. We investigated whether the levels of cytokines in the CVF could be a better predictive marker of PTB than that of fFN in CVF.

Materials and methods

1. Study population

A multi-center (Ewha Womans University Mokdong Hospital, Samsung Medical Center, Konkuk University Hospital, Seoul St. Mary's Hospital, and Seoul Metropolitan Government-Seoul National University Boramae Medical Center) prospective cohort study was conducted in singleton pregnant women suffering from complications of PTL and/or pPROM between 22 weeks and 36 weeks 6 days of gestation, from 2014 to 2015. The characteristics (age, parity, and body mass index at admission) of the mother were analyzed. To diagnose pPROM, sterile speculum exam was conducted for detecting amniotic fluid pooling in vaginal cavity and nitrazine test was done. Uterine activity was assessed by cardiotocography. PTL was diagnosed in patients with regular uterine contraction and 4 or more contractions in 20 minutes, or 8 or more in 60 minutes as detected by cardiotocography. A total of 65 women with singleton pregnancies were included in this study. Patients with preeclampsia, hemolysis, elevated liver enzymes, a low platelet count (HELLP) syndrome, incompetent internal os of cervix (IIOC), and placenta previa were excluded. Out of the 59 participants, 19 were diagnosed with pPROM, and the rest with PTL (Fig. 1); no patient was suspected of clinical chorioamnionitis, defined according to

the criteria of Gibbs et al. [23], which includes the presence of fever (>37.8°C) and two or more of the following associated clinical findings: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis, maternal tachycardia, and fetal tachycardia. Gestational age was estimated by fetal biometry, assessed using a sonogram during the first trimester. Cervical length was measured at the time of admission by transvaginal ultrasound. Vaginal transducers were placed in the anterior fornix to examine the endocervical canal. Calipers were used to measure the length of the cervical canal from internal to external os. Women with pPROM at less than 35 weeks of gestation were treated with corticosteroids, tocolytics, and antibiotics. After 34 weeks, all patients with pPROM underwent induced delivery, when there was no labor pain, or cesarean section, if it was indicated. Those with PTL were treated with corticosteroids and tocolytics. We checked pregnancy outcome in terms of gestational age at delivery, birth weight, and Apgar score of the newborn at 1 and 5 minutes.

2. Cervicovaginal fluid collection and laboratory study

CVF was collected from the posterior vaginal fornix with a sterile cotton swab and stored at –80°C within 30 minutes of collection, until further analysis. The levels of 13 inflammatory cytokines (MIP-1α, MIP-1β, TNF-α, IL-1β, IL-6, IL-8, IL-17α, granulocyte-colony stimulating factor [G-CSF], IL-7, IL-4, IL-5, IL-10, and IL-13) in the CVF were measured using the Human Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore, Burlington, MA, USA). The intra-assay and inter-assay variations of all analyses were less than 10% and 12%, respectively. fFN in CVF was also sampled similarly and measured using a human fFN enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO, Houston, TX, USA).

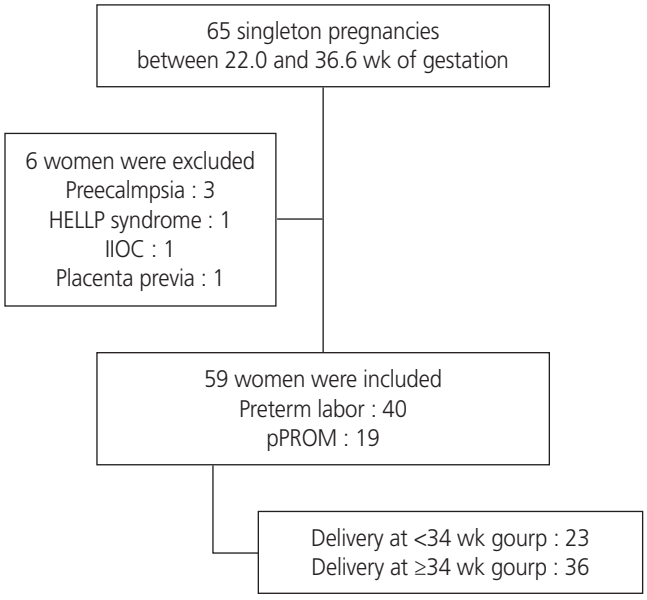

Fig. 1. Flow chart of participants in the cohort study. HELLP, hemolysis, elevated liver enzymes, and a low platelet count; IIOC, incompetent internal os of cervix.

3. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (version 20; SPSS Inc., Chicago, IL, USA). Clinical characteristics were compared using Student's *t*-test for continuous variables and χ^2 test for categorical variables. $P<0.05$ was considered statistically significant. The relationship between the level of cytokines and gestational age at delivery was analyzed using Pearson's correlation. The levels of cytokines were analyzed by the Mann-Whitney *U* test and compared between preterm delivery group (delivery at <34 weeks) and normal controls (delivery at ≥34 weeks). Receiver operating characteristic (ROC) curves of cytokine levels in early preterm birth were generated and the area under the curve (AUC) was determined.

Results

1. Characteristics of the study population

The demographic and clinical data of the study participants are presented in Table 1. The study group consisted of 23 pregnant women who delivered before 34 weeks of gestation and the controls (n=36) delivered after 34 weeks of gestation. There was no significant difference in age, parity, body mass index, and cervical length between the two groups ($P=0.519$, $P=0.730$, $P=0.487$, and $P=0.381$, respectively). fFN in the CVF showed a significant difference between the two groups ($P=0.017$). The study group was divided into PTL- and pPROM- subgroups. Frequency analysis was performed at the time of admission using the χ^2 test for the diagnosis. There was no statistical difference in the distribution between the two groups, with a *P*-value of 0.77. Gestational age at the time of diagnosis was significantly different for the two groups ($P=0.002$). The white blood cell count, birth weight, and Apgar score of the newborns showed sig-

nificant differences between the two groups (P<0.001).

2. Cytokine levels in the cervicovaginal fluid in the study and control groups

Among the 13 cytokines considered for the study, 9 (MIP-1β, MIP-1α, TNF-α, IL-1β, IL-6, IL-8, IL-17α, G-CSF, IL-7) were detected in the CVF of the 2 groups. Fig. 2 graphically shows the correlation of these 9 cytokines with the gestational age at delivery; 4 of these (MIP-1α, MIP-1β, IL-6, and IL-7) showed a significant negative correlation (P=0.003, P=0.010, P=0.035, and P=0.041, respectively). The levels of these 9 cytokines were compared between the study group and control group and are shown in Table 2. The levels of 3 cytokines (MIP-1α, IL-6, and IL-17α) were significantly higher in patients with PTL and/or pPROM who delivered before 34 weeks of gestation, compared to controls (P=0.028, P=0.002, and P=0.018, respectively). ROC curves were constructed for the 5 cytokines (MIP-1α, MIP-1β, IL-6, IL-7, and IL-17α) showing significant differences between the two groups and AUC values and optimal concentration thresholds for predictive utility were determined. Based on these thresholds, the sensitivities and specificities for the prediction of PTB were determined (Fig. 3 and Table 3). Sensitivities of MIP-1β, MIP-1α, IL-6, IL-17α, and IL-7 were 47%, 70%, 80%, 75%, and 35%, respectively, and their specificities of were 82%, 57%, 65%, 69%, and 94%, respectively; the areas under the ROC curves of these cytokines were 0.676, 0.677, 0.788, 0.715, and 0.643, respectively. The sensitivity and specificity of CVF fFN were 33% and 95%, respectively. Forty patients who were diagnosed with PTL at the time of admission were analyzed. The levels of IL-6 and IL-7 showed significant increase in the study group with P-values of 0.023 and 0.040, respectively (Supplementary Table 1). The ROC curves for these 2 cytokines were constructed and AUC values and optimal concentration thresholds for predictive utility were determined (Supplementary Table 2). Sensitivities of IL-6 and IL-7 were 83% and 83%, respectively, and their specificities were 76% and 64%, respectively. The sensitivity and specificity of CVF fFN were 33% and 70%, respectively. Subgroup analysis of 19 patients who were diagnosed with pPROM at the time of admission compared the cytokines of PTB and control group. IL-1β showed a significant difference with P-value 0.043 (Supplementary Table 3).

Table 1. Clinical characteristics of the study population (n=59)

Characteristics	Delivery at <34 wk (n=23)	Delivery at ≥34 wk (n=36)	P-value[a]
Maternal age (yr)	31 (23–38)	32 (19–46)	0.519
Nulliparity	6 (16.7)	3 (13.0)	0.730[d]
BMI (kg/m^2)	24 (20–33)	25 (18–32)	0.487
Fetal fibronectin (ng/mL)	133 (11–699)	40 (11–142)	0.017[b]
WBC (cell/mL)	13,596 (8,130–24,280)	10,063 (6,440–16,640)	<0.001[c]
Cervical length (mm)	20 (0–44)	23 (2–69)	0.381
<25 mm group	12 (57.1)	18 (54.5)	>0.990[d]
≥25 mm group	9 (42.9)	15 (45.5)	
Diagnosis at admission			
PTL group	12 (52.2)	28 (77.8)	0.077[d]
pPROM group	11 (47.8)	8 (22.2)	
Gestational age at admission (wk)	28 (22–33)	31 (20–36)	0.002[b]
Gestational age at delivery (wk)	29 (23–33)	36 (34–41)	<0.001[c]
Birth weight (g)	1,323 (570–2,320)	2,640 (780–3,740)	<0.001[c]
Apgar score at 1 min	6 (0–10)	8 (4–10)	<0.001[c]
Apgar score at 5 min	7 (0–10)	9 (8–10)	<0.001[c]

Data are expressed as median (range) for continuous variables and number (%) for categorical variables.
BMI, body mass index; WBC, white blood cell; PTL, preterm labor; pPROM, preterm premature rupture of membranes.
[a]Continuous variable were analyzed by Student's t-test; [b]Significant difference between 2 group (P<0.05); [c]Significant difference between 2 group (P<0.001); [d]Categorical variable was analyzed by χ^2 test.

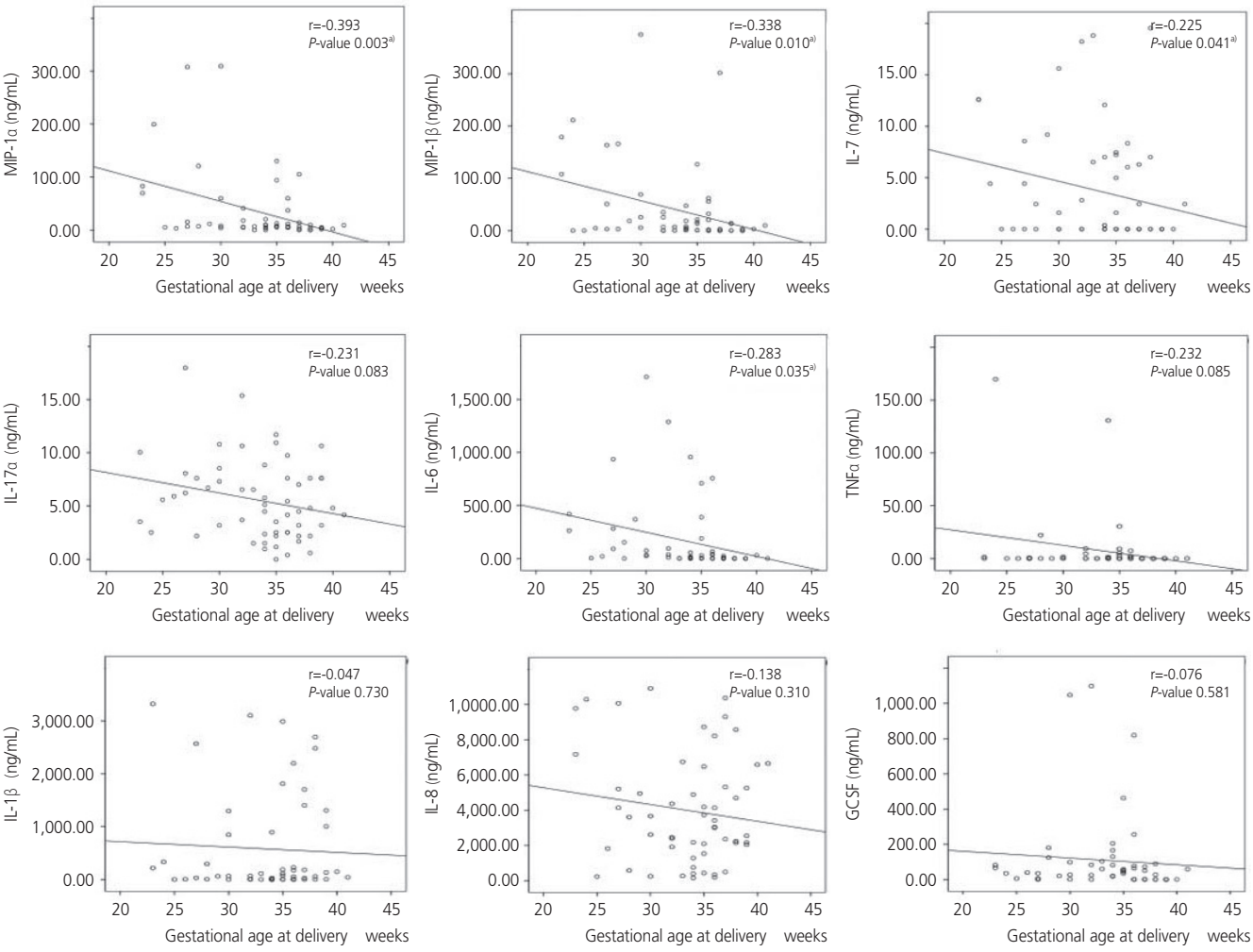

Fig. 2. The relationship between cytokine level and gestational age at delivery. Using Pearson correlation. MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; IL, interleukin; G-CSF, granulocyte colony stimulating factor. [a]Significantly negative correlated with the gestational age at delivery (P<0.05).

Table 2. Cervicovaginal fluid cytokine levels in patients in the study group and control group

Cytokine	Delivery at <34 wk (n=23)	Delivery at ≥34 wk (n=36)	*P*-value[b]
MIP-1β	18.56 (0–375.00)	2.89 (0–301.56)	0.067
MIP-1α	11.62 (0–309.47)	5.73 (0–130.48)	0.028[a]
TNF-α	0 (0–169.67)	0 (0–130.66)	0.661
IL-1β	65.35 (0–3,321.48)	67.08 (0–2,990.27)	0.738
IL-6	83.27 (0–1,712.41)	1.44 (0–956.15)	0.002[a]
IL-8	3,663.22 (234.78–10,911.85)	3,007.72 (154.79–10,365.90)	0.472
IL-17α	6.54 (1.51–17.97)	4.17 (0–11.69)	0.018[a]
G-CSF	38.80 (0–1,097.48)	38.74 (0–818.30)	0.225
IL-7	2.81 (0–18.83)	0 (0–19.55)	0.064

Data are expressed as the median (range) (pg/mL).
MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; IL, interleukin; G-CSF, granulocyte colony stimulating factor.
[a]Significant difference between 2 groups (P<0.05); [b]Using Mann-Whitney U test.

Discussion

Our study showed that elevated levels of multiple cytokines (MIP-1α, MIP-1β, IL-6, IL-7, and IL-17α) in the CVF were associated with PTB. Among these, IL-6 and IL-17α showed better sensitivity than the more commonly used marker fFN. This indicated that IL-6 and IL-17α can be used as predictive markers for PTB.

Fetal fibronectin, involved in maintaining the integrity of the fetal chorion and maternal decidua, predicts spontaneous PTB when detected in CVF between 22 and 34 weeks of gestation. Meta-analysis of Leitich et al. [24], found the sensitivity and specificity of fFN in predicting PTB in mothers with PTL to be 63% and 86%, respectively. In our study, however, fFN showed 33% sensitivity of and 95% specificity as a predictor of PTB. As shown in Table 3, the sensitivities of IL-6 and IL-17α were higher than those of fFN by 80% and 75%, respectively. The AUC of fFN was 0.662, while those of IL-6 and IL-17α were 0.788 and 0.715, respectively. The results for fFN may be confounded by unprotected vaginal intercourse, digital examination, bleeding, or contamination with amniotic fluid [1,12]. We did not consider the amniotic fluid contamination of CVF as a confounding factor and believe that it affected the outcome for pPROM patients. Therefore, in the subgroup analysis, the fFN and cytokine levels of the PTL patients, but not of the pPROM patients, were compared. In subgroup analysis, the sensitivity of IL-6 and IL-7 both were 83%, which was higher than the 33% sensitivity of fFN.

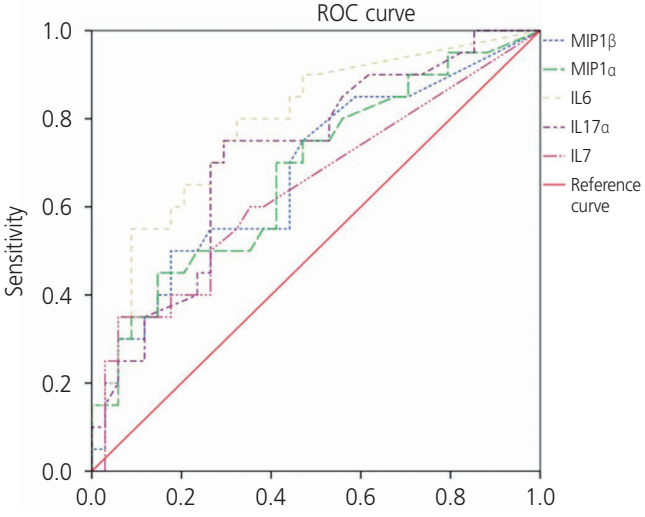

Fig. 3. ROC curves of 5 cytokines in the prediction of early preterm birth (delivery at <34 weeks). ROC, receiver operating characteristic; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; IL, interleukin; G-CSF, granulocyte colony stimulating factor.

Several protein mediators and cytokines have been studied in the amniotic fluid, blood, urine, and CVF [1,25,26]. Studies considering the association of elevated inflammatory cytokines with chorioamnionitis and PTL were initiated in the amniotic fluid [19]. However, since amniocentesis itself is an invasive method, many studies sought to measure the same non-invasively in the blood, urine, and CVF. The composition of human CVF reflects the local biochemical environment of the gestational tissues. Therefore, CVF proteins, such as fFN, IGFBP1, defensins, lactoferrin, sialidase, granulocyte elastase, human chorionic gonadotropin, IL-1β, IL-6, IL-8, IL-18, IL-1 receptor antagonists, and TNF have been utilized to predict PTB [27-32]. Particularly, IL-6 is strongly associated with intra-amniotic inflammation and microbe-associated intra-amniotic inflammation; hence, measurement of IL-6 in CVF can be used as an easy, noninvasive, rapid method for point-of-care assessment [33,34]. IL-6 induces T-lymphocytes to synthesize C-reactive protein and to promote the differentiation of B-cells. It is a pro-inflammatory cytokine seen widely in de-

Table 3. Receiver operating characteristic curve analysis

Cytokine	AUC	95% CI	*P*-value	Cut-off	Sensitivity	Specificity
MIP-1β	0.676	0.525–0.828	0.032	17.51	47	82
MIP-1α	0.677	0.527–0.828	0.031	6.46	70	57
IL-6	0.788	0.661–0.915	0.000	23.46	80	65
IL-17α	0.715	0.574–0.857	0.009	5.53	75	69
IL-7	0.643	0.485–0.802	0.081	0.98	35	94
Fibronectin	0.662	0.494–0.830	0.073	74.50	33	95

AUC, area under a receiver operating characteristic curve; CI, confidence interval; MIP, macrophage inflammatory protein; IL, interleukin.

cidual tissue, placenta, fetal membrane, and amniotic fluid, and regulates immune adaptation to allow the pregnancy to progress. Preterm labor and pPROM are thought be because of the inflammatory condition caused by infection. Our study has showed that IL-6 is superior to conventional fFN assays. IL-17α as a protective cytokine in host defense against bacterial and fungal infections, was shown to be associated with intra-amniotic inflammation [35]. No study has analyzed IL-17 in the CVF; however, IL-17α was significantly elevated in the amniotic fluid of patients with cervical insufficiency [16]. MIP-1α and MIP-1β are produced in the macrophages in response to infection and inflammation. They subsequently activate human granulocytes and the level of MIP-1α in the CVF is associated with amniotic fluid infection [21,36,37]. MIP-1α has been studied in the CVF of patients with pPROM, but not PTL. In this study, we found that elevation of the MIP-1α level in the CVF was associated with PTL. IL-7 is a cytokine that can stimulate IL-17 production by cells involved in both innate and adaptive immunity. Elevated serum IL-7 is also associated with PTL [38].

Although our sample size was small, the strength of this study is that it was conducted as a multi-center prospective study in 5 centers in Korea. This study directly compared cytokines and fFN in the CVF from the same cohort of patients. When selecting a patient group, the ones with multiple gestation, gestational diabetes, and gestational hypertension were excluded. However, factors such as cervical conization history, socio-economic status, pre-pregnancy body mass index, smoking, drug use, and anxiety that could be confounding factors could not be excluded. The gestational age at diagnosis is also a confounding factor that may affect the gestational age at delivery, but no analysis has been done to correct this.

In conclusion, CVF cytokines, especially IL-6, IL-17α, could be useful predictive markers of PTB in patients with PTL and/or pPROM; however, studies with larger cohort will be required for validation of these findings.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board Committee of Ewha Womans University Mokdong Hospital (certificate No. EUMC 2014-06-010).

Patient consent

The patients provided written informed consent for the publication and the use of their images.

Supplementary materials

Supplementary Tables associated with this article can be found online at https://doi.org/10.5468/ogs.19131.

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Obstetrics & Gynecology Science

Prognostic impact of p16 and p53 gene expressions in stage 1a epithelial ovarian cancer

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Objective
Epithelial ovarian cancer (EOC) is rarely detected at stage 1a. Most of the patients have a good prognosis and there are limited factors that affect their survival. In the present study, we evaluated the p16 and p53 gene expressions of stage 1a EOC patients. Prognostic effects of these gene expressions, as well as those of other factors on short term survival were analyzed.

Methods
Our study included 29 patients. The specimens of the ovary with cancer were stained for p16 and p53. Gene expressions and other prognostic factors were evaluated.

Results
The median age of the patients was 51 years (27–84). The mean numbers of dissected pelvic and paraaortic lymph nodes were 27 and 12, respectively. The mean follow-up time was 33.7±18.9 months. During this period, recurrence occurred in two patients. One of the patients had grade 2 mucinous carcinoma and died of the disease at month 12 after the recurrence occurred at month 7. The second patient had clear cell carcinoma and recurrence occurred at month 34. p16 and p53 gene expressions or other factors were not associated with overall survival (OS) or disease-free survival in the short term. The lower p16 positivity rate in the non-clear cell group was found to be statistically significant (P=0.003). Both p53 and p16 positivity rates were higher in the high-grade carcinoma.

Conclusion
The levels of none of the common prognostic factors, including those of p16 and p53 gene expression, were associated with the progression-free survival or OS of stage 1a in the short term. Appropriate surgical staging and non-omission of subclinical metastases seem to be of central importance.

Keywords: Disease-free survival; Genes, p16; Genes, p53; Progression-free survival

Introduction

Ovarian cancer is usually diagnosed at advanced stages. It is the most lethal gynecological cancer and accounts for 4.3% of all cancer-related deaths [1]. Advanced-stage epithelial ovarian cancer (EOC) is the leading subject of research in literature compared to early-stage disease. However, most early-stage disease-related studies evaluate the patients with stage 1 or 2 together, even though stage 1a/1b disease has a better prognosis than stage 1c or 2 [2].

Stage 1a EOC constitutes a minor group (up to 10%) of patients, with a 5-year survival rate of over 90% [3]. There are several prognostic factors associated with stage 1a

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disease, including age, histological factors (e.g., type and grade), and molecular factors such as abnormal oncogene expression (e.g., p53, p16, Her2/neu, PTEN, p21, and ki67).

p16 is a cyclin-dependent kinase (CDK) inhibitor that inhibits retinoblastoma protein levels by inhibiting CDK phosphorylation during cell cycle progression. Another mode of its action involves the inhibition of cyclin D1, which inactivates pRb through CDK4. Both p16 and pRb are important tumor suppressors in cell cycle regulation. p16 is a potent inhibitor of CDK4 and CDK6. p16, pRb, and cyclin are involved in the transition from the G1 to S phase, which is the most important control point of the D1 cell cycle. The p53 protein is localized in the nucleus and plays a role in the mechanisms underlying DNA damage repair. p53 controls the transition from the G1 to S phase. It is the most common site of genetic change in human tumors [4]. Approximately, 50% of human tumors contain a p53 gene mutation. Additionally, several studies have reported about the high p53 mutation rate in EOC [5-7]. Recently, mutation or promoter methylation of the p16 gene has also been detected in ovarian serous adenocarcinoma [8]. In the current study, we aimed to evaluate the effect of p16 and p53 expressions and conventional prognostic factors on stage 1a EOC.

Materials and methods

Our study included 29 patients who underwent staging surgery for EOC at the Department of Gynecologic Oncology, School of Medicine, Başkent University and were diagnosed with stage 1a disease. The study was conducted in accordance with the Declaration of Helsinki. Standard staging surgery included hysterectomy, bilateral salphingo-oophorectomy, bilateral pelvic and paraaortic lymph node dissection, omentectomy, and appendectomy. Uterus and one ovary were preserved and unilateral salphingo-oophorectomy was performed in fertility-sparing surgery. The International Federation of Gynecologists and Obstetricians (FIGO) system was used for disease staging [9]. Patients were evaluated in groups based on the histological type (clear and non-clear cell carcinoma) and the presence or absence of p16 and p53 gene expressions. p16 and p53 expressions were evaluated immunohistochemically in paraffin blocs. Nuclear staining in the epithelial cells was evaluated under a light microscope. Lack of staining, slight staining, or staining of less than 10% of the tumor cells was defined as negative, while others were defined as positive (Fig. 1). Data were analyzed using SPSS for Windows v.15.0 (SPSS Inc., Chicago, IL, USA). Descriptive and frequency analyses were performed. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The level of statistical significance was set at $P<0.05$.

Results

Our study included 29 patients. The median age of the pa-

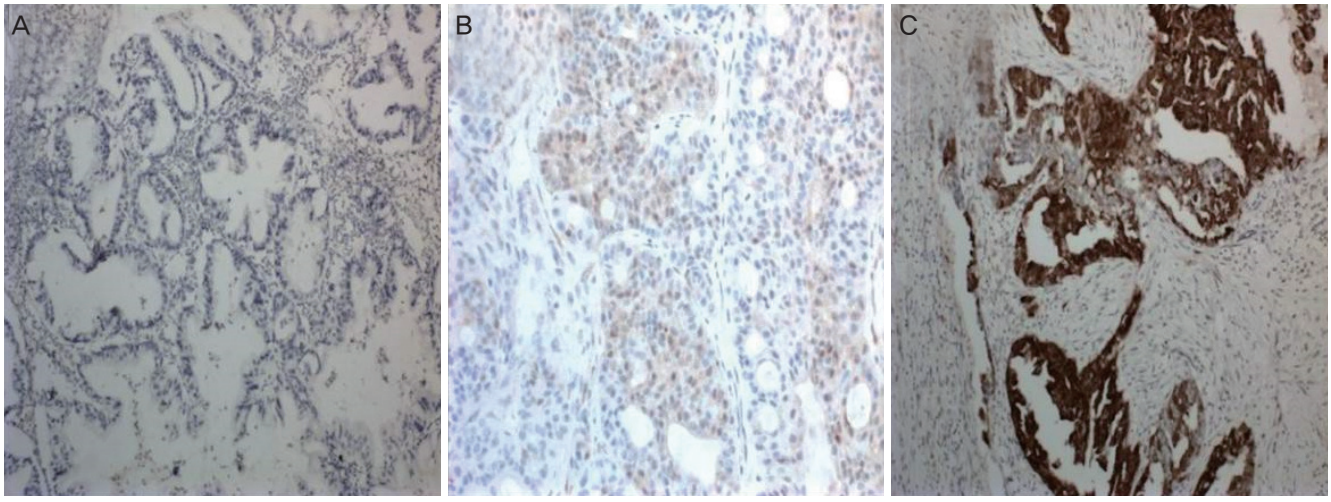

Fig. 1. (A) Weak staining pattern with p16 marker (×10 high-power field [HPF]); (B) Moderate staining pattern with p16 marker (×20 HPF); (C) Strong staining pattern with p16 marker (×10 HPF).

tients was 51 (27–84). The number of premenopausal and postmenopausal patients were 14 (48.2%) and 15 (51.8%), respectively. The general properties of the patients are summarized in Table 1. The mean numbers of dissected pelvic and paraaortic lymph nodes were 27 and 12, respectively. The mean tumor diameter was 11.5±1.1 cm.

Table 2 summarizes the staining patterns for p53 and p16 according to the histological types. p53 was positive in all specimens of patients with clear cell histology. The lower p16 positivity rate in the non-clear cell group was found to be statistically significant (*P*=0.003). Furthermore, both p53 and p16 positivity rates were higher in the high-grade tumor, and this was statistically significant for the p16 group (*P*=0.048).

The mean follow-up time was 33.7±18.9 months. During this period, recurrence occurred in two patients and one of these patients died of the disease. One of the patients had grade 2 mucinous carcinoma and died of disease at month 12 after a recurrence at month 7. The second patient had clear cell carcinoma and recurrence occurred at month 34. She was subjected to a combined chemotherapeutic regimen that included the administration of paclitaxel and carboplatin after a secondary cytoreduction. She was alive at t month 42 of the follow-up. The levels of none of the prognostic factors, including those of p16 and p53 gene expression, were statistically significant in the disease-free survival (DFS) or overall survival analyses in the short term (Table 3).

Table 1. General properties of patients

Variables	Values
Age	
≤60	22 (75)
>60	7 (25)
Histological type	
Clear cell	12 (41)
Non-clear cell	17 (59)
Menopausal status	
Premenopausal	14 (48)
Postmenopausal	15 (52)
Surgical approach	
Standard	26 (89)
Fertility sparing	3 (11)
Follow-up (mon)	
Min	12
Max	70

Values are presented as number (%).

Discussion

Stage 1a EOC is defined as the disease that is confined to one of the ovaries with intact surface epithelium and no other metastases. Thus, only a few prognostic factors are associated with the tumor. Histological type and grade are the globally well-accepted factors, and in addition, molecular patterns have been found to be related to tumor behavior.

Table 2. The staining patterns for p53 and p16 according to the histopathological evaluation

Variables	p53+	p53-	*P*-value	p16+	p16-	*P*-value
Histological type						
Clear cell	9 (75.0)	3 (25.0)	0.498	12 (100)	0 (0)	0.130
Non-clear cell	9 (56.3)	7 (43.8)		15 (88.2)	2 (11.8)	
Non-clear subtypes						
Endometrioid	3 (75)	1 (25)	0.279	4 (100)	0 (0)	0.003
Mucinous	0 (0)	8 (100)		6 (75.0)	2 (25.0)	
Serous	4 (80)	1 (20)		5 (100)	0 (0)	
Grade[a]						
1	6 (75)	2 (25)	0.06	3 (37.5)	5 (62.5)	0.048
2	5 (100)	0 (0)		1 (20.0)	4 (80.0)	
3	16 (100)	0 (0)		12 (75.0)	4 (25.0)	

Values are presented as number (%).
[a]Clear cell tumors were involved in grade 3 group due to the agressive nature.

Table 3. Survival analysis of prognostic factors

Variables	DFS	*P*-value	OS	*P*-value
Age		0.224		0.226
<60	22 (35.8±20.7)		22 (36.0±20.4)	
>60	7 (25.6±10.0)		7 (26.7±11.5)	
Histological type		0.182		0.161
Clear cell	12 (39±18.2)		12 (39.67±18.2)	
Non-clear cell	17 (29.3±19.1)		17 (29.6±18.8)	
Grade		0.536		0.489
1	8 (26.9±14.0)		8 (26.9±14.0)	
2	5 (34.0±22.3)		8 (35.0±20.9)	
3	16 (36.3±20.5)		16 (36.8±20.6)	
p16		0.078		0.103
Positive	16 (38.9±19.9)		16 (38.94±19.9)	
Negative	13 (26.3±16.0)		13 (27.38±16.1)	
p53		0.297		0.277
Positive	27 (34.3±19.3)		27 (34.8±19.1)	
Negative	2 (19.5±9.1)		2 (19.1±9.1)	

Values are presented as number (mean±standard deviation).
DFS, disease-free survival; OS, overall survival.

The aim of our study was to investigate whether p53 and p16 expressions affect survival in stage 1a EOC. In the current analysis, we did not find a statistically significant association between survival and the levels of any of the factors, including those of p53 and p16 expression, in the short term.

The initial approach in early-stage ovarian cancer treatment is surgical staging. These results constitute the basis of adjuvant treatment and prediction of prognosis [10,11]. Additionally, there is no difference in 5-year survival expectancy between fertility-sparing or radical surgery at stage 1 [12,13]. In stage 1a disease, classic staging procedure is sufficient and curative in a substantial proportion of patients. Observational studies have shown that 30% of patients, who were thought to have stage I or II disease during the initial surgery, were diagnosed with a more advanced stage disease after more comprehensive restaging laparotomy [14]. In a study comprising 138 patients, the recurrence rates of complete and incomplete staging were 10% and 28%, respectively [15]. At this point, the main targets of the surgery in patients who are thought to have early-stage disease should be appropriate staging procedure and the exclusion of occult and subclinical metastases [16]. In our study, all patients were subjected to a standard staging surgery with an adequate number of lymph nodes in both the pelvic and paraaortic regions.

Endometrioid histology is associated with a better prognosis and an earlier stage at the time of diagnosis [17]. Contrastingly, clear cell histology is associated with poor prognosis [3]. In addition, clear cell histology has an aggressive nature and can be considered as a high-grade tumor. Even stage 1a patients with clear cell carcinoma undergo adjuvant therapies. Clear cell histology was the most frequent histological type in our study and was evaluated as a separate group. All specimens were p53-positive and this was a conspicuous point.

In the cell cycle, DNA damage is controlled at the G1/S control point and DNA repair mechanisms are activated. Mutation of the p53 tumor suppressor gene results in a DNA repair failure leading to neoplastic development [18]. Mutation of p53 is frequently detected in high grade serous ovarian cancers, and this frequency may be up to 80% in advanced stages [19]. Over-expression of p53 is associated with poor prognosis [20-25] and high tumor grade [24]. Additionally, p53 was associated with lower DFS and poor prognosis in stages 1 and 2 [21,22]. Association of p16 and EOC is a relatively less evaluated issue compared to p53. In contrast, p16

gene mutations or promoter methylation has been detected in serous ovarian cancer in recent years [8]. Moreover, p16 over-expression is associated with high differentiation and tumor grade [26], advanced-stage [8], and poor prognosis [27]. Similar previously published studies have commonly focused on advanced stage disease or stage 1 and 2 disease together. In the current study, we investigated the effect of these molecular changes in stage 1a differently from other studies. We found some significant histopathological findings, whereas its effect on survival could not be well-established due to the short follow-up time.

Stage 1a EOC patients were included in the current study to minimize heterogeneity. Prognostic factors, including p53 and p16 expressions, were evaluated and initial reports of short-term results are reported herein. Standard surgical and medical management of a single-center and uniform patient selection should be considered the strengths of this study. However, the short follow-up time and a limited number of patients should be considered the limitations. The long-term results will be also presented in the future.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study was approved by the review board of the University of Medical Sciences.

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Effect of gynecological cancer and its treatment on bone mineral density and the risk of osteoporosis and osteoporotic fracture

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Objective
The purpose of this study was to evaluate the risk of osteopenia and osteoporosis by examining the bone mineral density (BMD) of the lumbar spine and femur in patients with gynecological cancer without bone metastasis and to evaluate the impact of treatment for different cancers on BMD.

Methods
This study retrospectively reviewed the medical records of 243 women with gynecological cancer and 240 controls between March 2010 and December 2016. Patients with cervical cancer (n=105), endometrial cancer (n=63), and ovarian cancer (n=75) were treated with total hysterectomy including bilateral salpingo-oophorectomy and/or chemotherapy and/or radiotherapy. For the control group, healthy post-menopausal women without gynecologic cancer were selected.

Results
Before anticancer treatment, the BMD of patients with cervical cancer and ovarian cancer was significantly lower than that of the controls, and the BMD of patients with endometrial cancer was not significantly different from that of the controls. However, the BMD of endometrial cancer significantly decreased after treatment. According to the treatment methods, there were significant differences in the BMD of L3, L4, and the femur neck. Changes in the BMD were lowest in patients who underwent surgical treatment only, and the highest bone loss was found in patients who underwent postoperative concurrent chemoradiotherapy.

Conclusion
Patients with cervical and ovarian cancer had lower BMD than those in the control group before treatment, and patients with endometrial cancer had decreased bone density after treatment. Therefore, during the treatment of gynecological cancer, strategies should be implemented to mitigate these risks.

Keywords: Bone density; Treatment-associated cancer; Osteoporosis

Introduction

Osteoporosis is the most common metabolic bone disease caused by reduced bone mineral density (BMD) and is associated with high risk of bone fractures. Fractures due to osteoporosis are closely related to the mortality rate of the elderly population, and there is an increased prevalence of osteoporosis due to increased life expectancy. Therefore, social and medical interest in the risk factors, diagnosis, and treatment methods of osteoporosis is increasing. Several systemic conditions and diseases may affect bone mineral density, includ-

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ing insufficient calcium intake, excessive sodium intake, lack of exercise, chronic kidney disease, parathyroid disease, hyperthyroidism, and gastrointestinal absorption disorder [1].

The mean BMD of women is lower than that of men, and bone mass accumulates between 20 and 30 years of age. The loss of bone density is most rapid after the age of 50, particularly 5 years after menopause in women [2,3]. A decrease in estrogen in postmenopausal women affects bone and lipid metabolism [4]. Estrogen plays an important role in the osteoclast–osteoblast balance related to maintenance of the BMD. It is known that BMD decreases when estrogen concentration decreases [5].

Previous studies have reported low BMD and high serum calcium levels in various types of cancer including prostate and breast cancer without bone metastasis [6-11]. The decrease in the BMD associated with cancer may be linked to the osteoclastic effect of the malignant cell itself as well as the cancer treatment-induced bone loss (CTIBL) caused by surgery, chemotherapy, and radiation therapy [12-14]. Gonadal dysfunction due to cancer treatment reduces hormone secretion, leading to decreased BMD.

The purpose of this study was to evaluate the risk of osteopenia and osteoporosis in patients with gynecological cancer according to the type of cancer and to analyze the BMD of the lumbar spine and femoral neck after various cancer treatments including surgery, chemotherapy, and radiation therapy.

Materials and methods

Between March 2010 and December 2016, a retrospective study was conducted by analyzing the medical records of women who were treated at Haeundae Paik Hospital Obstetrics and Gynecology. All subjects underwent physical examination, blood tests, ultrasonography, and bone mineral density measurements.

1. Study participants

A total 243 patients with gynecological cancer were selected for this study. Patients with cervical cancer (n=105), endometrial cancer (n=63), and ovarian cancer (n=75) were treated with total hysterectomy including bilateral salpingo-oophorectomy and/or chemotherapy and/or radiotherapy. Six patients with bone metastasis or those who did not undergo surgery due to an advanced stage of cancer were excluded. For the control group, 240 healthy post-menopausal women who did not have menstruation for 1 year were selected. The BMD was measured before and 1 year after cancer treatment. In the control group, the BMD was measured again 1 year after the initial test.

Patients with thyroid disease, parathyroid disease, kidney stones, hyperprolactinemia, malabsorptive disorder, or previous pathologic fractures that may affect bone metabolism were excluded from the study. Patients who were prescribed medication that may affect the study outcomes, such as hormones, bisphosphonates, calcitonin, vitamin D, or calcium, were also excluded.

2. Methods

1) Bone mineral density measurement

The BMD of the 1st–4th lumbar spine and femoral neck was determined by measurement with dual-energy X-ray absorptiometry (DXA, Lunar Radiation Corp., Madison, WI, USA). According to the criteria of the World Health Organization, the diagnosis of osteopenia was made with a T-score in the range of –1.0 and –2.5 and that of osteoporosis with a T-score of –2.5 or below [15,16].

2) Cancer treatment

Gynecological cancer patients were treated with total hysterectomy including bilateral salpingo-oophorectomy and/or chemotherapy and/or radiotherapy. Eighty-five patients received only surgical treatment, and 92 patients were treated with surgery and an adjuvant or palliative chemotherapy. Eight patients received surgery and radiotherapy, and 58 patients received surgical treatment with concurrent chemoradiation therapy (CCRT). Forty-four cervical cancer patients underwent only total hysterectomy with bilateral salpingo-oophorectomy, and 48 patients underwent postoperative CCRT. Thirty-eight endometrial cancer patients (60.3%) underwent surgical treatment only. Most ovarian cancer patients (90.7%) were treated with chemotherapy after surgery (Table 1).

3) Research variable factors

Age, height, weight, body mass index (BMI), and obstetric history were included as common demographic characteristics. In gynecological cancer patients, the cancer stage ac-

cording to the International Federation of Gynecology and Obstetrics (FIGO) classification, histological classification, and cancer treatment methods were included as research variables. Among them, the variables that showed a significant difference were set as covariates.

4) Statistical analysis

Statistical analysis was performed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) ver. 23.0 (IBM Corp., Armonk, NY, USA), and statistical significance was set as $P<0.05$. The independent *t*-test was used to compare continuous variables such as age, weight, and height, and the χ^2 test was used to compare the discrete variables such as obstetric history and prevalence of osteopenia and osteoporosis. According to cancer type, continuous variables were compared using analysis of variance. A multiple linear regression analysis was used to analyze the factors affecting BMD.

Results

1. Demographic characteristics

Table 1 shows demographic characteristics, cancer stage according to the FIGO classification, and cancer treatment methods of gynecological cancer patients. The mean age, height, weight, BMI, and obstetric history of patients with cervical cancer, endometrial cancer, and ovarian cancer were not significantly different between the groups. The body weight of the endometrial cancer group was greater than that of the other groups, and the BMI was slightly higher; however, there was no significant difference between the groups.

According to the FIGO classification, 25 (23.8%), 38 (36.2%), and 26 (24.8%) patients had cervical cancer at stages Ia, Ib, and IIb, respectively. Forty-five (71.4%) patients in the endometrial cancer group had stage Ia, and 24 (32.0%) and 19 (25.3%) patients in the ovarian cancer group had

Table 1. Demographic characteristics, cancer stage and treatment methods of patients with gynecologic cancer

Characteristics	Cervical cancer (n=105)	Endometrial cancer (n=63)	Ovarian cancer (n=75)	*P*-value
Age (yr)	57.2±11.6	56.2±9.7	57.2±11.6	0.739
Height (cm)	156.1±5.3	157.2±5.7	156.4±6.2	0.488
Weight (kg)	56.9±8.1	59.2±11.3	57.7±8.3	0.065
BMI (kg/m^2)	23.4±3.3	24.2±4.2	23.7±3.6	0.066
Parity	2.1±1.1	1.9±1.1	1.9±1.2	0.332
Cancer stage[a]				
Ia	25 (23.8)	45 (71.4)	9 (12.0)	
Ib	38 (36.2)	5 (7.9)	6 (8.0)	
Ic	-		9 (12.0)	
IIa	10 (9.5)	2 (3.2)	4 (5.3)	
IIb	26 (24.8)	0 (0.0)	4 (5.3)	
III	3 (2.9)	8 (12.7)	24 (32.0)	
IV	3 (2.9)	3 (4.8)	19 (25.3)	
Treatment methods				
Surgery	44 (41.9)	38 (60.3)	3 (4.0)	
Surgery + chemotherapy	10 (9.5)	14 (22.2)	68 (90.7)	
Surgery + CCRT	48 (45.7)	6 (9.5)	4 (5.3)	
Surgery + RT	3 (2.9)	5 (7.9)	0 (0)	

Continuous variables were compared by analysis of variance test and categorical variables by χ^2 tests. Values are presented as mean±standard deviation or number (%).

BMI, body mass index; CCRT, concurrent chemoradiation therapy; RT, radiation therapy; FIGO, International Federation of Gynecology and Obstetrics.

[a]The cancer stage follows the FIGO classification.

stages III and IV advanced stage, respectively.

2. Bone mineral density before and after cancer treatment according to cancer type

The BMD before and after cancer treatment in patients with cervical cancer, endometrial cancer, and ovarian cancer was compared with those of the control group (Table 2). There was no significant difference between the cancer type; however, the T-scores of ovarian cancer patients were lower than those of cervical cancer and endometrial cancer patients.

Before beginning cancer treatment, the BMD of cervical cancer patients was significantly lower in the 1st and 2nd lumbar spine and femoral neck than in the control group. Ovarian cancer patients showed significantly lower BMD from L1 to L4, at each level. The average T-score with standard deviation of the lumbar spine was –0.9±1.4, and the T-scores from the 1st to 4th lumbar spine were –1.2±1.4, –1.1±1.5, –0.9±1.5, and –0.7±1.5, respectively, which was significantly lower than those of the control group. However, the T-scores of endometrial cancer patients were not significantly different from those of the control group.

Among 243 women with gynecological cancer, 96 (39.5%) were in the normal range, 114 (46.9%) were diagnosed with osteopenia, and 33 (13.6%) were diagnosed with osteoporosis according to the criteria of the World Health Organization. In 240 control subjects, 118 (49.2%), or approximately half were in the normal range, 100 (41.7%) were diagnosed with osteopenia and 22 (9.2%) were diagnosed with os-

Table 2. The T-scores of the lumbar spine and femur neck according to the type of cancer compared with the controls and the prevalence of osteopenia and osteoporosis before and after cancer treatment

Variables	Cervical cancer (n=105)	*P*-value	Endometrial cancer (n=63)	*P*-value	Ovarian cancer (n=75)	*P*-value	Control (n=240)
At baseline							
Lumbar spine BMD	–0.6±1.5	0.056	–0.5±1.6	0.328	–0.9±1.4	0.001	–0.3±1.5
L1	–0.9±1.4	0.013	–0.8±1.6	0.158	–1.2±1.4	0.000	–0.6±1.3
L2	–0.8±1.5	0.029	–0.7±1.7	0.184	–1.1±1.5	0.001	–0.6±1.5
L3	–0.6±1.7	0.179	–0.5±1.7	0.355	–0.9±1.5	0.007	–0.4±1.6
L4	–0.3±1.6	0.209	–0.1±1.8	0.957	–0.7±1.5	0.004	–0.2±1.6
Femur neck BMD	–0.9±1.0	0.029	–0.7±1.2	0.643	–0.9±1.2	0.091	–0.6±1.1
Prevalence							0.079
Normal (%)[a]	44 (41.9)		24 (38.1)		28 (37.3)		118 (49.2)
Osteopenia (%)[b]	47 (44.8)		32 (50.8)		35 (46.7)		100 (41.7)
Osteoporosis (%)	14 (13.3)		7 (11.1)		12 (16.0)		22 (9.2)
After 1 years							
Lumbar spine BMD	–0.7±1.4	0.047	–0.9±1.4	0.019	–1.0±1.4	0.013	–0.3±1.5
L1	–1.0±1.4	0.028	–1.3±1.3	0.004	–1.2±1.4	0.010	–0.6±1.4
L2	–0.9±1.5	0.046	–1.1±1.5	0.029	–1.0±1.5	0.046	–0.5±1.6
L3	–0.7±1.7	0.110	–1.0±1.4	0.019	–0.9±1.5	0.044	–0.3±1.6
L4	–0.3±1.6	0.189	–0.3±1.6	0.264	–0.7±1.5	0.016	–0.0±1.6
Femur neck BMD	–1.0±1.0	0.002	–0.9±1.2	0.129	–1.0±1.3	0.071	–0.6±1.1
Prevalence							0.167
Normal (%)[a]	37 (35.2)		19 (30.2)		23 (30.7)		90 (37.5)
Osteopenia (%)[b]	51 (48.6)		35 (55.6)		37 (49.3)		122 (50.8)
Osteoporosis (%)	17 (16.2)		9 (14.3)		15 (20.0)		28 (11.7)

The T-scores are presented as mean±standard deviation, *P*-value by independent *t*-test.
BMD, bone mineral density; WHO, World Health Organization.
[a]Values are presented as number (%), *P*-value by χ^2 test; [b]Diagnosed by T-scores of lumbar spine or femur neck according to WHO criteria.

teoporosis. There was no significant difference between the groups (*P*=0.079); however, the rates of osteopenia and osteoporosis were 5.2% and 4.4% higher in cancer patients than in the control group, respectively.

After 1 year of cancer treatment, the average T-score of the lumbar spine, the T-score of the 1st to 2nd lumbar spine, and femoral neck in cervical cancer patients was significantly lower than those in the control group. In the ovarian cancer group, the average T-scores of L1-L4 and the 1st to 4th level of the lumbar spine were significantly lower than those of the control group. In patients with endometrial cancer, the T-score of the 1st to 3rd level of the lumbar spine and the average T-score of L1-L4 was significantly lower than those of the control group.

Changes in the BMD before and after cancer treatment according to the type of gynecological cancer are shown in Fig. 1. In endometrial cancer patients, the BMD was significantly lower than that in cervical cancer and ovarian cancer patients, and there was a significant difference in the lumbar spine (*P*=0.024).

3. Changes in bone mineral density according to treatment method

In 243 patients with gynecological cancer, changes in the BMD before and after cancer treatment were compared in relation to the different treatment methods (Table 3). The T-scores of the 3rd and 4th lumbar spine and the femoral neck were significantly different according to the treatment methods. The smallest change in the BMD was observed in 85 patients who had undergone only surgical treatment, including bilateral salpingo-oophorectomy. Conversely, the T-scores of the 58 patients who received surgical treatment with CCRT significantly decreased after treatment.

4. Factors associated with bone loss

The factors affecting the BMD in gynecological cancer pa-

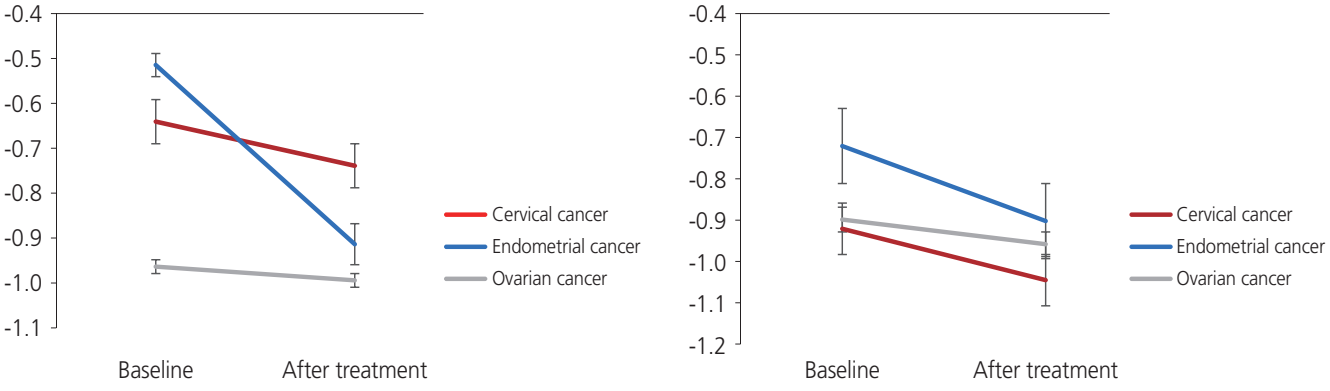

Fig. 1. The T-scores of the lumbar spine and femur neck in patients with gynecologic cancer before and after treatment. BMD, bone mineral density.

Table 3. The changes of bone mineral density in patients with gynecologic cancer according to treatment methods

Variables	Surgery only (n=85)	Surgery + chemotherapy (n=92)	Surgery + RT (n=8)	Surgery + CCRT (n=58)	*P*-value
Lumbar spine BMD (g/cm^2)[a]	0.02±0.47	–0.18±0.63	–0.09±0.56	–0.30±0.47	0.051
L1	–0.01±0.71	–0.12±0.95	–0.67±0.51	–0.36±0.58	0.052
L2	0.01±0.56	–0.16±0.80	–0.21±0.53	–0.28±0.51	0.228
L3	–0.02±0.54	–0.18±0.76	0.20±0.72	–0.34±0.57	0.043
L4	0.07±0.71	–0.17±0.74	0.37±1.33	–0.23±0.62	0.022
Femur neck BMD (g/cm^2)	–0.04±0.24	–0.03±0.26	–0.19±0.26	–0.27±0.30	0.026

Values are presented as mean±standard deviation, *P*-value by analysis of variance.
BMD, bone mineral density; CCRT, concurrent chemoradiation therapy; RT, radiation therapy
[a]Mean change of T-scores.

tients were analyzed by multiple linear regression analysis (Table 4). Age and BMI were found to affect the BMD in the lumbar spine and femur neck, and there was no significant relationship between height, weight, and obstetric history and the BMD. There was a negative correlation between age and the BMD, whereas there was a positive correlation between BMI and the BMD. According to the type of cancer, endometrial cancer showed a negative correlation with the BMD, but it was not a significant variable in other types of cancer. There was no significant difference when analyzing the influence of treatment methods and the stages of cancer.

Discussion

The life expectancy of gynecological cancer patients has improved due to the advances in medical technology for diagnosing and treating gynecological cancer. Quality of life is also important for patients with longer life expectancy, and osteoporosis can have a significant impact on quality of life. Previous studies have reported a decrease in bone density and hypercalcemia in patients with other malignancies without bone metastasis [6-11]. Studies have shown that malignant tumor cells secrete serum growth factors that promote bone resorption and bone destruction. Prostaglandins [17,18], tumor growth factor [19], osteoclast activator [11], and parathyroid hormone analogue [20,21] are known be involved in osteoclast activation [15].

The purpose of this study was to evaluate the risk of osteopenia and osteoporosis in patients diagnosed with gynecological cancer and to investigate the differences according to the type of cancer. Furthermore, we tried to identify the changes after cancer treatment. When the BMD before cancer treatment was compared according to the type of cancer, the BMD of cervical cancer patients was significantly lower in the 1st and 2nd lumbar spine and the femoral neck than that in the control group. Previous studies have reported a lower BMD of the lumbar spine in cervical cancer patients before cancer treatment [22-24]. Hung et al. [24] reported that premenopausal patients with cervical cancer had signifi-

Table 4. The factors associated with bone loss by a multiple linear regression analysis

Variables	Lumbar spine T-score				Femur neck T-score			
	β	Standardized error	t	*P*-value	β	Standardized error	t	*P*-value
Age (yr)	–0.050	0.003	2.055	0.041	–0.042	0.002	–1.904	0.058
Height (cm)	–0.006	0.031	0.194	0.846	0.036	0.027	1.347	0.179
Weight (kg)	0.013	0.041	–0.309	0.758	–0.054	0.036	–1.504	0.133
BMI (kg/m^2)	0.135	0.104	0.338	0.046	0.129	0.090	1.432	0.153
Parity	–0.160	0.085	–1.883	0.060	–0.058	0.073	–0.787	0.432
Cancer type								
Cervical cancer	0.088	0.062	1.406	0.160	0.024	0.054	0.436	0.663
Endometrial cancer	–0.311	0.078	–3.989	0.000	–0.104	0.067	–1.568	0.118
Ovarian cancer	–0.010	0.074	–0.139	0.889	–0.118	0.064	–1.837	0.067
Treatment methods								
Surgery	–0.134	0.067	–1.992	0.470	–0.076	0.057	–1.328	0.185
Surgery + Chemotherapy	0.237	0.100	2.375	0.180	0.148	0.087	1.694	0.091
Surgery + CCRT	–0.056	0.118	–0.476	0.063	–0.070	0.103	–0.679	0.059
Surgery + RT	0.204	0.189	1.077	0.282	0.067	0.165	0.408	0.684
Cancer stage								
Stage I	–0.069	0.051	–1.341	0.181	–0.072	0.049	–1.480	0.140
Stage II, III, IV	–0.152	0.087	–1.742	0.082	–0.041	0.056	–0.732	0.465

β and standardized error are unstandardized coefficients.
BMI, body mass index; CCRT, concurrent chemoradiation therapy; RT, radiation therapy.

cantly lower BMD in L2-L4 spines than in the controls. Lee et al. [22] suggested that postmenopausal patients with cervical cancer had a lower BMD and higher risk of osteoporosis in the lumbar spine before cancer treatment than patients with endometrial cancer. Therefore, this study supports the results of previous reports.

In the present study, ovarian cancer patients had a lower BMD in the 1st to 4th lumbar spine and a lower average T score of L1–L4 than those of the control group before treatment. More than half (57.3%) of the ovarian cancer patients were in advanced stages of the disease, with stages III and IV according to the FIGO classification. The long period of exposure to estrogen deprivation due to ovarian failure presumably influenced the low BMD before treatment. However, when analyzing the stage of cancer as a variable through multiple linear regression analysis, no significant relationship was found.

The study analyzed bone loss after one year of cancer treatment. The BMD of cervical cancer patients was lower in the L1, L2 spines, for average T-scores of L1–L4, and in the femoral neck than those in the control group. Ovarian cancer patients showed a significantly lower BMD from L1 to L4, and the average T score of L1–L4, than the controls. However, as bone density was already lower in cervical and ovarian cancer patients than in controls before cancer treatment, it cannot be concluded that treatment had an effect on the reduction of BMD. Alternatively, the BMD of endometrial cancer patients was not significantly different when compared with the control group before treatment. The BMD of the 1st to 3rd lumbar spine, the average T-score of the lumbar spine, and the femoral neck were decreased after 1 year of treatment compared with that in the control group. Further, in multiple linear regression analysis, only endometrial cancer showed a negative correlation to bone loss when the type of cancer was set to variable, and other gynecological cancers showed no significant correlation.

In a previous study, endometrial cancer patients who had undergone bilateral oophorectomy, followed by chemotherapy or radiation therapy, showed significantly lower BMD than other gynecological cancer groups [25]. In estrogen-dependent endometrial cancer, high levels of estrogen protect bone mass from osteoclasts and stimulate osteoblasts to maintain bone density [26,27]. Similarly, in the present study, the BMD of endometrial cancer patients before treatment was higher than those of cervical cancer and ovarian cancer patients. However, after cancer treatment, hypogonadism may cause a rapid decrease in estrogen levels, leading to abrupt changes in BMD in endometrial cancer patients. Previous studies have reported that cancer treatment-induced bone loss is associated with hypogonadism [12,13]. Conversely, persistent exposure to high levels of estrogen increases the accumulation of bone mass and lowers the risk of fractures. Previous studies verified low risk of endometrial cancer in patients with osteoporosis or osteoporotic fractures [28-30].

Several anticancer drugs used in chemotherapy have direct toxic effects on osteoblasts or can indirectly affect bone metabolism by inducing hypogonadism. In addition, immunosuppressive agents such as steroids and cyclosporine, used in combination with anticancer drugs, may increase the risk of osteoporosis [13,31]. When comparing the changes in the BMD according to the methods of cancer treatment, there was a significant difference in the BMD before and after treatment in the 3rd and 4th lumbar spine and the femoral neck (Table 3). The change in the BMD was lower in patients who underwent only surgical treatment including bilateral salpingo-oophorectomy, and the greatest decrease in the BMD was observed in patients who had undergone surgical treatment with CCRT. However, in the multiple linear regression analysis, no significant influence was found according to the treatment methods.

Previous studies have identified a decrease in bone density and an increase in bone turnover markers in cervical cancer patients treated with chemoradiotherapy [22,32-34]. In addition, cervical cancer has been found to reduce the BMD after radiotherapy and increase the incidence of osteoporotic fractures [35,36]. Radiation therapy leads directly to bone atrophy and indirectly to vascular changes of the bone, leading to destruction [13]. Radiotherapy of the pelvis is known as a risk factor for sacral fractures, and it has been reported that fractures of the femoral neck occur in approximately 2% of cervical cancer patients who underwent radiotherapy [14,35,36].

Hwang et al. [32] reported that the 4th lumbar spine and femoral bone density decreased in patients with cervical cancer after chemoradiotherapy. The 4th lumbar spine is an area affected by irradiation of the pelvis; thus, this area may experience a reduction in the BMD after irradiation. In the present study, patients who underwent chemotherapy with concurrent chemoradiation after surgical treatment had significant changes in their BMD, which is in line with the

results of previous studies. However, as a limitation of the study, the number of patients who underwent radiotherapy after surgery was small; therefore, this study cohort may not be truly representative of the population.

This study was a retrospective comparative analysis, with data extracted from patients' medical records. Through multiple linear regression analysis, the average age and BMI were found to affect the BMD in gynecological cancer patients. However, it was difficult to control all the factors that could affect the patients' bone metabolism, such as smoking, alcohol intake, nutrition, and physical exercise [1]. The study has limitations owing to the retrospective observational nature. Many types of covariates are expected to affect the dependent variable. Attempts to control these covariates would result in a reduced and insufficient number of subjects to represent the specific populations. Retrospective observational studies require a large population for reporting rare outcomes. In addition to the difficulty in controlling various factors, there are limitations related to various biases such as selection bias and information bias. Furthermore, we compared the changes in BMD before and after cancer treatment for 1 year in the study. However, the scale for detecting changes in the BMD was too small; therefore, the interpretation of the data was limited.

Nevertheless, this study has a large number of gynecological cancer patients and various analyses based on the type of cancer, cancer staging, and treatment methods. We tried to investigate the changes in the BMD before and after treatment and identify the risk of osteoporosis among gynecological cancer patients. In conclusion, the BMD of cervical and ovarian cancer patients before cancer treatment was significantly lower than that in the control group, and the BMD after cancer treatment was remarkably decreased in endometrial cancer patients.

The early diagnosis and treatment of cancer have improved the treatment rate and survival rate of gynecological cancer patients; however, osteoporotic fractures not only reduce the quality of life of long-lived patients but also increase their mortality. This study suggests that the risk of bone density loss and osteoporosis in gynecological cancer should be recognized, prevented, and diagnosed early to reduce the incidence of osteoporotic fractures. Proper calcium and vitamin intake, outdoor activity recommendations, and regular BMD measurements may improve the quality of life of gynecological cancer patients. In addition, larger scale prospective studies should be carried out to investigate the factors that affect the long-term, as well as the short-term side effects of cancer treatment in gynecological cancer patients.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study was approved by the Institutional Review Board of Haeundae Paik Hospital, Inje University (IRB No. 2018-04-019) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Patient consent

The patients signed written informed consent for the publication and the use of their images.

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Obstetrics & Gynecology Science

Knowledge and awareness about fertility preservation among female patients with cancer : a cross-sectional study

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Objective
The incidence of women in the reproductive age group diagnosed with cancer has recently increased. However, very few patients opt for or are offered fertility preservation (FP) strategies because of a significant lack in awareness. The present study was conducted to evaluate the knowledge of the effect of cancer treatment on fertility and available options for FP.

Methods
This was a cross-sectional study conducted at a tertiary care center from March 2019 through August 2019. One hundred female patients with gynecological or nongynecological cancer and 18-40 years of age were interviewed. The participants were categorized on the basis of the modified Kuppuswamy socioeconomic status (SES) scale and the responses of the patients in the different categories were compared.

Results
More than half the patients (63%) were in the 20–35-year age group. Most of the patients (71%) were married, and of them, 28 (39.4%) desired to have children. Only 32% of the patients were aware of the detrimental effect of cancer and its treatment on future fertility, and of them, only 28% could specify the gonadotoxic effect of chemotherapy. Knowledge was significantly higher in the upper and middle SES levels than it was in the lower SES level (P<0.001). More than half of the patients (68%) were not aware of the existing FP options, whereas one-third of the patients (32%) were given information about FP by their physicians.

Conclusion
The overall awareness of the gonadotoxic effect of cancer therapy and available FP options in the present study was poor. Awareness of FP among both patients and clinicians needs to be increased.

Keywords: Cancer; Fertility preservation; Knowledge; Awareness; Chemotherapy

Introduction

The global cancer burden is estimated to have increased to 18.1 million new cases and 9.6 million deaths in 2018 [1]. One in 5 men and 1 in 6 women worldwide develop cancer during their lifetime and 1 in 8 men and 1 in 11 women die from the disease [1]. With the advances in cancer treatment, including surgery, chemotherapy, and radiotherapy, the overall survival rate of patients with cancer has improved, especially in the last 2–3 decades. Survival rates are the highest in patients with cancer in the 15–44-year age group,

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with a 5-year survival rate ranging between 60% and 82% [2-5]. However, cancer treatment has many undesirable side effects, including impaired fertility, which occurs in approximately 80% of survivors and becomes an important quality-of-life issue in these patients [6,7]. Cancer itself affects fertility when it involves reproductive organs that need to be surgically removed, and when treatment involves gonadotoxic chemotherapy or radiotherapy in the pelvic field or the hypothalamus-pituitary area of the brain. Because of late marriages and delayed childbearing, the proportion of young patients with cancer who have yet to plan for their first pregnancy is increasing. Sometimes the only fertility option left for these cancer survivors is the use of donated gametes.

Oncofertility, a term coined by Woodruff in 2006, combines cancer treatment with fertility preservation (FP) for young women with cancer who desire future fertility [8]. Various FP methods have evolved over the last 2 decades. Among them, embryo and oocyte cryopreservation are established methods of fertility preservation with possibility of having own genetic offspring in future. Translocation of the ovaries to above the pelvic brim protects the ovaries from pelvic radiotherapy. However, translocation is not always successful because of radiation scatter and remigration of the ovaries and is of no benefit when treatment involves chemotherapy or whole-body irradiation. Ovarian tissue cryopreservation is still in the experimental stage and suppression of the ovaries with gonadotropin-releasing hormone agonist (GnRHa) has limited benefit for FP [9-11].

The American Society of Clinical Oncology (ASCO) and the American Society of Reproductive Medicine (ASRM) recommend that all possible FP options should be discussed with women diagnosed with cancer before starting any gonadotoxic therapy [12].

Despite the recommendations of international societies, more than half of patients with cancer are not given all the information they need about FP options and very few patients actually undergo any FP procedure [13-16]. The primary reason behind these facts might be a lack of awareness among patients and treating physicians regarding possible FP strategies [17]. In addition, sociodemographic disparities have been shown to affect access to FP services [18,19]. These disparities may be due to disparities in education status and the inability to afford FP. Other reasons why few patients undergo an FP procedure include time constraints, fear about the safety of the procedure, fear of the risk of cancer recurrence from a future pregnancy, and fear of the transmission of cancer to offspring.

The present observational study was conducted to evaluate the knowledge and awareness of female patients with cancer attending the OBGYN outpatient clinic and the infertility clinic at our institute regarding the effect of cancer treatment on future fertility and available methods of FP. We also categorized the participants according to their socioeconomic status (SES) using the recent classification system applicable to the Indian population and assessed the difference in knowledge among the SES groups.

Education of head of family	Score	Occupation of head of family	Score	Monthly income of family (Jan 2017)	Score
Profession or honours	7	Profession	10	>41430	12
Graduate or post graduate	6	Semi- professional	6	20715-41429	10
Intermediate or post high school diploma	5	Clerical, Shop-owner	5	15536-20714	6
High school certificate	4	Skilled worker	4	10357-15535	4
Middle school certificate	3	Semi-skilled worker	3	6214-10356	3
Primary school certificate	2	Unskilled worker	2	2092-6213	2
Illiterate	1	Unemployed	1	<2091	1

Socioeconomic class		Total Score
I	Upper	26-29
II	Upper middle	16-25
III	Lower middle	11-15
IV	Upper lower	5-10
V	Lower	< 5

Fig. 1. Modified Kuppuswamy socioeconomic status scale.

Materials and methods

This was a cross-sectional study conducted at a tertiary care referral center from March 2019 through August 2019. Ethical approval was obtained from the Ethics Committee of the All India Institute of Medical Sciences (Ref. No. IEC-9/04.01.2019, RP-50/2019) and informed consent was obtained from all the participants. Inclusion criteria were age from 18–40 years old, diagnosis of cancer, and attendance at the infertility clinic. The women who visited the infertility clinic were referred either by the OBGYN clinic (gynecological cancer) or by other specialties for FP. A total of 100 women participated in the study. Participation was completely voluntary, and a fertility counselor interviewed each participant. The questionnaire (Appendix 1) elicited information about the sociodemographic profile of the participants and contained questions to determine knowledge regarding the effect of cancer treatment on future fertility and the gonadotoxic effect of chemotherapy and radiotherapy, awareness of FP, whether information regarding the effect of cancer on fertility and FP was provided by the primary physician, and the reasons for not proceeding with FP. The study population was stratified into 5 SES categories according to the modified Kuppuswamy scale (Fig. 1) [20]. Because the number of patients in each group was small, the upper-middle and lower-middle SES categories were combined into a single middle SES category and the upper-lower and lower SES categories were combined into a single lower SES category. The knowledge levels of the three SES groups (upper, middle, and lower) were compared.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 19 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used for quantitative variables and are expressed as mean, standard deviation (SD) or frequency, and percentage. The responses of participants was stratified according to the SES scale. To compare the categorical data, Pearson's chi-square test or a Fisher exact test was performed as appropriate. A post hoc pairwise comparison was also performed using Bonferroni's correction. For all statistical tests, $P<0.05$ was considered to be statistically significant.

Results

A total of 100 women participated in the study. The sociodemographic characteristics of the study population are presented in Table 1. The mean (±SD) age of the study cohort was 28.8±7.36 years. Forty-eight percent (48%) of the patients came from the OBGYN clinic and 52% were referred by other departments in our hospital or by other hospitals.

Table 1. Baseline demographic profile of the study subjects (n=100)

Characteristics	Values
Age (yr)	
<20	16 (16)
20–35	63 (63)
>35	21 (21)
Type of family	
Nuclear	56 (56)
Joint	44 (44)
Marital status	
Married	71 (71)
Single	29 (29)
Education	
Illiterate	19 (19)
Primary	10 (10)
Secondary	26 (26)
Graduate and above	45 (45)
Socioeconomic status	
Lower	51 (51)
Middle	44 (44)
Upper	5 (5)
Fertility at the time of diagnosis	
Living child	43 (43)
No living child	28 (28)
Unmarried	29 (29)
Type of cancer	
Ca ovary	30 (30)
Ca breast	25 (25)
Ca cervix	11 (11)
Lymphoma	17 (17)
Ca endometrium	7 (7)
Others	10 (10)

Values are presented as number (%).

Seventy-one percent (71%) of the participants were married at the time of the cancer diagnosis and 29% were unmarried. When asked about the desire for bearing a child in the future, 57 patients (28 of whom were married) indicated they wanted children in the future, 6 patients had not yet decided, and the remaining 37 patients had at least one living child and were not interested in FP. Comparing the wish for future fertility among the SES classes, significantly more women in the upper SES class than in the lower SES class wished to have future fertility. This may be due to the increasing trend of delayed childbearing by the upper SES class (60% vs. 11.8%) (*P*=0.017).

When asked about their knowledge of the effect of cancer and its treatment on future fertility, only 32% of the women were aware that cancer treatment might impair future fertility. When specifically asked about the gonadotoxic effect of chemotherapy and radiotherapy, only 28% of the women had an awareness of the effect. As shown in Fig. 2A, significantly more women in the upper SES class were aware of the harmful effect of cancer therapy on future fertility and of the gonadotoxic effect of chemotherapy and radiotherapy. The pairwise comparison with Bonferroni correction showed that there was a significant difference in awareness of the harmful effect of cancer therapy between the upper and lower SES classes (*P*=0.003) and between the middle and lower SES classes (*P*=0.001). Similarly, with respect to awareness of the gonadotoxic effect, there was significant difference between the upper and lower SES classes (*P*=0.017) and between the middle and lower SES classes (*P*=0.001). However, there was no significant difference (*P*>0.05) between the upper and middle groups for the two parameters. Less than one-third (32%) of the women were aware of FP, and of them, 28% were aware of oocyte and embryo cryopreservation. This awareness was significantly higher in the upper and middle SES classes (Fig. 2A). We found that none of the patients in the lower education groups (illiterate and primary education) had knowledge regarding the effect of cancer and gonadotoxic effect of chemotherapy on fertility, whereas significantly more women in the higher education group (graduate and above) had more knowledge in this regard (Fig. 2B). Also, awareness of FP was significantly greater in the higher education group (Fig. 2B). More than half of the women (68%) were not given any information by their primary physician regarding the effect of cancer treatment on future fertility and about FP. Patients who desired future fertility cited lack of information, financial constraints, lack of family support, and fear of delay in cancer treatment as reasons for not pursuing treatment. The most common reason among 28.6% of the patients was lack of proper information from their physicians. Only 11 patients underwent the FP procedure and 5 patients had to undergo surgical removal of the uterus and ovaries because of cancer. Follow-up found that 2 patients who underwent FP died.

Discussion

The present cross-sectional study evaluated the knowledge and awareness of female patients with cancer regarding the gonadotoxic effect of cancer therapy and FP options.

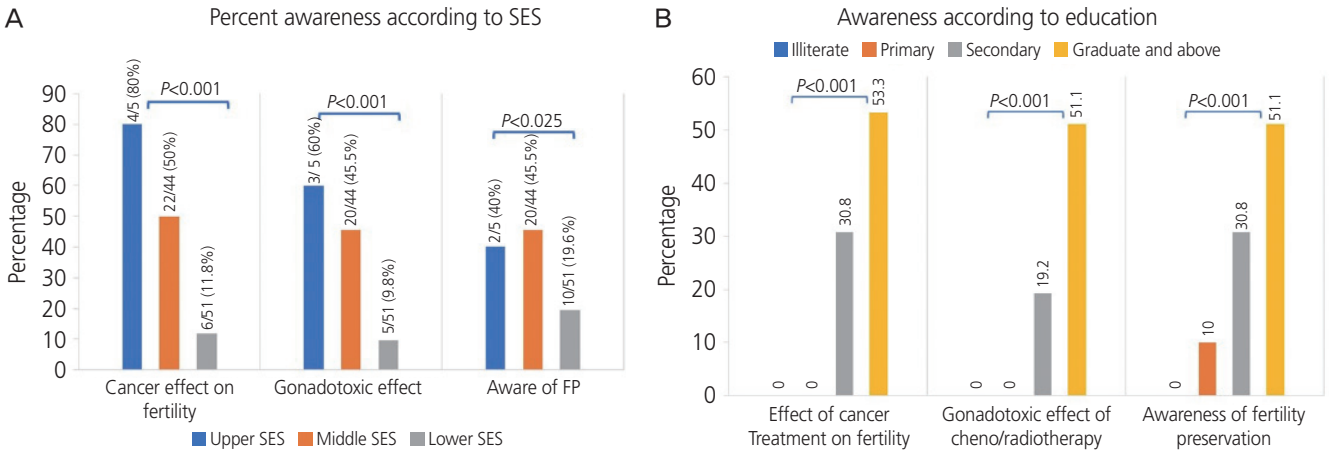

Fig. 2. Oncofertility awareness according to (A) SES and (B) education of the patient. SES, socioeconomic status.

The study was conducted at the infertility clinic of a tertiary care referral government institute so most of the women belonged the to lower and middle SES levels. The study concluded that overall knowledge and awareness about the harmful effect of cancer therapies and about FP before initiating gonadotoxic treatment was poor. Women in the lower SES class had significantly less knowledge than did women in the upper SES class. Similarly, knowledge was significantly low in the lower literacy groups.

About 10% of cancer patients are <45 years of age at the time of diagnosis [21]. Advances in cancer therapy have led to a remarkable improvement in the survival rates of patients with cancer. The overall cancer death rates have fallen by more than 1.6% per year between 2004 and 2008 [22]. Because of the trend of delayed childbearing, more women are diagnosed with cancer before they have planned their first pregnancy. This has led to the need for FP for which various options are available in accordance with the age of the patient, the type of cancer and prognosis, the type and dose of chemotherapy, marital status, and existing ovarian reserve.

Both radiotherapy and chemotherapy can impair fertility. Chemotherapy affects fertility via gonadotoxicity, and radiotherapy, if it involves the pelvis, affects the reproductive organs (ovary and uterus) [23]. Studies have shown that the rate of fertility and pregnancy of patients after cancer treatment is 38% lower than that of women in the general population [24].

Considering the improved survival rates among young patients with cancer and impaired fertility after cancer treatment, it is important to counsel these patients about the harmful effects of cancer treatment on future fertility and the available FP options. Lack of knowledge about FP among physicians and patients can lead to financial, legal, and psychological consequences.

The most common nongynecological cancers in our patient cohort were breast cancer and lymphomas. One of the main treatment modalities for these cancers is chemotherapy with alkylating agents, which are highly gonadotoxic because they are not cell cycle specific and affect other cells in the ovaries. If these cancers are detected at an early stage, patients have good overall survival, and with FP, they have a better chance of achieving pregnancy in the future. In our study, the awareness of FP did not differ much among patients with different types of cancer because overall knowledge was poor, but patients with breast cancer or lymphoma who have a good prognosis will benefit greatly from FP.

Our study found that approximately two-thirds of the patients were not aware of the effect of cancer treatment on fertility and of the gonadotoxic effect of chemotherapy and radiotherapy. A study conducted in Pakistan demonstrated a lack of awareness in more than 90% of young female patients with cancer about cancer treatment causing infertility [25]. Other studies have also demonstrated poor knowledge of the effect of cancer treatment on fertility [26,27].

Because knowledge about oncofertility among patients and physicians is poor, patients do not get the right information about the methods available to preserve their fertility and about the possibility of having their own genetic children in the future once cancer treatment is completed. In a study by Chin et al. [28], only 60% of women with cancer received fertility counseling before starting cancer therapy and only 13% of those women were referred to a fertility specialist. Our study showed comparable results whereby 68% of the study population was not counseled by their primary physician about the gonadotoxic effect of cancer therapy on future fertility.

This lack of knowledge about the harmful effect of cancer therapy may be due to low SES and low education level. The Chin et al. [28] study showed that women who had less education, were unmarried, or already had a child were less likely to receive fertility counseling because fertility is not considered a primary concern for these women. Our study also showed that women of low SES and those with a low education level had significantly less knowledge about the harmful effect of cancer therapy compared to that of their respective counterparts.

ASCO and ASRM guidelines recommend that patients with cancer should be given sufficient information about cancer treatment-related infertility and available fertility options [29]. However, studies have shown that physicians and patients have insufficient knowledge about FP [30,31]. Chehin et al. [32] assessed patients with cancer for their knowledge and awareness about FP and found that only 34% of the women were aware that cancer therapy could lead to infertility and only 22% were aware of FP options. Higher education was associated with more knowledge about the effect of cancer therapy on fertility and available FP options. Chehin et al. [32] concluded that patients with cancer had poor overall knowledge about FP. We found similar results in our study with only one-third of the women aware of the gonadotoxic

effect of cancer treatment and less than one-third (28%) having knowledge about FP. In addition, those with a higher education had better knowledge.

More than 90% of the patients in a study by Osmani et al. [25] wanted to discuss fertility issues with their primary oncologist before initiation of treatment, but other studies have found that less than half of the participants were offered a detailed consultation [21,17]. In our study, only 32% of the patients were informed about FP by their primary physician. Similarly, Ameri et al. [33] found that only 22.7% of their study patients received information regarding cancer treatment and infertility. However, they also found that significantly more patients with gynecological cancer received information compared to those with nongynecological cancer [33]. In contrast, studies conducted in the US and Switzerland found that more than 70% of the participants were informed by their healthcare professionals about fertility impairment after cancer treatment [31,34]. This regional difference in physician counseling may be due to a greater adherence to guidelines in countries like US and Switzerland in comparison to India, Brazil and Iran [32,33]. Reports have shown that only British and American physicians discuss fertility issues with their patients [30,35].

Some studies have shown that disparities in providing information depended on the gender of the patient [34,36]. In a study by Armuand et al. [36], only 48% of the women compared to 80% of the men received information about the risk of infertility with cancer treatment. The reasons behind this difference might be that cryopreservation of sperm is a relatively simple procedure and less costly compared to female FP methods, and for men there is no delay in starting cancer treatment. Female FP methods have many barriers including the time needed for hormonal medication to stimulate the ovaries, delayed cancer treatment, financial constraints, and the fear of risks associated with ovarian stimulation. To overcome these barriers, the oncologist and the reproductive endocrinologist could collaborate to prevent delay in referral, financial support arranged from government and nongovernment organization to cover expenses for FP, and ovarian stimulation protocols could be modified for minimal risk and overall less time required to complete the procedure.

Kim et al. [37] conducted a prospective study to evaluate the efficacy of the FP consultation process and predictors of decisional conflict among patients in need of FP. More than half (52%) of the participants requested their consultation, all participants answered that consultation was a helpful resource for information, and 73% of the participants made their decision about treatment after consultation. The consultation helped the patients formulate questions related to FP. Although consultation appeared to play a critical role in the decision-making of the patients about FP, the referral rate for consultation by oncologists was still poor [37].

Various reasons for not opting for FP may be the cost, a lack of proper information among both patients and treating physicians, fear of delaying cancer treatment and fear of risk of transmission of cancer to future offspring among patients with cancer.

To increase the knowledge of oncologists about FP, a Cancer and Fertility Platform was established in 2012 to provide a website with up-to-date information, communication tools, and ready-to-use script for fertility counseling [26]. After 3 years of use of the Cancer and Fertility Platform, the knowledge and practices of physicians were evaluated. The survey showed that referrals for FP by the cancer physicians was significantly higher after using the Cancer and Fertility Platform [38]. According to a recent systematic review of factors to improve oncofertility care, referrals for FP are very low worldwide, even after the publication of international guidelines on FP. International oncofertility models of care (MOC) are needed to train cancer and noncancer healthcare professionals to improve their knowledge about FP so they can convey that knowledge to patients requiring a referral [39]. A multidisciplinary team that includes an oncologist, a fertility specialist, a nurse counsellor, and a mental health professional is needed to provide high-quality cancer care to these patients.

One limitation of our study was its small sample size as a result of few patients being referred to us for FP. We assessed the knowledge of only female patients with cancer and not that of physicians. Another limitation was that the participants were patients with cancer who were consulting at a tertiary referral center. Knowledge and awareness of FP are expected to be poor among the general population. To our knowledge, this was the first study of Indian female patients with cancer.

Our study showed that the overall knowledge of young female patients with cancer regarding fertility impairment following cancer treatment and available FP options is poor. Because life expectancy after cancer treatment is increasing,

the option of FP should be a topic of interest among all oncologists. It is important to provide standardized up-to-date information and systematically offer oncofertility counseling to patients of reproductive age who are scheduled for cancer treatment. This can be accomplished by adopting a structured program that includes having a detailed face-to-face discussion with patients, providing them with written information about the various aspects of oncofertility, and organizing education programs for patients and concerned physicians.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

Ethical approval was obtained from the Ethics Committee of the All India Institute of Medical Sciences (Ref. No. IEC-9/04.01.2019, RP-50/2019).

Patient consent

Informed consent was obtained from all the participants.

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Appendix 1. Questionnaire

Name Age UHID No.

Phone number: Family size

Referred from

Marital status at the time of diagnosis: married/unmarried

Type of cancer:

Fertility status at the time of diagnosis (No. of abortions/living children):

Education: Income:

Profession: Socioeconomic status:

Q1. What was your age at the time of your cancer diagnosis?

Q2. Do you intend to have more children? Yes/no/not yet decided

Q3. Do you have any idea that cancer will affect your future fertility? Yes/no

Q4. Of these, what type of treatment have you received/are planning to receive for cancer?
a. Surgery b. Chemotherapy c. Radiotherapy d. Combination

Q5. Are you aware of the gonadotoxic effect of chemotherapy/radiotherapy on future fertility and ovarian function? Yes/no/don't know

Q6. Were you given proper FP options information before going for gonadotoxic treatment? Yes/no/don't know

Q7. What is/was the reason for not opting for FP before treatment?

Did not have information	Financial reason	Lack of family support	Was not informed by physician
Did not want to delay my cancer treatment	Risk of transmission of cancer to the future child	Already had desired children at that time	Any other

Q8. Are you aware of available FP options for you? Yes/no

Oocyte cryopreservation	Embryo cryopreservation	Ovarian cortex preservation
Shield from radiation field	GnRHa suppression	Ovarian transposition

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Is placental KISS-1 expression associated with first trimester abortion spontaneous?

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Objective

Several studies have examined biological markers during the first trimester to predict the maintenance of a healthy pregnancy. One such marker is kisspeptin, which is encoded by the KISS-1 gene. We aimed to determine whether first-trimester pregnancy losses were associated with levels of placental KISS-1 expression.

Methods

This prospective case control study was conducted at a tertiary center. The study group included 27 and 24 patients who underwent dilation and curettage at <10 weeks of gestation, due to first trimester spontaneous pregnancy loss and for elective termination (control), respectively. Placental and decidual tissues from all patients were sectioned and immunohistochemically analyzed for kisspeptin.

Results

Age, gravida status, parity number, gestational week, and number of previous abortions did not significantly differ between the groups. KISS-1 expression levels were significantly lower in the group with spontaneous abortion compared with the group with elective termination. The median staining intensity of KISS-1 expression in the elective and spontaneous termination groups were 3 (strong) and 2 (moderate), respectively (P=0.004). KISS-1 expression levels were significantly lower among patients with previous abortions in the elective termination group (P=0.002).

Conclusion

KISS-1 expression levels were found to be significantly reduced in patients with spontaneous pregnancy loss; KISS-1 plays an important role in the implantation and continuation of pregnancy.

Keywords: Genes, tumor suppressor; Abortion, spontaneous; Pregnancy, unwanted; Placenta

Introduction

The first trimester of pregnancy is a critical period for the continuity and progression of a healthy pregnancy. Approximately 15% of clinically confirmed pregnancies result in spontaneous abortion, and 80% of those occur during the first trimester of pregnancy [1]. The major etiological factors for spontaneous abortions during the first trimester of pregnancy are genetic, endocrine, anatomical, and immunological; however, the etiology of 50% of such abortions remains unknown. Regulation of the finely controlled process of trophoblast invasion plays a major role in preventing complications of pregnancy, including early spontaneous abortion [2,3]. The ability of markers such as mean arterial pressure,

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uterine artery pulsatility index, placental growth factor, pregnancy-associated plasma protein A, maternal serum alpha-fetoprotein, and free beta-HCG, in predicting healthy maintenance of pregnancy, have been examined during the first trimester [4,5]. However, studies of markers that could indicate the well-being of pregnancy during the first trimester, are under way.

Recent studies have focused on molecular mechanisms like those of tumor cells because trophoblast migration and invasion occur via such molecular mechanisms [6,7]. One such mechanism is that of kisspeptin.

Kisspeptin constitutes a group of peptide fragments encoded by the KISS-1 gene (a tumor metastasis suppressor) in humans and each fragment acts on various tissues via numerous mechanisms [8]. Kisspeptin-10 (KP-10) might play a role in the regulation of trophoblast invasion, puberty onset, and in the development of the hypothalamic–pituitary–gonadal axis [9,10]. Kisspeptins, especially KP-10, reportedly play important roles in trophoblast invasion through the regulation of placental angiogenesis. Trophoblast invasiveness reduced by KP-10 might induce the downregulation of matrix metallopeptidase activity [11]. Kisspeptin expression in syncytiotrophoblasts and cytotrophoblasts is reduced in women who experience recurrent spontaneous abortion compared with controls [12]. Sullivan-Pyke et al. [13] found lower serum kisspeptin levels in cases experiencing spontaneous abortions. Thus, kisspeptin might serve as a new serum biomarker to discriminate the likelihood of spontaneous abortion from viable intrauterine pregnancy [13].

The present study compared the expression of placental KISS-1 that encodes kisspeptin, between first-trimester pregnancy losses and elective pregnancy terminations to determine the differences at the tissue level.

Materials and methods

1. Study population

This prospective, case control study was conducted at the Department of Gynecology and Obstetrics of a tertiary center.

The sample size of the study was calculated using the G*Power (G*Power Ver. 3.1.9.2, Franz Faul, Üniversität zu Kiel, Kiel, Germany) statistical package. The required sample sizes for α=0.05 and Zα=1.96, and β=0.10 and Zβ=–1.28 was calculated for each group. The study and control groups included 27 and 24 consecutive patients who underwent dilation and curettage at <10 weeks of gestation due to first trimester spontaneous pregnancy loss and for elective termination, respectively, between July 2017 and January 2018. Elective pregnancy termination is possible before gestational week 10 in Turkey. Therefore, we included first-trimester pregnancy losses only before gestational week 10.

Spontaneous pregnancy loss was diagnosed via transvaginal ultrasound and clinical assessment. We excluded patients with a retroplacental hematoma, active maternal infection, systemic lupus erythematosus, hypertension, diabetes, chronic renal insufficiency, alcohol consumption, a smoking habit (cigarettes), or a history of ≥2 spontaneous abortions. According to the guidelines of the Royal College of Obstetricians and Gynaecologists, recurrent pregnancy loss is defined as a total of ≥3 abortions [14]. We analyzed the karyotypes of all patients, and excluded those with abnormalities to unmask and avoid possible effects of chromosomal abnormalities on kisspeptin expression between the groups. Patients with vaginal bleeding and hematomas were not included due to low risk. As much as possible, we aimed to select only patients with healthy pregnancies for the control group.

Curettage specimens from 27 spontaneous pregnancy losses and 24 elective pregnancy terminations histopathologically diagnosed as "decidua showing the Arias-Stella reaction and chorionic villi" at the Faculty of Medicine, Department of Pathology between 2001 and 2003 were collected and archival hematoxylin and eosin stained sections were reevaluated. Kisspeptin was immunohistochemically assessed in tissue samples containing decidua and chorionic villi without areas of hemorrhage and necrosis.

2. Immunohistochemistry

Sections were incubated at 56°C for 24 hours, deparaffinized in xylene, then rehydrated using a descending alcohol series (100–70%). Endogenous peroxidase activity was blocked by immersing the sections for 5 minutes in methanol containing 0.5% hydrogen peroxide. Antigens were retrieved by microwave-heating the sections in trisodium citrate buffer (10 mM sodium citrate, pH 6.0) for 10 minutes. The sections were rinsed 3 times in de-ionized distilled water, immersed in 0.3% hydrogen peroxidase for 30 minutes to block endogenous peroxidase activity, then washed in PBS for 2–3 minutes. Non-specific background staining was minimized

using Ultra V Block (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 5 minutes. The sections were then incubated for 1 hour at room temperature with the mouse monoclonal primary antibody to KISS-1, 24-Q (Santa Cruz Biotechnology Inc., Dallas, TX, USA) diluted 1:100. This antibody was raised against a partial recombinant protein mapped within amino acids 46–145 of KISS-1 of human origin. After a wash with PBS for 5 minutes, Goat Anti-Polyvalent (Lab Vision Corp., Fremont, CA, USA) was applied, then the sections were washed in PBS. The sections were incubated for 15 minutes at room temperature with streptavidin peroxidase (Lab Vision Corp.). Immunoreactivity was visualized using the chromogen, DAB (Thermo Fisher Scientific Inc.). The sections were counterstained with Mayer's hematoxylin solution and mounted. Staining specificity was confirmed by comparison with a positive control.

3. Analysis of immunohistochemical expression

Two pathologists who were blinded to the pregnancy stage and outcomes analyzed all sections. Immunohistochemical staining was evaluated by scanning whole sections for antibodies using a BX51 light microscope (Olympus Optical Co., Ltd., Tokyo, Japan) at 4×, 10×, 20×, and 40× magnification. KISS-1 proteins were mainly located in the glandular epithelium, decidualized stromal cells, cytotrophoblasts, and syncytiotrophoblasts.

The results are expressed as ratios (%) of positivity based on staining intensity scored as 0 (negative), 1+ (low), 2+ (moderate), and 3+ (strong). Ratios (%) of stained cells were: 0 (<10%), 1 (10–25%), 2 (10–50%), 3 (51–80%), and 4 (>80%). The final score was calculated as the product of the ratio of stained cells and staining intensity, resulting in weak (0–2), moderate (3–6), and strong (8) KiSS-1 expression [15].

4. Statistical analysis

Variables were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and MedCalc 14 (MedCalc Software Ltd., Ostend, Belgium) software. The normality of data distribution was evaluated using Shapiro-Wilk tests. The homogeneity of variances was assessed using Levene tests. Pairs of independent groups were compared using independent samples *t*-tests with Bootstrap results, and Mann-Whitney *U*-tests with Monte Carlo results. Categorical variables were compared using Pearson χ^2 tests with exact results. Correlations between KISS-1 and age, number of births, parity, and abortus were assessed using Spearman rho tests. The sensitivity and specificity of relationships between classifications based on cutoff values calculated according to the number of abortions and KISS-1 variables for the 2 groups of patients, and the actual classification were analyzed using receiver operating characteristics curves. Diagnostic-dependent variables with explanatory variables were estimated using logistic regression analysis, K-Nearest Neighbor, and Decision Tree. However, the results were not significant and are not shown. Quantitative variables are expressed as mean±standard deviation, range (maximum–minimum), and medians. Categorical variables are expressed as number (%). The variables were analyzed at 95% confidence intervals, and $P<0.05$ was considered statistically significant.

Table 1. Comparison of demographic data and KISS-1 levels between the 2 groups

Characteristics	Optional abortion (n=24)	Abortus (n=27)	Total (n=51)	*P*-value
Age	31.54±4.95 (41–24)	31.89±7.37 (43–19)	31.73±6.29 (43–19)	0.839
Parity	2 (8–1)	2 (7–1)	2 (8–1)	0.893
KISS-1	3 (3–2)	2 (3–2)	3 (3–2)	0.004
Gravida				
Primigravid	8 (33.3)	8 (29.6)	16 (31.4)	1
Multigravid	16 (66.7)	19 (70.4)	35 (68.6)	
Previous abortion				
None	21 (87.5)	19 (70.4)	40 (78.4)	0.182
1	3 (12.5)	8 (29.6)	11 (21.6)	

Values are presented as mean±standard deviation, median (maximum–minimum), or number (%). Data were compared using independent samples *t*-tests (Bootstrap), Mann-Whitney *U*-tests (Monte Carlo), and Pearson χ^2 tests (exact).

Results

Table 1 shows the demographic characteristics of the patients. Mean age (control vs. study group: 31.54±4.95 vs. 31.89±7.37 years), gravida status, parity number, previous abortions and gestational weeks (*P*=0.072) did not significantly differ between the 2 groups. These finding confirmed the homogeneous distribution of demographic characteristics within each group.

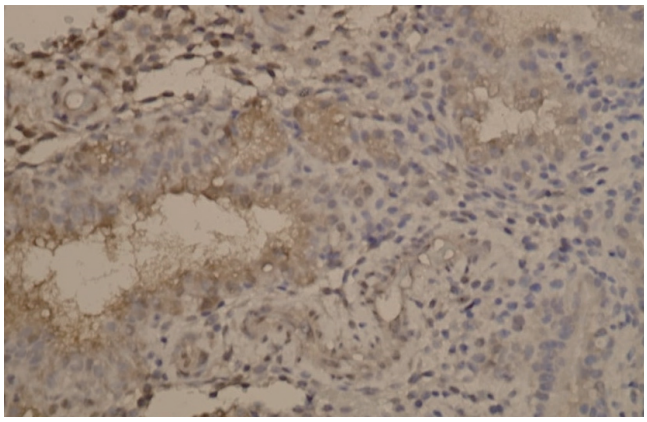

Fig. 1. Weak (+) staining intensity.

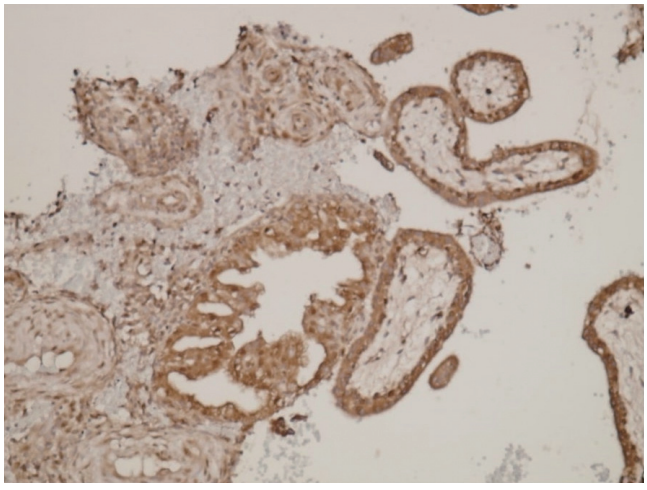

Fig. 2. Strong (+++) intensity of staining.

Median values of placental KISS-1 expression significantly differed between the two groups. Median values for staining intensity indicating KISS-1 expression in the control and study groups were 3 (strong) and 2 (moderate), respectively (*P*=0.004; Table 1). Fig. 1 shows a loss of KISS-1 in expression in the glandular epithelium and decidualized stromal cells. Fig. 2 shows strong KISS-1 staining intensity in the glandular epithelium and decidualized stromal cells.

The effects of previous abortions on placental KISS-1 expression were significantly lower in the group with elective termination (*P*=0.002), but did not significantly differ in the group with spontaneous abortion (Table 2).

We determined a cutoff value of 2 for KISS-1 expression based on the areas under receiver operator characteristics curves. The sensitivity and specificity were 59.3% and 83.3%, respectively (Table 3, Fig. 3).

The expression of KISS-1 was significantly lower in the patients with, than without a history of abortion (*P*<0.001; Table 4).

Table 2. Investigation of the relationship between KISS-1 levels and previous abortion in both groups

Previous abortion	No.	KISS-1	*P*-value
Optional abortion			
No	21	3 (3–2)	0.002
Yes	3	2 (2–2)	
Abortus			
No	19	2 (3–2)	1
Yes	8	2 (3–2)	

Values are presented as median (maximum–minimum). Data were compared using Mann-Whitney *U*-tests (Monte Carlo).

Table 3. Determination of cut-off, sensitivity and specificity values for KISS-1

Cut-off	Optional abortion (n=24)	Abortus (n=27)	AUC (95% CI)	*P*-value
KISS-1				
>2	20 (83.3)[a]	11 (40.7)	0.713 (0.856–0.570)	0.004
<2	4 (16.7)	16 (59.3)[b]		

Values are presented as number (%). Data were analyzed using ROC (Honley & Mc Nell - Youden index J).
AUC, area under the ROC curve; CI, confidence interval; ROC, receiver operating curve.
[a]Sensitivity; [b]Specificity.

Discussion

This study investigated whether placental KISS-1 expression differed between patients with pregnancy losses and those who had elective pregnancy terminations within ≤10 weeks of the first trimester.

Serum kisspeptin values during early gestation are found to be associated with abortive imminence, poor obstetric outcomes such as preterm birth, preeclampsia, and intrauterine growth retardation [16]. The same study also associated kisspeptin with recurrent pregnancy losses, indicating that serum kisspeptin could serve as a marker of placental function [12]. However, comparative information about KISS-1 expression between healthy pregnancies and non-recurrent abortion is scarce.

Our results showed significantly lower placental KISS-1 expression in patients with first-trimester pregnancy losses than in those requesting elective terminations. As far as we are aware, this is the first comparison of KISS-1 in materials derived from non-recurrent spontaneous abortions and elective pregnancy terminations.

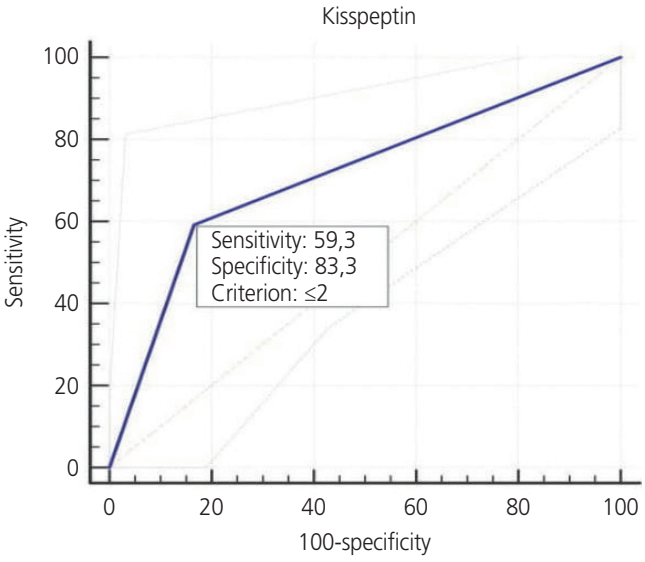

Fig. 3. Sensitivity and specificity for KISS-1.

Kisspeptin has many effects on the physiology of puberty, the neuroendocrine system, reproductive

physiology, and it plays a regulatory role in syncytiotrophoblasts [9,10]. Kisspeptin and its receptor system are expressed during implantation and placentation in the endometrial and placental tissues of various species, including humans other. A study of hysterectomy materials obtained from non-pregnant female dogs and dogs at different stages of pregnancy found a significant increase in KP-10 levels after implantation and around the middle of pregnancy [17]. Another study found higher levels of KISS-1R expression in the early placenta, where the invasive capacity of trophoblasts needs to be tightly controlled compared with term placenta in humans [18]. These studies suggested a potential role of the kisspeptin/KISS-1R system in the invasive and migratory properties of trophoblasts.

Levels of kisspeptin and its receptor GPR54 were also associated with spontaneous abortions due to their actions on trophoblast invasion. Wu et al. [12] compared the immunohistochemical expression of kisspeptin in decidua and trophoblasts between women with first-trimester pregnancy loss and those who requested legal termination. Levels of kisspeptin were significantly lower in decidual tissue and trophoblasts from women experiencing first-trimester pregnancy loss than in those requesting legal elective termination. We compared KISS-1 expression in decidual cells and trophoblasts between women with first-trimester pregnancy loss and those with elective pregnancy termination. Levels of KISS-1 expression were significantly lower in decidua and trophoblasts from patients with first-trimester pregnancy losses compared with elective termination. We found significantly lower KISS-1 expression in the group with spontaneous abortion, which supports published findings [12,19]. However, unlike the patients in those studies, none of our patients had anamnesis of recurrent pregnancy losses.

Table 4. Correlation analysis for KISS-1

KISS-1	Optional abortion		Abortus		Total	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Age	–0.243	0.252	0.170	0.397	0.005	0.97
Previous abortion	–0.845	<0.001	–0.043	0.832	–0.544	<0.001

Data assessed using Spearman's ρ test.

In contrast, we identified a negative correlation between KISS-1 expression and abortion in our cohort. The expression of KISS-1 did not significantly differ between patients with spontaneous abortion with and without a history of abortion. However, KISS-1 expression was significantly lower among the women with, than without a history of abortion who requested legal termination. We speculate that previous abortions did not change the KISS-1 expression value in the group with spontaneous abortion because they already had low expression, whereas previous abortions significantly changed the normal level of KISS-1 expression in women with unwanted pregnancies. This result suggests an association between reduced KISS-1 expression and recurrent abortions. Others have also found higher KISS-1 expression in preterm, than in term pregnancy, indicating that KISS-1 expression gradually decreases as the placenta matures [20,21]. Together with these findings, the present results suggested that KISS-1 is mainly expressed in the placenta during early pregnancy.

Kisspeptin concentrations have been determined plasma or serum, but few studies have immunohistochemically assessed kisspeptin expression in tissues. Our results showed that KISS-1 is mainly expressed in villous trophoblasts, and that reduced KISS-1 expression is associated with early pregnancy outcomes.

The limitations of our study are that KISS-1 expression was examined only in trophoblastic tissue, and plasma levels were not simultaneously measured. Future studies should investigate correlations between serum kisspeptin levels and trophoblastic KISS-1 expression. Consequently, whether serum kisspeptin could serve as a predictive or diagnostic marker of miscarriage should be investigated. Low expression of KISS-1 might be the result of abortions. Therefore, KISS-I expression should be analyzed before and after elective abortion to clarify a cause-and-effect relationship. In addition, detecting KISS-1 mRNA using RT-PCR might be useful to precisely determine whether the KISS-1 gene is affected. Our study did not include patients with recurrent pregnancy loss, which differed from previous similar studies. The reason is that many etiologies can cause pregnancy loss, and a kisspeptin deficiency might function as a cofactor instead of being a direct cause. Thus, we avoided reducing heterogeneity.

In conclusion, we assessed the expression of KISS-1, which is the gene that produces the protein kisspeptin in abortus tissues, and found that KISS-1 plays an important role in the implantation and continuation of pregnancy.

Acknowledgements

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study protocol was approved by the Institutional Review Board of Baskent University (KA16/132).

Patient consent

All patients provided written informed consent to participate in the present study.

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Prevalence of iron deficiency anemia in Asian female population and human development index (HDI): an ecological study

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Objective
Anemia is a major problem in developing countries, the purpose of the present study was to investigate the relationship between the human development index (HDI) and the prevalence of anemia among Asian women.

Methods
This was a descriptive-analytic ecological study. The study analyzed data including HDI and prevalence of anemia extracted from the World Bank. The correlation coefficient and analysis of variance were used for data analysis. The significance level was considered to be less than 0.05. Analyses were performed using Stata-14 software.

Results
The results revealed that the prevalence of anemia in women of reproductive age (R=–0.626, P<0.001), pregnant women (R=–0.576, P<0.001) and non-pregnant women (R=–0.623, P<0.0001) was significantly negatively correlated with HDI index. In the analysis of HDI components and the prevalence of anemia, a significant negative correlation was observed between the prevalence of anemia and the mean years of schooling, life expectancy at birth, and expected years of schooling indices in all 3 groups of women (non-pregnant, pregnant, and of reproductive age) (P<0.05).

Conclusion
Since there was a negative correlation between the prevalence of anemia in women and HDI, paying greater attention to factors which influence anemia may contribute to the prevention of anemia in developing countries.

Keywords: Prevalence; Anemia; Asia

Introduction

Anemia represents a global problem that affects people from all walks of life in all societies. Around a quarter of the world's population suffers from this disease. The problem is not constrained to the medical sector, and its roots can be traced to education, demography, and nutrition. Iron deficiency accounts for about half of the world's anemia. Anemia has caused 19.7 million years of life adapted with disability. The average annual economic loss incurred as a result of iron deficiency anemia in 10 developing countries is estimated at approximately $16.78 per capita or 4% of gross domestic

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product (GDP) [1].

Although adults only need 1 mg of absorbable iron daily, iron deficiency anemia remains a widespread issue. Women of reproductive age who have poor diets and suffer from malnutrition are at a greater risk for anemia [2,3]. The prevalence of iron deficiency anemia in women is higher than that of men. Despite the economic-scientific advances in recent years, anemia is still prevalent especially in pregnant women and children before school age [4].

The World Health Organization (WHO) estimates that iron deficiency anemia took the lives of 273,000 people in 2004. Of this figure, 45% were Southeast Asian, 31% African, 9% Eastern Mediterranean, 4% Oceania, and 3% European. Of all anemia cases, 97% are found in middle-income countries [5]. Anemia is particularly prevalent in women of reproductive age and children under the age of 5. Studies in developed countries such as Japan, Sweden, and the United States indicate the rising incidence of iron deficiency anemia in affluent societies. It has also posed a major problem in developing countries such as India over the last 50 years [6].

The results of a study in 185 countries from 1990 to 2011 exhibited that anemia was the most prevalent in Southeast Asia. Extreme cases of iron deficiency anemia can escalate to maternal mortality and preterm labor [3,7]. Several factors contribute to the widespread prevalence of micronutrient deficiencies in South Asia including iron deficiency in the diet, poor health, genetic causes, chronic infections, malaria and schistosomiasis [8,9]. The prevalence of anemia varies widely in different parts of the world and it is more salient in lower socioeconomic classes [10].

Moreover, as far as the relationship between anemia and the human development index (HDI) is concerned, in areas with a high HDI, anemia-related problems are less noticeable. HDI represents the essence of human development measures. This index measures the average success of a country in 3 main dimensions of human development, i.e. a long and healthy life, education and appropriate living standards [11].

Given the prevalence of anemia in Asian countries, it is necessary to raise awareness about its contributing factors in order to plan and manage the financial and human resources required to curb its incidence. It is essential for countries to introduce the necessary interventions by comparing their data with those of other countries with regard to the effect of socioeconomic status on the root causes of anemia [11].

The purpose of this study was to explore the impact of socioeconomic development (based on HDI) on the prevalence of anemia among Asian women based on data retrieved from the World Bank in 2018.

Materials and methods

The present research was a descriptive-analytic ecological study that investigated the relationship between anemia prevalence in 3 groups of women and its connection with HDI in Asian countries. The study data included HDI and the prevalence of anemia obtained from the World Bank [12].

1. Definition of anemia

Percentage of women aged 15–49 years with a hemoglobin concentration less than 120 g/L for non-pregnant women and lactating women and less than 110 g/L for pregnant women, adjusted for altitude and smoking [13,14].

2. Human development index

The HDI provided by the World Bank delivers the latest data on global development, which includes national, regional and global estimates. According to the Human Development Report, countries are divided into 4 groups: very high human development, high human development, medium human development, and low human development based on the level of the HDI. The numerical value of the HDI is between 0 and 1. It measures efforts taken by each country to reach the highest possible HDI value, and also allows comparisons between countries. HDI represents the essence of human development measures. It measures the mean success of a country in 3 main dimensions of human development: a long and healthy life, access to education and proper living standards [15].

3. Statistical analysis

In this study, data analysis was performed using correlation coefficients and an analysis of variance (ANOVA) to investigate the relationship between the prevalence of anemia and HDI. A *P*-value of less than 0.05 was considered significant. Analyses were performed using the Stata-14 software (StataCorp, College Station, TX, USA).

Results

According to the results reported in 2016, the global prevalence of anemia is 32.5% in non-pregnant women, 40.1% in pregnant women, and 32.8% in women of reproductive age. The highest prevalence of anemia in all 3 groups of women (non-pregnant, pregnant, and of reproductive age) was observed in South-East Asia and the lowest in America (Table 1). The analysis of trends in anemia prevalence in 3 groups of women between 2000 and 2016 demonstrates that the prevalence of anemia in all 3 groups was the highest in the South-East Asia region, which was above the global average during these years (Fig. 1).

According to the analysis of the prevalence of anemia, the highest prevalence of anemia in non-pregnant women was recorded in Yemen (70.2%), Pakistan (52.2%), and India (51.5%); the highest prevalence of anemia in pregnant women was recorded in Yemen (63%), Cambodia (55.8%), and Myanmar (53.8%); and the highest prevalence of anemia in women of reproductive age was recorded in Yemen (69.6%), Pakistan (52.1%), and India (51.4%) (Table 2).

The results revealed a significant negative correlation between the prevalence of anemia in women of reproductive age (R=–0.626, *P*<0.001), pregnant women (R=–0.576, *P*<0.001) and non-pregnant women (R=–0.623, *P*<0.001) and HDI index (Fig. 2).

In the analysis of HDI components and the prevalence of anemia in 3 groups of women, a significant negative correlation was observed between the prevalence of anemia in non-pregnant women and mean years of schooling (MYS) (r=–0.564, *P*<0.001), life expectancy at birth (LEB) (r=–0.552, *P*<0.001), and expected years of schooling (EYS) (r=–0.564, *P*<0.001). There was a significant negative correlation between anemia prevalence in pregnant women and MYS (r=–0.563, *P*<0.001), LEB (r=–0.439, *P*<0.001), and EYS (r =–0.534, *P*<0.01). Moreover, there was also a significant negative correlation between the prevalence of anemia in women of reproductive age and MYS (r=–0.567, *P*<0.001), LEB (r=–0.553, *P*<0.001), and EYS (r=–0.566, *P*<0.01). No significant correlation was observed between the prevalence of anemia and GDP in all 3 groups of women (*P*>0.05) (Table 3).

The results of the ANOVA suggested that in non-pregnant women, the highest mean prevalence (48.7±19.1) belonged to low human development and the lowest mean prevalence (27.4±9.2) to very high human development, and this differ-

Table 1. Prevalence of anemia in women estimates by World Health Organization (WHO) region

WHO region	Prevalence of anemia in non-pregnant woman (%)					Prevalence of anemia in pregnant woman (%)					Prevalence of anemia in woman reproductive age (%)				
	2000	2005	2010	2015	2016	2000	2005	2010	2015	2016	2000	2005	2010	2015	2016
Africa	44.8 (39.8–49.0)	42.6 (38.2–46.5)	39.1 (34.7–43.2)	38.0 (32.0–44.1)	38.2 (31.7–45.0)	52.1 (48.9–54.9)	50.5 (47.6–52.9)	48.1 (44.8–50.7)	46.3 (41.0–50.7)	46.2 (40.3–51.3)	45.6 (41.0–49.5)	43.4 (39.3–47.0)	40.1 (36.0–43.9)	38.8 (33.1–44.5)	39.0 (32.7–45.4)
America	19.8 (16.5–23.7)	18.7 (16.1–21.5)	17.5 (14.5–21.1)	18.2 (13.5–24.7)	18.8 (13.5–26.4)	28.9 (22.9–34.5)	26.8 (21.7–31.7)	25.4 (20.5–30.4)	25.3 (19.5–32.3)	25.5 (19.2–32.8)	20.2 (17.0–24.0)	19.1 (16.5–21.7)	17.8 (14.9–21.3)	18.4 (13.9–24.8)	19.1 (13.9–26.5)
South-East Asia	47.4 (42.3–52.2)	46.5 (41.1–51.2)	44.9 (39.4–50.2)	45.2 (39.0–50.2)	45.6 (38.8–51.2)	50.3 (45.6–55.1)	49.2 (43.8–53.6)	48.0 (42.5–52.8)	47.9 (42.3–53.1)	48.1 (42.1–53.7)	47.6 (42.7–52.2)	46.7 (41.4–51.2)	45.0 (39.7–50.2)	45.3 (39.3–50.2)	45.8 (39.1–51.2)
Europe	20.2 (16.1–25.5)	19.8 (16.0–25.0)	19.5 (15.7–24.5)	21.7 (16.2–28.8)	22.5 (16.4–30.7)	27.5 (22.0–33.6)	26.0 (21.2–31.5)	25.3 (20.6–31.1)	26.1 (20.3–33.9)	26.5 (20.3–35.0)	20.4 (16.3–25.6)	20.0 (16.2–25.1)	19.7 (15.9–24.5)	21.8 (16.4–28.7)	22.7 (16.6–30.7)
Eastern Mediterranean	39.3 (34.7–43.6)	38.4 (34.0–42.8)	37.4 (32.9–41.8)	39.0 (32.2–45.6)	39.8 (32.1–47.5)	41.1 (36.4–45.7)	40.5 (36.6–44.5)	40.1 (36.1–43.8)	40.6 (34.9–46.5)	40.9 (34.5–47.6)	39.5 (35.0–43.7)	38.6 (34.4–42.8)	37.6 (33.3–41.8)	39.1 (32.6–45.5)	39.8 (32.4–47.4)
Western Pacific	21.8 (15.7–30.7)	19.4 (13.8–28.3)	19.2 (12.5–29.4)	23.7 (12.5–38.8)	25.1 (12.5–41.8)	28.9 (23.4–36.6)	27.7 (22.1–35.4)	28.3 (20.9–39.1)	32.0 (20.5–49.3)	33.0 (20.5–51.1)	22.0 (16.0–30.8)	19.6 (14.2–28.4)	19.5 (12.8–29.6)	23.9 (12.9–38.6)	25.3 (13.0–41.8)
World	31.1 (28.5–34.2)	30.1 (27.6–33.1)	29.4 (26.6–32.9)	31.6 (27.4–36.4)	32.5 (27.8–37.8)	41.6 (39.1–44.0)	40.4 (38.1–42.8)	39.4 (36.9–42.1)	39.8 (36.4–43.8)	40.1 (36.4–44.6)	31.6 (29.1–34.7)	30.6 (28.2–33.5)	29.9 (27.2–33.3)	32.0 (27.9–36.6)	32.8 (28.3–38.0)

ence was statistically significant (F=4.73, *P*<0.001). In pregnant women, the highest mean (45.7±8.6) belonged to low human development and the lowest mean (33.8±6.6) to very high human development, and the difference was statistically significant (F=4.11, *P*<0.001). These results were comparable to those found in women of reproductive age, with the highest mean prevalence of anemia (48.8±18.8) belonging to low human development and the lowest mean prevalence to the very high human development (27.6±9.1). Here, the difference was also statistically significant (F=4.73, *P*<0.001) (Table 4).

Discussion

Iron deficiency anemia has been reported in 25% of mothers with malnutrition [3]. Pregnant women and young children are more likely to develop anemia. Geographically, people living in Asia and Africa are at a greater risk of anemia. According to the WHO, the prevalence of anemia in all women, pregnant women, and non-pregnant women was 37%, 51%, and 35%, respectively, in 1988 [16]. The results of this research suggested that, in general, the highest prevalence

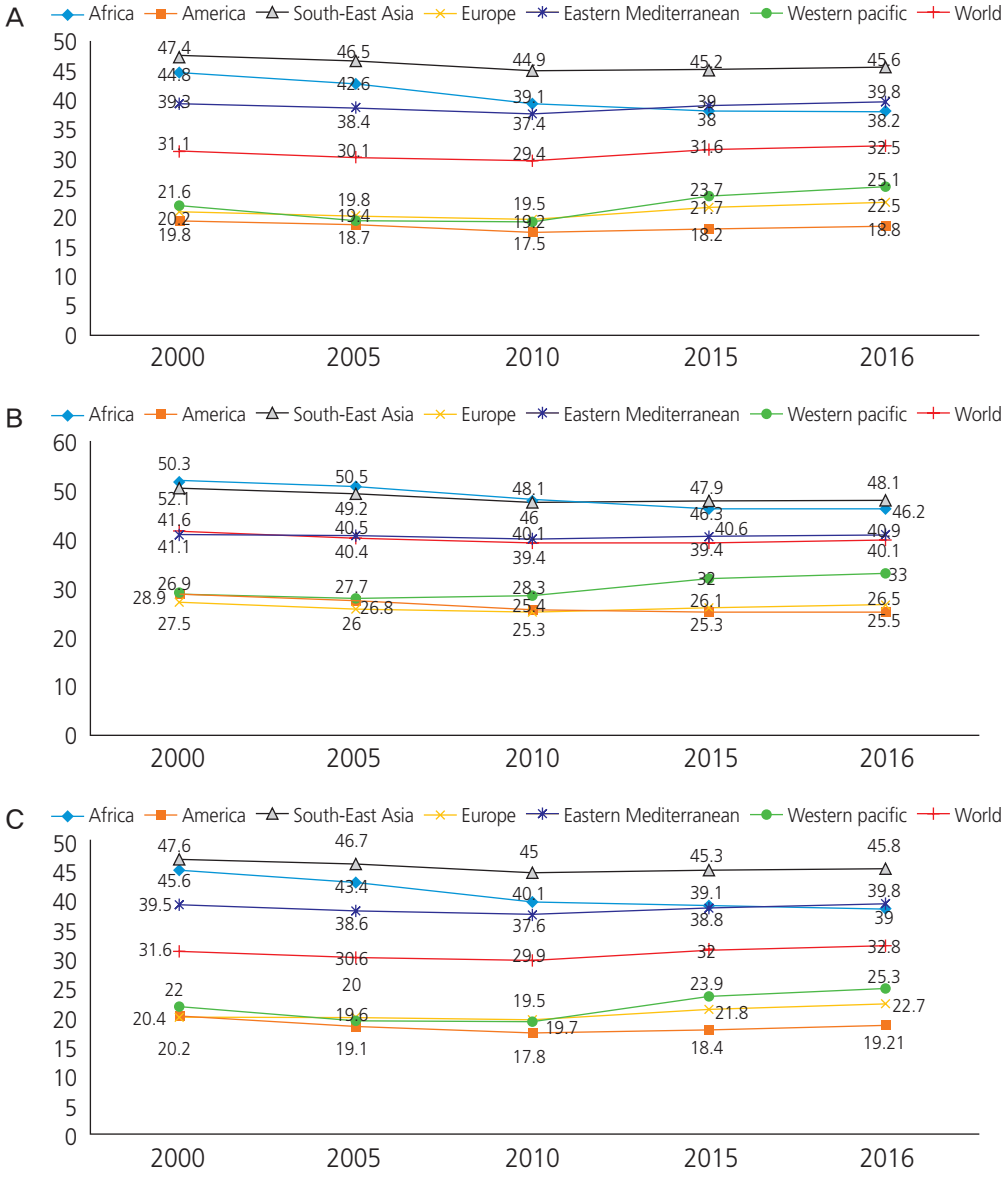

Fig. 1. Trend of anemia prevalence in 3 groups of women. (A) Non-pregnant woman, (B) pregnant woman, (C) woman reproductive age by region World Health Organization.

Table 2. Prevalence of anemia in Asian women (estimates by country in 2016)

Country	Prevalence of anemia in non-pregnant woman (%)	Prevalence of anemia in pregnant woman (%)	Prevalence of anemia in woman reproductive age (%)
Afghanistan	42.4 (27.7–55.8)	38.2 (24.7–53.3)	42.0 (28.2–54.9)
Armenia	29.2 (12.2–56.3)	35.5 (17.5–63.3)	29.4 (12.6–56.3)
Azerbaijan	38.4 (25.3–51.8)	40.1 (26.9–54.7)	38.5 (25.7–51.6)
Bahrain	41.9 (19.0–67.2)	42.8 (20.2–64.8)	42.0 (19.7–66.7)
Bangladesh	39.6 (21.1–55.5)	45.7 (28.9–60.1)	39.9 (22.0–55.5)
Bhutan	35.7 (24.8–47.8)	32.8 (21.3–47.9)	35.6 (25.0–47.3)
Cambodia	46.3 (36.3–54.2)	55.8 (46.0–63.3)	46.8 (37.2–54.6)
China	26.2 (9.8–47.7)	32.4 (15.6–57.8)	26.4 (10.2–47.5)
Cyprus	25.1 (9.6–50.6)	29.0 (15.2–57.0)	25.2 (9.9–50.2)
Georgia	27.4 (12.9–45.5)	30.1 (17.5–50.3)	27.5 (13.2–45.3)
India	51.5 (41.5–58.7)	50.1 (42.0–57.2)	51.4 (41.8–58.5)
Indonesia	28.2 (18.7–38.8)	42.0 (30.4–53.7)	28.8 (19.4–39.0)
Iran	30.3 (12.7–52.8)	34.1 (17.5–57.6)	30.5 (13.2–52.4)
Iraq	28.7 (17.2–44.3)	33.5 (19.3–51.6)	29.1 (18.0–44.1)
Israel	15.2 (7.0–38.0)	23.8 (12.7–49.9)	15.7 (7.5–37.3)
Japan	21.2 (14.4–31.0)	34.1 (18.2–57.6)	21.5 (14.8–31.0)
Jordan	34.5 (20.8–50.0)	37.1 (22.9–51.6)	34.7 (21.4–49.7)
Kazakhstan	30.7 (13.0–52.2)	30.4 (15.7–54.3)	30.7 (13.4–51.2)
Kuwait	23.5 (9.8–46.3)	31.2 (14.8–57.3)	23.8 (10.2–46.2)
Kyrgyzstan	35.9 (21.3–51.5)	39.8 (25.1–55.6)	36.2 (22.0–51.0)
Lao People's Democratic Republic	39.3 (19.5–60.7)	45.7 (23.8–65.0)	39.7 (20.7–60.7)
Lebanon	31.1 (13.2–55.0)	35.2 (17.8–60.1)	31.2 (13.6–54.8)
Malaysia	24.4 (9.4–49.8)	37.1 (15.8–63.0)	24.9 (10.0–49.7)
Maldives	42.4 (21.2–60.9)	46.9 (18.6–67.5)	42.6 (21.9–61.0)
Mongolia	18.9 (7.1–38.8)	30.3 (18.2–49.5)	19.5 (8.0–38.4)
Myanmar	46.0 (34.2–54.7)	53.8 (41.5–62.4)	46.3 (35.0–54.9)
Nepal	34.9 (19.2–50.9)	40.0 (22.9–57.7)	35.1 (19.9–50.9)
Oman	38.0 (17.4–62.2)	41.8 (20.8–62.5)	38.2 (18.1–62.1)
Pakistan	52.2 (35.3–67.4)	51.3 (36.4–62.8)	52.1 (35.7–67.0)
Philippines	14.9 (9.7–25.6)	30.3 (19.2–45.2)	15.7 (10.5–26.1)
Qatar	27.5 (10.2–52.8)	33.4 (15.2–58.5)	27.7 (10.6–52.9)
Saudi Arabia	42.8 (18.0–70.9)	45.5 (20.7–65.6)	42.9 (18.8–70.5)
Singapore	22.0 (8.5–50.3)	31.8 (15.2–59.6)	22.2 (8.7–50.3)
Sri Lanka	32.5 (14.1–51.3)	35.4 (19.2–55.6)	32.6 (14.7–51.1)
Syrian Arab Republic	33.5 (13.8–56.5)	36.1 (17.2–60.2)	33.6 (14.6–56.3)
Tajikistan	30.3 (14.0–49.3)	33.5 (17.3–57.4)	30.5 (14.9–48.5)
Thailand	31.6 (11.2–70.2)	40.2 (15.9–67.6)	31.8 (11.7–70.2)
Timor-Leste	41.2 (25.0–55.9)	42.6 (24.5–60.7)	41.3 (26.2–55.9)
Turkey	30.7 (12.0–56.7)	34.4 (16.5–60.2)	30.9 (12.5–56.6)
Turkmenistan	32.6 (13.3–54.9)	33.1 (16.7–58.1)	32.6 (13.8–54.4)
United Arab Emirates	27.6 (10.8–52.7)	33.2 (15.7–59.6)	27.8 (11.1–52.6)
Uzbekistan	36.8 (16.2–52.6)	24.9 (14.6–48.5)	36.2 (16.6–51.6)
Viet Nam	23.7 (10.4–43.7)	37.3 (19.3–58.5)	24.2 (11.0–43.8)
Yemen	70.2 (54.7–79.7)	63.0 (56.3–66.7)	69.6 (55.0–78.5)

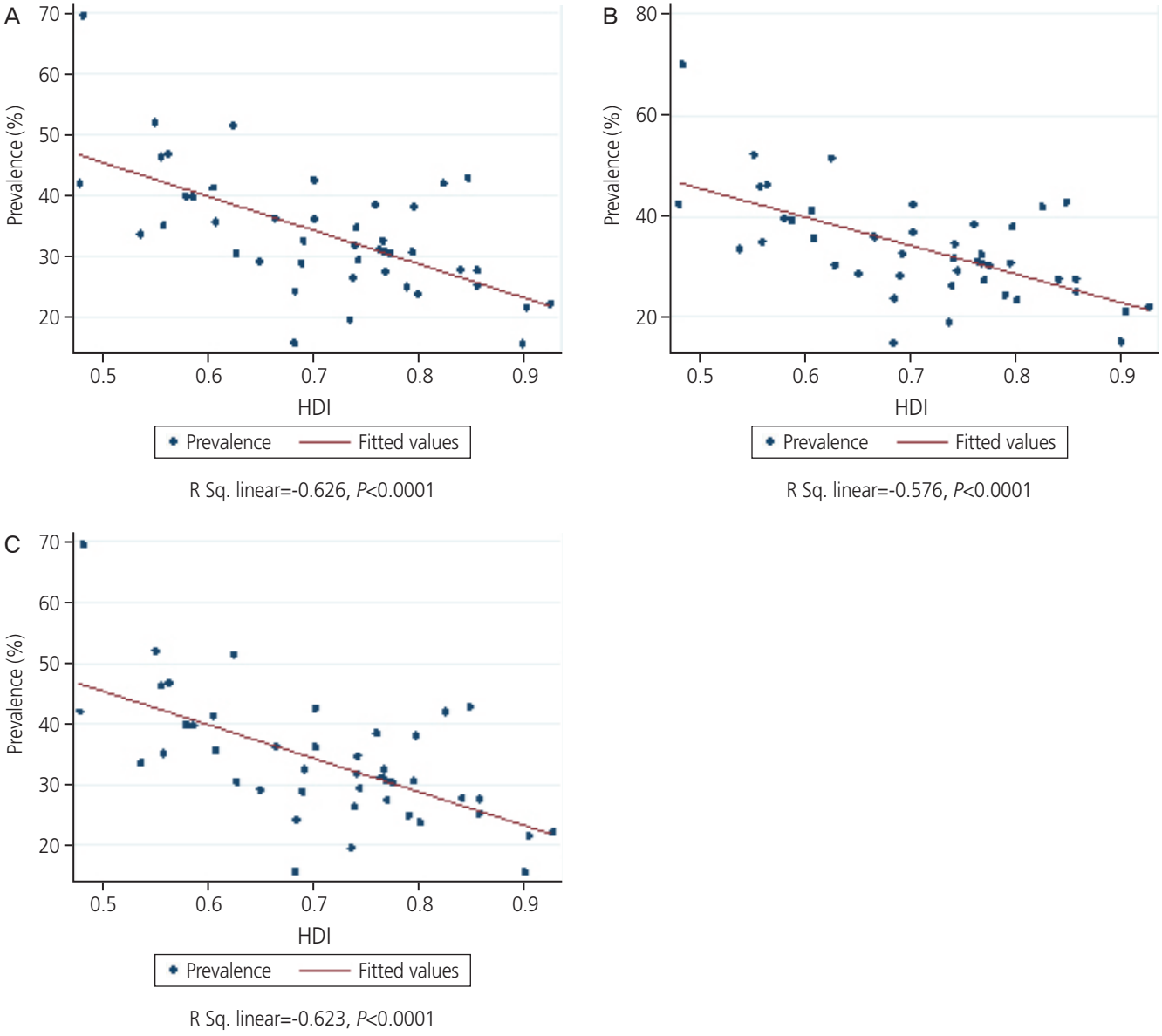

Fig. 2. Correlation[a] between the human development index (HDI) and prevalence of anemia in Asian women. (A) Women reproductive age, (B) pregnant woman, (C) non-pregnant woman in 2016. [a]Statistical method used: correlation coefficient.

Table 3. Pearson correlation between human development index (HDI) component and prevalence of anemia in Asian women in 2016

HDI component	Prevalence of anemia in non-pregnant woman (%)		Prevalence of anemia in pregnant woman (%)		Prevalence of anemia in woman reproductive age (%)	
	r[a]	*P*-value	r[a]	*P*-value	r[a]	*P*-value
Gross national income per 1,000 capita	–0.284	>0.05	–0.225	>0.05	–0.286	>0.05
Mean years of schooling	–0.564	<0.001	–0.563	<0.001	–0.567	<0.001
Life expectancy at birth	–0.552	<0.001	–0.439	<0.001	–0.553	<0.001
Expected years of schooling	–0.564	<0.001	–0.534	<0.001	–0.566	<0.001

[a]Statistical method used: Pearson correlation coefficient.

of anemia in non-pregnant women, pregnant woman, and women of reproductive age was observed in Asia, Africa, and Asia, respectively. Moreover, the highest prevalence of anemia in non-pregnant women (70.2%), pregnant women (63%), and women of reproductive age (69.6%) was recorded in Yemen.

The lowest incidence of anemia in non-pregnant women was recorded in the Philippines (14.9%); in pregnant women in Israel (23.8%); and in women of reproductive age in Japan and the Philippines (15.7%).

The difference in the prevalence of anemia in these areas can also be attributed to differences in the socioeconomic status of individuals. One indicator that reflects the status of countries is HDI. This index explores the status of a country in 3 key development dimensions, including health, education and living standards. Health is measured with LEB, education with the EYS, and living standards as gross national income per capita or GDP. In countries with high HDI, the prevalence of anemia is lower. The secondary causes of anemia prevalence include timely screening and diagnosis, elevated quality of food, early-stage treatment of malnutrition, and accurate estimation of vitamin intake in these countries [9].

In middle-income countries, the prevalence of anemia is higher; as a result, 97% of anemia-induced mortality is reported in developing countries [5]. The highest prevalence of anemia in the world is observed in countries with low HDI [17]. In this context, the highest prevalence of anemia has been recorded in Asian countries. Nutritional anemia is widespread not only in developing countries but also in affluent societies [2,16].

The results of a 2016 study by Petry et al. [4] demonstrated that anemia has declined in countries with moderate to high HDI. In this study, iron deficiency anemia in preschool children and women of reproductive age was 25% and 37% in high HDI communities, respectively, while this figure was higher than the 45% in low HDI communities. A 2011 study by Oliveira et al. [18] showed that there was an inverse association between the prevalence of anemia and HDI. This study revealed that the prevalence of anemia was significantly higher among blood donors in communities with lower HDI [18]. In their 2012 study, Laxmaiah et al. [19] exhibited that the incidence of HDI was inversely correlated with the prevalence of anemia. These studies demonstrated that anemia is still a major health issue and the prevention and control of malnutrition should be considered as a key strategy in curbing the prevalence of anemia.

Socioeconomic developments have wielded huge influence on the prevalence of anemia. In low- and middle-income countries, the risk of anemia is increasing. Socioeconomic growth is inversely related to the prevalence of anemia. Chronic infections, nutritional disorders, obesity, chronic noncommunicable diseases, thalassemia, and genetic susceptibility are among the risk factors for anemia. Anemia is linked to HDI in societies, so that according to statistics, the most anemia-related deaths take place in less developed countries [4].

The results of this study demonstrate a significant negative correlation between the prevalence of anemia in non-pregnant women and MYS (r=–0.564, P<0.001), LEB (r=–0.552, P<0.001), and EYS (r=–0.564, P<0.001); a significant negative correlation between anemia prevalence in pregnant women and MYS (r=–0.563, P<0.001), LEB (r=–0.439, P<0.001), and EYS (r=–0.534, P<0.01), and a significant negative correlation between the prevalence of anemia in women of reproductive age and MYS (r=–0.567, P<0.001), LEB (r=–0.553, P<0.001), and EYS (r=–0.566, P<0.01). The lower prevalence of anemia in countries with higher HDIs

Table 4. Prevalence of anemia in different human development index (HDI) regions Asian women in 2016

HDI	Prevalence of anemia in non-pregnant woman (%)	Prevalence of anemia in pregnant woman (%)	Prevalence of anemia in woman reproductive age (%)
Very high human development	27.4±9.2	33.8±6.6	27.6±9.1
High human development	31.4±5.8	35.3±5.3	31.6±5.6
Medium human development	36.3±10	41.7±8.1	36.5±9.8
Low human development	48.7±19.1	45.7±8.6	48.8±18.8
P-value (F-test)[a]	<0.001	<0.001	<0.001

Data are shown as mean±standard deviation.
[a]Statistical method used: analysis of variance.

could be attributed to factors such as the existence of micronutrients in foods, more accurate diagnostic methods and enhanced access to primary health care.

The major factors contributing to the high prevalence of anemia in most countries are low income levels and malnutrition. Hence, one effective step to mitigate the prevalence of anemia is to provide primary anemia prevention strategies such as increasing household per capita and providing food packages for families. Despite the interrelation of these factors, caution should be practiced when interpreting such studies because, in addition to the environmental factors of anemia prevalence, the limitations of descriptive studies should also be taken into consideration. Early prevention and epidemiological studies, especially for people in less developed countries, can help reduce the burden of disease and improve health status in these countries.

In women from different south-east Asian regions iron deficiency anemia should ideally be addressed through dietary diversification and improved access to foods that have high levels of bioavailable iron, including animal products. Daily or intermittent iron supplementation, alone or together with folic acid and other micronutrients, can be used for high-risk groups (children, pregnant women and women of reproductive age), to improve iron intake. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anemia in a given setting and population group, and should be built into the primary health-care system and existing programs.

In conclusion, the results of the present study suggest a negative correlation between the prevalence of anemia in women and HDI. Moreover, the prevalence of anemia in these countries was negatively correlated with LEB and education. Hence, a study of factors that raise the prevalence of anemia in countries with low HDI can go a long way in reducing its incidence rate. Further, raising the level of a mother's education and her awareness can also contribute to a reduced incidence.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study was approved by Ethic Committee (IR.KMU.REC.1398.505).

Patient consent

The patients provided written informed consent for the publication and the use of their images.

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Comparison of pain and proper sample status according to usage of tenaculum and analgesia: a randomized clinical trial

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Objective
Colposcopic biopsy is a discomfortable procedure. Additionally, it creates negative influence on sexuality. This study aimed to investigate the relationships among tenaculum, pain perception, and biopsy size during colposcopy.

Methods
In total, 228 patients who underwent colposcopy-directed biopsy were included, and randomized into 4 groups based on whether analgesic and tenaculum were used and replaced (tenaculum with n=58/without analgesic n=56, no tenaculum replacement with n=57/without analgesic n=57). Lidocaine hydrochloride (40 mg) plus adrenaline (0.025 mg) was administered in the analgesic groups. The pain was assessed using a linear visual analog scale. The biopsy specimen size was measured in millimeters.

Results
The mean age of the patients was 42.85±8.88 years. The most frequent colposcopy indications were atypical squamous cells of undetermined significance and human papilloma virus-positive results on cervical cytology (30.2%; n=69). Low- and high-grade intraepithelial lesions were noted in 14.91% (n=34) and 10.96% (n=25) women through colposcopy-directed biopsy results, respectively. Tenaculum replacement increased pain perception in the without analgesic group; however, no statistically significant differences were noted between of the groups with and without tenaculum replacement with analgesic. The size and number of biopsy specimens were not associated with tenaculum replacement and analgesic use.

Conclusion
Administration of analgesics decreased discomfort and pain in patients. Tenaculum replacement aided colposcopists in manipulating the cervix. Additionally, administration of analgesics relieved pain in the tenaculum replacement group.

Trial Registration
ClinicalTrials.gov Identifier: NCT03279666

Keywords: Cervix uteri; Biopsy; Pain perception; Human papilloma virus

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Introduction

Colposcopy is the cornerstone of cervical precancerous lesion evaluation [1]. It has been the standard of care for evaluating abnormal cervical cytology since the 1970s [2].

In conjunction with screening and treatment of precancerous lesions, colposcopy has played a crucial role in reducing the incidence and mortality due to cervical cancer for the past 50 years [1]. The current guidelines for initial management of high-grade cervical cytological abnormalities recommend an immediate diagnostic excisional procedure during the initial colposcopy that is critical to a "see-and-treat" approach in selected patients. In addition, it allows recognition of a subset of women with cervical disease and safe observation of the disease over time. Further, it plays an important role in reducing the over-treatment of low-grade lesions [3].

Procedure risks of colposcopy, such as significant bleeding, infection, and long-term morbidity, are low. Colposcopy-directed punch biopsies are associated with discomfort, cramping, and pain. Furthermore, some women reported a negative influence on sexuality [4]. A traumatic colposcopy experience may lead to some women refraining from obtaining adequate cervical screening in the future [5]. Both abnormal cervical cytology results and colposcopy experience can induce anxiety in women [6]. A study reported that women experienced worry and anxiety in the interim period—the time between being notified of an abnormal screening result and colposcopy appointment [7]. However, the procedure can usually be performed on an outpatient basis without anesthesia [8]. Researchers have studied the use of local anesthesia, topical spray, and forced cough method to reduce pain due to colposcopy-directed punch biopsy [8-10], and most of these studies indicated that these methods were effective.

Most gynecologic oncology centers worldwide use the International Federation of Gynecology and Obstetrics staging system for gynecological cancers such as cervical cancer. The clinical stage is used for cervical cancer staging; hence, it is based on the results of the physical examination by doctor, biopsies, imaging tests, and a few other tests such as magnetic resonance imaging, cystoscopy, and proctoscopy [11]. Stage 1A (microinvasive) cervical cancer can only be detected through microscopic examination of removed tissue, preferably a cone; however, several punch biopsies may be required for diagnosis. Stage 1A cervical cancer was defined as invasion limited to measured stromal invasion with a maximum depth of 5 mm and stage 1A1 was defined as measured stromal invasion with a maximum depth of 3 mm [12]. Hence, punch biopsy size is crucial.

This study aimed to investigate the relationship among tenaculum placement, pain perception, and biopsy size during colposcopy.

Materials and methods

Bakirkoy Dr. Sadi Konuk Training and Research Hospital (BTRH), University of Health Sciences, was the reference center in the cervical smear and human papilloma virus (HPV) screening program for 30–65-year-old women. The screening had been performed by primary level health staff (family physicians and screening center staff) under the Republic of Turkey, Ministry of Health, since 2014. These centers referred women with high-risk (HR) HPV positive results to specific hospitals. HR-HPV detection was performed using the commercially available Hybrid Capture 2 assay® (HC2; Digene Corp., Gaithersburg, MD, USA). The results were assessed in BTRH, and colposcopy was performed by gynecologic oncology faculty members or gynecologic oncology fellows in patients with indications for colposcopy.

This study was conducted on patients with indications for colposcopy and within the scope of the Republic of Turkey, Ministry of Health Cervical Cancer Screening program between June 15, 2017 and December 31, 2017. The study was approved by the ethical board of BTRH (number: 2017/47) and was registered in ClinicalTrials.gov (number: NCT03279666). Informed consent was obtained from all the patients before the procedure. Patients were randomized using a computer program. A statistical program (GPower version 3, Heinrich Heine University, Düsseldorf, Germany) was used to estimate the sample size through a 1-tailed hypothesis using an independent sample t-test with an α error of 0.05 and a power of 0.90. The total sample size required for a moderate effect size (d=0.50) was calculated to be 236. In the present study, 58 women were in group A, 56 women were in group B, 57 women were in group C, and 57 women were in group D. *Post hoc* analysis for moderate effect size indicated that the power of the test was reduced to 0.80. In addition to undergoing colposcopy, the inclusion criteria for the study were as follows:

- Being older than 21 years old

- No history of receiving colposcopy, loop electrosurgical excision procedure (LEEP), conization, or cryotherapy
- Did not use analgesics up to 6 hours before the procedure
- Did not experience chronic pain or did not use analgesics for a long-term
- Did not have advanced atrophic or hypertrophic cervix
- Did not have visible cervical mass (polyp, myoma, or locally advanced cervical cancer)

The exclusion criteria were as follows:

- Did not provide consent for participation in the study
- Had advanced orthopedic problem
- Had allergy to analgesics
- Were pregnant

This study enrolled 228 women. Patients were randomly assigned to 4 groups (Fig. 1).

- Group 1: patients with tenaculum replacement without analgesic (n=58)
- Group 2: patients without tenaculum replacement without analgesic (n=56)
- Group 3: patients with tenaculum replacement with analgesic (n=57)
- Group 4: patients without tenaculum replacement with analgesic (n=57)

The number of patients who underwent cervical biopsy as well as endocervical curettage (ECC), only cervical biopsy, and only ECC were 182, 42, and 4, respectively.

The patients were placed in the lithotomy position in a quiet colposcopy room and using a single-use vaginal speculum colposcopy was performed by the gynecologic oncology team, comprising a doctor and nurse. Colposcopy was started by cleaning the cervix with 5-mL saline. Cotton soaked in freshly prepared 5% acetic acid solution was applied on the cervix for 3 minutes, and subsequently, colposcopic examination was performed. Then, 2-mL lidocaine hydrochloride (40 mg) plus adrenaline (0.025 mg) was injected at the 8 and 4 o'clock positions of the cervix using a 22-gauge needle to

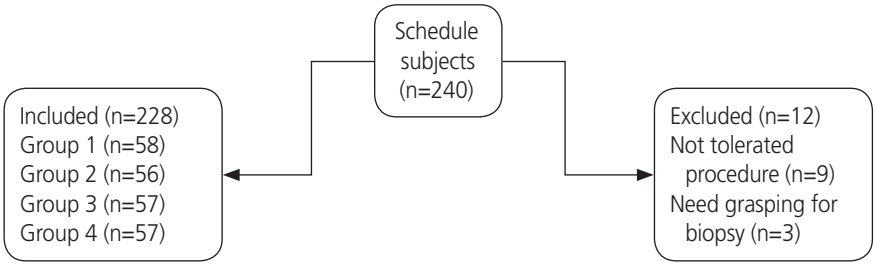

Fig. 1. Flow diagram of the study.

Table 1. General data of all groups

Variables	Group 1 (n=58)	Group 2 (n=56)	Group 3 (n=57)	Group 4 (n=57)	*P*-value[a]
Gravidy	4.57±4.9/3 (0–34)	3.2±1.9/3 (0–9)	3.1±1.9/3 (0–9)	3.6±2.6/3 (0–14)	0.327
Parity	3.02±2.06/2 (0–12)	2.7±1.8/2 (0–8)	2.3±1.4/2 (0–7)	2.4±1.5/2 (0–7)	0.297
SVD	2.6±2.2/2 (0–12)	2.1±1.9/2 (0–7)	1.9±1.6/2 (0–7)	2.02±1.7/2 (0–7)	0.540
SCA	0.5±0.9/0 (0–4)	0.46±0.8/0 (0–3)	0.35±0.7/0 (0–3)	0.3±0.6/0 (0–3)	0.681
Abortus	0.69±1.4/0 (0–9)	0.38±0.6/0 (0–2)	0.54±0.7/0 (0–3)	0.89±1.2/0 (0–5)	0.099
UC	0.88±3.9/0 (0–30)	0.13±0.5/0 (0–2)	0.21±0.8/0 (0–5)	0.28±0.9/0 (0–5)	0.112
Age	43.4±10.03/42 (28–72)	43.1±8.5/42.5 (29–64)	41.8±7.9/41 (27–60)	43.1±9.1/44 (25–63)	0.809
Number of biopsy	2.9±1.04/3 (1–5)	3.3±1.2/3 (1–5)	3.6±0.9/3 (1–6)	3.7±0.9/4 (2–6)	0.001[b]
Pain level	3.9±3.1/3 (0–10)	4±3.3/3 (0–10)	2.2±2.7/1 (0–10)	2.6±3.02/1 (0–10)	<0.001[b]
Size of biopsy_median	3.4±1.3/3.3 (1–8)	3.8±1.8/3.5 (1–8.3)	3.6±1.2/3.3 (1.7–7.5)	3.7±1.3/3.7 (1–7)	0.492

Values are presented as mean±standard deviation/median (minimum–maximum). Group 1, patients with tenaculum replacement without analgesic; Group 2, patients without tenaculum replacement without analgesic; Group 3, patients with tenaculum replacement with analgesic; Group 4, patients without tenaculum replacement with analgesic.

SVD, spontaneous vaginal delivery; SCA, sectio caserean abdominalis; UC, uterine curuttage for unwanted pregnancy.

[a]Kruskal-Wallis test; [b]$P<0.01$.

those in the groups 3 and 4. The doctor waited for 2 minutes for the analgesic to take effect before obtaining biopsy specimens. Biopsy specimens were obtained from necessary sites by using a biopsy equipment (Schumacher biopsy forceps 1/1 made in Germany), with or without tenaculum replacement on the anterior part of the cervix transversely in accordance with the group. ECC was performed using a Recamier uterine curette (width 3 mm made in Germany) if required. Subsequently, the linear visual analog scale was used to assess pain within 2 minutes after colposcopic examination.

1. Statistical analysis

Descriptive statistics were used to define continuous variables (mean; standard deviation; and minimum, median, and maximum). The Kruskal-Wallis test was used to analyze the association between more than 2 continuous variables that were independent and not normally distributed. The Mann-Whitney *U* test was used to analyze the association between 2 continuous variables that were independent and not normally distributed. The correlation between 2 continuous variables without normal distribution was analyzed with Spearman's rho correlation analysis. Chi-square (or Fisher exact test as applicable) was used to analyze the association between categorical variables. Statistical significance was set at *P*=0.05. The analyses were conducted using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

Results

A total of 228 of 240 patients were analyzed. Ten patients were excluded because they did not provide consent to participate and other 2 patients were excluded because their biopsy results indicated cervical cancer. The mean age of included patients was 42.85±8.88 years (range, 25–72 years; Table 1). The most frequent colposcopy indications were atypical squamous cells of undetermined significance and HPV positive results on cervical cytology (n=69; 30.2%). The most common HR-HPV type was HPV 16 (n=68; 29.8%). HPV 51 was the most frequently detected non-HPV 16-18 of the high-risk HPV types (Table 2). Normal cytology, low-grade intraepithelial lesions and high-grade intraepithelial lesions

Table 3. Relationship between pathological results and pain perception

Pathological result	Pain perception
Pathological result of ECC	
Inadequate (n=11)	3.4±2.6/2 (1–9)
Normal (n=162)	2.9±3.1/1 (0–10)
LSIL (n=6)	4.7±3.7/4 (1–10)
HSIL (n=7)	4±3.4/2 (0–9)
P-value[a)]	0.277
Pathological result of biopsy	
Normal (n=164)	3.1±3.1/2 (0–10)
LSIL (n=34)	3.7±3.5/2.5 (0–10)
HSIL (n=25)	3.1±3.03/2 (0–10)
Cancer (n=1)	-
P-value[a)]	0.653

Values are presented as mean±standard deviation/median (minimum–maximum).
ECC, endo cervical curettage; LSIL, low grade intraepithelial lesion; HSIL, high grade intraepithelial lesion.
[a)]Kruskal-Wallis test.

Table 2. Human papilloma virus status

HPV type	Group 1 (n=58)	Group 2 (n=56)	Group 3 (n=57)	Group 4 (n=57)	*P*-value[a)]
Negative	16 (27.6)	15 (27.3)	24 (42.1)	24 (42.1)	0.044
HPV type 16	13 (22.4)	10 (18.2)	14 (24.6)	8 (14.0)	
HPV type 18	2 (3.4)	2 (3.6)	3 (5.3)	2 (3.5)	
HPV type 16+18	6 (10.3)	8 (14.5)	3 (5.3)	2 (3.5)	
HPV type non 16-18	15 (25.9)	19 (34.5)	8 (14.0)	10 (17.5)	
HPV type 16 or 18+HPV-N	6 (10.3)	1 (1.8)	5 (8.8)	11 (19.3)	

Values are presented as number (%). Hybrid capture 2 technology was used for detection of 13 high-risk HPV types.
HPV, human papilloma virus; HPV-N, non 16-18 high-risk HPV types.
[a)]Ki-Kare test.

(HSIL) were noted in 73.68% (n=168), 14.91% (n=34), and 10.96% (n=25) women, respectively. Squamous cell cervical cancer was noted in one patient with HSIL who underwent conization (Table 3).

On the basis of *post hoc* paired comparisons, statistically significant differences were observed between groups 1 and 3 and between groups 1 and 4 for the distribution of biopsy count (Mann-Whitney *U*, $P<0.05$, Bonferroni correction). Group 1 had a lower mean for biopsy counts. The mean biopsy size did not differ between groups (Table 4).

Statistically significant differences were observed between groups 1 and, between groups 1 and 4, between groups 2 and 3, and between groups 2 and 4 for pain perception (Mann-Whitney *U*, $P<0.05$, Bonferroni correction). In groups 1 and 2, the mean level of pain was higher than that in the groups 3 and 4. Although few biopsy specimens were obtained in group 1, pain severity in group 1 was higher than that in groups administered with lidocaine hydrochloride (40 mg) plus adrenaline (0.025 mg; Table 5).

The pain was statistically higher among patients who underwent cesarean section once, who did not undergo curettage, and who were nulliparous. Menopause, smoking and alcohol consumption, educational degree, presence of chronic illness, pathological results of cervical smear, presence of HPV, ECC, and pathological results of ECC and biopsy did not have an influence on pain perception. However, pain perception differed among groups with 3 or 5 biopsy specimens (Kruskal-Wallis, $P<0.05$). Paired comparisons data indicated that pain perception in group 1 was higher than that in groups 3 and 4, wherein 3 biopsy specimens were obtained from patients, and that in group 2 was higher than that in group 3, wherein 5 biopsy specimens were obtained from patients (Mann-Whitney *U*, $P<0.05$). In all the groups, no statistically significant difference was noted in biopsy size and pain (Spearman's rho, $P>0.05$), and the sizes of the biopsy specimens were >3 mm, 3 mm tumoral tissue which is considered a stage 1A1-stage 1A2 threshold for cervical cancer.

Although pain during colposcopic biopsy was not severe, administration of the analgesic decreased discomfort and pain in patients, resulting in better cervical assessment with colposcopy. Although tenaculum placement increased pain perception mildly, it aided colposcopists to manipulate the cervix and obtain biopsy specimens easily. In addition, administration of analgesics relieved pain in patients of the tenaculum replacement group, without any influence on the biopsy size.

Table 4. *Post hoc* binary comparison between number of biopsy and pain perception

Post hoc binary comparison	Number of biopsy[a]	Pain perception[a]
Group 1 vs. Group 2	0.131	0.975
Group 1 vs. Group 3	0.001[b]	<0.001[b]
Group 1 vs. Group 4	<0.001[b]	0.004[b]
Group 2 vs. Group 3	0.135	0.001[b]
Group 2 vs. Group 4	0.054	0.007[b]
Group 3 vs. Group 4	0.495	0.679

[a]Mann-Whitney *U* test; [b]$P<0.01$.

Discussion

Colposcopy and related procedures have been recognized to be potentially painful [13]. Women experiencing pain during colposcopy are anxious, and interventions aimed at reducing the level of pain and anxiety before and during colposcopy are useful [14]. Studies have reported that anxiety levels be-

Table 5. Correlation between number of biopsy and pain level

Number of biopsy	1 (n=21)	2 (n=45)	3 (n=42)	4 (n=59)	5 (n=61)	*P*-value[a]
Group 1	4±3.3/3 (0–9)	3.1±2.9/1.5 (0–9)	4.2±2.8/4 (1–10)	4.3±3.5/3 (0–10)	4.3±4.9/2 (1–10)	0.588
Group 2	4.3±4.5/4 (0–9)	3.1±3.03/1 (0–10)	3.1±2.9/2 (0–10)	6.1±2.8/6.5 (1–10)	4.4±3.5/3 (0–10)	0.151
Group 3	-	1.2±0.9/1.5 (0–2)	2.2±2.8/1 (0–10)	3.1±3.1/1.5 (0–10)	0.7±1.4/0 (0–4)	0.065
Group 4	-	3±3.3/2.5 (0–7)	1.8±3.1/1 (0–10)	3.3±3.1/2 (0–10)	2.4±2.8/1 (0–8)	0.344
P-value[a]	0.830	0.757	0.002[c]	0.078	0.019[b]	-

Values are presented as mean±standard deviation/median (minimum–maximum).
[a]Kruskal-Wallis test; [b]$P<0.05$; [c]$P<0.01$.

fore and during the procedure are elevated in women. Particularly, anxiety can be attributed to the uncertainty regarding what the colposcopic examination entails, events during the procedure, and fear or experience of pain during the procedure [15,16]. Humor, imagination of pleasant scenes and experiences, music, and forced coughing have been reported to reduce pain through the gate control theory of pain based on partial inhibition of painful sensations traveling along slower nerve fibers by overriding stimuli that travel along fast nerve fibers [17,18]. However, to the best of our knowledge, no study has reported the relationship among the use of tenaculum, pain perception, and biopsy size to date.

In 2011, Naki et al. [10] found that local anesthesia was more effective than forced coughing for relieving pain during cervical biopsy, in contrast to Schmid et al. [9] in 2008, but the duration of the entire procedure was significantly longer in patients in the local anesthetic group [10].

Bogani et al. [19] observed that nulliparity, number of previous vaginal deliveries, and pain expectancy were associated with pain perception during colposcopic biopsy, and only pain expectancy independently correlated with operative pain levels. Factors such as educational level, obstetric history, and chronic pelvic pain may affect the anticipated pain score. Öz et al. [20] reported a significantly inverse relationship between patients' educational level and reported pain scores after both cervical biopsy and ECC procedures. On the contrary, in the present study, we found that women who were nulliparous, who underwent cesarean section abdominalis, and who did not undergo dilatation and curettage perceived a higher level of pain. However, education, menopause, smoking, history of ECC, and chronic diseases were found to be not associated with pain score.

The first study by Rabin et al. [21] investigated the use of local anesthesia for gynecological procedures by comparing 20% benzocaine gel with placebo for its ability to reduce pain associated with cervical biopsy, intrauterine device insertion, pain associated with injection of anesthetic drug, tenaculum placement, and ECC, and revealed that 20% benzocaine gel significantly reduced pain associated with these procedures. In the current study, it was found that local anesthesia reduced pain perception as a significant difference in pain perception was noted between the groups without tenaculum replacement and with or without local anesthesia.

Several scientific studies have indicated that women subjected to a passive, pleasing and simple visual distraction during colposcopy experienced less post procedural pain than those who were not subjected to visual distraction [22,23]. In the present study, the overall pain level in group 3 patients was low (2.2±2.7).

The procedure type may affect patients' pain perception. Kola-Palmer et al. [24] reported that a significant difference in sensory pain existed between treatment groups of women undergoing see-and-treat large loop excision of the transformation zone treatment and those undergoing colposcopy plus punch biopsy who reported significantly higher levels of pain perception than women who underwent diagnostic colposcopy.

The tenaculum is used to stabilize and supply counter traction on the cervix and uterus [25]. A study by Goldthwaite et al. [26] compared pain scores with tenaculum placement after injecting intracervical lidocaine and topically applying lidocaine gel. Lidocaine injection was more effective than lidocaine gel application in the study. Robinson et al. [27] indicated that a significant difference in pain perception existed after comparing intracervical lidocaine injection (saline injection), when traction was placed with tenaculum during hysterosalpingography, with no injection. Oyama et al. [28] revealed that lidocaine injection reduced pain score during cervical biopsies and endocervical curettage, and overall procedure scores. Additionally, Kiviharju et al. [29] found that local anesthesia decreased the pain sensation. In the present study, we found that intracervical lidocaine injection reduced pain during colposcopic biopsy.

Colposcopists speculate that the most suitable colposcopic assessment and biopsy method is the one that involves administration of analgesics and tenaculum use (group 3 in this study). This method can aid in reducing pain, and the physician can manipulate the cervix and obtain biopsy specimens easily during the procedure. Invasive cancer can be diagnosed with a single biopsy. However, generally, biopsy instruments are not sufficiently large to diagnose invasive cancer. In addition, further studies with more participants should be conducted to investigate whether the size of the biopsy specimen has an influence.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

All the procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patient consent

Informed consent was obtained from all the study participants.

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Obstetrics & Gynecology Science

Intravaginal isonicotinic acid hydrazide (INH) versus misoprostol for cervical ripening prior to hysteroscopy

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Objective
The aim of the present study was to compare the efficacy of vaginal isoniazid (isonicotinic acid hydrazide [INH]) and vaginal misoprostol in cervical ripening before hysteroscopic surgery.

Methods
This randomized controlled trial included patients scheduled for hysteroscopic surgery during April 2016 and June 2017. The inclusion criteria were as follows: postmenopausal women or those at premenopausal age who had not had a vaginal delivery and candidate for diagnostic or operative hysteroscopy with closed cervix before intervention. The INH group (intervention group) received 900 mg of vaginal isoniazid (three 300-mg pills) 6–8 hours before hysteroscopic surgery. The misoprostol group (control group) received 400 micrograms of vaginal misoprostol 6–8 hours before hysteroscopic surgery. Finally, the efficacy of the 2 agents was comparatively analyzed.

Results
Baseline characteristics were comparable between the groups. In 67 cases in the INH group (95%) and 45 in the misoprostol group (50%), hysteroscopic entry was successful without additional mechanical dilation, and this difference was statistically significant (*P*=0.001). The odds ratio (OR) obtained in this study was 0.57 for both INH and misoprostol groups (OR, 0.57; 95% confidence interval, 0.43–0.75). Further, 19 cases in the INH group vs. 45 cases in the misoprostol group did not respond to the intervention, indicating statistically significance (*P*=0.001).

Conclusion
Vaginal INH is more effective than misoprostol in cervical ripening before hysteroscopic surgery and can be a good alternative to misoprostol.

Trial Registration
Iranian Registry Clinical Trial (IRCT) Identifier: IRCT2015112821506N4

Keywords: Isonicotinic acid hydrazide; Misoprostol; Cervical ripening

Introduction

Since the advent of hysteroscopic surgery, anomalies of the uterine cavity such as endometrial polyps, sub-mucosal myomas, adhesions, and uterine septums are being effectively treated [1]. Diagnostic hysteroscopy is a standard method of diagnosing intrauterine lesions, which it makes possible to carry out biopsy and intraoperative lesion removal. The sensitivity of this method has been reported to range between 84% and 97%, and its specificity is between 88% and 93% [2-4]. Furthermore, hysteroscopic surgery reduces the need

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for major and unnecessary surgeries [5].

One of the major problems of hysteroscopic surgery is the difficulty in inserting a hysteroscope (especially a resectoscope) into the cervical canal [5]. Nearly half of the complications of hysteroscopic surgeries occur during entry into the cervix, such as cervical rupture, false path, and perforation of the uterus [6].

In the past, the role of laminaria and prostaglandins of the E-series in cervical dilatation and reducing intraoperative complications of cervical dilatation have been proven [7]. Misoprostol is a synthetic analogue of prostaglandin E1 used to treat and prevent gastric ulcers, which is now widely used in obstetrics and gynecology. Its applications in obstetrics and gynecology include medical abortion in the first and second trimesters of pregnancy, preparation of the cervix before vacuum aspiration or dilatation and curettage, labor induction, and prevention and treatment of postpartum hemorrhage [8-10]. Although misoprostol was manufactured for oral administration, numerous pharmacokinetic studies have shown that its active metabolite concentration is retained for longer in vaginal administration than oral [11]. The advantage of misoprostol to other prostaglandin analogues is that it is cheap, stable at room temperature, and available in the form of oral pills [4,12]. However, in our country, misoprostol is not always available due to its abortifacient action, which makes it the only agent for illegal medical abortion, prohibited by the government of Iran. Besides, its administration is restricted to inpatients. Therefore, finding an effective agent that can be used in the outpatient setting is seen as a priority in our country.

Isonicotinic acid hydrazide (INH) is an anti-tuberculosis agent, recently introduced for cervical ripening. It has been studied in term pregnancies, where it was as effective as misoprostol [13]. There is some evidence that the effect of INH on cervical ripening may be partially due to nitric oxide (NO) production in the cervix. It has previously been shown that injection of INH causes a significant increase in the NO levels in the red blood cells (RBCs) of rats, and it has been suggested that NO plays an important role in the pathogenesis of INH-induced oxidative stress in RBCs [14].

As the knowledge and data on the efficacy of this new agent on cervical ripening is still limited, especially regarding some adverse effects such as abdominal cramps, nausea, and vomiting, and because of the feasibility of using INH in the outpatient setting, we conducted the present study to assess the effect of vaginal INH on cervical ripening before hysteroscopic surgery and to compare the efficacy of this agent with that of misoprostol.

Materials and methods

This study was a prospective randomized controlled trial that included patients with intrauterine pathology referred to Hazrat e Rasoul e Akram Hospital, Tehran, who were candidates for hysteroscopic surgery between April 2016 and June 2017. Their medical, gynecologic, and obstetric histories were obtained one day before surgery.

The inclusion criteria were as follows: postmenopausal women or those at premenopausal age who had not had a vaginal delivery and candidate for diagnostic or operative hysteroscopy with closed internal os before intervention (no entrance of Hegar size 2). Patients were excluded if contraindications for receiving prostaglandins, such as severe asthma, glaucoma, heart disease, hypertension, or renal failure were present; severe utero-vaginal prolapse that interfered with the insertion of pills in the posterior vaginal fornix; history of cervical surgery or cervical insufficiency; simultaneous laparoscopy; and cervical myomas.

The participants were randomly assigned to 2 groups by means of computer-generated randomization, and sealed opaque medication packets were used consecutively. In order to prevent information bias, the patients and the surgeons who performed the hysteroscopic procedure were blinded to the treatment and the intervention.

The misoprostol group (n=90) received 400 micrograms of vaginal misoprostol (two 200-mg pills) (Cytotec, Searle Pharmaceuticals, Sutton, UK) 6–8 hours before hysteroscopy. The INH group (n=86) received 900 mg of vaginal INH (three 300-mg pills) (Daru Pakhsh, Tehran, Iran) 6–8 hours before hysteroscopy. The tablets were administered by one of the research team members after complete vaginal examination. Prior to general anesthesia, the patients were asked about adverse drug reactions such as nausea and vomiting, vaginal bleeding, diarrhea, and uterine cramps, and a questionnaire was filled out by the surgeon.

The surgeries were carried out on all the patients by one surgeon. The hysteroscopic surgeries were performed using a BETTOCCHI Hysteroscope (5 mm) based on a 2.9-mm rod-lens telescope with a 30-degree lens angle from KARL STORZ

(Tuttlingen, Germany). The primary outcome measure was hysteroscope entry with no force or mechanical dilation. Interventions were considered as no-response if we needed to perform mechanical dilation before hysteroscope entry. Secondary outcomes were as follows: non-responsive patients, maximum Hegar size in non-responsive patients, intrauterine pathology, side effects of cervical dilatation (bleeding, false path, uterine perforation, and cervical rupture), and adverse drug reactions before surgery. The outcome measures were compared between the misoprostol and INH groups using SPSS statistical tests.

1. Main outcome

The main outcome in this study was hysteroscope entry with no force or mechanical dilation.

2. Sample size

The sample size for this study was calculated according to previous studies [15]. The statistical power and significance level were 80% and 5%, respectively. Although 52 participants in each group was the computed result, to increase the power, 176 patients (90 patients in the misoprostol group and 86 in INH group) were enrolled in the study (Fig. 1) [16-19].

3. Statistical analysis

Statistical analysis was performed using SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). Data were presented as the mean±standard deviations for quantitative variables and compared using the *t*-test. Independent nominal data were analyzed using the χ^2 test and were reported as percentages. Furthermore, logistic regression was used to obtain the odds ratio (OR) and hazard prediction. The significance level in this study was set at $P<0.05$.

Results

The age, height, and weight of the women in the INH group and misoprostol group were comparable (38.59±10.57 vs. 40.42±10.81 years, 160.62±5.91 vs. 161.97±7.08 cm and 72.1±14.52 vs. 72.73±8.22 kg) ($P>0.05$). The body mass index of the INH group was approximately equal to that of the misoprostol group (27.79±3.29 vs. 27.98±5.58). Gravidity of the women in the INH and misoprostol groups was 1.39±0.49 and 1.27±0.44, respectively; the difference was not statistically significant ($P=0.087$) (Table 1).

Table 1 shows the comparison of the qualitative variables

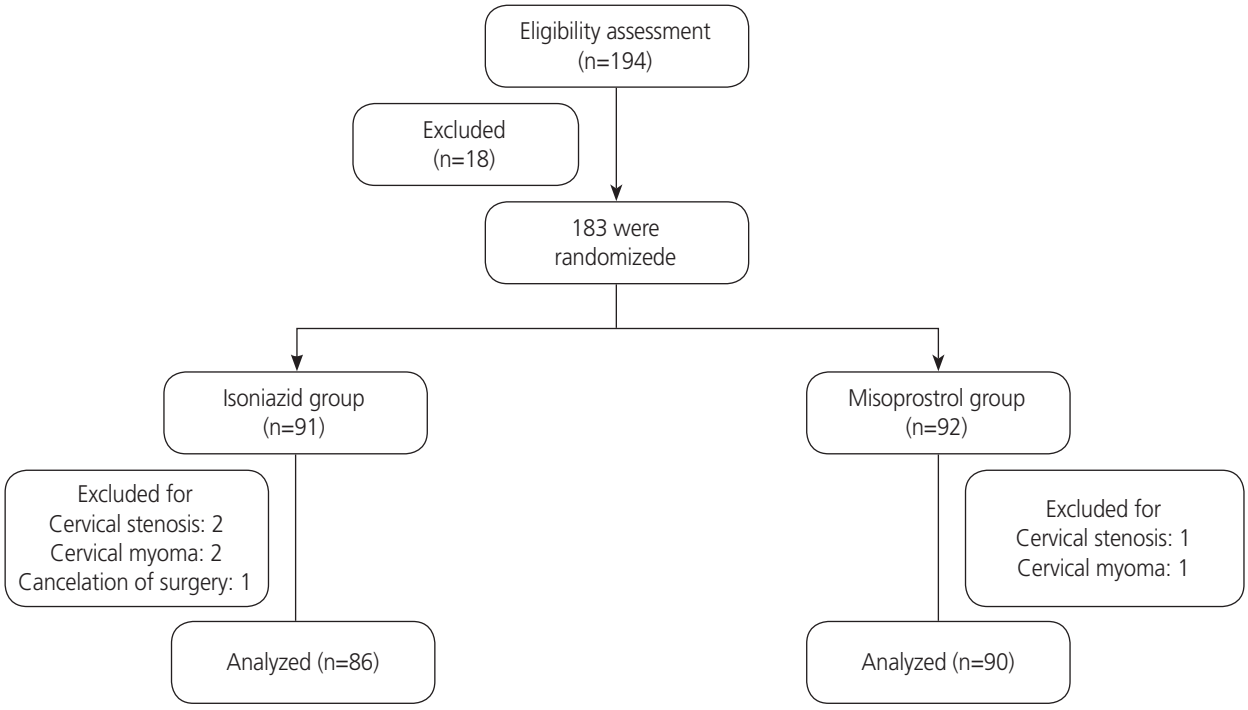

Fig. 1. CONSORT diagram of the trial.

between the misoprostol and INH groups. As can be seen, in 45 patients in the misoprostol group (50%) and 67 in the INH group (95%), hysteroscope entry was successful without additional mechanical dilation, and this difference was statistically significant (*P*=0.001) (Table 2).

The OR obtained in this study was 0.57 for both misoprostol and INH groups, indicating that the likelihood of hysteroscope entry without additional mechanical dilation was 43% lower in the misoprostol group than in the INH group (OR, 0.57; 95% confidence interval [CI], 0.43–0.75) (Table 2 and Fig. 2).

Further, the OR of non-response in the misoprostol group was 1.75 times that in the INH recipients, meaning 75% higher (OR, 1.75; 95% CI, 1.32–2.30) (Table 2). Comparison of intrauterine pathology between the 2 groups showed that the numbers of women with Asherman's syndrome in the misoprostol group and INH group were 4 (4.4%) and 0 (0%), respectively, while 12 women (13.3%) in the misoprostol group and 19 (22.1%) in the INH group had no notable intrauterine pathology, and this difference was statistically significant (*P*=0.001).

We did not encounter any adverse drug reactions in either group. However, we had one case of false path in INH group and one case of uterine perforation during hysteroscope entry in a very anteverted uterus in the misoprostol group; both were diagnosed and managed properly.

Discussion

The results of the present study show that INH is more effective than misoprostol in cervical ripening before hysteroscopic surgery. The aim of this randomized clinical trial was to compare the efficacy of vaginal misoprostol and INH on cervical ripening before hysteroscopy. There have been numerous studies on the effect of misoprostol on cervical preparation before hysteroscopy, but none have compared it with INH.

For example, in a study by Batukan et al. [11], the effect of

Table 1. Basic demographic factors in 2 groups

Variables	INH (n=86)	Misoprostol (n=90)	*P*-value	OR (95% CI) (misoprostol/INH)
Age	38.59±10.57	40.42±10.81	0.258	-
BMI	27.98±5.58	27.79±3.29	0.647	-
Gravidity	1.27±0.44	1.39±0.49	0.087	-
Parity				
No delivery	6 (31.6)	10 (22.2)	0.786	1.00
1 delivery	3 (15.8)	13 (28.9)	0.143	0.53 (0.23–1.23)
2 delivery	7 (36.8)	9 (20.0)	0.582	0.78 (0.33–1.83)
3 and more delivery	3 (15.8)	13 (28.9)	0.195	0.54 (0.22–1.27)
Menshis				
Regular	25 (29.1)	20 (22.2)	0.251	1.00
Menorrhagia	3 (3.5)	5 (5.6)	0.288	0.48 (0.10–2.25)
Metrorrhagia	19 (22.1)	22 (24.4)	0.262	0.69 (0.29–1.61)
Menometrorrhagia	14 (16.3)	20 (22.2)	0.150	0.56 (0.22–1.37)
Oligomenorrhea	3 (3.5)	1 (1.1)	0.421	2.40 (0.23–24.87)
Amenorrhea	1 (1.2)	1 (1.1)	0.699	0.80 (0.04–13.06)
Irregular	21 (24.4)	21 (23.3)	0.381	0.80 (0.34–1.86)
Menopause				
Yes	12 (14)	8 (8.9)	-	1.00
No	74 (86)	82 (91.1)	0.613	1.05 (0.94–1.17)

Values are presented as mean±standard deviation or number (%).
BMI, body mass index; CI, confidence interval; OR, odds ratio; INH, isonicotinic acid hydrazide.

Table 2. Outcome variables in 2 groups and predict of variables by logistic regression

Variables	INH (n=86)	Misoprostol (n=90)	*P*-value	OR (95% CI) (misoprostol/INH)
Primary outcomes				
Hysteroscopy entrance				
Yes	67 (77.9)	45 (50.0)	1.000	1.00
No	19 (22.1)	45 (50.0)	0.0001	0.57 (0.43–0.75)
Secondary outcomes				
Non responses				
Yes	19 (22.1)	45 (50.0)	1.000	1.00
No	67 (77.9)	45 (50.0)	0.0001	1.75 (1.32–2.30)
Intra uterine pathology				
Normal	19 (22.1)	12 (13.3)	-	1.00
Polyp	31 (36.0)	42 (46.7)	0.090	0.71 (0.26–1.95)
Myoma	17 (19.8)	15 (16.7)	0.513	0.75 (0.56–0.99)
Asherman's syndrome	0 (0)	4 (4.4)	0.035	0.94 (0.22–0.99)
Septum	6 (7.0)	4 (4.4)	0.612	0.63 (0.07–5.10)
Atrophy	2 (2.0)	2 (2.2)	0.530	0.90 (0.27–3.01)
Focal hyperplasia	10 (11.6)	7 (7.8)	0.554	1.05 (0.95–1.16)
Product of conception	1 (1.2)	4 (4.4)	0.035	0.75 (0.56–0.99)
Max Hegar size in nonresponses	3.73±1.28	3.84±1.04	0.726	-

Values are presented as number (%) or mean±standard deviation.
CI, confidence interval; OR, odds ratio; INH, isonicotinic acid hydrazide.

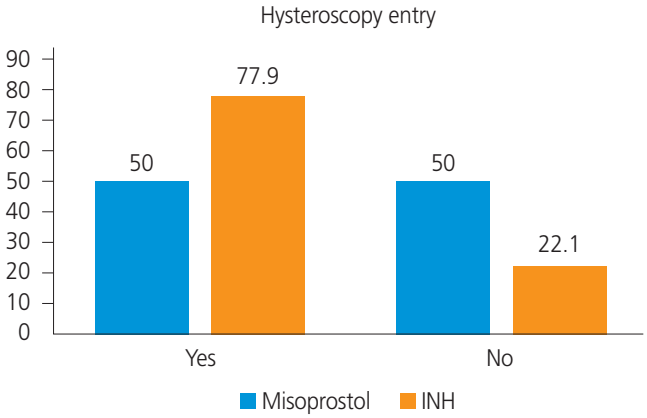

Fig. 2. The compare of frequency of hysteroscopy entry without mechanical dilation in 2 group. INH, isonicotinic acid hydrazide.

vaginal and oral misoprostol on cervical preparation before hysteroscopy was evaluated, and the results showed that oral administration of misoprostol was preferable to vaginal administration.

In the present study, the hysteroscope entered the uterus with no additional mechanical dilatation in 50% and 77.9% of the women in the misoprostol group and INH group, respectively. Moreover, the likelihood of hysteroscope entry was 43% lower in the misoprostol group than in the INH group. On the other hand, the OR of non-response in the misoprostol group was 75% higher than that in the INH group. The ORs show that the incidence of Asherman's syndrome in the misoprostol group was 0.94 times that in the vaginal INH group.

INH is a recently introduced agent for cervical ripening. The results of a study, conducted by Haghighi and Mohabatian [13] showed that vaginal INH is an effective agent for cervical ripening prior to labor induction in term pregnancies. The mechanism of action of INH on cervical dilatation is similar to that of NO donors. The results of some studies showed that INH affects cervical dilatation via production of NO [14]. El-Khayat et al. [20] showed that primary cervical dilatation and the duration of dilatation were higher with vaginal misoprostol than with Foley catheter plus vaginal isosorbide mononitrate, but there was no significant difference between the

2 groups in terms of the duration of surgery or difficult in dilatation. The lack of a statistically significant difference in their study might be due to different sample sizes or methods from those used in the present study. On the other hand, Waddell et al. [21] found that the use of vaginal misoprostol before hysteroscopy reduced the pain during and pressure required for cervical dilatation.

The results of numerous studies on the effect of misoprostol on cervical dilatation have shown that using vaginal misoprostol had a more pronounced effect on cervical dilatation than its sublingual form. Bisharah et al. [22] randomly assigned 40 women to an intervention group (100 mg of sublingual misoprostol) and a placebo group and found no significant difference between the 2 groups in terms of uterine dilatation. The results of a study by Mulayim et al. [23] showed that cervical dilatation time was longer in the control (placebo) group than in the intervention (sublingual misoprostol use) group; thus, cervical tearing was greater in the control group. In a study conducted by Preutthipan and Herabutya [24], vaginal misoprostol was found to reduce dilatation time in premenopausal women. Another study by Sordia-Hernández et al. [25] showed that the time of hysteroscopic surgery in the vaginal misoprostol group was significantly shorter than that in the placebo and sublingual misoprostol groups.

The strength of this study is that it compares the effect of vaginal misoprostol with that of other vaginal medications. In many studies, vaginal misoprostol groups have been compared with placebo groups or rectal, oral, or sublingual misoprostol administration. This method made it possible to compare the effect of 2 different drugs on vaginal dilatation.

A study conducted by Inal et al. [26] showed that dinoprostone had a greater effect on cervical dilatation than misoprostol. In the present study, the OR obtained for hysteroscopic entrance in the misoprostol and INH groups was 0.57 (0.43–0.75), which indicated that the likelihood of hysteroscope entry into the uterus in the misoprostol group was 43% lower than that of the INH group. This means that cervical dilatation in the women receiving misoprostol was less than in the INH recipients.

INH can be simply used as a ripening agent before hysteroscopy. It is easily available and does not seem to have any complications. However, its underlying mechanism of cervical ripening is unknown. Our study was limited by its small sample size. Further research is needed to determine the mechanisms of action and clinical efficacy of INH against that of other ripening agents to find the best dosage and timing of INH for ripening of cervix before hysteroscopy.

In conclusion, vaginal INH seems to be more effective than misoprostol in cervical ripening before hysteroscopic surgery and can be a good alternative to misoprostol.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This paper is derived from a medical doctorate thesis of Iran Medical Sciences University.

Ethical approval

The study was approved by the Local Ethics Committee of Iran University of Medical Sciences, Tehran, Iran and registered in the Iranian Registry of Clinical Trials (IRCT.ir); ID number: IRCT2015112821506N4.

Patient consent

In this study, informed consent was received from all the participants and their partners.

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Original Article

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Obstetrics & Gynecology Science

Serum vitamin D levels and ovarian reserve markers in secondary amenorrhea patients: Is there a link?

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Objective
To investigate whether serum 25-hydroxyvitamin D [25(OH)D] level is associated with ovarian reserve markers in secondary amenorrhea (SA) patients.

Methods
Sixty-three women diagnosed with SA were recruited during 12 months from the initiation of this prospective observational study. Serum 25(OH)D levels, serum anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) were estimated in study participants and ovarian reserve markers were compared between participants with vitamin D deficiency and those with normal vitamin D levels.

Results
Of the 63 participants, 27 (42.9%) were vitamin D deficient (<20 ng/mL) and 36 (57.1%) had normal vitamin D levels. The mean AMH levels and AFC were 10.86±8.94 μ/L and 15.23±7.65 in the vitamin D deficient group, and 7.24±5.62 μ/L and 12.30±6.95 in the normal vitamin D group. Univariate and multivariate linear regression analysis of $\log_{10}$ transformed AMH and AFC with serum 25(OH)D adjusted for age and body mass index confirmed no association between vitamin D levels and AMH levels or AFC. There was also no correlation between serum 25(OH)D and AMH levels or AFC in all participants. However, participants with vitamin D deficiency had an increased chance of having polycystic ovarian syndrome (PCOS) as cause of SA than those with normal vitamin D levels (adjusted odds ratio, 7.559; 95% confidence interval, 1.28–44.65; *P*=0.026) after adjustment for clinical factors by logistic regression model.

Conclusion
There was no correlation between serum 25(OH)D levels and ovarian reserve markers in SA patients, but vitamin D deficiency may be linked to PCOS patients.

Keywords: Anti-Mullerian hormone; Amenorrhea; Ovarian reserve; 25-hydroxyvitamin D; Polycystic ovarian syndrome

Introduction

Vitamin D is known to have a major influence on bone health through regulation of calcium phosphorus homeostasis. Recently, the female reproductive system has been defined as one of the non-classical target organs of vitamin D. Vitamin D receptor expression was identified in ovarian granulosa cells as well as other female reproductive organs, including endometrium and the uterus. These findings suggest that vitamin D may have a potential role in female reproduction [1-3]. Several studies have demonstrated a direct effect of vitamin D on ovarian folliculogenesis and steroidogenesis in animal and human cell-line studies; vitamin D receptor null mutant mice have impaired folliculogenesis and vitamin D

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stimulated steroidogenesis in human ovarian cells [4,5].

Anti-Müllerian hormone (AMH) is produced by granulosa cells of small antral and pre-antral follicles, and along with antral follicle counts (AFCs), is a well-known representative marker for ovarian reserve. AMH plays a crucial role in folliculogenesis and is not affected by the menstrual cycle, making it the most widely used ovarian marker. Although it is still unclear how vitamin D affects female reproductive function, interest in a potential relationship between vitamin D and ovarian reserve markers, particularly AMH, has increased since reports on the effect of vitamin D on follicular development have accumulated. Moreover, the presence of a functional vitamin D response element in the AMH gene promotor region was demonstrated [6] and experimental results in animal and human granulosa cells have also reported that vitamin D affects AMH signaling [7,8]. These basic research studies have indicated that vitamin D deficiency may alter gonadal function through abnormal AMH signaling. However, discrepancy exists in clinical study findings under various settings, where some suggest a significant association between vitamin D levels and ovarian reserve, while others have not found any correlation between vitamin D and ovarian reserve markers [9,10]. Considering these contradictory results, we performed this prospective observational study to examine serum 25-hydroxyvitamin D [25(OH)D] levels in patients with secondary amenorrhea (SA) and investigate the relationship between serum 25(OH)D levels and ovarian reserve markers (i.e., serum AMH and AFC) in these women.

Materials and methods

1. Study design and participants

This study was conducted as a prospective cohort study for 12 months from March 2018 to February 2019. The study population was comprised of patients evaluated by a single reproductive endocrinologist for symptoms of SA. A total of 78 participants were initially recruited to the study. Three participants refused to participate in the study and 12 participants were excluded due to the study's exclusion criteria. The exclusion criteria of this study were as follows: 1) women who had taken hormonal medication including oral contraceptives or vitamin D supplements within the previous 6 months; 2) women who had ovarian surgery, chemotherapy or radiotherapy; 3) women who took medication and/or were diagnosed with a systemic disease that can affect menstruation (e.g., psychoaffective medicine, thyroid hormone, diabetes, hyperprolactinemia); 4) women who refused to participate this study. Patients' information including age, parity, height, body weight, cause of SA, season of blood sampling (i.e., to account for seasonal changes in vitamin D), current medications and other past medical history were recorded. Gynecologic ultrasonography was also performed on their first study visit. To determine cause of SA, polycystic ovarian syndrome (PCOS) was diagnosed on the basis of Rotterdam criteria [11], and primary ovarian insufficiency (POI) was regarded as 2 recordings of serum follicular stimulating hormone (FSH) levels of more than 40 IU/L at least one month apart in a woman aged under 40 years (i.e., >2 standard deviation [SD] less than the mean menopausal age) [12]. All patients whose etiology of SA was not determined, excluding women with POI and PCOS, were deemed unexplained chronic anovulation [13]. PCOS in adolescents was only diagnosed in women with at least 2 years after menarche and who had SA, hyperandrogenemia, and increased ovarian volume on ultrasound [14].

Venous blood samples of all participants were taken for the measurement of serum 25(OH)D and AMH on the same day, prior to treatment for SA. Vitamin D deficiency was defined as serum 25(OH)D levels <20 ng/mL based on the Endocrine Society clinical practice guidelines [15].

2. Serum vitamin D, hormones and ovarian reserve markers measurements

1) Serum 25-hydroxyvitamin D

Each participant's venous sample was drawn into a serum separation tube (SST) and serum 25(OH)D was measured using a DIA source 25 OH Vitamin D3 radioimmunoassay kit (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) with immunoassay device (gamma 5) and presented in ng/mL. The total imprecision coefficient of variance was 5.38% at a concentration level of 6.35 ng/mL and 4.96% at 37.71 ng/mL.

2) Serum follicular stimulating hormone

Each participant's venous sample was drawn into an SST and serum FSH was measured using an Elecsys FSH electrochemiluminescence immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany) with immunoassay device (Cobas e 801) and presented in mIU/mL. The total imprecision coef-

ficient of variance was 3.1% at a concentration level of 48.8 mIU/mL and 2.3% at 17.4 mIU/mL.

3) Anti-Müllerian hormone and antral follicle counts

Blood drawn into the SST was centrifuged within 1 hour (3,000 rpm for 10 minutes). The separated serum was measured for AMH level by ECLIA method with Elecsys AMH kit (Roche Diagnostics GmbH) on a Cobas e 601 immunoassay analyzer and presented in ng/mL. The total imprecision coefficient of variance was 3.5% at a concentration level of 0.042 ng/mL and 3.4% at 0.20 ng/mL. An AFC was measured by one gynecologist on the participant's first visit in all individuals with gynecologic ultrasonography. AFC represented the total number of all antral follicles, from 2 mm to 10 mm in size, in both ovaries.

3. Statistical analysis

Categorical variable data are presented as a frequency with percentage and continuous variables are presented as a group mean±SD. Differences in study participants' characteristics were compared across subgroups with a χ^2 test or Fisher's exact test for categorical variables and independent *t*-test or Mann-Whitney's *U* test for continuous variables, as appropriate. An analysis of variance with Duncan's *post hoc* test or Kruskal-Wallis test with Dunn's *post hoc* test were also employed, as appropriate. To check if data were normally distributed, we used a Shapiro-Wilk test. Partial correlation coefficients controlling for age and body mass index (BMI) were estimated to investigate the linear relationship between 2 continuous variables. Univariate linear regression analysis between each variable (age, BMI, vitamin D, season), and multivariate linear regression analysis with all covariates, were performed to identify the regression coefficients for factors related to the $\log_{10}$ AMH, AFC, PCOS and POI. For data visualization, box plot and scatter plot were also displayed. Finally, logistic regression analysis was performed to identify whether vitamin D deficiency is associated with PCOS as a cause of SA. All statistical analyses were carried out using SPSS 24.0 (SPSS Statistics for Windows 24.0; IBM Corp., Armonk, NY, USA) statistical software and *P*-values less than 0.05 was considered statistically significant.

Table 1. Patients' demographic variables

Variable	Vitamin D (ng/mL)		*P*-value
	<20	≥20	
Age (yr)	22.54±5.33	27.81±8.65	0.014[a]
BMI (kg/m^2)	22.85±5.17	22.56±4.39	0.823[a]
Amenorrhea cause			
PCOS	21 (80.8)	19 (51.4)	0.454[b]
Unexplained chronic anovulation	2 (7.7)	12 (32.4)	
POI	3 (11.5)	6 (16.2)	
AFC	15.23±7.65	12.30±6.95	0.119[c]
AMH	10.86±8.94	7.24±5.62	0.130[a]
FSH	6.30±4.28	8.25±9.39	0.392[a]
Season of blood sample			0.128[b]
Autumn	6 (23.1)	2 (5.4)	
Spring	9 (34.6)	11 (29.7)	
Summer	6 (23.1)	16 (43.2)	
Winter	5 (19.2)	8 (21.6)	

Values are presented as mean±standard deviation or number (%).
BMI, body mass index; PCOS, polycystic ovarian syndrome; POI, primary ovarian insufficiency; AFC, antral follicle count; AMH, anti-Müllerian hormone; FSH, follicular stimulating hormone.
[a]*P*-values were derived from Mann-Whitney *U* test; [b]*P*-values were derived from Fisher's exact test; [c]*P*-values were derived from independent *t*-test.

Results

Sixty-three participants with SA were finally included in this study. The mean age of participants was 26.11±8.05 years and the mean BMI was 22.75±4.75 kg/m^2. Participants' baseline and biochemical characteristics of patients are shown in Table 1. Of all participants, 42.9% (n=27) were vitamin D deficient (<20 ng/mL) and 57.1% (n=36) had normal vitamin D levels (≥20 ng/mL). The mean AMH levels and AFC were 10.86±8.94 μ/L and 15.23±7.65 in the vitamin D deficient group, and 7.24±5.62 μ/L and 12.30±6.95 in the normal vitamin D group, respectively. The difference in BMI, cause of amenorrhea, season of blood sample and FSH levels between the 2 groups did not reach statistical significance, but age was significantly higher in the normal vitamin D group.

There was no correlation between serum vitamin D and AMH levels (r=–0.142, *P*=0.279) or AFC (r=–0.101, *P*=0.433) in all participants (Fig. 1). After univariate linear regression analysis of $\log_{10}$ transformed AMH or AFC with all covariates, multivariate linear regression analysis of $\log_{10}$ transformed AMH or AFC with vitamin D, age and BMI was performed with adjustment for confounding variables. While there was a significant association between serum AMH levels and age, there were no significant relationships between serum AMH levels or AFC and serum vitamin D levels: regression coefficient for vitamin D predicting $\log_{10}$ AMH: –0.002, standard error (SE): 0.004, *P*=0.557 (Table 2), regression coefficient for vitamin D predicting AFC: –0.042, SE: 0.040, *P*=0.304 (Table 3). In the univariate and multivariate linear regression of PCOS modelled with vitamin D levels, there was also no significant association between PCOS and serum vitamin D

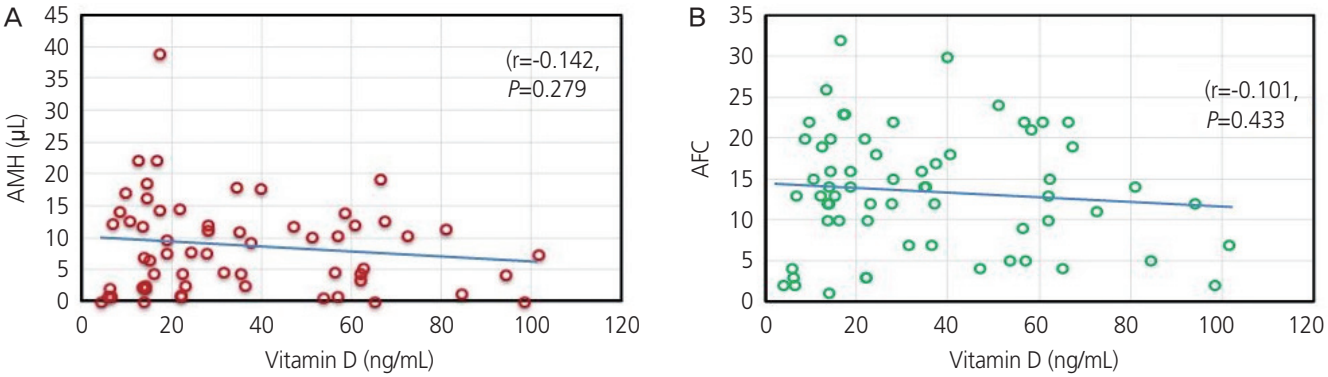

Fig. 1. No correlation between serum vitamin D and anti-Müllerian hormone (AMH) levels (A) or antral follicle counts (AFCs) (B).

Table 2. Regression coefficients in univariate and multivariate linear regression analysis between all covariates and serum anti-Müllerian hormone (AMH)

Covariates	Univariate			Multivariate		
	Estimate±SE	t	*P*-value	Estimate±SE	t	*P*-value
Vitamin D	–0.007±0.004	–1.962	0.055	–0.002±0.004	–0.591	0.557
Age (yr)	–0.031±0.011	–2.867	0.006	–0.034±0.013	–2.541	0.014
BMI (kg/m^2)	0.008±0.021	0.370	0.713	0.020±0.022	0.937	0.353
Season of blood sample						
Winter	-	-				
Spring	–0.177±0.247	–0.715	0.478			
Summer	0.301±0.237	1.272	0.208			
Autumn	–0.310±0.309	–1.002	0.320			

Dependent variable: $\log_{10}$ AMH.
SE, standard error; BMI, body mass index.

levels (Table 4). The results of univariate and multivariate linear regression of POI modelled with vitamin D levels was also similar: regression coefficient±SE: 0.001±0.002, *P*=0.641, –0.001±0.002, *P*=0.557, respectively). However, serum vitamin D levels for each SA cause group demonstrated lower tendency for vitamin D levels in the PCOS or POI groups than in the unexplained chronic anovulation group (31.07±22.05, 31.37±31.89, 48.92±27.69, respectively, *P*=0.056) (Fig. 2). In the logistic regression analysis, women with vitamin D deficiency had an increased chance of having PCOS as cause of SA than those with normal vitamin D levels (adjusted odds ratio [OR], 7.559; 95% confidence interval, 1.28–44.65; *P*=0.026) after adjusting for BMI, age and seasonal variation of vitamin D.

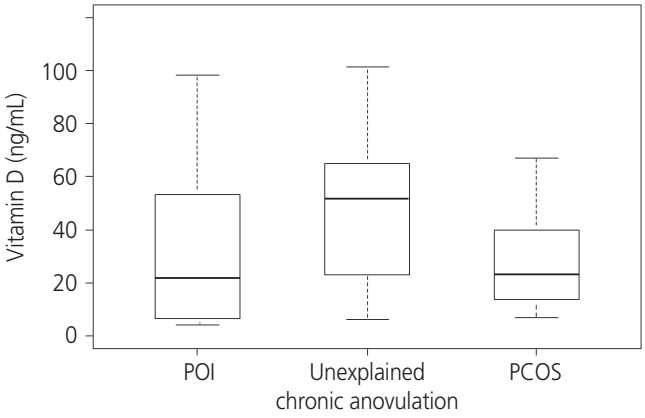

Fig. 2. Comparison of serum vitamin D levels among primary ovarian insufficiency (POI) group, unexplained chronic anovulation group and polycystic ovarian syndrome (PCOS) group.

Table 3. Regression coefficients in univariate and multivariate linear regression analysis between all covariates and antral follicle counts (AFCs)

Covariates	Univariate			Multivariate		
	Estimate±SE	t	*P*-value	Estimate±SE	t	*P*-value
Vitamin D	–0.066±0.036	–1.836	0.071	–0.042±0.040	–1.038	0.304
Age (yr)	–0.188±0.110	–1.720	0.090	–0.178±0.137	–1.294	0.201
BMI (kg/m^2)	0.121±0.189	0.636	0.527	0.191±0.207	0.920	0.361
Season of blood sample						
Winter						
Spring	1.400±2.394	0.585	0.561			
Summer	4.374±2.327	1.880	0.065			
Autumn	–2.300±3.069	–0.749	0.456			

Dependent variable: AFCs.
SE, standard error; BMI, body mass index.

Table 4. Regression coefficients in univariate and multivariate linear regression analysis between all covariates and polycystic ovarian syndrome (PCOS)

Covariates	Univariate			Multivariate		
	Estimate±SE	t	*P*-value	Estimate±SE	t	*P*-value
Vitamin D	–0.006±0.002	–2.521	0.014	–0.005±0.003	–1.815	0.075
Age (yr)	–0.011±0.007	–1.425	0.159	–0.008±0.009	–0.916	0.363
BMI (kg/m^2)	0.015±0.013	1.158	0.251	0.017±0.013	1.228	0.224
Season of blood sample						
Winter	-					
Spring	0.067±0.165	0.404	0.687			
Summer	0.249±0.160	1.556	0.125			
Autumn	–0.033±0.211	–0.158	0.875			

Dependent variable: PCOS.
SE, standard error; BMI, body mass index.

Discussion

Our study demonstrated that serum 25(OH)D levels were not related with ovarian reserve markers among SA patients, even after adjustment for age, BMI, cause of amenorrhea, and seasonal variation of vitamin D. However, mean serum vitamin D levels were different depending on the cause of amenorrhea, however the difference did not reach statistical significance (Fig. 2). In particular, vitamin D levels in the participants with PCOS or POI demonstrated a tendency towards lower serum vitamin D levels than those in participants with unexplained chronic anovulation. In addition, women with vitamin D deficiency had an increased chance of having PCOS than those with normal vitamin D levels, after adjustment for clinical factors by logistic regression model. We did not calculate the adjusted OR for POI since participants with POI were a very small portion of the study participants. The lack of correlation between serum vitamin D levels and ovarian reserve markers across all participants in our study is in agreement with several recent studies that also demonstrated no correlation. However, the result of lower vitamin D levels in participants with PCOS in this study needs to be taken with caution, considering the heterogeneous results of previous studies [16,17]. To date, the role of low vitamin D levels on AMH regulation or the pathophysiology of PCOS is unclear, with some previous reports suggesting that this vitamin D deficiency is likely due to obesity or a metabolic syndrome that is frequently associated with PCOS [18,19].

While basic research has reported that vitamin D affects ovarian folliculogenesis and steroidogenesis, there is limited clinical information on how vitamin D affects ovarian markers such as AMH. Wojtusik et al. [9] have reported a dose-dependent decrease in AMH mRNA levels in granulosa cells after treatment with vitamin D. In another recent study, human granulosa cells treated with vitamin D exhibited altered AMH signaling and they demonstrated an inverse correlation between vitamin D status in follicular fluid and AMH receptor-II (AMHT-II) mRNA gene expression [8]. Binding AMH to AMH receptor-II (AMHR-II) is known to suppress follicular maturation, by inhibiting primordial follicle recruitment into the growing follicle pool, and by decreasing the sensitivity of follicles to FSH [10]. Since vitamin D had an effect on down-regulation of AMHR-II gene expression, the phosphorylation process, and nuclear localization after AMH-AMHR-II binding [8], it may be involved in promoting follicle development by altering AMH production pattern and FSH sensitivity in ovarian granulosa cell [20,21].

Previous studies on the relationship between vitamin D and ovarian reserve markers, particularly AMH, have had inconsistent results. For instance, Merhi et al. [22] found a positive relationship between serum vitamin D and AMH levels and suggested vitamin D deficiency might be associated with lower ovarian reserve in late reproductive aged women. Dennis et al. [23] also suggested that vitamin D might have a positive effect on AMH production in adults, and the extent of seasonal variation in women's AMH levels correlated with the extent of their variation in vitamin D levels. In another study of 1,430 premenopausal women, a negative correlation was found between serum vitamin D levels and urinary FSH levels, suggesting that low vitamin D levels may influence ovarian reserve and earlier menopause [24]. Meanwhile, a recent cross-sectional study that included 283 infertile women, revealed no significant association between vitamin D and ovarian reserve markers (AMH, AFC) [25], which is in agreement with a prospective study that failed to demonstrate any beneficial effect of vitamin D supplementation on normalization of serum AMH levels [26].

In this study, generally healthy women should be included as controls. However, it was difficult to recruit healthy women to volunteer to have their AMH and vitamin D levels measured, so we conducted this study in patients with SA who required AMH measurements for diagnosis. These SA patients included women with various levels of ovarian reserve, and it was assumed that unexplained chronic anovulatory patients were similar to healthy women with normal ovarian reserve. Indeed, participants with unexplained chronic anovulatory in our study demonstrated normal ovarian reserve (data not shown). In addition, cholecalciferol, the main natural source of vitamin D, is affected by sun exposure and outdoor activity, so participants' duration of outdoor activity should also be measured. However, we did not conduct a survey of patients' outdoor activity as subjective factors could have been reflected in this evaluation. Although the validity of AMH measurements in those younger than 20 years of age has not been established, we included participants in this age group and their AMH measurement, as the small number of girls in their late teens in this study were considered to have reached sufficient maturity.

This study is the first report on the relationship between serum vitamin D levels and ovarian reserve in Korean women.

This study was possible because it was conducted in patients with SA, which included women with various levels of ovarian reserves. Furthermore, this study is strengthened by its prospective design, which has a low risk of confounding bias, the fact that both AFC and AMH were measured as a marker of ovarian reserve, and finally AMH was measured on the same day as 25(OH)D.

However, this study has several limitations. Firstly, this study has small study population to then analyze by subgroup(s) of amenorrhea causes, which increases the risk of selection bias or type II error. Secondly, healthy women with normal menstrual cycles, who were not SA patients, were not included as controls. Thirdly, this study was observational in nature, so we cannot interpret these results in terms of causality. Despite of these limitations, this study is the first prospective observational study of the relationship between vitamin D and ovarian reserve in Korean women with SA, suggesting no association between them. In this regard, it is not necessary to routinely measure serum vitamin D levels in SA patients with abnormal ovarian reserve. However, it would be meaningful to provide vitamin D supplementation, after measuring serum vitamin D concentration in PCOS patients, considering the low vitamin D levels in PCOS patients in this study. Nevertheless, given the uncertainty of how vitamin D affects female reproduction and the limitations of this study, no clear conclusion can be drawn. Further study is required on the relationship between vitamin D levels and obesity or metabolic abnormalities in patients with PCOS.

In conclusion, this observational study demonstrated that there was no correlation between serum vitamin D levels and ovarian reserve markers in SA patients, but that vitamin D deficiency may be linked to PCOS patients. However, given the limitations of this observational study, further prospective research with a larger population and age-matched control group should be investigated to draw more definite conclusions on the relationship between vitamin D levels and ovarian reserve markers.

Acknowledgements

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board of Inje University Haeundae Paik Hospital, Busan, Korea (approval number: 2017-01-018).

Patient consent

All participants provided written informed consent.

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Case Report

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Obstetrics & Gynecology Science

Postnatally diagnosed coexisting congenital diaphragmatic hernia with pulmonary sequestration: a report of two cases

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While the associations between pulmonary sequestration (PS) and congenital diaphragmatic hernia (CDH) are known, CDH may be obscured by PS and thus, overlooked on prenatal ultrasonography when coexisting with PS. We present 2 cases of postnatally diagnosed CDH combined with PS. In both cases, PS was prenatally diagnosed as an isolated lung mass, while CDH was confirmed only after birth. Both newborns were sufficiently stable that management was not required immediately after birth. PS may function as an "anatomical barrier" to prevent herniation of the abdominal contents into the chest, thus acting as a "protector" providing normal lung maturation throughout pregnancy. If PS is suspected prenatally, coexisting CDH may be obscured; thus, close prenatal care and counseling of the parents regarding the possibility of CDH are essential. These infants should be delivered at a tertiary center, and imaging should be performed to exclude coexisting CDH.

Keywords: Pulmonary sequestration; Congenital diaphragmatic hernias; Prenatal ultrasonography

Introduction

Pulmonary sequestration (PS) is a nonfunctioning mass of lung parenchyma that lacks communication with the normal tracheobronchial tree. It has an aberrant systemic arterial blood supply, usually from the thoracic or abdominal aorta [1]. Congenital diaphragmatic hernia (CDH) affects 1 in 2,500 newborns and is associated with high rates of morbidity and mortality [2]. While the association of PS with CDH is as high as 30%–40%, the prognosis of their coexistence is unknown [3-5]. We present 2 cases of coexistent PS with CDH. In both cases, only the isolated lung mass was diagnosed prenatally; the coexisting CDH was diagnosed only after birth.

Case report

1. Case 1

A 31-year-old multigravida was referred to our hospital for a fetal lung mass at 27.5 weeks of gestation. On detailed ultrasonography examination, a hyperechoic, homogeneous, well-defined mass measuring 2.5×1.8 cm was found posterior to the right lower lobe of the fetal lung (Fig. 1A). Because no aberrant systemic arterial blood supply was observed, the initial diagnosis was congenital pulmonary airway malformation (CPAM). The fetal right kidney was slightly elevated, raising suspicion of an ectopic kidney (Fig. 1B). Although the diaphragm deviated upward on the right, it was well

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maintained despite the right ectopic kidney. In the follow-up ultrasonography performed at 34.4 weeks of gestation, persistent left superior vena cava (PLSVC) was noted, but there were no changes in the size of the lung mass and no evidence of CDH.

The female neonate was delivered vaginally at 39.5 weeks of gestation and weighing 2,720 g. Apgar scores were 8 at 1 minute and 9 at 5 minutes. During workup in the neonatal intensive care unit (NICU), her vital signs were stable and oxygen saturation was maintained above 95% in room air. However, the right lung aeration was slightly decreased upon physical examination. Chest radiography demonstrated decreased lung volume and diffuse haziness in both lung fields. One day after birth, right CDH was identified on chest computed tomography (CT) conducted due to the upper deviation of the liver and right kidney seen on an abdominal sonogram. The liver, right kidney, and some bowel loops were located in the right lung field (Fig. 1C). Lung hypoplasia at the right lower lobe and PLSVC were observed. There was no evidence of CPAM on the postnatal chest sonogram and CT. The CDH was repaired 3 days after birth. A 5-cm Bochdalek's hernia with a sac was present on the posterior side of the right diaphragm. The herniated organs were anatomically reduced into the abdomen. The pediatric surgeon attempted to determine the lung mass based on the prenatal history even though there was no evidence of lung mass on the chest CT. A 5×4-cm lung mass was observed in the upper right diaphragm and the area adjacent to the right of the aorta, and excision of the mass was performed. The origin of the lung mass was unclear, as was its association with other organs. Histopathological examination showed mature and immature lung tissues, which was consistent with extralobar pulmonary sequestration (ELS; Fig. 1D). Two months after the surgery, the patient had no postoperative complications and had appropriate growth for her age.

2. Case 2

A 29-year-old primigravida was referred to our hospital for

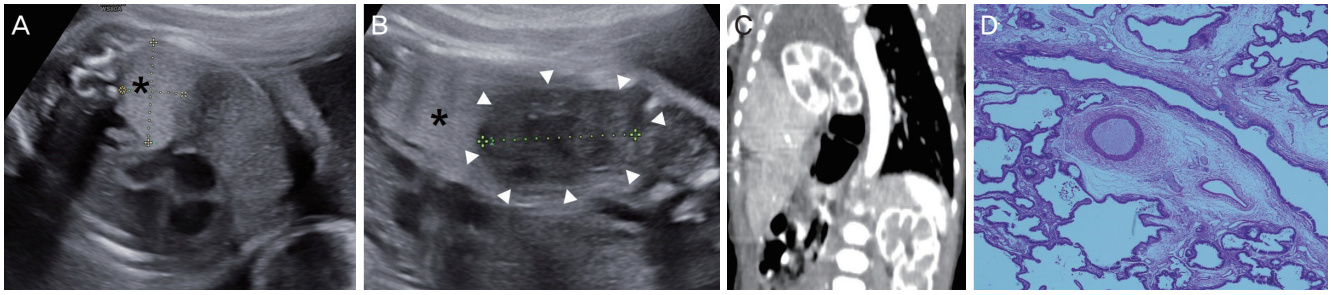

Fig. 1. Prenatal and postnatal findings in Case 1. Prenatal ultrasonographic image showing (A) a hyperechoic mass (asterisk) on the posterior right lower lung on the transverse view and (B) elevated right kidney (arrowheads) beneath the lung mass on the coronal view. (C) Postnatal computed tomography image showing the liver, right kidney, and some bowel loops in the right lung field as well as right lung hypoplasia. (D) Postoperative histopathological findings of the extralobar lung sequestration showing immature pulmonary parenchyma with bronchiole-like structures with pseudocystic dilatation.

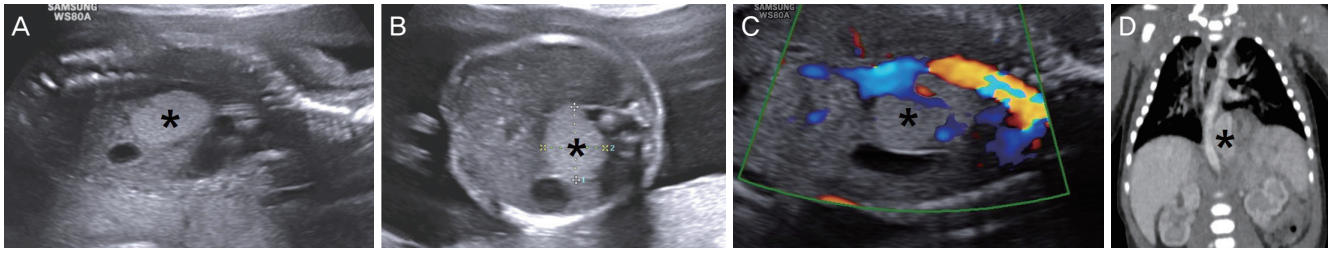

Fig. 2. Prenatal and postnatal findings in Case 2. Prenatal ultrasonographic image showing (A) a hyperechoic mass (asterisk) in the left upper abdominal cavity in the sagittal view and (B) posterior to the stomach in the transverse view. (C) Color Doppler ultrasonographic image showing that the lung mass received blood supply from the descending aorta. (D) Computed tomography image after birth showing the stomach fundus and presumed extralobar lung sequestration positioned below the diaphragm herniated into the left thorax.

a suspected fetal lung mass at 22.2 weeks of gestation. Detailed ultrasonography examination showed a hyperechoic, homogeneous, well-defined mass measuring 2.0×1.8 cm in the left upper abdominal cavity, posterior to the stomach (Fig. 2A and B). The lung mass was thought to be PS, as its blood supply was from the descending aorta (Fig. 2C). Although the stomach was slightly above its normal position, the diaphragm looked intact. On follow-up ultrasonography performed at 35.3 weeks of gestation, the lung mass was unobservable.

The male neonate was delivered through a cesarean section on maternal request at 37.3 weeks of gestation and weighed 2,580 g. Apgar scores were 8 at 1 minute and 9 at 5 minutes. After birth, mild chest retraction was observed; thus, a high-flow nasal cannula (HFNC) was applied. Oxygen saturation was maintained above 95%. Initial chest radiography did not show any abnormal findings. A 2.4×0.5×2.5-cm well-defined, wedge-shaped mass was observed within the left lower thorax on chest CT performed 8 days after birth. The mass was thought to be an ELS because of its arterial supply from the aorta and drainage into the portal vein. A hiatal hernia was suspected because the stomach fundus and presumed ELS (positioned below the diaphragm) herniated into the left thorax (Fig. 2D). Because HFNC was needed for only 2 days after birth and the infant's general condition had improved after ventilatory support, the pediatricians decided to avoid immediate surgery and discharged the infant to the outpatient department. The infant did not undergo the surgery, and no acute illnesses related to the diagnosis was found several months after birth.

Discussion

PS is a discrete mass of nonfunctioning lung parenchyma that does not communicate with the tracheobronchial tree and has an aberrant systemic arterial blood supply, usually from the thoracic or abdominal aorta [1]. Based on the pleural covering, it is classified as intralobar pulmonary sequestration (ILS) or ELS. While ELS is uncommon (15–25% of cases) compared to ILS (75–85% of cases), most prenatally diagnosed cases are ELS, above the diaphragm, and most frequently occur in the lower left side of the chest [6,7]. ELS lesions also occur below the diaphragm and can be confused with neuroblastoma or adrenal hemorrhage [8,9].

ELS is more frequently associated with other anomalies than ILS, including CDH, cardiac abnormalities, pulmonary hypoplasia, and foregut anomalies [1,10]. Among these abnormalities, CDH is the most common coexisting anomaly, occurring in 30–40% of cases [3-5]. CDH is characterized by incomplete diaphragm formation and is complicated by pulmonary hypoplasia and pulmonary hypertension, which may require assisted ventilation or extracorporeal membrane oxygenation (ECMO) in severe cases [11].

The relationship between PS and CDH may be explained by the embryogenetic concept that PS develops at 4–5 weeks of gestation. This occurrence can interfere mechanically with diaphragm fusion and pleuroperitoneal canal closure occurring at 10 weeks of gestation, resulting in CDH [12]. ELS or CDH, when present alone, is often diagnosed prenatally by ultrasonography. However, when both conditions are present, CDH may be missed in prenatal diagnosis. In such cases, an accurate diagnosis may not be possible because the mass effect of PS is speculated to cause an "anatomical barrier" in concomitant CDH, blocking the herniation of the abdominal contents; this may serve as a "protector," allowing normal development of both lungs throughout the pregnancy [4,13]. CDH may be missed until delivery and then appear dramatically after delivery, depending on the size of the defect in the diaphragm, pressure in the abdominal cavity after labor, delivery mode, respiration of the infant after delivery, resuscitation of the infant, and/or positive pressure ventilation [13]. PS often decreases in size before birth and may not need treatment unless complications such as fetal hydrops or pleural effusion are present after birth [14,15]. The prognosis is generally good if there are no complications; however, the prognosis for cases with coexisting CDH is not established [13].

There were several reasons to explain why CDH was overlooked in our patients. In Case 1, the defect was large enough that the right kidney could herniate into the right lung field. However, the PS was also large enough to prevent herniation of the bowel contents through the diaphragmatic defect. Although the right kidney appeared to be somewhat elevated on prenatal ultrasonography, no evidence of herniation was found. After spontaneous vaginal delivery, CDH was immediately discovered as the anatomical barrier function of the PS was lost; the intra-thoracic pressure was believed to have decreased as the neonate cried, while the increasing intra-abdominal pressure pushed the abdominal contents

into the thoracic cavity. The respiratory function of the infant appeared to be stable considering the degree of herniation. This may be because the protector function of the PS allowed lung maturation. In Case 2, CDH was confirmed later when compared to Case 1 because the infant had no obvious respiratory difficulty and there were no specific findings on initial chest radiography. Chest CT was performed based on the prenatal history of PS; it unexpectedly confirmed accompanying CDH. On prenatal examination, only the stomach appeared to be slightly elevated above the normal position. Considering the PS, we should have suspected the presence of CDH. Because the condition of the infant was stable and he did not show any symptoms of CDH, careful monitoring was performed without immediate surgery.

If a lung mass, especially PS, is suspected on prenatal ultrasonography, coexisting CDH may be obscured. Thus, careful prenatal care and counseling of parents regarding the possibility of postnatal CDH are necessary. Postnatally, imaging should be performed immediately to determine whether surgical treatment is necessary, even if the respiratory function of the infant is stable. Although the natural regression of PS is expected, these patients should be delivered at a tertiary center and coexisting CDH should be excluded.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study was approved by the Institutional Review Board of Kyungpook National University Chilgok Hospital (IRB No. KNUCH 2020-06-026) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Patient consent

The patients provided written informed consent for the publication and the use of their images.

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Case Report

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Obstetrics & Gynecology Science

Retroperitoneal Erdheim-Chester disease without skeletal bone involvement mimicking uterine sarcoma with multiple organ involvement

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Erdheim-Chester disease (ECD) is a rare type of non-Langerhans cell histiocytosis and is characterized by the diffuse histiocytic infiltration of multiple organs. Retroperitoneal ECD, especially with uterine involvement, is extremely rare. We report about a 73-year-old woman who presented with vaginal spotting for a month and experienced abdominal pain along with pus-like urine. Computed tomography revealed an irregular mass-like lesion in the uterus, possibly a uterine sarcoma, invading the ureter, rectosigmoid, and bladder. A tissue biopsy of the retroperitoneal mass revealed typical morphological and immunohistochemical features of ECD. However, clinical features, especially long bone involvement, did not coincide with ECD, and BRAF V600E gene mutation was not detected. We made a diagnosis of atypical retroperitoneal ECD mimicking uterine sarcoma with multiple organ involvement.

Keywords: Erdheim-Chester disease; Uterus; Ureter

Introduction

Histiocytosis is a complex and heterogeneous group of disorders of unknown etiology. Moreover, differentiating the types of histiocytosis can be quite challenging and requires a combination of clinical, radiological, and immunohistochemical features [1]. Erdheim-Chester disease (ECD) is characterized by the accumulation of foamy macrophages, chronic inflammation, fibrosis, and organ failure [2]. ECD tumors contain lipid-laden macrophages that are CD68-positive and CD1a-/S100-negative [3]. ECD primarily affects the bones and may involve virtually every organ and tissue with protean clinical manifestations, ranging from asymptomatic bone involvement to multisystemic, life-threatening forms [2].

We describe a unique case of multi-retroperitoneal organ involvement, mainly the ureter, kidney, rectum, bladder, and uterus, with no skeletal bone involvement.

Case report

A 73-year-old woman visited our hospital with vaginal spotting for a month. She had also been experiencing abdominal pain along with pus-like urine. Transvaginal ultrasonography revealed a 5-cm mass on the anterior uterus. The mass was suspected to have invaded the bladder and rectum. Laboratory studies revealed an elevated white blood cell count of 12.5 10^3/μL and a C-reactive protein level of 23.17 mg/dL. However, other tumor markers (CA125, CA19-9, carcinoembryonic antigen) were within the normal range. Magnetic resonance imaging also revealed a 5.2×5.8×6.8-cm^3

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irregular mass lesion in the uterus that invaded the right pelvic sidewall, bladder, rectosigmoid colon, and right ureter (Fig. 1A and B).

A computed tomography urography scan of her abdomen also revealed a lobulated soft tissue mass in the uterus that invaded the right mid ureter, severe hydronephrosis, and diffuse wall thickening at the rectosigmoid junction of the colon (Fig. 1C).

Explorative laparotomy was performed based on the suspicion of a uterine sarcoma that invaded the right ureter, rectum, and bladder. A lobulated uterine mass approximately 7 cm in size was agglomerated with the rectum and bladder and invaded the right ureter and pelvic wall. The right ureter was filled with pus-like fluid. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, low anterior resection and small bowel resection, appendectomy, right nephrectomy, and partial cystectomy were performed. Frozen sections of the uterus, kidney, and rectum showed diffuse histiocytic infiltration. The final histopathology showed diffuse histiocytic infiltration within the entire myometrium, rectum, right ureter, right kidney, and bladder. Tumor cells had large amounts of cytoplasm with a foamy appearance; however, there were

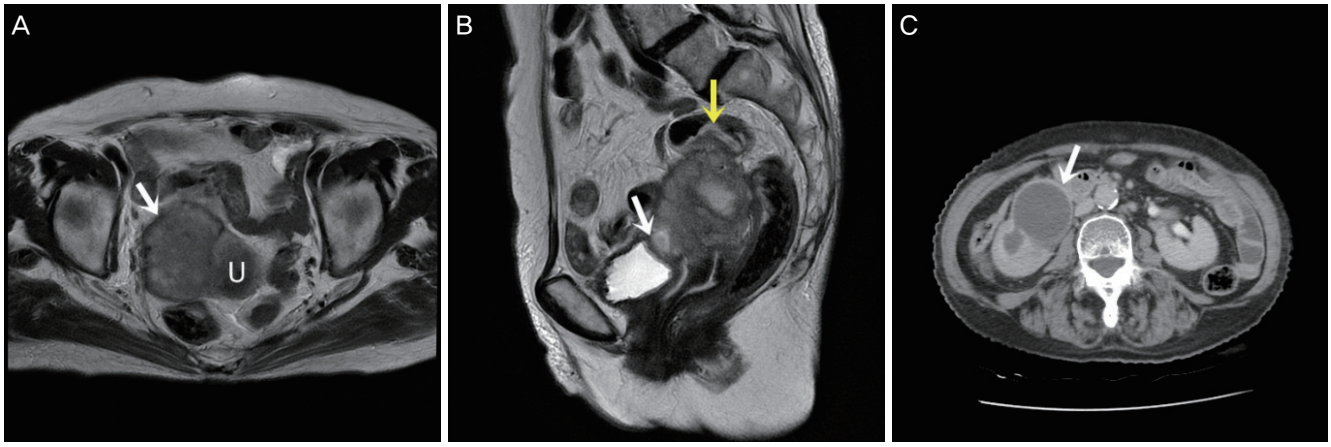

Fig. 1. (A) Magnetic resonance image showing a lobulated irregular mass (white arrow) at the left side of the uterus (U: uterus). (B) Magnetic resonance image showing bladder invasion (white arrow) and rectosigmoid invasion (yellow arrow). (C) Computed tomographic image showing severe hydronephrosis and hydroureter (white arrow).

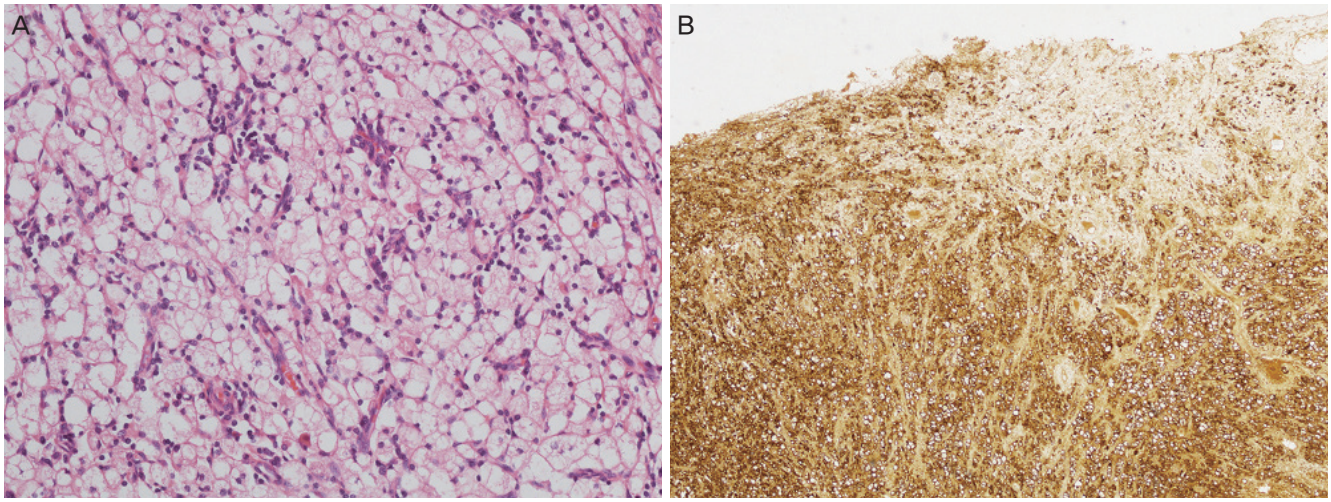

Fig. 2. (A) Image of histopathologic features of the uterus demonstrating clear and foamy histiocytes (hematoxylin and eosin stain, ×200). (B) Immunohistochemical staining image demonstrating the strong positive reactivity of histiocytic markers (CD68, ×40).

no atypia or mitosis (Fig. 2A). For differential diagnosis of histiocytic proliferation in the female genital tract, further diagnostic workup including immunohistochemistry (CD68, S100, and cytokeratin) and molecular pathology (BRAF V600E gene) was performed. Immunohistochemical staining was positive for CD68 (Fig. 2B) and negative for S100 and cytokeratin. BRAF V600E gene mutation was absent in real-time PCR. The bone scan revealed no uptake in long bones.

The patient was regularly followed up at 3-month intervals with no medical therapy, and she remained without evidence of recurrence 8 months after surgery.

Discussion

ECD is an extremely rare type of non-Langerhans cell histiocytosis involving multiple organs including the bones, central nervous system, heart, lung, retroperitoneum, and skin. Skeletal involvement is the most frequent manifestation, with 96% of patients displaying the characteristic radiological finding of bilateral symmetric long bone sclerosis [4]. However, ECD has heterogeneous and diverse clinical manifestations. Atypical presentations can be challenging for the diagnosis of ECD. ECD with no skeletal involvement is extremely rare, and only a few cases have been reported [5]. Our patient had no skeletal involvement.

Histologically, there is proliferation of foamy lipid-laden histiocytes surrounded by Touton giant cells. Due to the lack of cytological atypia in the cells, the neoplastic nature of the proliferation is often missed. Immunohistochemical staining for CD68, CD163, and FACTOR XIII is positive, with CD1a, Langerin, and S100 being negative. The BRAF V600E mutation is present in more than 50% of ECD cases, with other mutations such as N/KRAS and PIK3CA described in BRAF-negative ECD [6].

In a prospective investigation of 60 adults with ECD conducted at the National Institutes of Health (NIH) clinical center, one-third of patients had a history of renal impairment before their NIH visit [7]. A total of 65% of patients exhibited encasement of the kidney and involvement of the retroperitoneal space, causing renal artery stenosis in 54% of cases and ureteropelvic junction obstruction in 51% of cases [7]. However, a study investigating abdominal involvement during ECD reported that there was no uterine involvement [8].

ECD has no standard therapy, although consensus guidelines for clinical management were recently reported [9]. Empiric treatments include anti-inflammatory, immunosuppressive, and chemotherapeutic agents, and recent reports of BRAF V600E and MAPK pathway gene mutations in ECD have suggested treatment with BRAK and MEK inhibitors [7].

Our patient had multiple retroperitoneal organ involvement without skeletal bone involvement. Tissue biopsy and immunohistochemical studies suggested ECD diagnosis. However, the BRAF V600E gene mutation was not found. To the best of our knowledge, uterine involvement in ECD has not been reported. Differential diagnosis of ECD in the female genital tract includes a variety of infectious, inflammatory, and neoplastic disorders. Xanthogranulomatous inflammation involving the uterus is typically centered in the endometrium and involves a combination of neutrophils, plasma cells, lymphocytes, and hemosiderin-laden histiocytes [10]. Unlike ECD, uterine Rosai-Dorfman disease involves lymphocytophagocytosis or emperipolesis [11].

Our patient presented atypical ECD with pathologic and immunohistochemical characteristics that coincided with ECD and clinical characteristics such as skeletal bone involvement and BRAF V600E gene mutation that did not coincide with ECD. To the best of our knowledge, this is the first case report of retroperitoneal ECD with mainly uterine involvement. To correctly diagnose atypical ECD, communication among clinicians, radiologists, and pathologists is vital.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

The study was performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Patient consent

The patients provided written informed consent for the publication and the use of their images.

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Case report

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Obstetrics & Gynecology Science

Pelvic malakoplakia presenting as endometrial cancer: a case report

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Malakoplakia is a rare granulomatous, inflammatory disease generally manifesting as ulcers of the urogenital tract, especially in the bladder, but it can occur in any part of the body. Because of its varied clinical presentations, malakoplakia is considered for differential diagnosis upon suspicion. The final diagnosis is confirmed by the presence of Michaelis-Gutmann bodies. We report a case of pelvic malakoplakia accompanied by left lower quadrant pain that was misdiagnosed as endometrial cancer with pelvic mass based on imaging studies. The patient underwent dilatation and curettage, and the pathology report revealed no malignancy. Because of persistent pain and septic shock, she underwent a debulking operation to remove the mass. Histopathologic examination revealed malakoplakia. For postoperative management, she received broad-spectrum antibiotics, but abdominal pelvic computerized tomography performed on postoperative day 9 revealed pelvic mass recurrence. To the best of our knowledge, this is the only rare case report of pelvic malakoplakia mimicking endometrial cancer.

Keywords: Malakoplakia; Endometrial cancer; Pelvic inflammatory disease; Menopause

Introduction

Malakoplakia was first announced by Michaelis and Gutmann in 1902 and named the lesion malakoplakia from Greek malakos (soft), and plaka (plaque) by Vom Hansemann in 1903. Malakoplakia is a rare non-malignant inflammatory granulomatous disease that most commonly affects the genitourinary tract, but the involvement of other organs has also been reported [1]. Even though the pathogenesis is not completely known, a number of cases accompany bacterial infection in the pelvic and as a result, is diagnosed as tubo-ovarian abscess (TOA) at first. The theories about etiology include bacterial infection and abnormal immune response such as abnormal function of macrophage. The immunologic theory also explains the fact that the most commonly affected patients are the immune-suppressed ones [2,3].

The treatment of malakoplakia includes medical treatment such as antibiotics, surgical treatment, and sometimes withdrawal of immunosuppressive therapy [3]. Prognosis depends on not only the severity, location and extent of the disease but also the general health of the patients before the diagnosis.

We present a case which was first suspected as a severe infection involving left ovary and salpinx and as endometrial cancer later. After coming to our hospital, debulking surgery was done and the pathologist diagnosed her as malakoplakia. With conservative management including proper antibiotics after the surgery, she was fully recovered and discharged without her chronic pelvic pain.

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Case report

A 52-year-old woman presented with intermittent left lower quadrant pain that had started in 2017. She was diagnosed with systemic lupus erythematosus 19 years ago and had been taking oral corticosteroids since then. Her past medical history also showed steroid-induced diabetes mellitus, hypertension, and hypothyroidism, which was treated with levothyroxine. Menopause occurred when she was in her thirties.

On her first visit to the emergency department in another tertiary hospital in May 2018, she underwent abdominal pelvic computerized tomography (APCT), which showed a multiloculated lesion on the left adnexa, with left ovary involvement and hydrosalpinx or pyosalpinx. Under the impression of a TOA, she was admitted and received conservative care including intravenous (IV) antibiotics for 15 days. However, the pain in her left lower quadrant persisted. She visited the emergency department for the second time in July 2018. APCT was performed again, which revealed a slightly increased multiloculated, rim-enhancing lesion compared with the previous APCT findings. Again, the impression was TOA; however, APCT showed a newly developed wall enhancement of the left ureter due to either secondary change or combined ureteritis. Because of persistent pain, she agreed to undergo surgery. On August 3, 2018, the patient underwent laparoscope-guided bilateral salpingo-oophorectomy. The pathology report showed chronic active inflammation with foam cell collection in her left ovary and both salpinges.

Because the surgery did not alleviate her pain, she underwent both APCT and magnetic resonance imaging in April 2019, which revealed a 7-cm irregular mass in her left adnexa and an infiltrative lesion in her uterus, suggestive of endometrial cancer. Imaging results also showed left ureter invasion by the left adnexal tumor. As prophylactic management, a left ureter stent was inserted. Furthermore, she underwent dilatation and curettage for diagnosis. Pathological examination showed chronic active inflammation with foam cell collection, consistent with the finding in the previous laparoscopic surgery.

The patient visited the emergency department of our hospital on May 20, 2019. She had sepsis and a body temperature of >39°C; blood culture revealed the presence of extended-spectrum beta-lactamase–producing *Escherichia coli*. The cervix culture taken at the emergency department also showed the presence of the same organism. Her initial blood urea nitrogen level was 45.6 mg/dL, and her creatinine level was 2.75 mg/dL, which implied septic postrenal acute kidney injury. For septic shock evaluation, she underwent APCT without contrast, which revealed newly developed mild hydronephrosis of the left kidney. The left adnexal mass remained the same as that seen in April 2019 (Fig. 1). We started initial shock management in the emergency department and conservative care including an IV antibiotic (meropenem) for bacteremia and left percutaneous nephrostomy (PCN) for left hydronephrosis and acute kidney injury.

After her recovery from septic shock, the patient underwent debulking surgery on June 4, 2019. During the surgery, severe adhesion in the pelvis involving the peritoneum, rectum, uterus, and left ureter was noted. Multiple hard and catheter-shaped foreign body–like materials were observed in the left parametrium and left pararectal space and near the left internal iliac artery. Caseous necrotic materials had spread deep into the left pelvic and paravaginal space. Total hysterectomy and debulking operation of the left pelvic mass, including left pelvic lymph nodes, were performed.

The final pathology showed foamy histiocyte aggregation

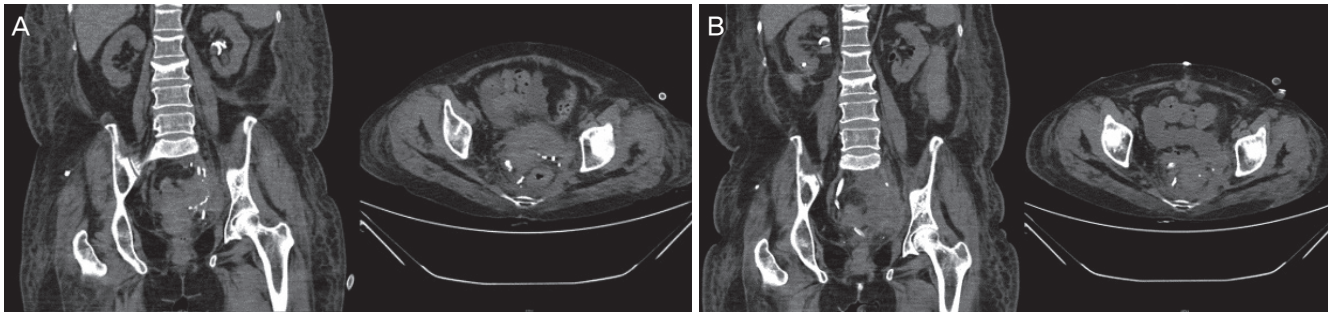

Fig. 1. (A) The patient's preoperative abdominal pelvic computerized tomography (APCT) depicting the pelvic mass (coronal view and horizontal view). (B) Postoperative day #9 APCT depicting the recurred pelvic mass (coronal view and horizontal view).

and acute and chronic inflammation with numerous Michaelis-Gutmann bodies, consistent with malakoplakia (Fig. 2). According to the recommended treatment for malakoplakia, we continued IV administration of tazoperan after the surgery. After the culture from peritoneal fluid cytology showed ceftazidime-sensitive *E. coli*, we changed the antibiotic to ceftazidime 1 g twice a day.

However, her creatinine level increased gradually to 4.48 mg/dL after the surgery. A retrograde pyelogram showed patent left ureter and dye spillage from the right ureter. Right PCN was performed. A computerized tomography scan performed on postoperative day 9 showed recurrence of a 9-cm mass in her left pelvis. Hence, we again administered tazoperan. On postoperative day 21, the patient was referred to a general hospital for further conservative care with IV antibiotics.

On July 24, approximately 1.5 months after surgery, she underwent left PCN removal and right PCN clamping. On August 2, the patient returned to the emergency department with complaints of fever and right flank pain that had started on the previous day. She presented with right costovertebral angle tenderness and pyuria and was admitted to the urology department with a diagnosis of right acute pyelonephritis. On August 9, the anterograde pyelogram showed leakage at the right ureter, which was similar to the previous retrograde pyelogram finding. Right PCN exchange was performed. After conservative care with IV antibiotics, the patient was discharged on August 12. On September 10, direct ureteroneocystostomy was attempted; however, only right PCN exchange was performed because the ureter was short.

The patient has been closely followed up through outpatient visits to both urology and obstetrics and gynecology departments.

Discussion

Malakoplakia is a rare chronic inflammatory disease that usually affects the genitourinary tract [1], but it can affect any part of the body. However, its pathogenesis remains unknown. The disease usually occurs in immunosuppressed patients such as those with diabetes mellitus or those taking oral corticosteroids for rheumatic disease or immunosuppressants due to organ transplantation and autoimmune disease. Because of its characteristics, malakoplakia is assumed to be related to a defect in macrophage function against bacterial infection [2,3].

In this case, malakoplakia resulted from bacterial infection in the pelvis that was first diagnosed as TOA and immunosuppression due to oral steroids and diabetes mellitus. Malakoplakia can affect various organs including the skin, lungs, and bones [4,5]. A literature review showed several reports of malakoplakia occurring in female pelvic organs involving the ovary and fallopian tube [6,7]; however, this is a very rare case of pelvic malakoplakia in the uterus without adnexa due to previous surgery and involving the full depth of the uterus, thus mimicking endometrial cancer as detected in imaging studies.

The diagnosis of malakoplakia is confirmed histologically after biopsy or surgery based on its pathognomonic features of von Hansemann histiocytes and Michaelis-Gutmann bodies, which are monocytes or macrophages containing undigested bacterial components including calcium [8].

The bladder is one of the organs most commonly affected with malakoplakia. Immunosuppressed patients such as those who underwent renal transplantation initially show hematuria or irritative symptoms [9]. However, malakoplakia affecting the female pelvis may not show disease-specific symptoms but rather general symptoms such as abdominal

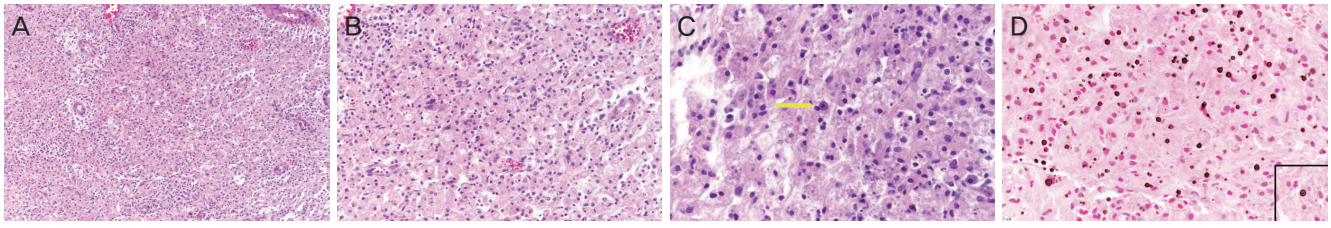

Fig. 2. (A, B) Foamy epithelioid histiocytes and some lymphocytes in endomyometrium and pelvic cavity (A: hematoxylin and eosin [H&E] stain, ×100, B: H&E stain, ×200). (C) Histiocytes with basophilic bodies containing calcium (H&E stain, ×400). (D) Michaelis-Gutmann bodies noted (Von Kossa stain, ×400).

pain and mild fever. Without clinical suspicion in immunosuppressed patients, diagnosis can be delayed, thus increasing morbidity, as noted in this case.

The treatment of choice for malakoplakia is conservative care with IV antibiotics as soon as possible to prevent disease progression. Reduction of immunosuppression in addition to IV antibiotics should be considered if possible. The treatment period or regimen is not yet established. However, recent case studies have reported the use of IV broad-spectrum antibiotics for longer than 2 months [9-11]. If the lesion is localized to one organ, medical treatment is recommended; however, surgical resection followed by IV antibiotics should be considered if the disease forms a mass.

Because malakoplakia is a rare disease, physicians should have clinical suspicion for precise diagnosis. Without such awareness, examinations and imaging studies may lead to an incorrect diagnosis, as in this case. In this case, the patient showed only low abdominal pain, and she was initially diagnosed with TOA. Her pathologic report from the first surgery showed only chronic inflammation, and she was treated with IV antibiotics for only 2 weeks. The short treatment period allowed disease progression. Therefore, careful history review and physical examination are necessary. Malakoplakia presents as a hard and solid mass containing nodules, which is similar to malignant tumors in imaging studies. However, it can be differentiated by pathologic examination.

This report illustrates a case that was assumed to be endometrial cancer based on imaging studies but was diagnosed as pelvic malakoplakia after surgical resection. Because complications and morbidity increase with incorrect treatment, malakoplakia should be carefully diagnosed and treated. Malakoplakia should be suspected in immunosuppressed patients showing symptoms of infection, and differential diagnosis is therefore required.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board of Severance Hospital (4-2019-0988).

Patient consent

The Institutional Review Board approved the exemption of patients consent under the restriction that no identifiable personal information is revealed in the process.

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Case Report

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Obstetrics & Gynecology Science

Granulomatous peritonitis caused by iatrogenic spillage of ovarian dermoid cystectomy: a case report and literature review

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A 39-year-old nulliparous woman experienced continuous mild fever and abdominal pain since undergoing laparoscopic ovarian dermoid cystectomy 3 months previously in a local hospital. Abdominal computed tomography revealed diffuse heterogeneous fat infiltrations with numerous micronodules in the greater and lesser omentum, combined with ascites with thickening of the parietal peritoneum. The patient underwent exploratory laparoscopy, which included partial pelvic peritonectomy, excision of granulomas, and adhesiolysis with massive irrigation. The patient was treated successfully with laparoscopic surgery and all reproductive structures were spared without operative complications. To avoid peritonitis, complete removal of cyst contents and massive irrigation should be performed during ovarian dermoid cystectomy. Conservative surgical treatment may be a good choice for treating granulomatous peritonitis induced by iatrogenic rupture.

Keywords: Iatrogenic disease; Teratoma, ovarian; Peritonitis

Introduction

Ovarian dermoid cyst (ODC) is one of the most common benign ovarian neoplasms [1]. Ovarian cystectomy or oophorectomy is usually performed to treat associated pain symptoms to exclude malignancy and avoid potential complications such as torsion and spontaneous rupture. Laparoscopic ovarian cystectomy has become a routine procedure and has replaced traditional laparotomic approaches [2]. However, despite the advantages of laparoscopic treatment, there is an increased risk for intraperitoneal spillage of cyst contents that can potentially result in chemical peritonitis. Prolonged chemical peritonitis rarely results in the formation of granuloma in the peritoneum, otherwise known as granulomatous peritonitis (GP). To our knowledge, there has been only one reported case of prolonged GP, which was treated with repeated laparotomic surgeries [3]. The case described herein would be the second of iatrogenic GP due to spillage of ODC contents. However, it is the first to describe successful treatment of iatrogenic GP using conservative laparoscopic surgery.

Case report

A 39-year-old nulliparous woman visited the department of infection with symptoms of daily night fever and abdominal pain. She had a history of laparoscopic ovarian cystectomy, performed 3 months previously in a local hospital, due to an ODC. She also complained of other gastrointestinal symptoms including nausea, dyspepsia, intermittent constipation, and abdominal discomfort; however, she did not experience pelvic pain. Physical examination revealed mild tenderness

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noted throughout the abdomen. Laboratory investigations revealed increased levels of inflammatory markers, including the tumor marker cancer antigen 125 (CA-125), which was elevated to 189.8 U/mL.

Abdominal computed tomography (CT) revealed diffuse heterogeneous, smudged, fat infiltrations with numerous micronodules in the greater and lesser omentum, along with ascites with thickening of the parietal peritoneum, and there was tubular enhancing granulation tissue in the right lower quadrant abdominal wall (Fig. 1A). The differential diagnosis included tuberculous peritonitis, malignant peritonitis, and GP, which in all cases involve elevated levels of CA-125. Consequently, the patient was referred to the department of general surgery and gynecology for simultaneous evaluation of the peritoneal and pelvic cavities, including biopsies of the nodules revealed on abdominal CT.

The first laparoscopic surgery was performed in the department of general surgery. The operation revealed the presence of multiple nodular lesions, 1–3 mm in size, in the peritoneum and omentum. Laparoscopic findings revealed pelvic adhesion and approximately 500 mL of serous ascites (Fig. 1B). In addition to the peritoneum and omentum excision biopsies, ascites fluid was sent for cytological examination.

The patient experienced persistent fever, even after the first laparoscopic surgery, and was transferred to the department of gynecology for exploration of the pelvic cavity and subsequent gynecological surgical management. The second laparoscopic surgery was performed 2 weeks after the first operation. Operative findings included severe adhesions in the omentum, bowel to the peritoneum, uterus, and tube to ovary, with multiple granulomatous nodules in the lesser and greater omentum (Fig. 1C). Peritoneal and pelvic adhesiolysis was performed, in which prominent granulomas were removed, and the peritoneal and pelvic cavities were copi-

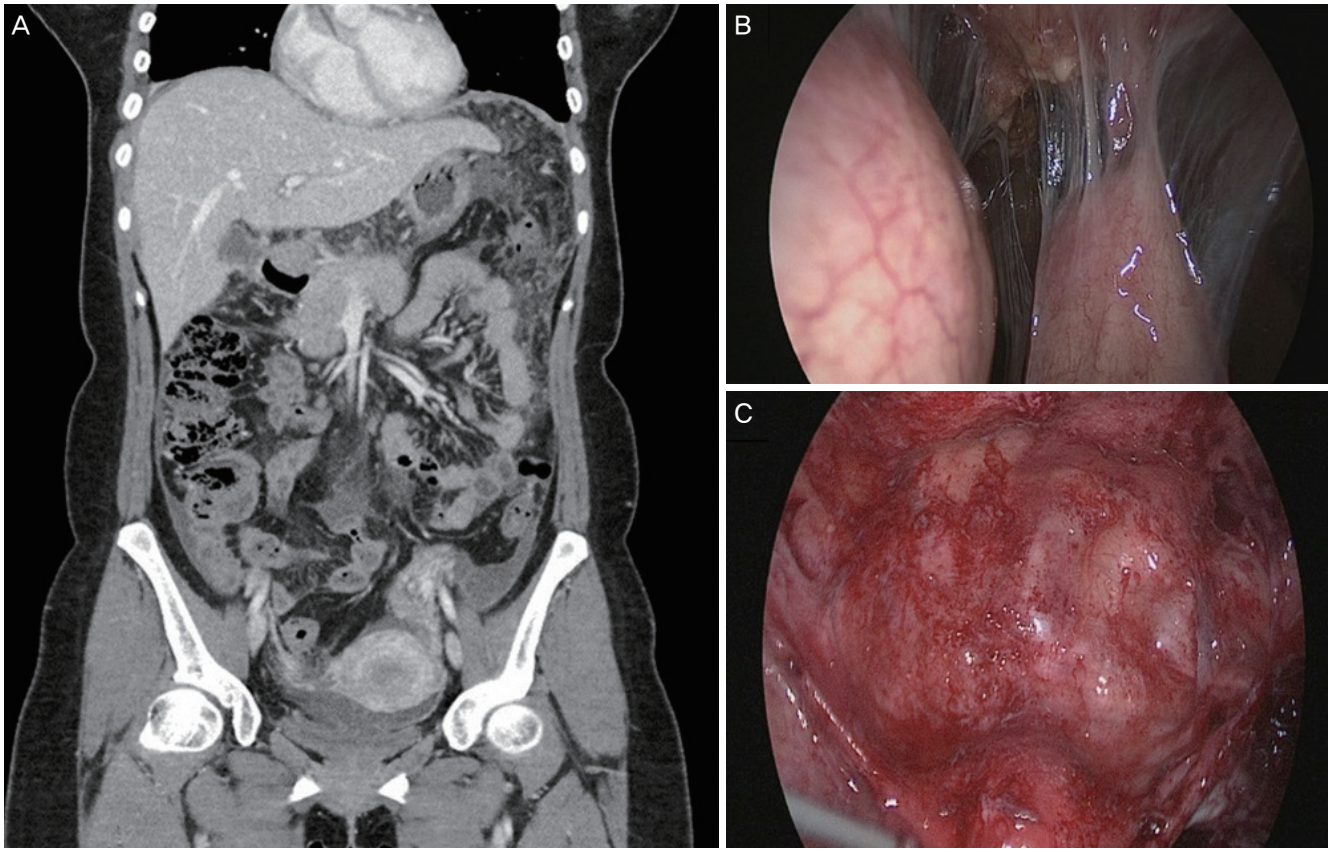

Fig. 1. Preoperative abdominopelvic computed tomography (CT) findings and intraoperative findings of granulomatous peritonitis. (A) Preoperative CT revealing diffuse moderate heterogeneous, smudged fat infiltrations with numerous micronodules in the greater and lesser omentum, combined with diffuse mild ascites with diffuse thickening of the parietal peritoneum, with tubular enhancing granulation tissue in the right lower quadrant abdominal wall. (B) Laparoscopic view revealing profuse sebaceous material in the entire pelvic cavity. (C) Laparoscopic view revealing multiple nodular lesions in the greater and lesser omentum.

ously irrigated. Frozen biopsy of the omental tissue revealed granulomatous inflammatory processes consistent with foreign-body reaction. However, no spillage of ODC materials trapped within the operative field was suspected.

Laboratory investigations revealed no evidence of malignancy or signs of tuberculosis in the ascites. The surgical specimens exhibited multifocal fibrotic changes with inflammatory cell infiltration (Fig. 2A), foreign-body type granulomas encompassing hair-like foreign materials (Fig. 2B and C), multinucleated giant cells within the granulomas (Fig. 2C), and non-caseating granulomas with chronic inflammatory cells (Fig. 2D). Biopsies of prominent nodules collected from the right pelvis and right para-colic gutter demonstrated a microscopic appearance similar to the surgical specimens. Interestingly, any suspected spillage of ODC contents was not observed either in laparoscopic exploration or in the irrigated ascites fluids. However, microscopic examination revealed hair-like materials in the resected granulomas.

The patient was started on a soft diet on postoperative day 3 and experienced significantly decreased gastrointestinal symptoms. The level of C-reactive protein increased to 7.77 mg/dL on postoperative day 1 but returned to the normal range (0.98 mg/dL) 1 week later. The patient was discharged on postoperative day 4 of the second laparoscopic surgery. She remained free of symptoms at the 3-month follow-up, with no additional postoperative management.

Discussion

ODC is one of the most common benign ovarian neoplasms present during childbearing years [1]. Such cysts are usually benign teratomas that often contain diverse types of tissue including hair, teeth, thyroid parenchyma, and cystic components. Ovarian cystectomy is used to treat pain and avoid complications such as torsion and spontaneous rupture. Spontaneous rupture and rupture during cystectomy can lead to chemical peritonitis, as observed in the present case.

Laparoscopy is regarded to be a safe and efficient method for the diagnosis and treatment of ODCs by proficient laparoscopic surgeons. This procedure offers many advantages including less postoperative pain, lower risk for wound complication, earlier ambulation, and more rapid convalescence. The risk for adhesion formation is also reduced using this procedure. However, in the case of an ODC, the laparoscopic approach could result in chemical peritonitis caused by spilled contents of a ruptured dermoid cyst. Gynecological laparoscopy is generally performed with the patient in the Trendelenburg position, and there can be difficulty in suctioning contents after rupture, which can be hidden in the upper abdominal space [4]. If spillage of ODC contents is suspected, irrigation and changing patient position to the reverse Trendelenburg will help spilled contents flow downward from the upper abdomen.

Leakage of sebaceous material and hair follicles from the intraperitoneal rupture of mature cystic teratoma causes an aseptic inflammatory peritoneal reaction, specifically, chemical peritonitis. Although we could not find materials that induced GP on gross inspection in the second operation, microscopic examination revealed hair-like materials, from which we inferred the possibility of a small amount of spillage of ODC contents during laparoscopic ovarian cystectomy.

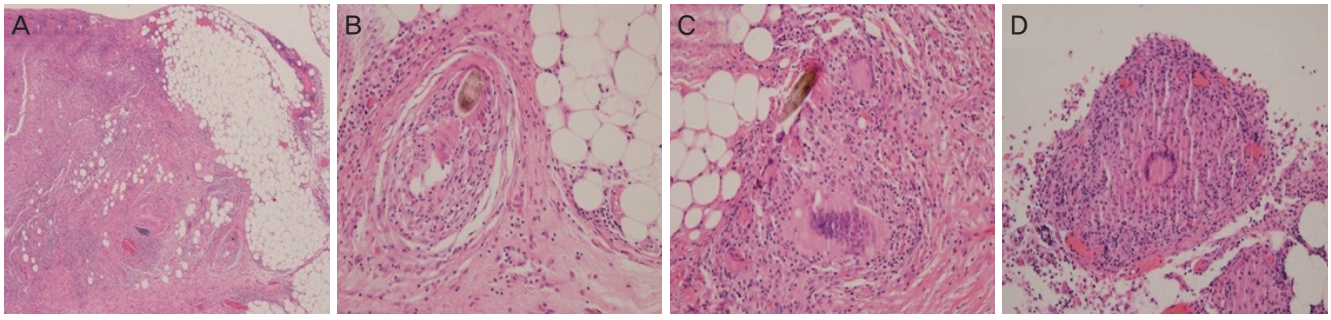

Fig. 2. Histological findings of peritoneal nodules. (A) Chronic inflammation and fibrosis of the omentum (hematoxylin and eosin [H&E] staining, original magnification ×40). (B) Hair follicle-like structure in the omentum (H&E staining, original magnification ×200). (C) Damaged hair follicle-like structure with multinucleated giant cells (H&E staining, original magnification ×200). (D) Foreign-body type granuloma (H&E staining, original magnification ×200).

Prolonged chemical peritonitis leads to granuloma formation in the peritoneum [4]. Chronic GP, however, is one of the catastrophic complications caused by intraoperative leaking of dermoid cyst contents. Moreover, the condition often appears along with severe adhesions, and ascites that resemble tuberculosis peritonitis or malignancy [5].

The occurrence of GP is very rare. Shawki et al. [6] reviewed 496 laparoscopic dermoid cystectomies in which spillage occurred in 324 (65.3%) cases. Despite the high incidence of spillage, however, chronic GP developed in only 1 (0.2%) patient. There are a few reports describing chronic GP due to spontaneous rupture of ODC [7,8]. To manage chronic GP due to spontaneous dermoid cyst rupture, hysterectomy with salpingo-oophorectomy or omentectomy using a laparotomy approach have been performed due to a clinical pattern that mimics advanced ovarian malignancy [7,8].

To the best of our knowledge, the first case of iatrogenic GP caused by the spillage of ODC contents during laparoscopic surgery was reported by Huss et al. [3]. They treated the patient with 2 laparotomies: one trans-rectal and the other via xipho-pubic access.

The signs and symptoms of GP may be subtle and marginal in the early period of chemical peritonitis; however, the patient would complain of progressive abdominal distention, lower abdominal pain, and gastrointestinal disturbances such as anorexia, nausea, vomiting, and diarrhea [7].

Chronic GP usually exhibits unusual radiological findings such as ascites and omental infiltration and thickening of parietal peritoneum on CT scan. These findings may be confused with the peritoneal spread of advanced malignancy and tuberculosis [5]. Thus, ascites fluid analysis includes acid-fast bacillus culture and staining, and/or cytology to rule out the presence of other malignancies; in addition, bacterial or fungal culture tests should also be evaluated. Significant elevation in serum CA-125 levels occurs in patients with tuberculous peritonitis and aseptic GP [9].

In the present case, conservative laparoscopic surgery—sparing the uterus and both ovaries by only removing the prominent inflammatory tissues and copious lavage—proved to be sufficient treatment for prolonged chemical peritonitis induced by dermoid cyst spillage. Granulomas encompassing foreign materials are relatively large and the surface mucosa is contagious and should be removed. If a patient experiences persistent gastrointestinal or pelvic symptoms and has suspicious findings of aggravated peritonitis on ultrasonography, repeated laparoscopic surgeries should be performed. Ruptured teratomas with peritonitis may be difficult to discriminate from malignancies [10]. In case of malignancy, thorough rinsing can reduce the possibility of direct seeding of tumor cells. As such, massive irrigation is essential when malignant tumor cannot be absolutely excluded [11]. In addition, copious saline lavage has been shown to decrease the risk for adhesion and inflammation [12]. Another technical point to prevent GP is careful dissection of the cyst and use of an endoscopic retrieval bag to remove cysts via a port site to prevent spillage [13].

In conclusion, surgeons should perform the technique in a less traumatic manner and be cautious about cystic rupture to prevent spillage. If, however, spillage occurs, complete removal of cyst contents and massive irrigation should be considered key measures to avoid peritonitis. It is apparent that an early conservative surgical approach with laparoscopy, involving removal of peritoneal granuloma and copious lavage, can result in successful treatment of advanced chemical peritonitis.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by Institutional Review Board of Ulsan University Hospital (approval number 2018-03-035).

Patient consent

Written informed consent was obtained from each participant.

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Video Article

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Obstetrics & Gynecology Science

Use of a ring retractor to facilitate specimen removal in laparoscopic surgery

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Objective
To introduce a new surgical technique for specimen removal during laparoscopic surgery.

Methods
The surgical technique was described, pictured, and recorded. The surgery was performed in a tertiary hospital.

Results
During laparoscopic surgery, the specimen is resected from the surrounding tissues and contained in a specimen bag to prevent spillage. The edges of the bag are then pulled through the trocar site, and the specimen—protected by the bag—is removed. To facilitate the removal process and to prevent spillage, assistants will normally hold the edges of the bag during the process. To mitigate the need for assistants to hold the edges of the bag, we wrapped the pulled edges of the bag around a ring retractor, which is a plastic ring, to straighten the bag. This technique enabled the operator to remove the specimen without needing an assistant.

Conclusion
The technique we describe here, using a ring retractor for specimen removal, is useful when assistants are unavailable to help during laparoscopic surgery.

Keywords: Laparoscopy; Specimen handling; Operative surgical procedure; Endoscopy

Laparoscopic surgery for benign gynecologic diseases consists of trocar insertion, tissue excision/repair, and specimen extraction. Specimen extraction can be challenging if the specimens are large or solid. For example, in a study of laparoscopic myomectomy, the mean specimen extraction time was 28 minutes, despite the use of a power morcellator [1]. During specimen extraction, the use of a specimen bag is recommended to prevent spillage.

Solo surgery is defined as an operation performed by the surgeon alone, without assistants. Lack of experienced assistants and advances in technology have promoted the adoption of solo surgery, and reports of its use in various fields have shown it to be feasible [2-5]. For example, in a study that included 25 solo surgeries for hernia repair, none of the procedures required an assistant [6]. In addition, in solo laparoscopic surgeries for benign gynecologic diseases, the tissue excision/repair step can be performed using a robotic uterine manipulator and a robotic laparoscope holder [5].

However, without assistants, a key issue is whether specimen extraction is possible. When we extract specimens, assistants pull the specimen bag and facilitate the extraction by straightening the bag; this helps in preventing spillage. If

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we can extract specimens without assistants, then the surgery can be performed completely solo from start to finish. In addition, we can reduce injuries associated with specimen extraction. For example, knife morcellation may injure assistants who are holding the specimen bag.

Herein, we report a technique that enables specimen extraction without the need for assistants. After the specimen is placed in the specimen bag, the edges of the bag are pulled through the trocar wound. The pulled edges of the bag are wrapped around a ring retractor, which is a plastic ring, to straighten the bag. By repeatedly turning the retractor inside out, the bag is straightened by the friction between the bag and retractor. Once the bag is straightened, the operator can extract the specimen alone. When the bag is loosened, the operator can additionally turn the retractor to re-straighten the bag.

By adopting this technique, specimens can be extracted alone, without assistants. One practical tip is to use a bag one size larger than one would normally use because extra length is required for wrapping around the retractor.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB No. B-1906-547-303).

Patient consent

The need for informed consent was waived.

Video clip

Video can be found with this article online at https://doi.org/10.5468/ogs.20025.

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Erratum

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Obstetrics & Gynecology Science

Correction of Phrase: Medically unfit women with early-stage endometrial cancer treated with the levonorgestrel intrauterine system

Manolis Nikolopoulos, MBBS, Michelle A.L. Godfrey, MBBS, MRCOG, Rekha Wuntakal, MBBS, MRCOG

In the published article, "Manolis Nikolopoulos, Michelle A.L. Godfrey, Rekha Wuntakal. Medically unfit women with early-stage endometrial cancer treated with the levonorgestrel intrauterine system. Obstet Gynecol Sci 2020;63(3):337-345. https://doi.org/10.5468/ogs.2020.63.3.337," we found an error in the above published article, and correct the error as below. The authors apologize for any inconvenience that it may have caused.

Old phrase on the page 339:
Change of the LNG-IUS was advised no later than thrice a year.

Corrected phrase:
Change of the LNG-IUS was advised every three years.

Erratum

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Obstetrics & Gynecology Science

Correction of the name of author: Pre-implantation genetic diagnosis and pre-implantation genetic screening: two years experience at a single center

Seyeon Won, Hannah Kim, Woo Sik Lee, Ji Won Kim, Sung Han Shim

In the published article, "Se Yeon Won, Hannah Kim, Woo Sik Lee, Ji Won Kim, and Sung Han Shim. Pre-implantation genetic diagnosis and pre-implantation genetic screening: two years experience at a single center. Obstet Gynecol Sci 2018;61(1):95-101. https://doi.org/10.5468/ogs.2018.61.1.95," the name of author was published incorrectly. The authors apologize for any inconvenience that it may have caused.

Se Yeon Won, Hannah Kim, Woo Sik Lee, Ji Won Kim, Sung Han Shim

The name of author should be corrected as follows. Changes are marked by underlines:

Seyeon Won, Hannah Kim, Woo Sik Lee, Ji Won Kim, Sung Han Shim

Obstetrics & Gynecology Science

Instructions for Authors

Enacted in January 1958
Revised in December 2019

Aims and Scope

Obstetrics & Gynecology Science (NLM title: Obstet Gynecol Sci) is an international peer-review journal that published basic, translational, clinical research, and clinical practice guideline to promote women's health and prevent obstetric and gynecologic disorders. The journal has an international editorial board and is published in English on the 15th day of every other month. Submitted manuscripts should not contain previously published material and should not be under consideration for publication elsewhere.

The journal has been publishing articles since 1958. The aim of the journal is to publish original articles, reviews, case reports, short communications, letters to the editor, and video articles that have the potential to change the practices in women's health care.

The journal's main focus is the diagnosis, treatment, prediction, and prevention of obstetric and gynecologic disorders. Because the life expectancy of Korean and Asian women is increasing, the journal's editors are particularly interested in the health of elderly women in these population groups. The journal also publishes articles about reproductive biology, stem cell research, and artificial intelligence research for women; additionally, it provides insights into the physiology and mechanisms of obstetric and gynecologic diseases.

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It is primarily for obstetricians & gynecologists. They will be able to obtain tailored information to adopt the information for their patients care. Its readership can be expanded to other positions:

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- Clinicians in the other fields can get the recent progress of obstetrics and gynecology so that they can refer their patients for more specific consultation to obstetricians & gynecologists.
- Administrators of the hospital or health center can access recent info and adopt a variety of data in the management of the institutes.
- Medical health students can understand the recent innovation and trends of obstetrics and gynecology so that they are able to learn those information during their study.
- Policy makers may be able to reflect the results of the articles to

the health policies especially for maternal health.
- The public will be able to read the advancement in the obstetrics and gynecology fields that they have a confidence in visiting obstetricians & gynecologists to consult their health problem.

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Ethical Considerations

1. Research Ethics

All of the manuscripts should be prepared based on strict observation of research and publication ethics guidelines recommended by the Council of Science Editors (http://www.councilscienceeditors.org/), International Committee of Medical Journal Editors (ICMJE, http://www.icmje.org/), World Association of Medical Editors (WAME, http://www.wame.org/), and the Korean Association of Medical Journal Editors (KAMJE, http://www.kamje.or.kr/intro.php?body=eng_index). All studies involving human subjects or human data must be reviewed and approved by a responsible Institutional Review Board (IRB). Please refer to the principles embodied in the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/) for all investigations involving human materials. Animal experiments also should be reviewed by an appropriate committee (IACUC) for the care and use of animals. Also studies with pathogens requiring a high degree of biosafety should pass review of a relevant committee (IBC). The approval should be described in the Methods section. For studies of humans including case reports, state whether informed consents were obtained from the study participants. The editor of OGS may request submission of copies of informed consents from human subjects in clinical studies or IRB approval documents. The OGS will follow the guidelines by the Committee on Publication Ethics (COPE, http://publicationethics.org/) for settlement of any misconduct.

2. Conflict of Interest

The corresponding author of an article is asked to inform the Editor of the authors' potential conflicts of interest possibly influencing the research or interpretation of data. A potential conflict of interest should be disclosed in the cover letter even when the authors are confident that their judgments have not been influenced in preparing the manuscript. Such conflicts may include financial support or private connections to pharmaceutical companies, political pressure from interest groups, or academic problems. Disclosure form shall be same with ICMJE Uniform Disclosure Form for Potential Conflicts of Interest (http://www.icmje.org/coi_disclosure.pdf). The Editor will decide whether the information on the conflict should be included in the published paper. In particular, all sources of funding for a study should be explicitly stated. The OGS asks referees to let its Editor know of any conflict of interest before reviewing a particular manuscript.

Statement of Informed Consent

Copies of written informed consent and institutional review board (IRB) approval for clinical research should be retained for reference as necessary. Please insert a sentence in the Materials and Methods section stating that the study was approved or exempt from approval and include the name of the IRB.

Statement of Human and Animal Rights

All human investigations must be conducted according to the principles expressed in the Declaration of Helsinki. All studies involving animals must state that guidelines of the authors' institution, or any applicable national law, regarding the use and care of laboratory animals were followed.

Selection and Description of Participants

Clearly describe the selection of observational or experimental participants (healthy individuals or patients, including controls), including eligibility and exclusion criteria and a description of the source population. Because the relevance of such variables as age, sex, or ethnicity is not always known at the time of study design, researchers should aim for inclusion of representative populations into all study types and at a minimum provide descriptive data for these and other relevant demographic variables. Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases, (e.g., prostate cancer)." Authors should define how they determined race or ethnicity and justify their relevance.

Originality and Duplicate Publication

All submitted manuscripts should be original; further, they should not be under consideration for publication by other scientific journals. Any part of the accepted manuscript may not be duplicated in any other scientific journal without the permission of the editorial board. If duplicate publication related to a paper in this journal is detected, the author(s) will be named in the journal, and the respective institute(s) of affiliation will be informed; additionally, there will be penalties for the author(s).

Secondary Publication

It is possible to republish manuscripts if they satisfy the conditions of secondary publication in the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals."

Data sharing statement

OGS accepts the ICMJE Recommendations for data sharing statement policy (http://icmje.org/icmje-recommendations.pdf). All manuscripts reporting clinical trial results should submit a data sharing statement following the ICMJE guidelines from 1 Jan 2020.

Evidence-Based Medicine

For the specific study design, such as randomized control studies, studies of diagnostic accuracy, meta-analyses, observational studies and non-randomized studies, it is recommended that the authors follow the reporting guidelines listed in the following table.

Initiative	Type of study	Source
CONSORT	Randomized controlled trials	http://www.consort-statement.org
STARD	Studies of diagnostic accuracy	http://www.stard-statement.org
PRISMA	Preferred reporting items of systematic reviews and meta-analyses	http://www.prisma-statement.org
STROBE	Observational studies in epidemiology	http://www.strobe-statement.org
MOOSE	Meta-analyses of observational studies in epidemiology	http://www.consort-statement.org/resources/downloads/otherinstruments/moose-statement-2000pdf

Registration of Clinical Trial Research

It is recommended that research dealing with a clinical trial be registered with a primary national clinical trial registration site such as https://cris.nih.go.kr/cris, or other sites accredited by the World Health Organization (WHO) or the International Committee of Medical Journal Editors.

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The journal also follows the open access policy of PubMed Central at United States National Library of Medicine (http://www.ncbi.nlm.nih.gov/pmc/).

All contents of the journal are available immediately upon publication without embargo period.

SUBMISSION PROCEDURES FOR PEER REVIEW

Manuscripts Submission

Manuscripts should be submitted online at (https://www.editorialmanager.com/ogs). Submission instructions are available on the website. All articles submitted to the journal must comply with these instructions. Failure to do so will result in return of the manuscript and a possible delay in publication.

Screening Before Review

If the manuscript does not fit the aims and scope of the journal or does not adhere to the Instructions to Authors, it may be returned to the author immediately after receipt and without a review. Before reviewing, all submitted manuscripts are inspected by Similarity Check powered by iThenticate (https://www.crossref.

org/services/similarity-check/), a plagiarism-screening tool. If the similarity score is too high, the editorial board will conduct a more profound content screening. If the similarity rate is 15% or more, further screening is usually performed; furthermore, every manuscript may be checked for excessive similarity in specific sentences. The settings for Similarity Check screening are such that the following are excluded: quotes, bibliography, small matches (e.g., six words), small sources (1%), and the Methods section.

Peer Review Process

The editor selects peer referees by recommendation of the editorial board members or from the specialist database owned by the editorial board. Acceptance of the manuscript is decided based on the quality and originality of research and its clinical and scientific significance by the referees. This journal uses a double-blind review, which means that identities of both the reviewer and author are concealed from the reviewers, and vice versa, throughout the review process. A referee's decision is given as "accept," "minor revision," "major revision," and "reject." If there is a marked discrepancy in the decisions between two referees or in opinions between the author and referee(s), the editor may send the manuscript to another referee for additional comments and a recommended decision. An initial decision will normally be made within four weeks of receipt of a manuscript, and the reviewers' comments will be sent to the corresponding authors by e-mail. Revised manuscripts must be submitted online by the corresponding author, who must indicate the alterations that have been made in response to the referees' comments item by item. Failure to resubmit the revised manuscript within eight weeks of the editorial decision is regarded as a withdrawal.

Article Processing Charges

There is no page charges for submission or publication.

MANUSCRIPT CATEGORIES

The journal focuses on clinical and experimental studies, reviews, case reports, and short communications. Any physician or researcher throughout the world can submit a manuscript if the scope of the manuscript is appropriate. However, manuscripts should be submitted in English.

[1] Original articles

Original articles are reports of basic or clinical investigations. The maximum length of a manuscript is 3,500 words of body text, excluding the abstract, references, figures, and tables. These articles are limited to 40 references. The manuscript should be organized in the following sequence: title page, the abstract and keywords, introduction, materials and methods, results, discussion, acknowledgments, references, tables, and figures with their legends.

[2] Reviews

Reviews are invited by the editor and should be comprehensive analyses of specific topics. Authors who wish to submit unsolicited reviews should contact the editor-in-chief to determine appropriateness of reviews for publication in OGS. These articles are organized as follows: title page, the abstract and keywords, introduction, body text, conclusion, acknowledgments, references, tables, and figures with their legends. The maximum word count is 4,500 words of body text, excluding the abstract, references, tables, and figures. The editors also suggest a limit of 150 references.

[3] Case reports

Case reports will be published only in exceptional circumstances (i.e., when they illustrate rare occurrences of clinical importance). Up to three cases may be described, and case reports should be organized in the following sequence: title page, the abstract and keywords, introduction, case report, discussion, acknowledgments, references, tables, and figures with their legends. The abstract should be unstructured, and its length should not exceed 150 words. The manuscript should not exceed 1,500 words; further, no more than two figures (including tables) should be included, and the number of references is limited to 15.

[4] Short communications

A short communication is a definitive report of highly significant findings in the field; it receives a very rapid review and, if accepted, is published within an average of 12 weeks from receipt. A manuscript should not exceed 1,500 words and must contain an unstructured abstract of approximately 150 words, a one-paragraph introduction, an abbreviated materials and methods section, a results section, and a concise discussion section. There should be no more than 20 references and no more than two tables (including figures).

[5] Letter to the Editor

A letter to the editor provides brief comments in response to a

specific published article in OGS. A letter addressing an article published in one of the three previous issues will be considered. The editor-in chief may invite the author(s) of the published article to reply in writing. A published letter is accompanied by either a reply from the original author(s) or the statement, "Reply declined." A letter must include a title page (including your affiliation, full address, and e-mail address), conflict of interest disclosure, and a Statement of Authorship signed by all authors. A letter can be signed by no more than four authors and must not exceed 1,000 words (excluding references); only one table or figure may be included (if essential). Additionally, no more than five references are allowed. Letters to the editor should deal with short clinical cases of medical interest or innovation. All letters should be recommended by the journal's editors. Please do not upload your case report as a letter on the submission website. No abstract or keywords are required, and text should be formatted in one continuous section.

[6] Video articles

Video articles are published in full online and include the abstract, video file, and still image. Video authors have the ability to present their scientific findings through visual media. All submitted files should be properly labeled so that they directly relate to the video articles' content. The maximum file size is 350MB (after conversion to MP4) and the video should not exceed 10 minutes. Formats accepted for conversion include MPG, AVI, MOV, WMA, WMV, SWF, RM, and FLA. An audio narration in English must accompany the video without music soundtracks. Please provide a video still image file as well. It can be any frame from the video or may be a separate. There should be a manuscript submitted with the video that includes a title page, structured abstract, body of text, and disclosures, as well as references (if needed). The abstract should not exceed 250 words and must describe concisely, in a paragraph, the following: Objective, Methods, Results, and Conclusion. The body text should not exceed 1,000 words, and there should be no more than 20 references. A video file should be submitted by using a URL/URI/External Resource.

MANUSCRIPT PREPARATION

Manuscripts for submission to OGS should be prepared according to the following instructions. The journal follows the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" (http://www.icmje.org/recommendations/), commonly known as "the Vancouver style," if not otherwise described below.

General Guidelines

After entering information about the authors, the manuscript title, abstract, keywords, and other details, you will be prompted to upload your files. The main document with manuscript text and tables should be prepared with in Microsoft Word.

- The main document should be organized in the following order: title page, the abstract and keywords, introduction, materials and methods, results, discussion, acknowledgments, references, tables, and figures with their legends.
- The manuscript should be written in 10-point font with double spacing on A4-sized paper (21.0×29.7 cm) with 2.5 cm margins (top, bottom, right, and left).
- Manuscript pages are to be numbered consecutively, centered at the bottom of each page and beginning without the title page as page 1.
- The use of acronyms and abbreviations is discouraged and should be kept to a minimum. Acronyms and abbreviations cannot be used in the title. When used, they are to be defined where first used, followed by the acronym or abbreviation in parentheses.
- Drug and chemical names should be stated in standard chemical or generic nomenclature. Units of measure should be presented according to the International System (SI) of units.

1) Title page

Include the following items on the title page: title of the article, full names of authors, academic degrees, and institutional affiliations of all authors. A short running head must also be provided, consisting of fewer than 40 characters including spaces. When addresses of authors differ, begin with the name of the organization where the primary research was conducted and follow with the names of the other organizations along with the authors' names, listed in numerical order. At the bottom of the title page, identify the corresponding author and include his/her postal address and e-mail address.

2) Abstract and Keywords

The abstract should not exceed 250 words and describe concisely, in a paragraph, the following: Objective, Methods, Results, and Conclusion. Up to five keywords should be listed below the abstract as index terms. For the selection of keywords, refer to Medical Subject Headings (MeSH, http://www.ncbi.nlm.nih.gov/

mesh) in Medline.

3) Introduction

Briefly describe the purpose of the investigation, including relevant background information.

4) Materials and Methods

Describe the research plan, materials (or subjects), and methods used, in that order. Explain in detail how the disease was confirmed and how subjectivity in observations was controlled. When experimental methodology is the main issue of the paper, describe the process in detail so as to recreate the experiment as closely as possible. The sources of the apparatus or reagents used should be given along with the source location (name of company, city, state, and country). Information regarding institutional review board/ethics committee approval or waiver and informed consent should be stated. Methodology for statistical analyses and criteria for statistical significance should be described.

5) Results

Results should be presented in a logical sequence in the text, tables/figures , and illustrations. Do not repeat in the text all data that appear in the tables or figures; you may, however, describe important points and trends.

6) Discussion

Observations pertaining to the results of research and other related materials should be interpreted for your readers. Emphasize new and important observations; do not merely repeat the contents of the introduction or results. Explain the meaning of observed opinions along with their limits; within the limits of the research results, connect the conclusion to the purpose of the research.

7) Acknowledgments

If necessary, persons who have made substantial contributions but who have not met the criteria for authorship are acknowledged here. All sources of funding applicable to the study, disclosure of potential conflicts of interest (including financial interests, activities, relationships, and affiliations), information on previous presentations, and any important disclaimers should be stated explicitly here.

8) Ethical approval

Clinical studies or experiments using laboratory animals or pathogens should mention approval of the studies by relevant committees in this section. The sources of special chemicals or preparations should be given along with their location (name of company, city and state, and country). Method of statistical analyses and the criteria for determining significance levels should be described. An ethics statement should be placed here when the studies are performed using clinical samples or data, and animals.

9) Patient consent

All authors are required to follow the ICMJE requirements (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/protection-of-research-participants.html) on privacy and informed consent from patients and study participants. Confirm that any patient, service user, or participant in any research, experiment, or clinical trial described in the paper has given written consent to the inclusion of material pertaining to themselves; and that authors have fully anonymized them.

10) References

In the text, references should be cited with Arabic numerals in brackets in the order cited. In the References section, the references should be numbered in order of appearance in the text (in English). List all authors if there are less than or equal to six authors. List the first six authors followed by "et al." if there are more than six authors. If an article has been published online but has not yet been given an issue or pages, the digital object identifier (DOI) should be supplied. Journal titles should be abbreviated in the style used in Medline. If the reference is in Korean, then provide the English version in the references list. Other types of references not described below should follow Citing Medicine: The NLM Style Guide for Authors, Editors, and Publishers (http://www.nlm.nih.gov/citingmedicine).

• Journal articles:

1. Park JH, Chung D, Cho HY, Kim YH, Son GH, Park YW, et al. Random urine protein/creatinine ratio readily predicts proteinuria in preeclampsia. Obstet Gynecol Sci 2013;56:8-14.
2. Reed SD, Newton KM, Garcia RL, Allison KH, Voigt LF, Jordan CD, et al. Complex hyperplasia with and without atypia: clinical outcomes and implications of progestin therapy. Obstet Gynecol 2010;116:365-73.

• Entire book:

3. Korean Society of Obstetrics and Gynecology. Gynecology. 4th ed. Seoul: Korean Medical Book Publisher; 2007.

• ***Part of a book:***

4. Holschneider CH, Berek JS. Valvar cancer. In: Berek JS, Novak E, editors. Berek & Novak's gynecology. 14th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2007. p.1549-80.

• ***Conference paper:***

5. Rice AS, Brooks JW. Canabinoids and pain. In: Dostorovsky JO, Carr DB, editors. Proceedings of the 10th World Congress on Pain; 2002 Aug 17-22; San Diego, CA. Seattle (WA): IASP Press; 2003. p.437-68.

• ***Online publication:***

6. Dieci MV, Barbieri E, Piacentini F, Ficarra G, Bettelli S, Dominici M, et al. Discordance in receptor status between primary and recurrent breast cancer has a prognostic impact: a single-Institution analysis. Ann Oncol 2012 Sep 20 [Epub]. https://doi.org/10.1093/annonc/mds248.

• ***Online sources:***

7. American Cancer Society. Cancer reference information [Internet]. Atlanta (GA): American Cancer Society; c2012 [cited 2012 Oct 20]. Available from: http://www.cancer.org/docroot/CRI/CRI_0.asp.
8. National Cancer Information Center. Cancer incidence [Internet]. Goyang (KR): National Cancer Information Center; c2012 [cited 2012 Oct 20]. Available from: http://www.cancer.go.kr/cms/statics.

11) Tables

- Each table should have a title, begin on a new page, and be numbered with an Arabic numeral in the order in which it is cited in the text.
- The title and contents of a table should be written in concise and clear English so that the reader can understand the table without referring to the text.
- The total number of tables shall not exceed five.
- Within a table, if a non-standard abbreviation or description is necessary, elaborate with an annotation below the table. Insert lower case, superscript letters a), b), c), etc., to the right of terms that need explanation. The annotation (preceded by the respective lower case letter) should appear below the table.
- Statistical measures, such as SD or SEM, should be identified.
- Vertical or horizontal lines between entries should be omitted.

12) Figures

- Upload each figure as a separate image file.
- The figure images should be provided in EPS or TIF format—although the JPEG format is allowed for color figures—in high resolution (preferably 300 dpi for figures and 600 dpi for line art and graphs).
- If figures are not original, the author(s) must contact each publisher to request permission to reprint; include information regarding permission to reprint in a footnote below the figure.
- Figures should be numbered, using Arabic numerals, in the order in which they are cited in the text.
- In the case of multiple images within the same figure, use capital letters after the numeral to indicate the correct order (e.g., Fig. 1A, Fig. 1B).
- The total number of figures shall not exceed five.
- A figure legend should be a one-sentence description (rather than a phrase or a paragraph) in English.

Submission Checklist

Obstetrics & Gynecology Science

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Instructions for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address

All necessary files have been uploaded, and contain:

- Abstract, keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'.
- References are in the correct format for this journal.
- All references mentioned in the Reference list are cited in the text, and vice versa.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print.
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes.

Conflict of Interest Form

Obstetrics & Gynecology Science

Corresponding Author Name _____

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_____

As an integral part of the online submission process, the corresponding authors are required to confirm whether they or their co-authors have any conflicts of interest to declare, and to provide details of these. At the point of submission, Korean Society of Obstetrics & Gynecology policy requires that each author discloses any financial interests or connections, direct or indirect, or other situations that might raise the question of bias in the work reported or the conclusions, implications, or opinions stated.

Author Signed Declarations

Use more forms if needed to add names.

By signing this conflict of interest form, each and every undersigned author agrees as follows: To the best of my knowledge, I have no relevant financial relationships except as follows (please list any possible exceptions below the author name).

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