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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
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유효성과 안전성이 입증된 정맥철분주사제입니다.[1-3]

Reference 1. Froessler et al. BMC Pregnancy and Childbirth 2014, 14:115, 2. Seid et al., Am J Obstet Gynecol 2008;199:435.e1-435.e7, 3. Braymann et al. ,J Perinat Med. 2017 May 24;45(4):443-453

[제품요약정보] 페린젝트 주 2mL, 10mL, 20mL [전문의약품] ■ **성분** 1mL 중 수산화제이철카르복시말토오스복염 180mg (철로써 50mg) ■ **효능효과** 경구용 철분제제의 효과가 불충분하거나 복용이 불가능한 철 결핍환자 ■ **용법 · 용량** ● 이 약의 총 투여량은 헤모글로빈 수치 및 체중에 따라 개인별로 결정되며, 과량 투여해서는 안된다. ● [환자의 체중: 35kg이상 70kg 미만] Hb<10g/dL 경우 1,500mg, Hb≥10g/dL 경우 1,000mg [환자의 체중: 70kg 이상] Hb<10g/dL 경우 2,000mg, Hb≥10g/dL 경우 1,500mg ● 1회 투여량은 1일 철로서 1,000mg(20mL) 또는 체중 kg당 철로서 20mg(0.4mL)을 초과하여서는 안된다. ● 철로서 1,000mg(20mL)의 투여는, 1주 1회를 초과하여서는 안된다. ● 혈액투석-의존성 만성 신장질환 환자에의 투여: 1일 1회 최대 투여 용량은 철로서 200mg(4mL)을 초과하여서는 안된다. ● 정맥으로만 투여되어야 한다. 이 약은 피하 또는 근육주사 할 수 없다. 정맥직접주사 또는 정맥점적주사로 투여할 수 있으며, 정맥직접주사시 혈액투석 동안에는 투석기의 정맥측 가지에 희석하지 않고 바로 투여할 수 있다. 1. 정맥직접주사: 이 약은 철로서 1,000mg까지 희석하지 않은 용액으로 정맥주사할 수 있다. 철로서 200mg까지의 용량은, 정해진 투여 시간이 없다. 철로서 200mg 초과 500mg 이하의 용량은, 이 약을 100mg 철/min 이하의 속도로 투여해야 한다. 500mg 초과 1,000mg 이하의 용량은, 이 약을 15분 이상에 걸쳐 투여해야 한다. 2. 정맥점적주사: 이 약은 정맥점적주사로 1회 최대 철로서 1,000mg(20mL)까지 투여할 수 있다. 이 약을 정맥점적주사 시에는, 0.9% m/V 멸균생리식염액을 이용하여 희석해야 한다. ■ **주요 사용상의 주의사항** [다음 환자에는 투여하지 말 것] 1) 이 약 또는 이 약의 구성성분에 과민반응 환자 2) 다른 비경구 철 제제에 대한 중대한 과민반응이 알려진 환자 3) 철 결핍증 이외의 빈혈 환자 4) 철분 과다 또는 철 이용 장애 환자 5) 진행형 세균혈증 환자 [다음 환자에는 신중히 투여할 것] 1) 간 기능 장애가 있는 환자 2) 급성 또는 만성 감염 환자 3) 천식, 습진, 아토피알러지 환자 및 그 병력이 있는 환자 4) 약물 알러지를 포함한 알러지 환자 5) 면역 또는 염증 질환 환자 6) 임부 ■ **이상반응** 흔하게(1%~10%미만): 두통, 어지러움, 고혈압, 오심, 주사부위반응, ALT의 증가, 저인산혈증 ■ **일반적 주의** ● 이 약을 투여할 때 혈관 밖으로 약액이 누출되지 않도록 해야 한다. 이 약이 주사부위에 누출되는 경우 피부를 갈색으로 변색시키거나 자극을 줄 수 있다. 약액 누출시 이 약의 투여를 즉시 중단해야 한다. ■ **포장단위** 2mL, 10mL, 20mL x 1 vial ■ **저장방법** 밀봉 용기, 실온(1~30℃)보관, 냉장보관하거나 얼리지 않아야 한다.

최종허가변경일 : 2017년 7월 26일

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2020 Congress Events

2020.09.27

The 106th Annual Congress of Korean Society of Obstetrics and Gynecology

On-line

2020.09.27

The 25th Seoul International Symposium

On-line

2020.11.07

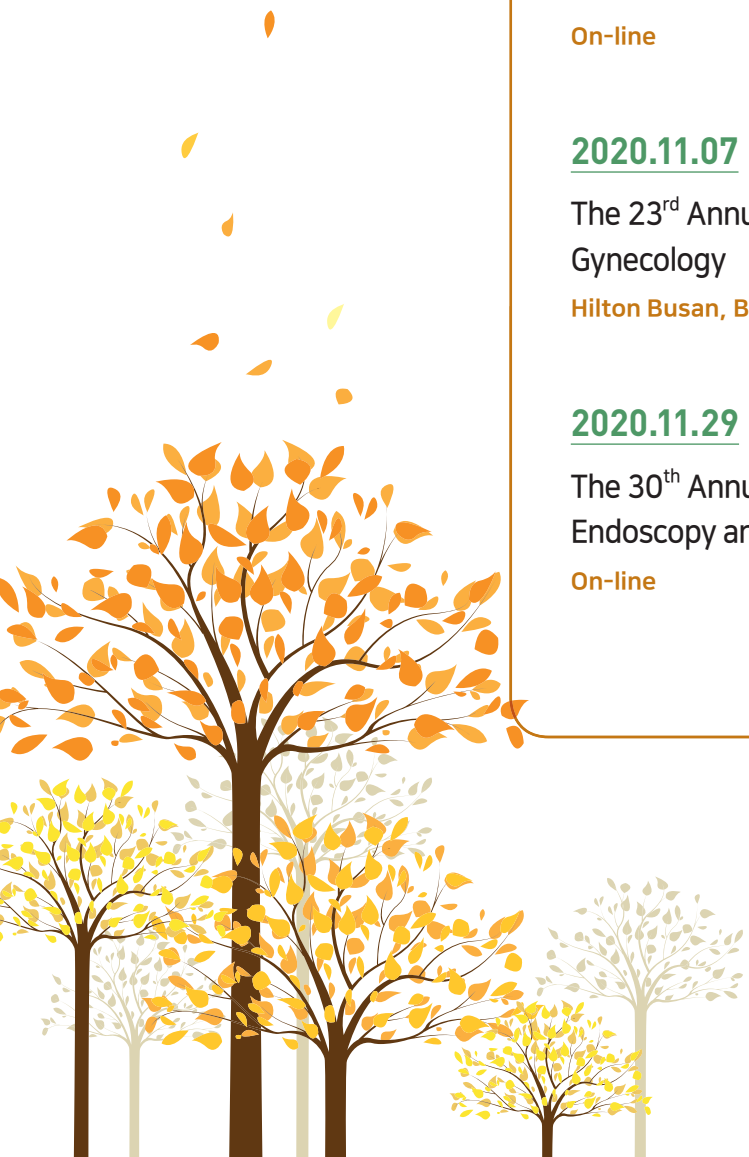
The 23rd Annual Congress of Ultrasound in Obstetrics and Gynecology

Hilton Busan, Busan, Korea

2020.11.29

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

On-line



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* Mirena® is contraindicated when congenital or acquired uterine anomaly including fibroids if they distort the uterine cavity.

** HMB with no identified pathology or fibroids less than 3 cm in diameter, which are not causing distortion of the uterine cavity or suspected or diagnosed adenomyosis.
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)

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[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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한쪽으로만 치우칠 수 있는 우리 아기의 영양이 균형 잡힐 수 있도록 도와줍니다.

식물성 영양 | 성장기용 조제식 | 빈틈 없는 영양설계 | 간편한 액상타입

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돌 이전에 생우유, 산양유, 콩으로 만든 영·유아식이 아닌 일반 콩 음료, 철분 함량이 낮은 유아식은 권장하지 않는다. [출처 : Bright futures, AAP, 2017]

돌 이전에는 성장기용 조제식 '베지밀 인펀트'를 주세요.

제품 선택 시 식품의 유형을 꼭 확인하세요!

	일반 조제식 (6~12개월)	베지밀 인펀트
제품	조제식	인펀트 프리미엄 1, 인펀트 우리콩 1
식품의 유형	성장기용 조제식	

돌 이후에는 균형영양을 위해 '베지밀 토들러'를 주세요.

식물성 단백질을 기초로 하여
성장, 발육에 필요한 영양을 꽉꽉 채웠습니다.

	일반 우유	베지밀 토들러
제품	MILK	토들러 프리미엄 2, 토들러 프리미엄 3, 토들러 우리콩 2, 토들러 우리콩 3
주요 원재료	동물성 단백질	식물성 단백질
	원유	대두

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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.

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- 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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Background

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Letter to the Editor

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Obstetrics & Gynecology Science

Safety and feasibility of robotic surgery in selected ovarian cancer patients undergoing interval debulking surgery

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With a great deal of interest, we read the article entitled "Robotic-assisted interval cytoreductive surgery in ovarian cancer: a feasibility study" by Carbajal-Mamani et al. [1]. The study retrospectively examined 12 patients who underwent interval cytoreductive surgery, with complete cytoreduction achieved in 75% of patients. The main advantages of the approach were minimal blood loss (100 mL), and length of hospital stay (2 days). Two robotic cases with upper abdominal disease required conversion to open laparotomy to achieve optimal cytoreduction. Regarding short-term outcomes, only one patient had a postoperative port-site hernia. No long-term outcomes or outcomes related to safety were presented because of the small median follow-up time (9.5 months).

The INTERNATIONAL MISSION study concluded that minimally invasive techniques could be used in patients with ovarian cancer undergoing interval cytoreductive surgery; however, this approach was feasible only for low-complexity standard cytoreductive procedures [2]. Moreover, a recent meta-analysis revealed that the minimally invasive approach resulted in less estimated blood loss and a shorter hospital stay, but the authors failed to clarify the oncological safety of the technique, and rates of disease recurrence via a sub-analysis based on stage or histologic type [3]. Another meta-analysis showed that complete cytoreduction could be achieved in 74.5% of patients in the minimally invasive surgery group compared to 53.10% in the laparotomy group. Questions could be raised regarding potential patient selection bias in the minimally invasive group since some individuals demonstrated a complete clinical response to chemotherapy and lower tumor loads on diagnostic laparoscopy [4].

The well-designed Carbajal-Mamani et al. [1] study is similar and focused on 57 patients with ovarian cancer who underwent robotic interval cytoreductive surgery. Eighty-two percent achieved complete cytoreduction [5]. This study showed that the robotic approach did not adversely affect overall survival. The median survival in the pre-robotic era was 37.9 months versus 42.8 months in the robotic era. Progression-free survival was 11.9 months in the pre-robotic era versus 16.5 months in the robotic era group. The conversion rate was 10.5% and no port-site metastases were described.

Traditionally, debulking surgery is performed via laparotomy. However, patients with a complete response to neoadjuvant chemotherapy may achieve complete cytoreduction with less-invasive surgery and may, therefore, be selected for minimally invasive techniques. We agree that complete cytoreductive surgery, using a robotic approach, is safe and feasible in these patients when performed by highly trained gynecological oncologists in selected tertiary care centers. However, concerns that require further clarification may include intra-operative spillage, port-site metastases, suboptimal cytoreduction in cases of upper abdominal disease, and the adequacy of lymph node dissection, bowel surgery,

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diaphragmatic stripping, splenectomy, or widespread upper abdominal surgery.

Based on the encouraging data of the above-mentioned studies, well-powered multicenter randomized trials should be considered. Such trials would overcome the limitations inherent to retrospective single-center findings. However, based on the recent negative results of Laparoscopic Approach to Cervical Cancer (LACC) trial, it is questionable if and when the Gynecological Oncology Society would proceed with such an effort.

Conflict of interest

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

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Review Article

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Obstetrics & Gynecology Science

Sonographic measure techniques of fetal penile length

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Postnatal penile length is a reliable, standardized, and widely used marker for the diagnosis of genitourinary pathology, as well as genetic and hormonal disorders. In contrast, prenatal diagnosis has not been developed equally and there is a lack of relevant literature. Our objective is to review the studies on fetal penile length, and apply findings to clinical practice. Although the most used technique is the outer penile length, there is no consensus regarding the appropriate technique for prenatal measurement. Several reports have provided reference data with high correlation. However, important issues like poor correlation with post-natal measures or presence of confounding variables are still present. Diagnosis of both a micropenis and macropenis can indicate related pathologies, and this information may benefit parental counseling and facilitate fetal management. Therefore, it is necessary to carry out prospective studies that provide reliable normative data.

Keywords: Fetal ultrasonography; Micropenis; Hypospadias; Fetal therapy; Genital system

Introduction

For more than 80 years, systematic measurement of penile length has been done in newborns [1]. This reliable measure is the basis for the diagnosis of micropenis, a medical condition that in turn is a marker for many genetic syndromes, endocrinological disorders or structural malformations, which may require more complex studies, hormonal treatments or corrective surgeries [2]. Given its importance, all the information regarding measurement techniques, normative data, spectrum of anomalies, associated pathologies as well as clinical management has been developed for decades [3]. In contrast, prenatal diagnosis has not been developed equally and there is a lack of an equivalent source of knowledge. Therefore, our ability to make a diagnosis of micropenis and its related pathologies using this method is much more limited. Our objective was to review the existing literature on fetal penile length, focusing on measurement technique, practical challenges and limitations, and prenatal counseling.

Embryology

Typical development of the male external genitalia requires the presence of a normal Y chromosome, an intact fetal hormonal axis, and correct placentation that provides the necessary stimuli to operationalize the whole system. An aberration in any of these variables may cause genital anomalies.

During the initial stages of organogenesis, 3 basic structures are formed: bipotential gonads, genital passages, and indistinct external genitalia including the phallus and genital folds. Stimulation provided by the placental human chorionic gonadotropin causes the Leydig cells to produce testosterone as early as the 7th week of gestation [4]. The influence of this androgen results in sexual differentiation, due to which the phallus elongates to become a penis; genital folds fuse to form the scrotum, and the bipotential gonads form the testicles [5]. Placental function continues until the 14–15th

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week of gestation, after which the production of testosterone becomes dependent on the stimulation of the fetal luteinizing hormone. External genitalia are completely formed by the 17–18th week [6], and continues to grow regularly until birth of the fetus [7].

Anomalies in penile length

1. Micropenis

Micropenis is defined as a penile length less than 2.5 standard deviations (SDs) from the mean [8]. This condition is distinguishable from other similar anomalies such as the small penis (above 2.5 SDs), penis agenesis, and buried penis. The last case is defined as a normal-sized penis surrounded by prepubic tissues that interfere with its measurement [9].

All these conditions indicate a wide variety of pathologies. We included alterations in urethral development, placental insufficiency, and the alterations of the hormonal axis or genetic syndromes. Urethral alterations are frequently associated with anomalies of the corpus spongiosum and/or corpora cavernosa that lead to an abnormal curvature of the penis, interfering in the measurement and giving a value lower than the real one [10]. Poor placentation can lead to an inadequate secretion of testosterone, causing the abnormal development of the genitals. There is also an association between delayed intrauterine growth and male genital anomalies [11]. In the series by Nemec et al. [12], all fetuses affected by CIR showed significant penile shortening. Regarding hormonal or genetic abnormalities, an alteration in the size of the penis can be the clinical manifestation of fetal endocrinopathies or genetic syndromes. Winter and Baraitser [13] reported in their genetic database a total of 369 syndromes, wherein the main finding was a micropenis.

2. Macropenis

Unlike the micropenis, there is no standardized term for a penis that is pathologically larger than normal, which also indicates possible pathologies. Terms such as "macropenis", "megapenis", or "enlarged penis" have been used. Additionally, there is no consensus regarding a cutoff point in reference tables. However, these cases do occur and indicate fetal pathology, as in the case of congenital megalourethra or congenital adrenal hyperplasia.

Congenital megalourethra is a rare urogenital malformation characterized by dilatation of the urethra due to defects in the anterior urethral valves or hypoplasia of the corpora cavernosa. The review by Moaddab et al. [14] shows that, to date, approximately 50 cases have been published in literature Ultrasound shows a macropenis with an irregular dilation of the urethra, accompanied by other signs of urinary obstruction such as a megacystis, bilateral hydronephrosis, and keyhole sign. The prognosis can be severe, with severe pulmonary hypoplasia and renal impairment. However, it can resolve spontaneously in up to 10% of cases and is susceptible to fetal therapy [15].

Congenital adrenal hyperplasia is a disorder of the adrenal gland due to an enzymatic deficiency, and is characterized by a deficiency of cortisol and an excess of androgens. It's clinical presentation occurs over a broad spectrum with a classical form that can trigger a potentially lethal crisis in the first hours of life [16]. Excessive androgens are the primary cause of ambiguous genitalia in female fetuses, which is why the study of the hormonal profile in amniotic fluid is indicated [17]. In the case of male fetuses, Merke and Bornstein [18] describe in their review that a macropenis is a possible consequence, and include a photograph of a newborn aged 7–10 days with an enlarged penis. Despite this evidence, there are no fetal cases published to date.

Measurement technique

1. Postnatal

The methodology for measuring penile length during postnatal examination is fully established. First reports date back to 80 years ago, and since then there have been hardly any changes [1].

The main issue in measuring the penis in newborns and infants is the impossibility of getting an erect length, so instead the so-called stretched penis length or stretched penile length (SPL) is used [19]. It should be measured from the base of the penis at the pubic symphysis to the tip of the glans, gently stretching it to its maximum resistance, depressing the adjacent pubic fat, and measuring the entire dorsal aspect of the penis [20]. As with most anthropometric measures, there may be several confounding factors in the measurement. These are due to the extensibility and elasticity of the penis, so its length can vary significantly in response to thermal, tactile, and environmental changes [1].

2. Prenatal

In comparison to postnatal measurement, there is no consensus regarding the techniques, measurements, and values for prenatal measurement [21]. Although we cannot make a physical examination like pediatricians, obstetric ultrasound allows the detailed evaluation of the male genitalia, including the size of the penis, the size of the scrotum, and the size and location of the testicles [22]. It is possible to measure variables of the penis such as its length, width, and diameter, as well as that of the comprising structures such as the corpus cavernosum, corpus spongiosum, and glans [23].

The most important question when it comes to measuring fetal penile length seems to be the features that should be used for measurement. Since the beginning, the classic measurement has been from the tip of the glans to the edge of the scrotum, also called the outer penile length (OPL) [12]. It is the measurement of the visible part, which is the simplest and most feasible to obtain. The vast majority of published studies use this measure to create their reference tables [12,21,24-27]. Its main drawback is that it is a partial measurement of the penis, which does not correspond to the postnatal measurement that accounts for the whole length. It can also lead to error in situations in which the fetal penis is normal in length but hidden by prepubic tissues such as the buried penis, penis palmatus, and in the presence of hernias or hydroceles [28].

Other authors have reported measurement strategies using alternative features. The length of the fetal penis has been measured from the tip of the glans to the pubic symphysis [12], to the proximal edge of the corpus cavernosum [29], and to the proximal edge of the corpus spongiosum [23]. Although correlation between OPL and these measures of "total penile length" was high and authors provided instructions for simplifying these measurements, no later work has repeated these methods. In fact, some authors have criticized these measurements as "impractical" due to the difficulty in correctly identifying said structures [24].

Technical issues such as angle of visualization, timing, frequency of measurement, and imaging modalities have barely been addressed. Most publications work with ultrasound using an axial plane; as explained by Johnson and Maxwell [26] a transducer that was angulated was used so that the penis was as horizontal as possible, allowing a clear view for measurement. On the other hand, Nemec et al. [12] criticized the use of axial and coronal planes since they did not allow sufficient visualization of the penis due to its curvature. Instead, the author recommended the measurement in a sagittal plane. It is noteworthy that his work on measurements of fetal penises is based on magnetic resonance imaging, rather than ultrasound [12].

There may be several confounding factors that affect the measurement such as the filling state of the bladder or fetal erections. However, Johnson and Maxwell [26] reported no significant differences between measurement with a full or empty bladder. In case of fetal erection, it is a phenomenon that has not been studied in detail. It is known that fetal penises can have erections due to factors such as changes in blood flow or contraction of the pelvic muscles [30], and that it is a relatively frequent finding in the third trimester to observe an average of 1 to 3 erections hourly [23] No work has specified whether the measurements in their references tables were made with relaxed or erect fetal penises, and the scope of bias that this introduces is unknown.

Reference tables

1. Postnatal tables

Postnatal reference tables have been fully consolidated over decades of research, starting from the works of Schonfield and Beebe [1] in the 1940s to the Feldman and Smith [3] regulations in the 1970s. The average SPL at birth is 35 mm and cutoff point for diagnosis of micropenis is a difference of more than 2.5 SDs or 25 mm [8]. In recent years, efforts have focused on individualizing normative data based on ethnicity due to important differences attributable to the place of birth [31-35] The nomogram of fetal length in Iranian neonates proposed by Soheilipour et al. [36], for example, shows a mean SPL of 22.48 mm at term, standing below the cutoff for a micropenis, as previously described.

Other equally important factors would be the gestational age at birth and the individual anthropometric measurements themselves. Penis growth closely follows the growth pattern of other measures such as body length, foot length, and neonatal weight ; the length of the penis can be predicted based on foot length and neonatal weight taken jointly [37]. As with other growth curve studies, the focus in the future would on making more individualized predictions instead of reference populations tables.

2. Prenatal tables

1) Penile length measurements using ultrasound

Reference tables published to date are shown in Table 1. Mean penile length calculated using gestational age is shown in Fig. 1 [12,21,23-27,29].

The studies that have followed the OPL methodology, which represent more than half of all reports, show a very high correlation in the results. On the 20th week of gestation, for example, the maximum range of difference between means is less than 1 mm, from average mean of 7.27 mm to 8.25 mm average mean. As the gestational age increases, so does the variability. The maximum difference at the 37th week of gestation is 6 mm, between average mean of 18.9 mm and 25 mm. Correlation observed between the OPL measurement and gestational age is high in all cases. We also note that the majority of studies report end at the threshold of 25 mm, which is considered the traditional cut-off point for postnatal diagnosis of micropenis [8]. This reinforces the idea that OPL measurement, even if it is the most practical, does not represent the total length of the postnatal penis. However, direct measures in anatomic studies of Shen et al. [6] seem to correlate with OPL curves, challenging the previous hypothesis.

The series by Perlitz et al. [29], Vuillard et al. [23], and the second report by Nemec et al. [12] are not based on the OPL measure, but rather use alternative techniques. At first glance the results of Nemec et al. [12] come closest to reference measurements, since both are based on the measurement from the tip of the penis to the edge of the pubic symphysis. Measurement of the corpora cavernosum or corpus spongiosum, on the other hand, use very specific structures of the penis and do not seem to be useful for correlation with SPL.

2) Challenges and limitations

Correlation between prenatal and postnatal measures

Prenatal ultrasound measurements and direct postnatal measurement using SPL study the same parameter but utilize different approaches and methodologies. To date, only one work has made the comparison between pre and postnatal measures [38]. With a small sample of 46 male fetuses and using the OPL measure technique, penile lengths were

Table 1. Measurement of fetal penile length

Population	Size study	Gestational age (wk)	Measurement technique	Year	Study group	Mean at each week (mm)					
						15	20	25	30	35	40
Turkey	179	17–37	OPL	2016	Akpinar et al. [24]	-	7.9	10.7	13.5	17.0	-
Israel	104	22–36	OPL	2012	Danon et al. [21]	-	-	10.9	14.7	18.0	-
US	494	14–41	OPL	2003	Pinette et al. [25]	-	7.3	10.8	14.6	18.6	23.1
Israel	419	14–38	OPL	2001	Zalel et al. [9]	4.3	7.2	10.9	15.26	20.3	-
UK	95	16–38	OPL	2000	Johnson and Maxwell [26]	-	8.2	11.9	16.66	22.4	-
Austria	194	18–34	OPL, total penile length	2012	Nemec et al. [12]	-	7.7	9.9	14.13	-	-
						-	18.2	23.9	33.13	-	-
France	486	18–40	OPL, corpus spongiosum length	2011	Vuillard et al. [23]	-	7.7	12.4	17.1	21.8	26.6
						-	19.4	31.3	43.2	55.1	67.1
Israel	242	14–35	Corpus cavernosum length	2011	Perlitz et al. [29]	8.1	18.6	26.5	38.0	50.2	-
Australia	188	24–36	SPL	1998	Tuladhar et al. [27]	-	-	17.5	25.5	33.5	-
Iran	587	30–41	SPL	2018	Soheilipour et al. [36]	-	-	-	18.0	21.0	25.9
US	-	8–22	Anatomic study	2018	Shen et al. [6]	3.9	8.0	-	-	-	-

OPL, outer penile length; SPL, stretched penile length.

compared both prenatally and postnatally. The result was a significant although weak correlation between pre and postnatal length, with a 0.32 coefficient of determination.

Fig. 1 shows the early measurements in the anatomic studies of Shen et al. [6] as well as the prenatal cutoff points, suggesting the real normative data should be a curve that met both scales. Further, different ultrasound measurements also shown in this figure do not resolve this problem [6,8].

The inclusion of 2 reference tables of preterm and term newborns further complicates the situation [27,36]. Tuladhar et al. [27] show a parallel curve to the OPL series, with a maximum difference of approximately 10 mm that remains constant throughout all gestational ages, finishing at the 36th week of gestation with a mean of 34.7 mm, which is very close to the postnatal mean in full-term infants at 35 mm [8]. However, Soheilipour et al. [36] show a similar curve to that of the OPL series, finishing at term with a mean of 22.9 mm, considered as a micropenis. The first series corresponds to a western population, whereas the second one is for an Iranian population. Similarly, it should be considered if the data obtained for preterm newborns is biased due to premature birth.

Lower limit of growth curve

Comparison of the lower limit is difficult due to the heterogeneity of units of measurement and results. Ideally, the lower limit could be set at the 2.5 SDs used to diagnose a micropenis during the postnatal period. However, most of the studies use other references, including SDs, interquartile range, and the 2.5, 3.5, and 10 percentiles. Among those works that have used SDs, small sized samples have provided anomalous results and distorted curves. Only Pinette et al.

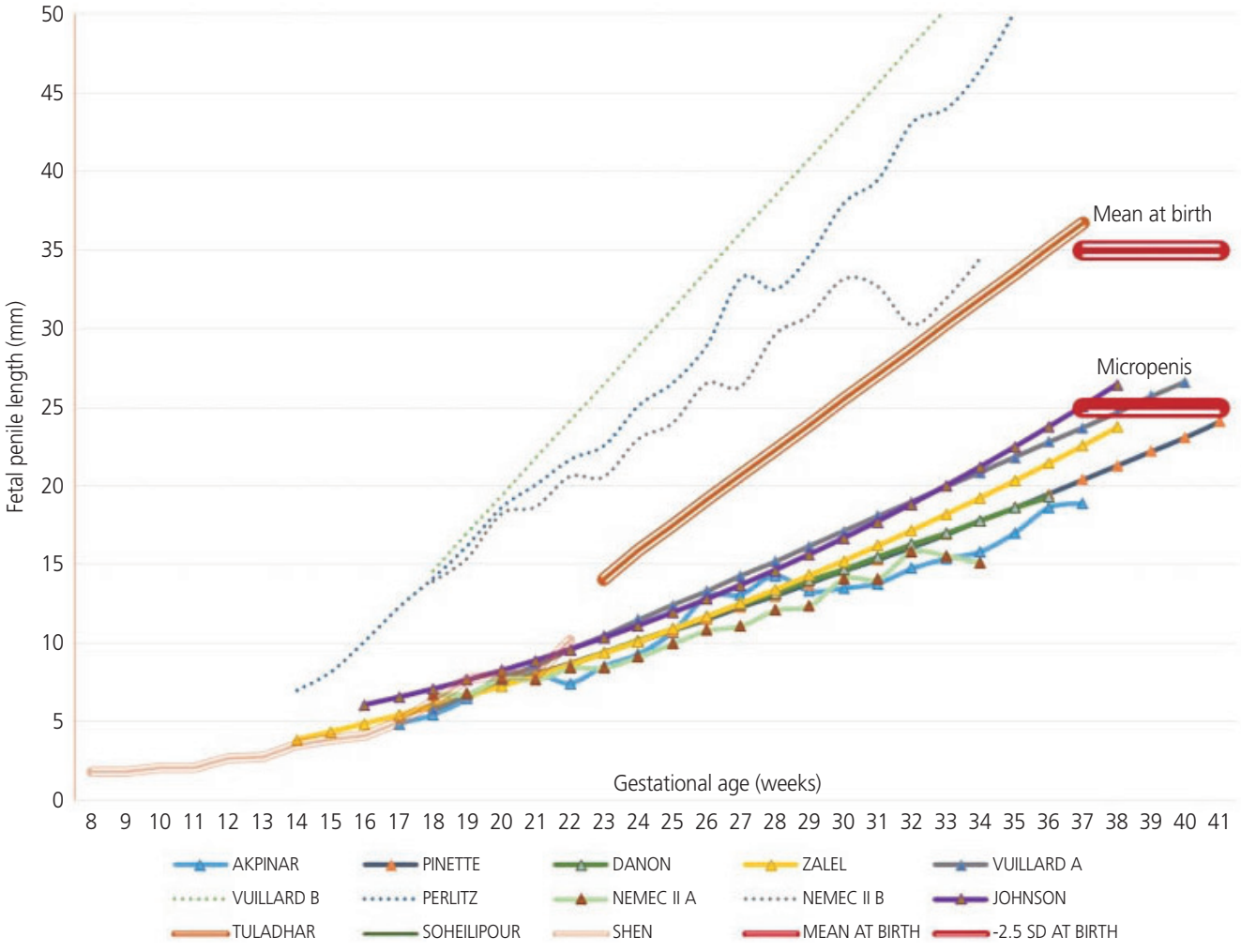

Fig. 1. Mean fetal penile length according to gestational age and study.

[25], which uses the largest sample, has provided a reliable lower limit based on the SDs.

Size of the samples

No study has exceeded 500 cases. Study designs are not based on cohorts that are measured weekly, and rather are retrospective studies in which measurements are added based on gestational age and relationship formulas. Akpinar et al. [24], for example, uses a total sample of 179 fetuses with measurements distributed over 21 weeks. The result is that half of the calculated means are based on measurements of 6 fetuses or less. Although the results of most of the references tables are similar and appear to be reliable, it would be necessary to carry out prospective studies with a large cohort, wherein the measurements were longitudinal for each fetus.

Gestational age

Most studies start measuring in the second trimester, at which time fetal sex can be determined with certainty. The work of Zalel et al. [9] began early, with measurements in the 14th week of gestation. Most studies finalize the measurements before reaching term, and only the works of Akpinar et al. [24] and Pinette et al. [25] continued until the 40th week of gestation.

Ethnic group

This is an important factor when establishing reference values, with significant differences between groups such as Caucasians and Asians [8,31,32]. However, there is no ethnic diversity in the samples used to date, with the exception of Akpinar et al. [24], who used a sample population of Turkish origin. The remaining studies are based on Caucasian populations from western countries and Israel.

Other measurements

The use of a single measure for the assessment of the penis poses problems such as the distinction between a small penis or a prominent clitoris [25]. Various complementary measurements have been proposed such as width, diameter, or even curvature of the penis, associated with the presence of hypospadias [10]. Katorza et al. [39] proposed the dynamic use of ultrasound and Doppler mode, which would allow the observation of male urination by locating the urine stream at the tip of the penis, ruling out the diagnosis of hypospadias.

The most promising measure would be the measurement of the width of the penis, introduced by Danon et al. [21]. Authors describe it as a measurement that is easily performed, with good correlation with length; they also provided the first reference table [21]. Akpinar et al. [24] replicate the same methodology with similar results. The main reason for the width measurement is that is provides greater specificity for the micropenis diagnosis, since a micropenis has a normal tubular shape, such that reduced length is accompanied by proportional reduction in width.

Clinical practice

Due to the limitations of the available studies and the current technical challenges, it is not possible to establish normative values or implement the routine measurement of the fetal penis using a mid-trimester ultrasound; the same has been concluded in different international guidelines [40,41]. Nevertheless, we could use the available evidence as guidance or in selected cases (Fig. 2). In these situations, the OPL measurement technique may be used, measuring the penis from the tip of the glans to the edge of the scrotum. The axial plane seems to be the most used and it would be appropriate for measuring the penis as horizontal as possible. As cutoff points, average length of the penis between 19 weeks and 22 weeks would be 7 to 10 mm, and a micropenis diagnosis would be below 4 mm. There is no consensus on the measure for diagnosing a macropenis. The corpora cavernosa and/or corpus spongiosum could be measured according to the Vuillard et al. [23] or Perlitz et al. [29] technique, respectively, to rule out a normal-sized penis buried between prepubic tissues. Penile width could also be measured using the Danon et al. [21] reference table.

A diagnosis of macropenis would mainly suggest a congenital megalourethra, so an anatomical scan of the urinary system should be done to observe signs such as a dilated and irregular urethra, megacystis, hydronephrosis, and anhydramnios. Genetic amniocentesis is a mandatory test prior to being a candidate for fetal therapy, even though irreversible damage and poor performance of fetal cystoscopy results in a high rate of termination of pregnancy [42]. The finding of a large penis with normal morphology and no other ana-

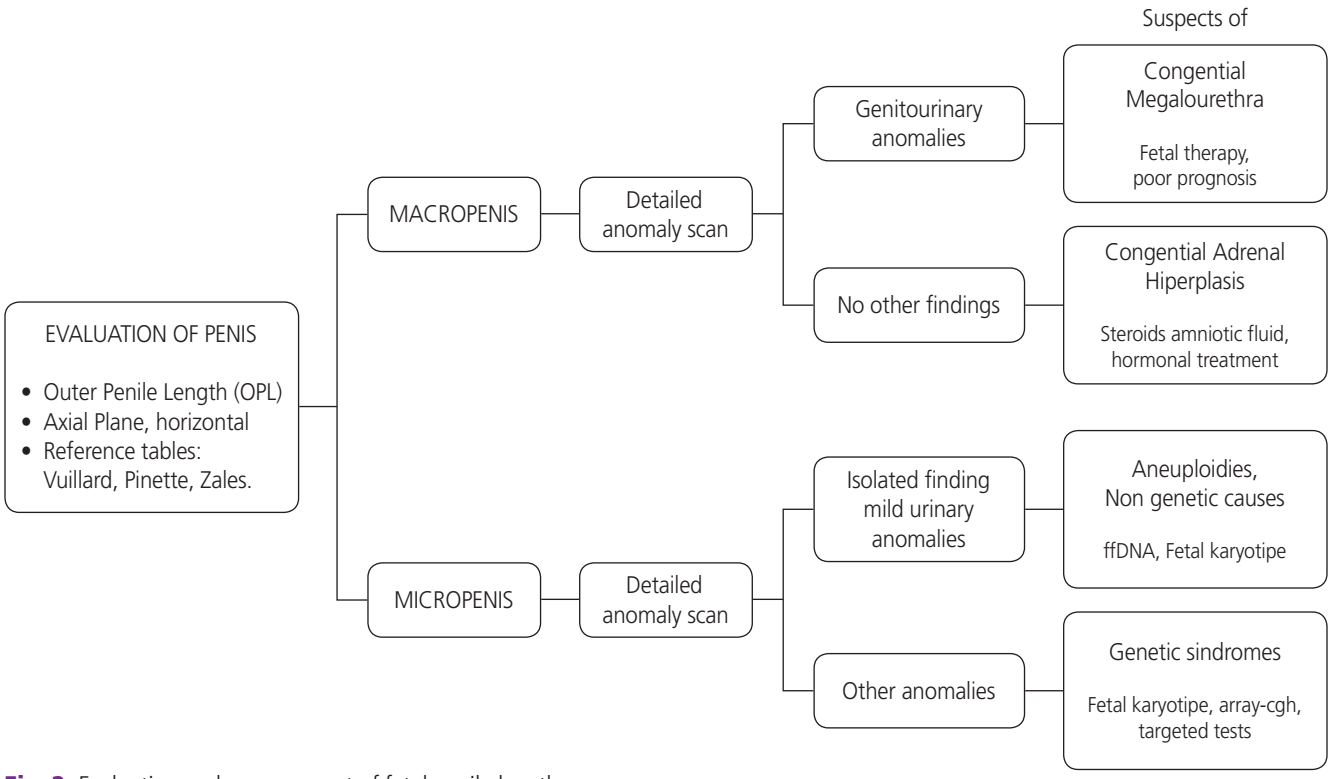

Fig. 2. Evaluation and management of fetal penile length.

tomical findings in the scan could guide us to a diagnosis of congenital adrenal hyperplasia. In this situation, amniocentesis for fetal karyotyping should be considered, as well as a study of steroid metabolites in the amniotic fluid [17]. This diagnosis requires the evaluation of an endocrinologist, use of hormonal treatment, and such diagnoses in severe cases can help prevent sudden death in the first days of postnatal life [43].

The diagnosis of micropenis is related to a much greater range of pathologies, as we have previously seen. In this case, the diagnostic work-up proposed by Pajkrt et al. [17] that includes a detailed scan and the use of genetic tests would be useful. The first step includes a detailed anomaly scan, focusing on the urinary system, and an assessment of fetal growth. The second step consists of the selection of a genetic test based on previous findings, to determine if the isolated findings or mild urinary anomalies are more related to chromosomal abnormalities or non-genetic pathologies; the finding of various anatomical abnormalities could indicate several genetic syndromes [17].

Depending on the results, it may be necessary to refer the patient to urology, endocrinology, clinical geneticist, or even to engage a multidisciplinary team to manage disorders of sexual development [44]. Isolated genitourinary anomalies such as anterior hypospadias would result in an early consultation with a pediatric urologist, facilitating a correct postnatal follow-up plan [45]. Endocrinopathies such as congenital adrenal hyperplasia could require a change in the birth plan, or reference to a specialized center to enable access to multidisciplinary teams in case of emergency issues such as gender of rearing [46]. Prenatal interventions like hormonal manipulation or fetoscopy for lower urinary tract obstructions are not being used systematically, and are currently limited to investigations [47]. The diagnosis of genetic syndromes with high morbidity and mortality could lead to parents choosing to terminate the pregnancy [48]. Therefore, incorrect diagnosis of the fetal penis length could have important short- and long-term implications.

However, due to poor knowledge in this area of prenatal medicine, it is crucial to be cautious when making a diagnosis and providing counseling information. Such diagnoses can cause considerable anxiety in parents, and may be a cause for the termination of pregnancy [38].

Conclusion

Postnatal penile length is a standardized, widely used, and a reliable marker for the diagnosis of genitourinary pathology, as well as genetic and hormonal disorders. Although the most studied technique is the OPL, there is no consensus regarding the technique appropriate for prenatal measurement. Even though it is not considered normative data, we can assume OPL cutoff points in the second trimester, as seen using an ultrasound, to be an average of 7–10 mm with a lower limit of 4 mm. The presence of both a micropenis and a macropenis can be associated with relevant pathologies. It is necessary to carry out prospective studies with sufficient sample sizes and using a common measurement system, so as to create more comprehensive reference tables.

Conflict of interest

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

Ethical approval

The study was approved by the Hospital General Santa Lucia Ethical Committee 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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Role of potassium channels in female reproductive system

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Potassium channels are widely expressed in most types of cells in living organisms and regulate the functions of a variety of organs, including kidneys, neurons, cardiovascular organs, and pancreas among others. However, the functional roles of potassium channels in the reproductive system is less understood. This mini-review provides information about the localization and functions of potassium channels in the female reproductive system. Five types of potassium channels, which include inward-rectifying (Kir), voltage-gated (Kv), calcium-activated (K_{Ca}), 2-pore domain (K_{2P}), and rapidly-gating sodium-activated (Slo) potassium channels are expressed in the hypothalamus, ovaries, and uterus. Their functions include the regulation of hormone release and feedback by Kir6.1 and Kir6.2, which are expressed in the luteal granulosa cells and gonadotropin-releasing hormone neurons respectively, and regulate the functioning of the hypothalamus–pituitary–ovarian axis and the production of progesterone. Both channels are regulated by subtypes of the sulfonylurea receptor (SUR), Kir6.1/SUR2B and Kir6.2/SUR1. Kv and Slo2.1 affect the transition from uterine quiescence in late pregnancy to the state of strong myometrial contractions in labor. Intermediate- and small-conductance K_{Ca} modulate the vasodilatation of the placental chorionic plate resistance arteries via the secretion of nitric oxide and endothelium-derived hyperpolarizing factors. Treatment with specific channel activators and inhibitors provides information relevant for clinical use that could help alter the functions of the female reproductive system.

Keywords: Potassium channels; Gonadal steroid hormones; Ovary; Uterus; Placenta

Introduction

The maintenance of extracellular potassium concentration within a narrow range is vital for numerous cellular functions, particularly for maintaining the electrical excitability of heart and muscle cells [1]. The plasma potassium concentration is usually maintained within narrow limits (typically, 3.5 to 5.0 mmol/L) through multiple mechanisms [2]. The functions of potassium channels are critical for urinary excretion of potassium ions. The long-term maintenance of potassium homeostasis is achieved by alterations in the renal excretion of potassium in response to variations in intake [3]. Fig. 1 shows that 90% of potassium that is derived from dietary intake is excreted by the kidney (up to 80 mEq/day intake vs. 72 mEq/day in the urine) to maintain normal potassium concentrations in the extracellular fluid (up to 70 mEq/day in adults) [1]. In Korea, the recommended dietary intake of potassium in individuals above 12 yr is 3,500 mg; the World Health Organization recommends a similar intake. Intracellular fluids contain approximately 98%, or rather 3,600 mmol (140 g) of the potassium ions in the body, while the other 2% present in the extracellular compartment is usually maintained within a narrow range (3.8–4.5 mEq/L) in serum [4,5]. Since our ancestors could easily obtain potassium-rich fruit and vegetables, the recommended content of 15 g/day was

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easily exceeded. There are several types of highly evolved potassium channels in the kidney [4]. The balance in potassium ion concentration across the cell membrane is essential for maintaining the resting membrane potential and signal conduction in nerve and muscle cells via the repolarization of action potential. Potassium channels are pore-forming transmembrane (TM) proteins and are classified into 4 major families: calcium-activated, inward-rectifier, voltage-gated, and 2-pore-domain potassium channels [6]. Certain researchers classify ATP-sensitive potassium channels as a fifth family independent of inward-rectifier potassium channels [7,8].

Potassium channels consist of 2 subunits: primary α-subunits and auxiliary β-subunits. The pore-forming subunits have 2–6 TM domains and the sensitivity of the channels to calcium, ATP, voltage, and oxygen among others is conferred by the α-subunits. Voltage-gated potassium channels (K_V channels) comprise of 6 TM domains, calcium-activated potassium channels (BK_{Ca} channels) have 6 or 7 TM segments, and inward-rectifying potassium channels have 2 TM segments as α-subunits [9]. In contrast, β-subunits regulate the activities of the channels; *e.g.*, SUR-subunits in Kir6.X channels are involved in the regulation of insulin secretion. The various types of potassium channels are involved in controlling different phases of the action potential—while the majority of K_V channels allow K^+ ion efflux when the membrane is depolarized, Kir channels conduct K^+ ions during hyperpolarization [10]. Table 1 outlines the details of calcium-activated (K_{Ca}) and inward-rectifying (Kir) K_{ATP} potassium channel families and Table 2 outlines the details of voltage-gated (K_v) and the 2-pore domain (K_{2P}) potassium channel families. The well-known activators and inhibitors are also listed in the tables [11].

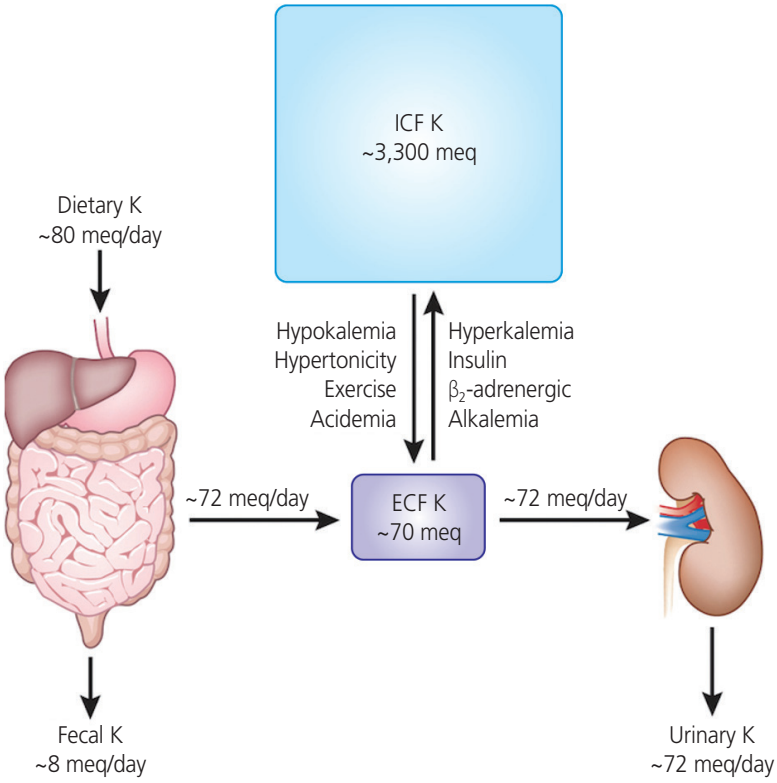

Fig. 1. The amounts of potassium of daily dietary intake, distribution in intracellular fluid (ICF) and extracelluler fluid (ECF), and daily fecal and urinary excretion. Most dietary potassium is excreted by the kidney (up to 80 mEq/day intake vs. up to 72 mEq/day in the urine) to maintain normal potassium concentrations. Approximately 98% of potassium is present in the ICF (up to 3,300 mEq), while the other 2% present in the extracellular compartment (up to 70 mEq) which is usually maintained within a narrow range (3.8–4.5 mEq/L) in serum. Hypokalemia has many causes including: excessive potassium loss due to diarrhea, excessive sweating from exercise, abuse of alcohol, some diuretics, or laxatives. In contrast, hyperkalemia can be occurred due to acute kidney failure, chronic kidney disease, medication such as angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, or beta blockers, and dehydration or excessive potassium supplements.

Notably, the potassium channels participate not only in the regulation of membrane potential but also in various cellular functions, including volume regulation, cell proliferation, cell migration, angiogenesis, and apoptosis [6]. This mini-review focuses on the multiple potassium channels associated with the cells of the female reproductive system, and discusses their action on the hypothalamus–pituitary–ovarian axis for the regulation of progesterone production from granulosa

Table 1. Summary of calcium activated (K_{Ca}) and inwardly-rectifying (Kir) K_{ATP} potassium channel families, activators and inhibitors

K_{ca} channels (8 isoforms & 5 subfamilies): BK_{Ca}, SK, IK		Kir channels (15 isoforms & 7 subfamiles): K_{ATP}	
HGCN	**IUPHAR**	**HGCN**	**IUPHAR**
KCNMA1	$K_{Ca}1.1$	KCNJ1	Kir1.1
KCNN1-3	$K_{Ca}2.1$–$K_{Ca}2.3$	KCNJ2, J12, J4 & J14	Kir2.1–Kir2.4
KCNN4	$K_{Ca}3.1$	KCNJ3, J6, J9 & J5	Kir3.1–Kir3.4
KCNT1 & T2	$K_{Ca}4.1$ & $K_{Ca}4.2$	KCNJ10 & J15	Kir4.1 & Kir4.2
KCNU1	$K_{Ca}4.1$	KCNJ16	Kir5.1
		KCNJ8 & J11	Kir6.1 & Kir6.2
		KCNJ13	Kir7.1
Channel opener for K_{ca}: NS1619		Channel opener for K_{ir}: Pinacidil, cromakalim, aprikalim (Kir6.x)	
Channel blocker for K_{ca}: Iberiotoxin, apamin, charybdotoxin		Channel blocker for K_{ir}: Tetraethylammonium (TEA), 4-aminopyridine (4-AP), glibenclamide, tolbutamide	

The table is adopted from reference [11].

Table 2. Summary of voltage gated (K_v) and the 2-pore domain (K_{2P}) potassium channel families, activators and inhibitors

K_V channels (42 isoforms & 12 subfamilies)		K_{2P} channels (15 isoforms)	
HGCN	**IUPHAR**	**HGCN**	**IUPHAR**
KCNA1–A0	$K_V1.1$–K_V 1,10	KCNK1	$K_{2P}1.1$
KCNB1–B2	$K_V2.1$ & $K_V2.2$	KCNK2	$K_{2P}2.1$
KCNC1–C4	$K_V3.1$–$K_V3.4$	KCNK3	$K_{2P}3.1$
KCND1–D3	$K_V4.1$–$K_V4.3$	KCNK4	$K_{2P}4.1$
KCNF1	$K_V5.1$	KCNK5	$K_{2P}5.1$
KCNG1–G4	$K_V6.1$–$K_V6.4$	KCNK6	$K_{2P}6.1$
KCNQ1–Q5	$K_V7.1$–$K_V7.5$	KCNK7	$K_{2P}7.1$
KCNV1& V2	$K_V8.1$ & $K_V8.2$	KCNK9	$K_{2P}9.1$
KCNS1–3	$K_V9.1$–$K_V9.3$	KCNK10	$K_{2P}10.1$
KCNH1 & H5	$K_V10.1$ & $K_V10.2$	KCNK12	$K_{2P}12.1$
KCNH2, H6, and H7	$K_V11.1$–$K_V11.3$	KCNK13	$K_{2P}13.1$
KCNH8, H3, and H4	$K_V12.1$–$K_V12.3$	KCNK15	$K_{2P}15.1$
		KCNK16	$K_{2P}16.1$
		KCNK17	$K_{2P}17.1$
		KCNK18	$K_{2P}18.1$
Channel opener for K_V: Correolide ($K_V1.5$), Stromatoxin-1 ($K_V2.1$), Flupirtine (K_V7) PD118057, NS1643 (KCNH)		Channel opener for K_{2P}: Arachidonic acid (TREK-1), volatile anesthetics (isoflurane)	
Channel blocker for K_V: 4-AP (K_V), phrixotoxin-2 (K_V4), Linopirdine (K_V7), dofetilide, E4031, Be-KM1 (KCNH)		Channel blocker for K_{2P}: Fluphenazine, L-methionine (TREK-1), SSRI, antipsychotics (haloperidol)	

The table is adopted from reference [11].

cells (GCs) in the ovary, myometrial relaxation in the uterus during pregnancy, the induction of uterine contractions in labor, and the modulation of human chorionic gonadotropin (hCG) production in the syncytiotrophoblast in the placenta [5,12,13].

Expression and roles of potassium channels in the ovary

The ovary facilitates the production and maintenance of oocytes and supports the secretion of female sex hormones, which is essential for the regulation of puberty and pregnancy and determines the reproductive lifespan [14]. The ovary is protected by 3 outer layers: germinal epithelium, connective tissue capsule, and tunica albuginea. The outer cortex and inner medulla are located inside the ovary. Ovarian follicles at various stages of maturation are present in the ovarian cortex, and oocytes or female germ cells are present in each follicle. From puberty, the development and degeneration of follicles from the primordial follicle stage to the corpus luteum is influenced by follicle-stimulating hormone (FSH). The follicle primarily consists of oocytes and granulosa; progesterone is produced from luteinized GCs and by the corpus luteum in the ovary in the non-pregnant state. Progesterone concentrations peak 7–8 days after ovulation and rapidly reduce along with the degeneration of the corpus luteum. Progesterone inhibits the synthesis of gonadotropin-releasing hormone (GnRH) to prevent the maturation of other follicles and prepares the body for a possible pregnancy. If fertilization does not occur, the corpus luteum degenerates and the follicle is excreted via menstrual bleeding [15]. The ovulation of mature follicles in the ovary is induced by a preovulatory surge in the levels of luteinizing hormone (LH), and subsequently, estrogen is secreted by the GCs of the ovarian follicles and the corpus luteum. The concentrations of LH and FSH secreted by the pituitary gland and estradiol secreted by the follicles peak during ovulation in the menstrual cycle [15].

In the ovary, the K_{ATP} potassium channels and the BK_{Ca} channels participate in the regulation of progesterone secretion, and the intracellular potassium and calcium concentrations affect the secretion of progesterone [16]. BK_{Ca} channels induce the repolarization of the plasma membrane and play an important role in the cellular response depending on the Ca^{2+} concentration. The acetylcholinergic agonist carbachol and oxytocin increase intracellular Ca^{2+} concentration in cultured human granulosa-lutein cells. The intracellular calcium signal affects progesterone release in lutein-GCs via BK_{Ca} [16-18]. hCG stimulates the release of oxytocin, which, along with acetylcholine, acts as an ovarian signaling molecule. Kunz et al. [16] reported that the activation of the BK_{Ca} channels in GCs is generally necessary for endocrine function, and BK_{Ca} channel blockade using iberiotoxin causes a marked reduction in hCG-stimulated progesterone secretion. Notably, hCG does not play a role in BK_{Ca} activation; acetylcholine and oxytocin, which are local neuroendocrine substances released by GCs, participate in this process by causing a transient increase in the intracellular Ca^{2+} concentrations [19]. In summary, the activities of the BK_{Ca} channel in GCs is mediated by intra-ovarian signaling involving neurotransmitters (*e.g.*, acetylcholine) and peptide hormones (e.*g.*, oxytocin). Traut et al. [20] reported the expression (*in vitro* and *ex vivo*) of several classes of K_{Ca} channels (IK, SK, and BK) in human GCs, which participate in gonadotropin-stimulated sex steroid hormone production.

In addition to the intracellular calcium levels, the intracellular potassium levels also affect progesterone secretion from luteal cells [21]. Typical K_{ATP} channels consist of an inward-rectifier K^+ ionophore (Kir6.x) and a sulfonylurea receptor (SURx); there are 2 Kir6.x isoforms (Kir6.1 and 6.2), and 3 SUR isoforms (SUR1, SUR2A, and SUR2B). Although 6 combinations of Kir6.x and SURx can be formed, only 4 types of K_{ATP} channel have been reported: those found in pancreas β-cells (SUR1/Kir6.2), cardiac and skeletal muscles (SUR2A/Kir6.2), smooth muscles (SUR2B/Kir6.2), and vascular smooth muscles (SUR2B/Kir6.1) [22]. Of these, only Kir6.1/SUR2B is detected in the corpus luteum of the ovary and myometrium of the rat, while the placenta expresses Kir6.1 with SUR1 and SUR2B [23]. These ovarian K_{ATP} channels are involved in the production of progesterone in luteinized GCs [5]. The K_{ATP} inhibitor glibenclamide triggers the depolarization of the plasma membrane induced by the blockage of the K_{ATP} channels; it reduces hCG-induced progesterone production in ovarian GCs [5]. However, the exact underlying mechanism remains unclear. Other ion channels, including $K_V4.2$ (KCND2) and L-type and T-type voltage-dependent Ca^{2+} channels and a K_{ATP}-independent progesterone production process are also involved in hormone regulation [24,25]. Therefore, progesterone production is likely regulated via complex physiological processes.

Expression and role of K_{ATP} channels in pulsatile secretion of gonadotropin-releasing hormone in the hypothalamus

Progesterone secretion is mediated by the Kir6.1/SUR2B K_{ATP} channel subtype in the ovary. K_{ATP} channels are also involved in modulating the excitability of GnRH neurons in an estrogen-sensitive manner (Fig. 2). The pancreatic β-cell subtype of channels containing Kir6.2/SUR1 subunits is the K_{ATP} channel subtype that is predominantly expressed in GnRH neurons in the hypothalamus [26]. The K_{ATP} channel Kir6.2/SUR1 modulates the pulsatile release of GnRH, and negative feedback from ovarian steroids affects the release of hormones in the hypothalamus and pituitary by altering the activity of the K_{ATP} channels. The Kir6.2 mRNA levels in the preoptic area responds to treatment with both E2 and progesterone. The K_{ATP} channel blocker tolbutamide enhances the frequency of pulsatile GnRH release under steroid treatment [27]. In contrast, the K_{ATP} channel activator diazoxide prevents the increase in the frequency of the LH pulse. As the Kir6.2/SUR1 subtype has a sulfonylurea receptor, this channel is activated by diazoxide, while it is blocked by sulfonylureas, including glibenclamide and tolbutamide [28]. Zhang et al. [26] reported that the glucose concentration and the presence of glucokinases also influence the excitability of GnRH neurons; whereas GnRH neurons are excited in high glucose concentrations, neuron excitability is inhibited in conditions of low glucose concentrations, such as starvation. Glucokinase

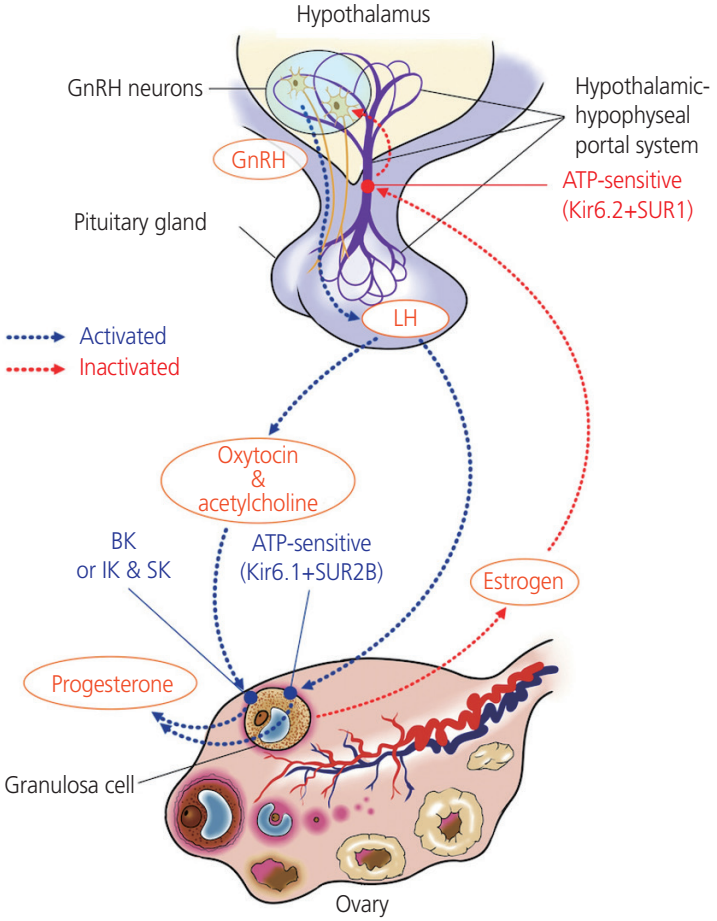

Fig. 2. Involvement potassium channels in hypothallus-pituitary-ovarian axis. While progesterone secretion is mediated by the Kir6.1/SUR2B, K_{ATP} channels subtype in the ovary, Kir6.2/SUR1 subunits is predominantly expressed in gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus. The K_{ATP} channel Kir6.2/SUR1 modulates the pulsatile release of GnRH, and activity of the K_{ATP} channels responds to negative feedback from ovarian steroids-E2 and progesterone. The acetylcholine and oxytocin increase intracellular Ca^{2+} concentration in lutein-granulosa cells, the intracellular calcium signal affects progesterone release via $BK_{Ca.}$ human chorionic gonadotropin stimulates the release of oxytocin, which, along with acetylcholine, acts as an ovarian signaling molecule. LH, luteinizing hormone.

facilitates the phosphorylation of glucose to glucose-6-phosphate and is expressed in GnRH neurons. While Kir6.2 and SUR1 transcripts were found to be expressed in all neuronal cells, 66.7% of neuronal pooled cells expressed glucokinase mRNA. This suggests that glucokinase plays a role in regulating GnRH release via the K_{ATP} channels [26].

Potassium channels in GnRH neurons form a rich substrate for the modulation of activity. As 2 main components of voltage-gated potassium channel currents in cells, both the slowly inactivating potassium current (I_K) and the fast inactivating A-type potassium current (I_A) are generated in GnRH neurons. In recent studies, estradiol was shown to suppress both I_K and I_A, these voltage-gated potassium conductance are largely responsible for the overall excitability and discharge activity in GnRH neurons [29-31]. The expression of genes encoding potassium channel proteins is altered in GnRH neurons at different stages of estrus in mice [32].

Expression and role of potassium channels in the uterus

The human uterus expands drastically during pregnancy owing to uterine smooth muscle hypertrophy, and the myometrium is one of the strongest uterine muscles that facilitates birth [33,34]. The uterus does not play any major role during pregnancy. Uterine quiescence during pregnancy is necessary for preventing preterm labor. Potassium efflux leads to the repolarization of the plasma membrane, which is responsible for maintaining the resting membrane potential. Inadequate repolarization of smooth muscle cells in the myometrium can lead to aberrant uterine activities, such as dystocia, preterm labor, and post-term labor [12]. The myometrium expresses several types of potassium channels, including BK_{Ca}, K_{ATP}, small-conductance Ca^{2+}-sensitive, voltage-gated, and 2-pore potassium channels [12]. The role of each type of potassium channel in the regulation of basal myometrial contractility and their contribution to molecular expression varies depending on the gestational stage [35].

BK_{Ca} channels play a prominent role in inducing smooth muscle relaxation as they mediate the depolarization of the plasma membrane and contribute to the generation of approximately 35% of the total cell repolarizing potassium current [36]. The potassium current density in term mouse myometrium was observed to decrease significantly. The functional importance of the BK_{Ca} channels decreases as a result of several factors, including a change in surface negative charges, decreased channel density, a positive shift in voltage-activation relation, and reduced sensitivity to calcium ions [24,37]. Furthermore, BK_{Ca} channels are replaced by smaller conductance delayed rectifier channels in the abovementioned period; these remove the noises in the current and generate a smooth current that requires limited rectification [36]. However, there is another explanation for the turnover from uterine quiescence to the state of uterine contraction. First, the sensitivity of BK_{Ca} to the intracellular calcium and voltage levels changes owing to alternative splicing of transcripts of the BK_{Ca} channel, which is accompanied by post-translational modifications as well. Second, protein kinases exert different modulatory effects based on the gestational stage. Third, Ca^{2+} levels increase during pregnancy [37,38]. Notably, the differences in the regulation of channel transcripts implies that different populations of channels exist in the myometrium; this alteration in transcripts during pregnancy may be regulated by sex hormones [21,38]. In particular, the expression of β1-subunit transcripts is partially regulated by estrogen and it is observed to peak in early pregnancy [39]. The regulatory β-subunit helps modulate uterine excitability by exhibiting sensitivity to both Ca^{2+} and voltage during gestation [37].

As BK_{Ca} channels play a prominent role in myometrial contraction, several agents that activate and inhibit these channels, including β-adrenoceptor agonists, relaxin, hCG, and nitric oxide (NO), have been studied based on their role in controlling myometrial contraction [35]. Notably, although the BK_{Ca} channel is the most abundant potassium channel in the myometrium and contributes to the regulation of myometrial functions, it does not play a role in the regulation of basal or uterine relaxation during late pregnancy or labor [35,40]. The BK_{Ca} channels are activated by NS1619, a benzimidazole derivate that promotes the activation of BK_{Ca} channels; the administration of this agent did not significantly affect the contractile activity in human term-pregnant myometrium when oxytocin was secreted [41]. These results suggest that although BK_{Ca} channels play a more significant role than other potassium channels in myometrial relaxation during the non-pregnancy period and in early gestation, the significance of their regulation is lesser in late gestation.

Instead of the BK_{Ca} channel, the K_{ATP}, K_V, and Slo2.1 (rapidly-gating sodium-activated) potassium channels affect the

transition from uterine quiescence in late pregnancy to the state of active contractions that aids the expulsion of the fetus in labor [35,41,42]. The intracellular Na^+, K^+, and Ca^{2+} ions and the ion channels are involved in the regulation of uterine relaxation and contraction. The Kir6.1/SUB2B K_{ATP} channel is the predominant subtype in the myometrium and ovary [43]. The expression patterns of the BK_{Ca} and K_{ATP} channels are similar in each gestational stage, while the expression of Kir6.1 and SUR2B transcripts is significantly higher in the non-pregnant state than in late pregnancy before or during labor [43]. The K_{ATP} channel activator pinacidil inhibits oxytocin-induced uterine contractions (both in terms of amplitude and frequency) in the myometrium in late pregnancy and in non-pregnant women; its effect was attenuated in the laboring myometrium owing to the differences in expression during gestation and labor [41].

The hyperpolarization of the plasma membranes of myometrial cells depends on the K^+ efflux for the maintenance of uterine quiescence, while the depolarization of the membrane potential owing to increased Na^+ influx is essential for countering uterine contractions by myocytes. K_{Na} channels, which are high-conductance K^+ channels activated by Na^+, require high concentrations of intracellular Na^+ instead of Ca^{2+} ions. Recently, Ferreira et al. [42] reported that oxytocin could regulate myometrial smooth muscle cell excitability via the Na^+-activated Slo2.1 K^+ channel. The peptide-hormone oxytocin regulates the transition from uterine quiescence to contraction. Slick, a rapidly-gating sodium-activated potassium channel, induces K^+ efflux and opposes Na^+ influx via NALCN (Na^+ leak channel, non-selective) at low levels of oxytocin [42]. However, when the levels of oxytocin increase at the end of pregnancy and it binds to its receptors, protein kinase C is activated and SLO2.1 expression is inhibited. As a result, after K^+ efflux is reduced, the voltage-dependent Ca^{2+} channels are activated. When Ca^{2+} influx occurs and actin–myosin cross-bridging is initiated, myometrial contractility is augmented [42].

Small-conductance Ca^{2+}-sensitive and voltage-insensitive potassium channels (SK), voltage-gated potassium channels (K_V), and 2-pore potassium channels are also expressed in the myometrium [44,45]. While small-conductance K^+ (SK_{Ca}) channels and intermediate-conductance calcium-activated K^+ (IK_{Ca}) channels are expressed in smooth muscle cells, including those of the myometrium, IK_{Ca} channels are primarily expressed in immune/inflammatory cells. The role of IK_{Ca} channels in the myometrium has not been studied thoroughly [46-48]. Dimethylamine-nitric oxide (DEA/NO) inhibits myometrial contraction via a NO-induced relaxation mechanism. Apamin and scyllatoxin specifically block Ca^{2+}-dependent apamin-sensitive K^+ channels ($SK_{Ca}1$ to $SK_{Ca}3$); the administration of 10 nM apamin or scyllatoxin could completely inhibit the DEA/NO-induced relaxation of the myometrium [45]. This indicates that the participation of the SK_{Ca} channels in NO-induced myometrial relaxation, especially in the overexpression of the $SK_{Ca}3$ isoform, results in uterine dysfunction and delayed parturition [49]. In mice, the expression of the $SK_{Ca}3$ channel decreases from the mid-gestational stages; sensitivity of this channel to apamin also reduces from late gestation. $SK_{Ca}3$ immunoreactivity was also observed in telocytes, formerly referred to as interstitial cells of Cajal or interstitial Cajal-like cells (ICLC), in non-pregnant myometrium, as well as in the glandular and luminal endometrial laminal epithelia in rats [46,50,51]. Although similar ICLC have been observed in rodent and human myometrial tissue, the functional role of these cells in the uterus remains unclear [52].

K_V channels are the most diverse of potassium channels. The α-subunits of voltage-gated K_V channels form the conductance pore and there are 12 classes and 40 types of α-subunits of K_V channels. Voltage-gated K_V channels are blocked by 4-aminopyridine (4-AP) or tetraethylammonium (TEA). The administration of both 1 and 5 mM 4-AP significantly enhanced myometrial contractility in the non-pregnant, as well as mid- and late-pregnant myometrium [35]. These results indicate that voltage-gated K_V channels contribute to uterine quiescence during mid to late pregnancy. Although treatment with TEA and 4-AP did not affect the contraction interval, the duration and amplitude of myometrial contractions were increased in both cases. The blockade response of Kv channels by 4-AP is not mediated by BK_{Ca} channels of the endometrium and nerves in the myometrium [44]. It has been suggested that several different K_V channel subtypes are present in the myometrium of both non-pregnant and term-pregnant mice. Myometrial contractions in non-pregnant mice were induced even at surprisingly low concentrations of 4-AP [53]. The $K_V4.3$ channel is not expressed in term-pregnant tissues. Phrixotoxin-2, which is a $K_V4.2$/ $K_V4.3$ blocker, induced contractions in non-pregnant myometrium, whereas the same was not induced in pregnant myometrium [44]. The genes encoding voltage-

gated K_V channels can be categorized into 9 families, named KCNA to KCNV gene families (Table 2). Two types of K_V channels encoded by members of the KCNQ (K_V7) and KCNH (K_V11) gene families appear to act as key regulators of uterine contractility and may serve as novel therapeutic targets [52]. All isoforms of KCNQ are expressed in non-pregnant mice, with KCNQ1 (Kv7.1) as the dominant form [54]. The KCNQ and KCNE isoforms are expressed in early and late gestational stages in mice as well, the majority of KCNQ isoforms are upregulated in late pregnancy [54]. KCNE is a type of β-subunit associated with voltage-gated KCNQ α-subunits. These findings suggest that instead of facilitating the maintenance uterine quiescence in early pregnancy, these proteins primarily regulate myometrial contractility in the late stages of pregnancy. All KCNQ genes, except KCNQ5, were also observed to be expressed in human term myometrium. Flupirtine and retigabine, which are activators to the KCNQ-encoded K^+ channel (K7), rapidly inhibited spontaneous and oxytocin-mediated myometrial contractility by 40–70% [54]. *Ether-à-go-go*-related genes or ERGs (ERG1–3) are members of the KCNH gene family (KCNH1–3). The ERG-encoded channel blockers dofetilide, E4031, and Be-KM1 increased oxytocin-mediated contractions in mouse myometrium, while the ERG activators PD118057 and NS1643 inhibited spontaneous contraction [55]. In summary, although several types of K_V channels are expressed in uterine myometrium, there are contradictory results with respect to their upregulation or downregulation based on the gestational stage. K7 (KCNQ) and K11 (KCNH) channels play central roles in regulating myometrial activity at term and are possible targets for tocolytic agents in preterm labor [54].

The TWIK-related K^+ (TREK-1) channel is a 2-pore K^+ channel. TREK-1 is a stretch-activated tetraethyl ammonium-insensitive K^+ channel that is sensitive to pH, hypoxia, stretching, temperature, phosphorylation, and NO [56]. This channel is expressed in human myometrium, particularly during pregnancy. Its expression is upregulated during pregnancy and is induced upon stretching [57]. The TREK-1 channel plays a role in the regulation of uterine contractions. The TREK-1 activator arachidonic acid was observed to reduce uterine contractions, while the TREK-1 blocker L-methionine exerted the opposite effect. Interestingly, this response is related to the effect of progesterone on uterine relaxation. Both progesterone and arachidonic acid were observed to exert similar effects on TREK-1 activity. The progesterone-induced inhibition of uterine contraction was reversed in the presence of the TREK-1 inhibitor L-methionine [33]. Furthermore, since the expression and activity of this channel are essentially induced in response to uterine wall stretching, TREK-1 may play a significant role in the determination of the type of pregnancy. Different TREK-1 expression patterns and activities in preterm versus term and in singleton versus twin pregnancies may help explain the differences in the uterine contractile response in each case [58].

Expression of potassium channels in the placenta

The placenta partakes in gas exchange, nutrient delivery, and waste transfer between the mother and fetus and has immunological and metabolic functions. Chorionic villi are in contact with maternal blood and are covered by a continuous layer of syncytiotrophoblasts. Two different lineages of undifferentiated cytotrophoblastic stem cells differentiate into villous and interstitial cytotrophoblastic cells. Syncytiotrophoblastic cells secrete pregnancy-associated hormones, including hCG and progesterone. hCG is a glycoprotein hormone and shares a common α-subunit with LH and FSH. hCG is produced by placental syncytiotrophoblasts after implantation in a process modulated by the partial pressure of oxygen, the presence of reactive oxygen species, and potassium channels [13]. It stimulates progesterone production, decidualization, angiogenesis, and cytotrophoblast differentiation [59]. $K_V1.5$ and $K_V2.1$ are classic delayed rectifier K_V channels that are sensitive to oxygen and TEA [7]. These channels are expressed in the syncytiotrophoblast. Díaz et al. [13] reported that the expression oxygen-sensitive K_V channels could be downregulated under hypoxic conditions (1% PO_2) and lead to lower hCG secretion from the placenta. Oxidative stress mediates hCG secretion and K^+ permeability, and the regulation of this mechanism depends on the partial pressure of oxygen [13].

Other potassium channels, including $K_V9.3$, Kir6.1, TASK-1, and BK_{Ca} channels are expressed in the human placental vasculature. It was also reported that all calcium-activated potassium channels (BK_{Ca}, IK_{Ca}, and SK_{Ca}) and K_V channels are expressed in the smooth muscle cells of chorionic plate resistance arteries (CPAs) [60,61]. The low-resistance feto–placental circulation and vasodilatation are essential for the suc-

cessful maintenance of pregnancy. Local vasodilators such as NO, prostacyclin, and endothelium-derived hyperpolarizing factors (EDHFs) mediate vasorelaxation [62-64]. EDHFs are as potent as NO and prostaglandin as blood pressure regulators. Endothelial intermediate and small-conductance K_{Ca} channels (IK_{Ca} and SK_{Ca}) are major components of EDHFs [65]. IK_{Ca} and SK_{Ca} are primarily expressed in the endothelial and smooth muscle cells of placental CPAs. In the endothelium, these channels may play a more important role in vascular function. Endothelial K^+ currents were observed to be inhibited in hypoxic conditions owing to the downregulation of IK_{Ca} and SK_{Ca} expression in the ovine uterine arteries and in porcine coronary arteries [66,67]. Endothelium-derived NO may regulate IK_{Ca}- and SK_{Ca}-dependent vasodilatation; the expression of endothelial nitric oxide synthase (NOS) and inducible NOS was observed to be significantly downregulated in women with preeclampsia. Therefore, the abnormal expression or dysregulation of IK and SK channels in the endothelium and smooth muscle cells of CPAs, NO, and EDHFs may play a crucial role in the pathogenesis of preeclampsia [62]. In human placental mitochondria, the potassium channel is formed by the subunit Kir6.1, which contributes to placental mitochondrial steroidogenesis by facilitating cholesterol uptake and intermembrane translocation through a mechanism independent of the transport of K^+ ions inside the mitochondria [68].

Conclusion

This mini-review provides insight into the roles of potassium channels, which form the largest and most diverse ion channel family, in the relaxation of smooth muscle cells and vasodilatation via the regulation of cell membrane potential as well as in specialized cellular functions, such as the regulation of female sex hormone secretion, relaxation of uterine muscles in pregnancy, turnover into labor, and the successful maintenance of pregnancy mediated by the regulation of vascular tone in placental CPAs. Among the ATP-sensitive potassium channels, Kir6.1/SUR2B in GCs and Kir6.2/SUR1 expressed in GnRH neurons in the hypothalamus modulate progesterone release and a negative feedback mechanism. All 5 types of potassium channels are expressed in uterine myometrium. Calcium-activated potassium channels play a major role in the relaxation of smooth muscles in nonpregnant state as well as in early to mid-term pregnancy. Other channels affect the transition from uterine quiescence to active myometrial contractions in late pregnancy, including voltage-activated and rapidly-gating sodium-activated potassium channels. The prevention of hypoxia in the placenta is important for treating preeclampsia. Endothelial intermediate- and small-conductance calcium-activated potassium channels mediate the vasodilatation of placental CPAs via the secretion of NO and EDHFs.

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Conflict of interest

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

Ethical approval

This study does not require approval of the Institutional Review Board because no patient data is contained in this article. The study was performed in accordance with the principles of the Declaration of Helsinki.

Patient consent

Written informed consent and the use of images from patients are not required for the publication.

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The effect of parity on the function of pelvic floor musculature in the long term: cross-sectional study

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Objective
Parity is associated with an increased risk of pelvic floor muscle dysfunction. The aim of this study was to evaluate the long-term effects of parity on this musculature.

Methods
This cross-sectional study was completed at the Department of Physical Therapy, Federal University of São Carlos, Brazil. In total, 143 women participated in the study and were classified into three groups according to parity: nulliparae, primiparae, and secundiparae women. All parous participants had last given birth between 1 and 6 years prior. Pelvic floor muscle function was assessed through unidigital vaginal palpation using the PERFECT scheme, with the contraction grade classified according to the Modified Oxford Scale and through manometry.

Results
There was no difference in scores on the Modified Oxford Scale (the means and standard deviations were 2.5±0.8 in nulliparae women, 2.3±0.9 in primiparae women, and 2.2±0.9 in secundiparae women; *P*=0.482) and manometry findings (the means and standard deviations were 42.3±22.7 in nulliparae women, 35.0±21.8 in primiparae women, and 33.2±20.0 in secundiparae women; *P*=0.144) among the assessed groups.

Conclusion
Parity had no effect, regardless of mode of birth, on the function of pelvic floor muscles and the presence of urinary symptoms, such as long-term urinary incontinence after birth.

Keywords: Muscle strength; Pelvic floor; Parity; Urinary incontinence; Postpartum period

Introduction

The structures of the pelvic floor (PF), such as muscles, connective tissue, and peripheral nerves, are influenced by hormonal, anatomical, and morphological changes during pregnancy [1,2], contributing to functional and structural modifications to the PF. This may result in long-term dysfunction, such as urinary incontinence (UI) [3]. The growth and increasing weight of the uterus and fetus increase the load on the pelvic floor muscles (PFMs). Moreover, the rise in levels of progesterone and relaxin contributes to a decline in musculature function [1].

In a recent systematic review, it was reported that vaginal delivery was related to a higher risk of long-term (greater than 1 year) stress urinary incontinence (SUI) after birth than a cesarean section [4]. However, the difference between

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This study was conducted at the Federal University of São Carlos - São Carlos, São Paulo, Brazil.

these groups seemed to decrease with increasing age and time after birth [4,5]. Barbosa et al. [6] verified that 2 years after birth, the mode of delivery was not a risk factor for PFM dysfunction in primiparae women.

Parity itself has also been associated with an increased risk of PFM dysfunction [2,4]. However, most studies that investigated the function of these muscles and urinary symptoms after birth included nulliparae and primiparae women only [7-9]. Even though total global fecundity rates are decreasing, there is a need for studies that involve women who have experienced more than one pregnancy, considering that in most developed countries, this index is approximately 1.7 children per woman, and in developing countries, 4.2 children per woman [10].

Other factors that may affect long-term PFM function, such as constipation, urinary symptoms, and previous PFM training, have been scarcely investigated. Therefore, the primary aim of this study was to evaluate the long-term effect of parity on the function of the PFMs. The secondary objective was to investigate if any other variables, such as constipation, urinary symptoms, and previous PFM training during pregnancy, were associated with long-term PFM function. The hypothesis tested in this study was that women with 1 or 2 children would show greater impairment in PFM function than nulliparae women.

Materials and methods

This was a cross-sectional study completed at the Women's Health Research Laboratory (Laboratório de Pesquisa em Saúde da Mulher - LAMU) of the Federal University of São Carlos (Universidade Federal de São Carlos - UFSCar), São Carlos SP, Brazil, between August 2015 and August 2016.

Women were recruited in the city of São Carlos through flyer distribution, magazine and social network ads, referrals from public health units, and direct phone contact with women who sought out the laboratory due to their interest in evaluating their PFMs. The selected volunteers were nulliparae, primiparae, and secundiparae women. The parous volunteers had last given birth 1–6 years prior to the study. The exclusion criteria were neurological or cognitive dysfunctions that could impair the understanding of the proposed procedures, motor or neurological dysfunctions in the lower limbs, urinary tract or vaginal infections at the time of assessment, previous multiple pregnancies (twins, triplets, etc.), intolerance to vaginal palpation or manometry, and postmenopausal status.

An anamnesis was performed through an assessment form containing questions about maternal sociodemographic and anthropometric information—the body mass index was calculated from the information given by the woman regarding her weight and height, intestinal constipation—investigated using ROMA III criteria [11], urogynecologic and obstetric history, previous PFM training, and neonatal anthropometric information. In addition, the heights and weights of the volunteers were collected using a bioimpedance scale (Tanita IronMan® InnerScan BC-558). In order to verify current urinary symptoms, two questions from the King's Health Questionnaire (KHQ) [12] were asked: "Do you ever experience urinary urgency with urinary loss before reaching the toilet?" and "Do you ever experience urinary loss during physical efforts such as coughing, sneezing, running?" When urinary loss was confirmed, the full KHQ was applied. This questionnaire is composed of eight domains in which the score varies from 0 to 100, wherein the higher the score, the worse the quality of life [12]. Through the Baecke Habitual Physical Activity Questionnaire (BQHPA), the level of physical activity of the subjects in the last 12 months was evaluated [13].

The assessment of PFM function was performed by 2 experienced physical therapists using visual inspection, digital palpation, and manometry. Intraclass correlation coefficients (ICCs) were used to measure inter-rater reliability of the Modified Oxford Scale (ICC, 0.98) and manometry (ICC, 0.94). Two physiotherapy assessments were performed on 8 women within the same day, with a 15-minute interval between each assessment. Examiners were blind to each other's results and to the inter-rater reliabilities of the MOS (examiner A: ICC, 0.96; examiner B: ICC, 0.87).

The assessments were conducted with the volunteer in a supine position, hips and knees flexed, and feet flat on the stretcher. A visual inspection and a stress test were performed by simulating a cough, aiming to verify the presence of simultaneous PFM contraction, and to check for any loss of urine. Next, unidigital vaginal palpation was performed, in which the therapist introduced the index finger lubricated with gel approximately 4 cm into the vaginal canal. The volunteers were instructed to contract the PFM as hard as they could while minimally using the accessory musculature (abdominal, gluteal, and hip adductor musculature). The instruc-

tion was to achieve an "in and upward movement" to be able to the obtain the needed degree of contraction (referred to as "Power" in the PERFECT scheme) [14]. The contraction was classified according to the Modified Oxford Scale of the PERFECT scheme. Three PFM contractions were performed and only the highest value was counted. The other items of the PERFECT scheme (E=endurance; R=repetition; F=fast) were also assessed. Although the Modified Oxford Scale may seem to be a subjective assessment method, it is 1 of the most widely used tools in clinical practice and scientific research, used to assess the function of PFM as part of the PERFECT scheme. This method presents satisfactory inter-rater reliability and validity, and a strong correlation with the contraction assessed by manometry in nulliparae women using Peritron equipment [15,16].

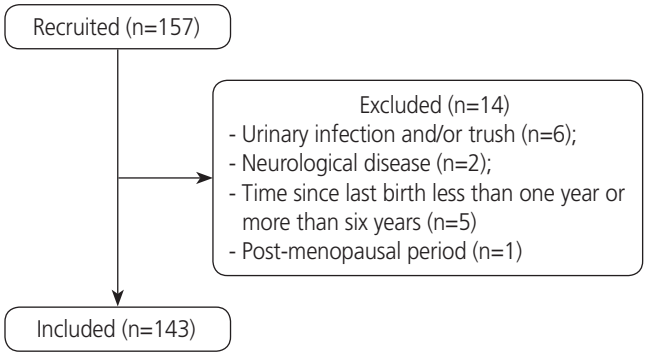

Fig. 1. The study flowchart.

Five minutes after completing digital palpation, the PFM contraction was assessed with Peritron (Cardio Design PtyLtd, Oakleigh, Victoria, Australia) equipment, and were graded from 0 to 300 cmH_2O and coupled to a vaginal probe (28×55 mm). The probe was covered with a non-lubricated condom, with its center positioned 3.5 cm into the vaginal introitus—after which the vaginal resting pressure was collected [17]. The device was reset to 0 for each contraction, and the volunteer received verbal commands and motivation during the PFM contractions, each lasting 5 seconds. They were also instructed to achieve an "inward and upward

Table 1. Sociodemographic and anthropometric data and level of habitual physical activity of volunteers in three study groups

Variables	NG (n=40)	PG (n=73)	SG (n=30)	*P*-value
Current age (yr)	29.3±4.4	32.4±4.5[a)]	37.0±4.8[a,b)]	<0.001
Current BMI (kg/m^2)	23.8±3.1	23.6±3.3[a)]	26.2±4.9[a,b)]	0.011
BMI ranges				
Low weight	1 (2.5)	2 (2.7)	0	
Eutrophic	27 (67.5)	50 (68.5)	14 (46.7)	
Overweight	11 (27.5)	19 (26.0)	10 (33.3)	0.008
Obesity	1 (2.5)	2 (2.7)	6 (20.0)	
Greatest lifetime body weight (kg)	69.6±9.7	75.9±10.2	81.5±15.8	0.061
BQHPA score	8.1±1.3	7.7±1.0	8.1±1.3	0.162
Ethnicity				
White	36 (90.0)	60 (82.2)	24 (80.0)	
Black	2 (5.0)	2 (2.7)	2 (6.7)	0.684
Asian	0	1 (1.4)	1 (3.3)	
Brown	2 (5.0)	10 (13.7)	3 (10.0)	
Highest level of education				
High school	0	6 (8.2)	7 (23.3)	
Undergraduate degree	7 (17.5)	8 (11)	3 (10.0)	0.154
Graduate degree	33 (82.5)	59 (80.8)	20 (66.7)	

Values are expressed as mean±standard deviation or number (%).
NG, nulliparae group; PG, primiparae group; SG, secundiparae group; BMI, body mass index; BQHPA, Baecke Habitual Physical Activity Questionnaire.
[a)]$P \leq 0.05$ in relation to NG; [b)]$P \leq 0.05$ in relation to PG.

movement" with maximal possible strength, while avoiding the activation of accessory muscles. The therapist visually confirmed the correct performance of the contractions by observing the movements of the probe, and by checking for minimal contractions of the accessory musculature [17]. Three contractions were performed with 1-minute intervals between them. In order to analyze the data, the mean value of the 3 contraction peaks was used. Vaginal manometry performed with Peritron equipment is considered to be a method with good intra-[18,19] and inter-reliability for the assessment of pelvic floor musculature [20].

The statistical analysis was performed using Statistica 7.0, and data normality was verified through a test of residuals. Next, the Kruskal-Wallis (for 3 groups) and Mann-Whitney tests (for 2 groups) were applied. The Fisher's exact test was performed to verify the association between qualitative variables and groups. A significance level of 5% was utilized. The sample calculation for this study was not performed at first, due to the lack of parameters in the literature on which to base calculations. A post hoc test was performed on the G*Power program using a 1-way analysis of variance with effect size 0.25 (small), 5% error, and considering a sample of 140 volunteers. A power of 0.76 was obtained.

Results

For this study, 157 volunteers were recruited, 14 of whom were subsequently excluded (Fig. 1). The 143 volunteers included women who were grouped according to parity. There were 40 in nulliparae group (NG), 73 in primiparae group (PG), and 30 in secundiparae (SG). Among the primiparae women, 36 (49.3%) underwent a vaginal delivery, and 37 (50.7%) underwent a cesarean section. Among the secundiparae women, 6 (20%) had had 2 vaginal deliveries, 19 (63.3%) had undergone 2 cesarean sections, and 5 (16.7%) had had a vaginal delivery and a cesarean section. The mean time from the last delivery until the time of assessment was 2.5±1.5 years for PG and 3.7±1.7 for SG women (shown as mean and standard deviation) (P<0.001).

Table 1 shows sociodemographic and anthropometric data from the subjects included in the study, as well as their level of habitual physical activity in the last 12 months (BQHPA Questionnaire). Their ages ranged from 21 to 48 years, body mass index (BMI) ranged from 16.9 to 38 kg/m², and the greatest body mass achieved during the women's lives ranged from 48 to 109 kg.

PFM function assessed through the PERFECT scheme and manometry showed no significant difference in relation to

Table 2. The mean and standard deviation of PFM function assessed by the PERFECT scheme and manometry according to parity

Variables	NG (n=40)	PG (n=73)	SG (n=30)	*P*-value
Power	2.5±0.8	2.3±0.9	2.2±0.9	0.482
Endurance	3.4±2.3	4.2±2.7	3.3±1.7	0.243
Repetition	2.8±1.5	2.8±2.0	2.4±1.5	0.172
Fast	6.4±3.7	6.0±3.2	5.1±2.9	0.391
Manometry (cmH_2O)	42.3±22.7	35.0±21.8	33.2±20.0	0.144

Values are expressed as mean mean±standard deviation (%).
NG, nulliparae group; SG, secundiparae group; PG, primiparae group.

Table 3. Occurrence of intestinal constipation, presence of current UI, and previous pelvic floor muscle training

Variables	NG (n=40)	PG (n=73)	SG (n=30)	*P*-value
Constipation	4 (10.0)	9 (12.3)	2 (6.7)	0.542
Urge urinary incontinence	1 (2.5)	9 (12.3)	1 (3.5)	0.100
Stress urinary incontinence	7 (17.5)	14 (19.2)	10 (33.3)	0.212
Previous training of the pelvic floor muscles	6 (15.0)	22 (30.1)	5 (16.7)	0.120

Values are expressed as number (%).
NG, nulliparae group; PG, primiparae group; SG, secundiparae group.

parity, as shown in Table 2. Nineteen volunteers (47.5%) from the NG group, 29 (39.7%) from the PG group, and 10 (53.3%) from SG group (*P*=0.572) presented contraction grades of 3 or 4 on the Modified Oxford Scale.

Data regarding intestinal constipation, pelvic floor muscle training, and the presence of current UI is shown in Table 3. There was no significant difference in KHQ domains among groups. The "UI impact" domain presented high scores in all the groups, averaging 57.1±41.7 in the NG group, 40.5±32.5 in the PG group, and 38.1±12.6 in the SG group (shown as mean and standard deviation) (*P*=0.501).

Table 4 presents information about pregnancy. There were significant differences in maternal age at the beginning of pregnancy among groups, with maternal age being higher in the SG group (*P*<0.001). In contrast, PFM preparation for birth was higher in the PG group (*P*=0.022).

Obstetric data, which were directly dependent on the volunteers' memories, were collected. At the time of the last birth, 7 (6.8%) women reported having undergone episiotomy, with 3 (4.1%) from the PG group and 4 (13.3%) from the SG group. Thirty-one (30.1%) women reported having some degree of perineal tearing, 26 (35.6%) from the PG group and 5 (16.7%) from the SG group.

Neonatal data were collected from their children's health booklets. The mean body mass of the biggest newborn from the PG group was 3.3±0.5 kg, and it was 3.5±0.6 kg in the SG group (shown as mean and standard deviation) (*P*=0.243). The mean head circumference in the PG group was 33.5±1.7 cm, and it was 35.6±4.7 cm in the SG group (shown as mean and standard deviation) (*P*=0.112). There was no significant difference between groups for both variables analyzed.

No correlation was found among the age at the time of assessment and BMI, parity, time between last birth and assessment, level of physical activity (BQHPA score), UI severity measure (KHQ score), maternal age and body mass at the beginning of last pregnancy, body mass gain during first and second pregnancies, neonatal data (body mass, length and head circumference), and PFM function as assessed through the P ("Power") item of the PERFECT scheme and vaginal manometry.

Discussion

The present study could verify that PFM function was similar among the nulliparae, primiparae, and secundiparae groups. Studies have shown that regardless of parity and mode of birth, there could be a decrease in PFM function after pregnancy. Nonetheless, the musculature has the capacity to recover its contractility up until 1 year after birth [21-23].

Normal PFM function has been described as the capacity to perform a contraction around the pelvic orifices, with an inward and upward movement of the perineum [24,25]. Less than half of the volunteers in the present study were able to perform a contraction graded 3 or 4 according to the Modified Oxford Scale. With regards to vaginal manometry, there are no minimum recommended values. However, our study showed a higher absolute mean manometry value in the nulliparae group, but there was no significant difference when compared to the other groups. This may be due to the large standard deviation observed in the values of manometry for all groups, which could be explained by the difficulty in performing a PFM contraction correctly. According to the literature, approximately 30% of women cannot contract these muscles properly [26]. Furthermore, only a few women reported having performed any kind of training for the pelvic floor muscles, and they were mainly nulliparae. Training for the pelvic floor muscles has been recommended during

Table 4. Information about the gestational period, urinary symptoms, and PFM preparation for birth

Variables	PG (n=73)	SG (n=30)	*P*-value
Maternal age at the beginning of last pregnancy (yr)	29.6±4.3	32.9±4.2	<0.001
Body mass gain in last pregnancy (kg)	13.1±5.2	12.2±4.6	0.622
Urge urinary incontinence during pregnancy	12 (16.4)	3 (10.0)	0.402
Stress urinary incontinence during pregnancy	23 (31.5)	9 (30.0)	0.881
Pelvic floor musculature training during pregnancy	35 (47.9)	8 (26.7)	0.022

Values are expressed as mean±standard deviation or number (%).
PFM, pelvic floor muscle; PG, primiparae group; SG, secundiparae group.

pregnancy and after birth, to strengthen the PFM, contribute to its recovery, and prevent future dysfunctions such as UI [27,28].

Pregnancy itself can be considered a risk factor for alterations in PFM function and a contributor to the occurrence of urinary symptoms resulting from the hormonal, anatomical, and functional modifications in the urinary tract during this period [1]. Other studies have demonstrated that the second pregnancy and delivery do not influence the structure and function of the pelvic floor. Rather, the first birth is a major contributor predisposing women to greater pelvic floor impairments [29,30]. In contrast, Jundt et al. [30] verified that secundiparae women had significantly poorer PFM function than primiparae women 27 months after birth, but there was no difference in the presence of UI and bladder neck hypermobility between the groups. Özdemır et al. [31] also demonstrated that PFM function, assessed through vaginal manometry, decreases with the increase in parity.

In the present study, both women that have had a vaginal and/or cesarean delivery were placed together in the PG and SG groups. During vaginal delivery, there may be compression and stretching of the neural, muscular, and connective structures, contributing to the changes in PFM function after birth [32]. Friedman et al. [33] found that vaginal and forceps deliveries were associated with a greater impairment in PFM function in multiparous women, 6 to 11 years postpartum. Driusso et al. [34] found through a systematic review that there was no difference in short-term PFM strength after childbirth between primiparae women who underwent cesarean section and those who underwent vaginal delivery. The difference in the results reported by the studies may be explained by the chosen assessment method, period after birth in which the assessment was conducted [27], classification adopted in studies referring to the type of birth, utilization of unwanted instruments and interventions (such as forceps, vacuum, Kristeller maneuver, and episiotomy), and the lack of standardization for PFM function classification parameters.

A few studies investigated other factors associated with the impairment of PFM function in a period considered long term after birth. In the present study, neonatal, sociodemographic, and anthropometric data were similar among groups. There were differences in current maternal age and BMI, maternal age at the beginning of the last pregnancy, and the performance of physical therapy techniques to prepare the PFM for birth among groups. The SG group had higher greater maternal age and BMI and had fewer members who underwent PFM preparation for birth; however, these factors do not influence long-term PFM function. Bocardi et al. [35] showed that aging is not a determinant of reduced function and electromyographic activity of the pelvic floor musculature. In the short term, Mendes et al. [27] verified that maternal age, marital status, ethnicity, and newborn body mass did not influence the PFM function in primiparae women, 50 to 70 days postpartum. It is thus expected that further studies on the influence of these factors on PFM function would not find associations, although these data should still be collected.

UI can lead to a reduction in a woman's quality of life [2]. Valeton and do Amaral [3] found a reduction in the quality of life related to UI symptoms and the domains assessed through KHQ after birth. In the current study, there was no explicit difference among groups in terms of impairment in the quality of life, with UI complaints assessed by the domains of the KHQ questionnaire. However, the domain that presented a higher absolute score was "Incontinence Impact." In addition, in terms of UI during pregnancy and current UI, no significant difference was found among groups. Fritel et al. [28] reported that the risk factors for UI are multifactorial, but that the second birth did not increase the risk of SUI. Other studies imply that parity is a risk factor for UI [2,4]. Rortveit et al. [36] verified, through a questionnaire, an association among parity, SUI, and mixed UI among women aged less than 65 years. Additionally, they found a high prevalence of UI among nulliparae women, indicating that other risk factors may be related to this dysfunction [36]. The type of birth has also been found to be associated with UI, with studies suggesting that vaginal delivery increases the long-term risk of UI after birth when compared with cesarean delivery [4]. Nevertheless, Qian et al. [37] demonstrated through questionnaires that cesarean surgery does not confer long-term protection against SUI after birth. Furthermore, women who presented with UI during pregnancy or puerperium had a higher long-term risk of symptom occurrence [30]. Since the present study did not find an association between parity and UI, with prior research being controversial and implicating a multifactorial explanation for UI, these results may help improve quality of life for the population with UI by providing evidence that can lead to the identification of possible risk factors.

Additionally, current prospective studies involving the assessment of function before and during pregnancy, with long-term follow-up after birth, may contribute to the elucidation of the factors associated with PFM function impairment.

Our study had some limitations, such as the smaller number of women in the secundiparae group. A larger sample for this specific group may have allowed for a better analysis of the influence of the variables affecting PFM function. Another limitation was that the obstetric and intervention data depended on maternal memory, which could have influenced the accuracy of this information. However, it is worth emphasizing that our study included the use of validated questionnaires and instruments, standardization of subjective assessments and high reliability between examiners conducting such assessments, careful collection of data on time elapsed since the last delivery and the time of assessment, and the inclusion of secundiparae women.

In conclusion, parity was not found to have any effect on long-term PFM function and urinary symptoms, such as UI, after birth, regardless of the mode of delivery.

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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 Research Ethics Committee of the Federal University of São Carlos (resolution 1.034.342).

Patient consent

All volunteers were informed about the proposed procedures, and those who agreed to participate voluntarily signed an informed consent form.

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Obstetrics & Gynecology Science

Management of isolated oligohydramnios in Korea: a questionnaire-based study of clinical practice patterns among the members of the Korean Society of Maternal Fetal Medicine

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Objective
The aim of this survey was to investigate the recommendations and clinical practice patterns of the Korean Society of Maternal and Fetal Medicine (KSMFM) members, regarding management of isolated oligohydramnios (IO).

Methods
From December 2018 to February 2019, questionnaires were e-mailed to the KSMFM members at 257 institutes that are listed by the Korean Statistical Information Services (KOSIS) as suitable labor premises. Responses to the seven questions on the management of IO, from diagnosis to treatment, were evaluated.

Results
A total of 72 KSMFM members responded to this survey. Nearly all participants (90.1%) used the amniotic fluid index (AFI) as the primary method for estimating amniotic fluid volume. The majority of the participants (73.6%) believed that IO was a risk factor for adverse pregnancy outcomes, including abnormal fetal heart rate (73.6%), need for cesarean delivery (58.3%), intrauterine fetal demise (52.8%), and meconium aspiration syndrome (50%). Almost 70% of the participants believed that induction of labor might decrease perinatal morbidities, and that late-preterm to early-term period (36–38 gestational weeks) was a suitable timeframe for delivery, if the fetus was sufficiently grown and antenatal testing revealed reassuring results. Less than half of the participants (47.2%) believed that maternal oral or intravenous hydration was a useful intervention for IO management.

Conclusions
KSMFM members preferred labor induction at late-preterm to early-term, to decrease perinatal morbidity in cases of IO, although it was still uncertain whether labor induction improved the outcomes. Further prospective studies are needed regarding IO management.

Keywords: Oligohydramnios; Questionnaire; Clinical practice pattern

Introduction

Amniotic fluid protects the fetus, reflects the fetal condition, and provides an environment for various fetal movements, including breathing [1,2]. Oligohydramnios is associated with maternal and fetal hypertension, diabetes, congenital anomalies, fetal growth restriction, and preterm rupture of membranes. Independently, it has been reported to reflect

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uteroplacental insufficiency and chronic fetal hypoxia [3,4]. When oligohydramnios is diagnosed by chance during the routine second and third trimester pregnancy examination, clinicians tend to recommend a variety of tests to identify its causes, such as the presence of congenital malformations and the size of the fetus, and to conduct fetal surveillance including Doppler studies.

Oligohydramnios without any evidence of either maternal or fetal abnormalities is known as "isolated" oligohydramnios (IO) [5]. In low-risk pregnancies, the incidence of IO has been estimated as 1.5% [6]. IO management guidelines are not well defined as its effect on perinatal outcomes remains controversial. However, a recent meta-analysis has suggested that IO in low-risk pregnancy was associated with adverse outcomes such as neonatal intensive care unit (NICU) admission, cesarean delivery, and meconium aspiration syndrome [7]. A systematic review and meta-analysis suggested that oral or intravenous maternal hydration improves amniotic fluid volume; however, further evaluation of its clinical application is required [8].

Given the potentially severe complications and lack of clear management guidelines, the diagnosis of IO can affect pregnancy management and the decision to induce labor. In fact, a 2009 survey of Society for Maternal Fetal Medicine (SMFM) has revealed that approximately 80% of the participants declared either that induction of labor in cases of IO did not reduce perinatal morbidity or that they were uncertain whether it did. However, despite being unsure of its benefits, 96% of the practitioners leaned towards intervention [9].

In the absence of IO management guidelines, insights into obstetricians' clinical practice patterns are required. This survey aimed to investigate the knowledge and clinical practice patterns associated with IO management among the members of the Korean Society of Maternal Fetal Medicine (KSMFM).

Materials and methods

From December 2018 to February 2019, questionnaires were sent to 272 labor units listed by the Korean Statistical Information Services (KOSIS) as suitable labor premises. The institutes included 41 obstetrics departments within university hospitals, 86 obstetrics departments within general hospitals, and 145 private obstetric hospitals. The survey comprised of seven questions and was distributed by e-mail.

IO was defined as an ultrasonography-based diagnosis of abnormally low amniotic fluid with intact membranes in the absence of a fetal growth restriction, fetal anomalies, or significant maternal comorbidity. The participants were asked to report on their management of such cases, based on their personal and their unit's standard protocols. The questionnaire was composed of three parts: 1) measurement and related perinatal outcomes of IO, 2) preferences for induction of labor in presence of IO atthird trimester and 3) preferences of oral or intravenous hydration and intraamniotic fluid infusion in IO. In this questionnaire-based survey, respondents were asked to provide details of their individual characteristics, such as medical practice setting, age, sex, and the year of their certification as obstetrical specialists. The questionnaire was adapted from an earlier survey for SMFM members regarding the same matter [9].

Table 1. Characteristics of obstetricians who responded to the survey, regarding their clinical practice patterns in oligohydramnios management (n=72)

Characteristics		Values
Sex	Male	33 (45.8)
	Female	39 (54.2)
Age (yr)		45.6±6.8
	30–39	15 (20.8)
	40–49	37 (51.4)
	50–59	18 (25.0)
	≥60	2 (2.8)
Type of labor unit	OB/GYN clinic	3 (4.2)
	OB/GYN hospital[a]	16 (22.2)
	OB/GYN department at a general hospital	7 (9.7)
	OB/GYN department at a university hospital	46 (63.9)
Duration of experience (yr)		15.2±7.4
Average number of monthly hospital-based deliveries	≤50	27 (37.5)
	51–100	20 (27.8)
	101–150	14 (19.5)
	151–200	6 (8.3)
	≥201	5 (6.9)

Data presented as mean±standard deviation or number (%).
OB/GYN, obstetrics and gynecology.
[a]With more than one staff obstetrician.

Results

1. Participants' characteristics

A total of 72 KSMFM members responded to this survey. The mean age of the respondents was 45.6±6.8 years. Among the sample, 45.8% and 54.2% respondents were males and females, respectively. The average duration of clinical obstetric experience was 15.2±7.4 years. About two-thirds of the participants (45/72) were working at hospitals with >50 deliveries each month (Table 1).

2. Management patterns of isolated oligohydramnios cases

Questionnaires and responses are summarized in Table 2. Most participants used the amniotic fluid index (AFI) as the primary method for estimating amniotic fluid volume (Question 1). The majority of participants (73.6%) declared that they believed IO was a risk factor for significant adverse pregnancy outcomes (Question 3). Moreover, over 50% of the participants believed that abnormal fetal heart rate (73.6%), cesarean delivery (58.3%), intrauterine fetal demise (52.8%), and meconium aspiration syndrome (50.0%) were adverse outcomes associated with IO. Similarly, approximately 70% of the participants considered IO to indicate a need for delivery induction in the third trimester (Question 4). Over 50% of the participants thought early-term (37–38 gestational weeks) delivery was appropriate in cases of IO, when the fetus was sufficiently grown and the results of antenatal testing were reassuring (Question 5). Less than half of the participants (34/72, 47.2%) believed that oral or intravenous hydration was a useful intervention in patients with IO. Among these, 50.0% (17/34) selected oral hydration for a short-term effect (Question 6). The majority of participants (63/72, 87.5%) did not perform amniotic infusion in pregnancies with IO (Question 7).

Discussion

Amniotic fluid physiologic dynamics during pregnancy are complex. Abnormal volumes of amniotic fluid are associated with various adverse pregnancy outcomes [10]. Oligohydramnios is estimated to occur in 0.5–5.5% of all pregnancies, depending on the method of amniotic fluid volume measurement [11-13]. Oligohydramnios without any evidence of anatomical, functional, or chromosomal abnormalities is called "isolated" oligohydramnios. Since IO indicates a low-risk pregnancy and the oligohydramnios measurements have a low accuracy, the IO management protocols are debatable [5,14].

In the present questionnaire-based survey, the majority of respondents used AFI to measure amniotic fluid volume. The gold standard for the amniotic fluid volume estimation is the dye dilution test [15]. However, this test is invasive, and is rarely required or used. Thus, single deepest pocket (SDP), measuring ≤2 cm, or AFI ≤5 cm are the commonly used indirect criteria for confirmation of oligohydramnios [16]. However, SDP and AFI often grossly under- or over-estimate oligohydramnios [17,18]. Although, neither of the two assessments have been proved to be superior to the other; when the AFI was used, significantly more cases of oligohydramnios were diagnosed, and more women underwent interventions [19].

Patients with oligohydramnios have high-risk pregnancies, associated with adverse outcomes such as admission to the NICU, meconium stained amniotic fluid, increased rate of cesarean section, low Apgar score at birth, fetus that is small for gestational age, low umbilical cord pH, and respiratory distress syndrome. However, since IO indicates a low-risk pregnancy, its evaluation as a risk factor for adverse pregnancy outcomes is still controversial [20]. In a recent meta-analysis of 12 studies, Shrem et al. [10] reported that IO at term was associated with significantly higher rates of labor induction, cesarean section, and short-term neonatal morbidity. Moreover, Rabie et al. [7] reported that IO in low-risk pregnancy increased the risk of NICU admission, cesarean delivery for fetal distress, and meconium aspiration syndrome. In the present survey, over 50% of the participants reported cesarean delivery, abnormal fetal heart rate, and meconium aspiration syndrome in neonates as adverse outcomes associated with IO. This was in accordance with the previous SMFM survey (Fig. 1). However, the proportion of the response of "no significant attributable risks" differed in the two surveys. This may be attributed to the increased understanding of IO in perinatal outcomes during the last 10 years.

Due to the associated adverse outcomes, interventions, such as labor induction, are important considerations when treating patients with confirmed IO in the third trimester of pregnancy. In 2009, the SMFM questionnaire-based survey revealed that approximately 80% of the participants believed

Table 2. Isolated oligohydramnios survey and responses

Questionnaire item	No. (%)
1. Which methods do you currently use most frequently to evaluate the amniotic fluid volume?	72 (100.0)
Amniotic fluid index (AFI)	65 (90.1)
Maximal vertical pocket	7 (9.9)
Subjective assessment	0
2. In your opinion, is isolated oligohydramnios a risk factor for any of the following outcomes: (Check all that apply)	72 (100.0)
Cesarean delivery	42 (58.3)
Abnormal fetal heart rate	53 (73.6)
Intrauterine fetal demise	38 (52.8)
Neonatal acidosis	15 (20.8)
Meconium aspiration syndrome	36 (50)
Low Apgar score	33 (45.8)
Low birth weight	21 (29.2)
NICU admission	34 (47.2)
Neonatal brain injury	8 (11.1)
No significant attributable risk	1 (1.4)
3. Does induction of labor in cases of isolated oligohydramnios at term decrease perinatal morbidity associated with the above-listed outcomes?	72 (100.0)
Yes	53(73.6)
No	15 (20.8)
Unknown	4 (5.6)
4. Is a case of isolated oligohydramnios in third trimester an indication for labor induction?	72 (100)
Yes	50 (69.4)
No	22 (30.6)
5. For a sufficiently grown fetus with isolated oligohydramnios and reassuring antenatal testing results, what is the maximum gestational age before recommending inducing labor?	72 (100.0)
34 weeks	5 (6.9)
35 weeks	3 (4.2)
36 weeks	8 (11.1)
37 weeks	26 (36.1)
38 weeks	15 (20.8)
39 weeks	7 (9.7)
40 weeks	7 (9.7)
No significant risk attributable to gestational age	1 (1.4)
6. Should oral or intravenous hydration be used to gain additional amniotic fluid before intervening in a patient with isolated oligohydramnios?	72 (100.0)
Yes	34 (47.2)
Oral hydration for short-term effect	17 (23.6)
Intravenous hydration for short-term effect	8 (11.1)
Oral hydration for long-term effect	8 (11.1)
Intravenous hydration for long-term effect	1 (1.4)
No	38 (52.8)
7. In your practice, is amniotic infusion used in patients with isolated oligohydramnios?	72 (100.0)
Yes	9 (12.5)
No	63 (87.5)

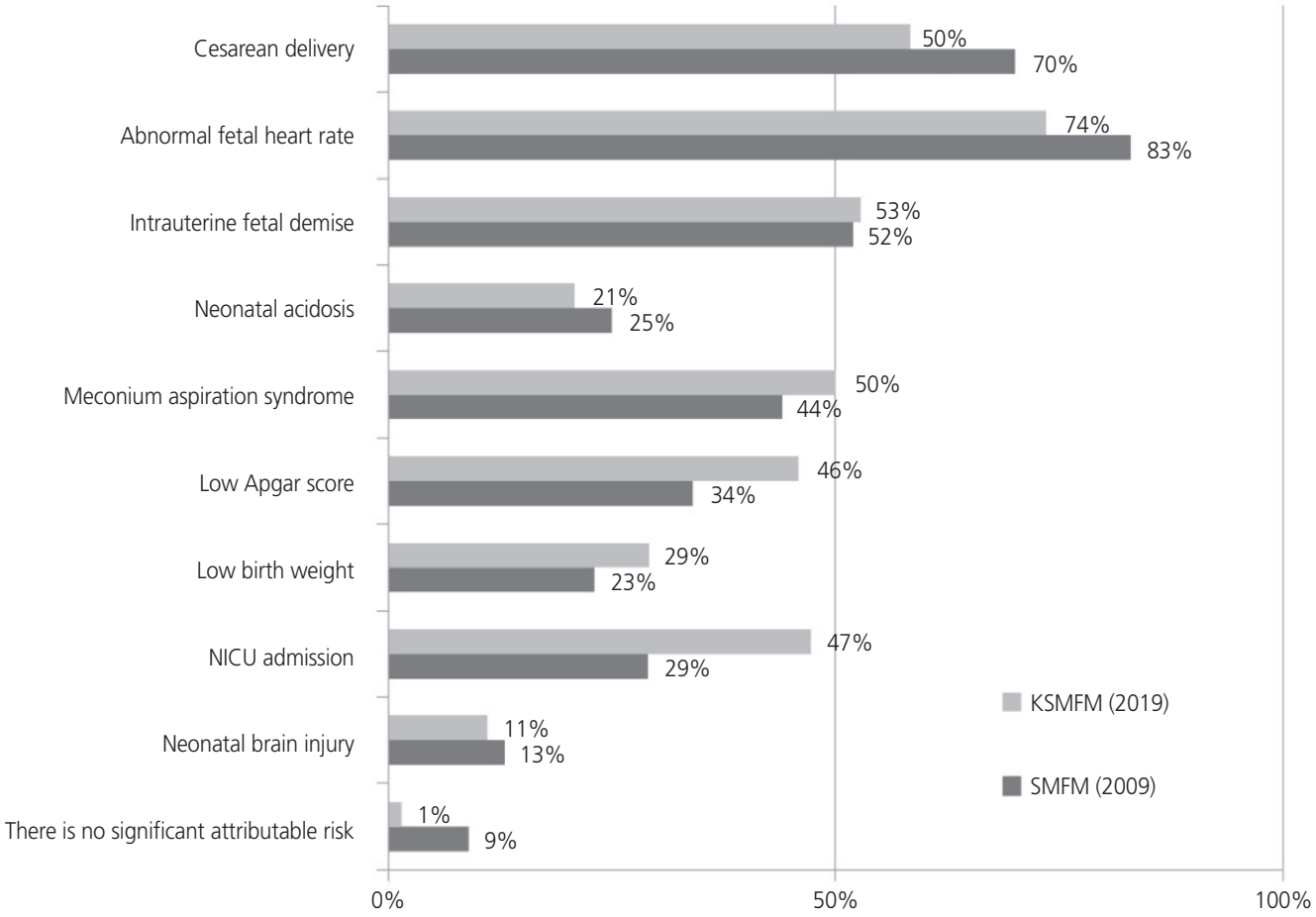

Fig. 1. Comparison of Society for Maternal Fetal Medicine (SMFM; 2009) and Korean Society of Maternal Fetal Medicine (KSMFM; 2019) survey regarding the relation between perinatal outcomes and isolated oligohydramnios. NICU, neonatal intensive care unit.

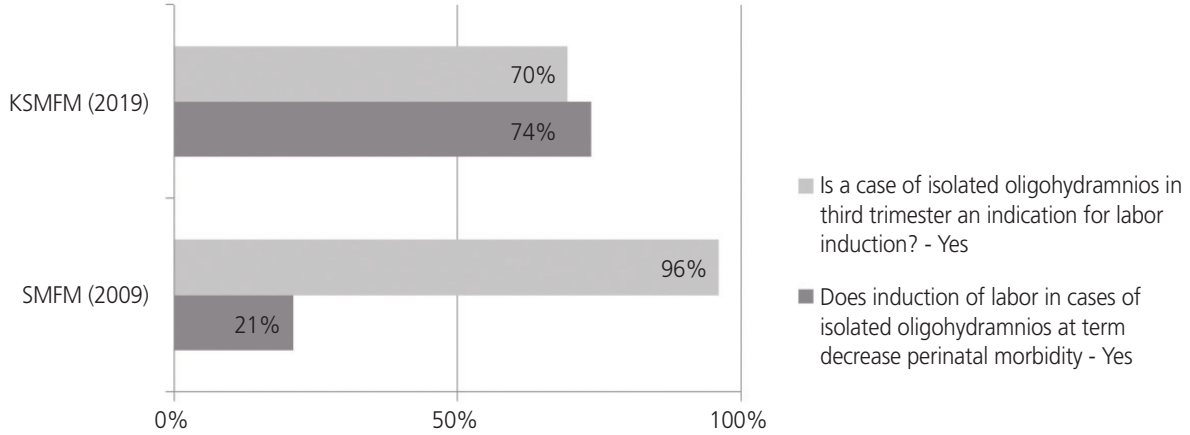

Fig. 2. Comparison of Society for Maternal Fetal Medicine (SMFM; 2009) and Korean Society of Maternal Fetal Medicine (KSMFM; 2019) survey regarding labor induction in isolated oligohydramnios.

that induction of labor in cases of IO did not reduce perinatal morbidity or that they were uncertain whether it did. However, despite being unsure of its benefits, 96% of the practitioners recommended this intervention [9]. Conversely, in the present survey, nearly 74% of the participants believed that induction of labor reduced perinatal morbidity and almost 70% of them indicated considering induction of labor in the third trimester of pregnancy with IO (Fig. 2). These results show that the perceptional uncertainty of labor induction for IO has reduced in the last 10 years. Furthermore, 68% of the participants declared that late pre-term to early-term period (36–38 gestational weeks) was a suitable timeframe for delivery. In a recent study, Rabies et al. have reported that the lack of required data precludes any conclusions regarding optimal timeframe for delivery in pregnancies with IO [7]. Conversely, Brzezinski-Sinai et al. [14] have reported that in cases of IO in late pre-term, induction of labor may reduce the risk of perinatal morbidities despite a premature birth. The American College of Obstetricians and Gynecologists (ACOG) recommendations state that 36.0–37.7 weeks of gestation are a suitable timeframe for delivery in cases with IO [21]. Given these considerations, until 35.6 weeks of gestation, conservative management is required. Further, until delivery, intensive fetal surveillances including fetal biophysical profile and Doppler analysis to evaluate placental dysfunction are needed [22,23]. In the KSMFM survey of 70 members in 2013, Oh reported that fetal surveillances were performed twice a week (20%), every week (43%) or every other week (35%), and 46% of respondents preferred induction after 37 gestational weeks in IO [24]. This result was similar to that of our survey.

Previous meta-analyses suggest that maternal hydration appeared to increase amniotic fluid volume and might be beneficial in the management of oligohydramnios [8,25]. In the SMFM survey, 51% of respondents thought that hydration (oral or intravenous) should be performed to gain additional amniotic fluid [9]. In the present survey, 47% of the participants believed that hydration had an effect in conservative management of IO. Based on this result, maternal hydration may be considered in clinical practices for IO management. Concurrently, majority of the survey participants (87.5%) declared that they did not consider amnioinfusion as a necessary practice in IO management.

This study had some limitations, including a low response rate, which was probably due to a reduced number of obstetricians in the Korean Society. The low response rate might have introduced different kinds of bias to the findings. Further, this survey showed results similar to the previous KSMFM survey of 2013 regarding the preferred induction time [24]. However, we believe that several additional questions including perception of hydration and perinatal outcomes are worth investigating than the previous study to understand additional clinical practice patterns. Additionally, 64% and 83% of participants worked for tertiary hospitals in this study and the previous study, respectively [24]. This suggests that this study represented the general practices in Korea. In conclusion, KSMFM members preferred inducing labor at late-preterm to early-term, to decrease perinatal morbidity in cases of IO, in accordance with the recent ACOG recommendation, although it is still uncertain whether labor induction improves the outcomes. Future prospective studies are needed regarding IO management.

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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 the Institutional Review Board of Konkuk University Hospital (IRB No.2020-07-028) and performed in accordance with the principles of the Declaration of Helsinki.

Patient consent

Written informed consents were obtained.

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Isolation of mesenchymal stem cells from Pap smear samples

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Objective

Exploiting their ability to differentiate into mesenchymal lineages like cartilage, bone, fat, and muscle, and to elicit paracrine effects, mesenchymal stem cells (MSCs) are widely used in clinical settings to treat tissue injuries and autoimmune disorders. One of accessible sources of MSC is the samples used for Papanicolaou (Pap) test, which is a cervical screening method for detecting potentially pre-cancerous and cancerous alterations in the cervical cells and to diagnose genetic abnormalities in fetuses. This study aimed to identify and isolate the stem cells from Pap smear samples collected from pregnant women, and to trace the origin of these cells to maternal or fetal tissue, and characterize their stem cell properties.

Methods

To investigate the possibility and efficiency of establishing MSC lines from the Pap smear samples, we were able to establish 6 cell lines from Pap smear samples from 60 pregnant women at different stages of gestation.

Results

The 3 cell lines randomly selected among the 6 established in this study, displayed high proliferation rates, several characteristics of MSCs, and the capacity to differentiate into adipocytes, osteocytes, and chondrocytes. Our study identified that the stem cell lines obtainable from Pap smear sampling were uterine cervical stromal cells (UCSCs) and had 10% efficiency of establishment.

Conclusion

Despite their low efficiency of establishment, human UCSCs from Pap smear samples can become a simple, safe, low-cost, and donor-specific source of MSCs for stem cell therapy and regenerative medicine.

Keywords: Papanicolaou test; Mesenchymal stem cells; Regenerative medicine

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Introduction

Adult mesenchymal stem cells (MSCs) are defined as undifferentiated multipotent cells that are capable of self-renewal, differentiating into several distinct tissue lineages, such as bone, adipose tissue, and cartilage, and *in vitro* expansion. To date, many types of MSCs, derived from bone marrow (BM), adipose tissue, and amniotic fluid, show tremendous potential to be used for treating conditions ranging from organ failures to immunological diseases [1]. Although the therapeutic mechanisms of MSCs have not been fully explored, accumulated data demonstrate that these cells hold promise for clinical applications [2]. Various approaches have been tried for MSC-mediated tissue regeneration. As these cells have the potential for multilineage differentiation, and can secrete soluble factors that enhance cell survival and function, they can be used for patient-specific tissue regeneration without ethical restrictions, and are immune privileged. These special properties of MSCs have encouraged the researchers to find the best sources of these cells, preferably using non-invasive procedures. MSCs from various sources share many characteristics and generally meet the accepted criteria for MSCs, like multilineage differentiation potential, self-renewal capacity, expression of specific surface markers, and adherence to plastic surfaces. However, each of these cells also exhibit a set of unique and individualistic properties in their differentiation potentials, expression of specific surface markers, cytokine production, gene expression profiles, and ability to establish cell lines *in vitro*. Moreover, the ease with which biopsies are obtained differs between sources [3].

The Papanicolaou (Pap) smear test is a cervical screening method to detect potentially pre-cancerous and cancerous abnormalities in the cervix. At 1942, Dr. Papanicolaou first discovered a deformed nuclear morphology in benign cells collected by scraping the cervix, indicating their cervical and uterine cancer potentials [4]. In addition, because Pap smear samples from pregnant women contain fetal trophoblastic cells, this test is also used for non-invasive prenatal diagnosis since the 1950s [5]. Majority of the cells in Pap smear samples are epithelial cells, such as cervical cells from the mother and trophoblasts from the fetus. Because of the wide use and simplicity of the Pap smear test, the isolated human uterine cervical stromal cells (hUCSCs) have been recently proposed as a source of adult MSCs [6]. However, their reliability and accessibility for clinical applications are not clear and their potential as a source of MSC should be further explored by comparing it with well-established MSCs. This study is designed to verify the hUCSCs harvested from Pap smear as a reproducible and expandable source of MSCs for their clinical application. The Pap smear test is non-invasive and has no consequent complications, and thus can be performed on a wide population of women. These advantages have greatly facilitated the establishment of MSCs in the current study. Supported by our previous experience in identifying and characterizing potential sources of adult stem cells [7], we isolated and expanded hUCSC lines from Pap smear samples, and identified, assessed, and compared their MSC properties in terms of self-renewal and multilineage differentiation potentials compared with MSCs from amniotic-fluid. Importantly, although the Pap smear test was originally developed to collect cervical epithelial cells for cancer screening in women, it has been reported that the samples from pregnant women also contain fetus-derived cells [8]. We therefore investigated whether the isolated cell population contain fetus-derived trophoblast stem cells, using human leukocyte antigen (HLA)-G, from a family of non-classical HLA class I molecules, typically expressed in embryos, embryonic stem cells, and trophoblasts [9].

Materials and methods

1. Isolation and culture of human uterine cervical stromal cells and amniotic fluid-derived mesenchymal stem cells

Pap smear samples were collected with informed consent, from 60 pregnant women at random, regardless of the length of their pregnancy. The collection procedure was approved by the Institutional Review Board of Korea University (KUGH16060-001). These samples were washed twice with phosphate-buffered saline (PBS) containing 100 U penicillin/streptomycin and centrifuged at 500 g for 10 minutes, and digested with 0.25% Trypsin/ethylenediaminetetraacetic acid (EDTA) (Hyclone, Waltham, MA, USA) for 30 minutes at 37°C. These were washed and centrifuged before the pellet was seeded into a 100 mm plate and incubated for 72 hours in low-glucose Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U penicillin/streptomycin, and 1% L-glutamine at 37°C and 5% CO_2. For expansion, adherent

cells obtained from Pap smear samples, and amniotic fluid-derived mesenchymal stem cells (AF-MSCs) were cultured in low-glucose DMEM containing 10% FBS, 100 U penicillin/streptomycin, 1% L-glutamine, 4 ng/mL basic fibroblast growth factor (R&D Systems, Minneapolis, MN, USA), and 50 μg/mL ascorbic acid (expansion medium). AF-MSCs were isolated and cultured in strict adherence to the guidelines issued by the Institutional Review Board of Korea University. These cells were previously confirmed to possess characteristics of MSCs, based on their differentiation, proliferation, and immunological phenotypes [7].

2. Proliferation assay

To determine the growth rates of hUCSCs and AF-MSCs, cells were seeded in 12-well plates at a density of 3×10^4 cells/well in expansion medium, cultured for 3 days, stained with 0.01% crystal violet solution, and de-stained with 10% acetic acid. Finally, absorbance at 600 nm was spectrophotometrically determined.

3. Adipogenic differentiation

Adipogenic differentiation was induced using a previously described protocol [7]. Briefly, cells were seeded in 6-well plates at a density of 3×10^4 cells/well, and cultured until they reached 100% confluency. Thereafter, cells were grown for 7 days in high-glucose DMEM (Invitrogen) supplemented with 1 mM dexamethasone (Sigma-Aldrich, St. Louis, MO, USA), 0.5 mM 3-isobutyl-1-methyl-xanthine (Sigma-Aldrich), 10 ng/mL recombinant human insulin (Sigma-Aldrich), 100 mM indomethacin (Sigma-Aldrich), and 10% FBS, to induce/maintain differentiation. After this, the cells were fixed in 10% formalin (Sigma-Aldrich) and stained with 2% (w/v) Oil Red O (Sigma-Aldrich) for 20 minutes at room temperature to detect oil droplets in the cytoplasm.

4. Osteogenic differentiation

Osteogenic differentiation was induced according to a previously described protocol [7]. Briefly, cells were seeded in 6-well plates at a density of 3×10^4 cells/well, cultured in low-glucose DMEM (Gibco/Invitrogen) containing 10% FBS until they reached 100% confluency, and fed twice a week with osteogenic induction medium (high-glucose DMEM [Invitrogen] supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, 0.2 mM ascorbate, and 10% FBS). Osteogenic differentiation was assessed by von Kossa staining.

5. Chondrogenic differentiation

To induce chondrogenic differentiation, cells were detached, transferred to a 15 mL polypropylene tube, pelleted via centrifugation at 200 g for 5 minutes, and cultured in high-glucose DMEM supplemented with 0.1 M dexamethasone, 50 μg/mL ascorbic acid (Sigma-Aldrich), 100 μg/mL sodium pyruvate (Sigma-Aldrich), 40 μg/mL proline (Sigma-Aldrich) 10 ng/mL transforming growth factor-1 (R&D Systems), 50 mg/mL ITS premix (Gibco/Invitrogen), 6.25 μg/mL insulin, 6.25 μg/mL transferrin (Sigma-Aldrich), 6.25 ng/mL selenious acid (Sigma-Aldrich), 1.25 mg/mL bovine serum albumin (BSA; Sigma-Aldrich), and 5.35 mg/mL linoleic acid (Sigma-Aldrich). After 4 weeks of culture, cells were fixed in 4% paraformaldehyde and stained with Alcian Blue (Sigma-Aldrich).

6. Reverse transcription polymerase chain reaction

RNA was isolated and purified using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using Reverse Transcriptase II (Invitrogen). To amplify target genes, 25 ng cDNA was mixed with forward and reverse primers (Bioneer, Daejeon, Korea). The PCR conditions were as follows: 24–30 cycles of denaturation at 99°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final amplification step at 72°C for 10 minutes. The levels of target genes amplified by 30 cycles of PCR were within the linear range. Primer sequences are listed in Supplementary Table 1.

7. Fluorescence-activated cell sorting analysis

hUCSCs and AF-MSCs were trypsinized and transferred to fluorescence-activated cell sorting (FACS) tubes (BD Biosciences Clontech, Palo Alto, CA, USA) at a density of 1×10^6 cells/tube. Cells were rinsed twice with cold Dulbecco's PBS containing 1% BSA (pH 7.4), and incubated with a primary antibody against CD29, CD31, CD34, CD44, CD45, CD73, CD90, or CD120a (BD Biosciences) or against human leukocyte antigen-G (HLA-G; Santa Cruz Biotechnology, Dallas, TX, USA) for 1 hour at 4°C. Thereafter, cells were washed twice with PBS containing 1% BSA, resuspended in 100 μL PBS containing 1% BSA and a fluorescein isothiocyanate-labeled secondary antibody diluted 1:100, and incubated for 40 minutes at 4°C. Finally, cells were washed twice with PBS containing 1% BSA and fixed in 4% paraformaldehyde for FACS analysis. To identify nonspecific signals, control cells

were incubated with isotype-matched immunoglobulins.

8. Colony-forming unit assay

hUCSCs and AF-MSCs were seeded in 6-well plates at a density of 100 cells/well, cultured for 14 days, washed twice with PBS, and fixed in 10% formalin for 20 minutes at room temperature. To visualize the colonies, cells were stained with 0.01% crystal violet solution for 20 minutes at room temperature, washed with deionized water, and air-dried. Colonies were typically 5–8 mm in diameter, and were scored macroscopically.

9. Karyotype analysis

Karyotyping was performed by the Cytogenomic Services Facility of Samkwang Medical Laboratories. hUCSCs were cultured in expansion medium as described above. Cell were treated with 0.05 μg/mL colcemid (Gibco/Invitrogen) for 1–2 hour to block the dividing cells at metaphase. Chromosomes were visualized by G-banding. At least 100 metaphase cells were analyzed, and a minimum of ten cells were karyotyped per line.

10. Immunofluorescence staining

To detect HLA-G, hUCSCs and AF-MSCs were incubated for 1 hour at 4°C with anti-HLA-G antibody (sc-21799; Santa Cruz Biotechnology) diluted in PBS containing 1% BSA, washed twice with PBS containing 1% BSA, and incubated for 40 minutes at 4°C with a fluorescein isothiocyanate-labeled secondary antibody diluted 1:100 in PBS containing 1% BSA. Nuclei were stained with DAPI diluted 1:1,000 in PBS containing 1% BSA.

11. Statistical analysis

All values are expressed as means±standard deviation. Data were compared using 1- or 2-way analysis of variances with *post hoc* Tukey's test and paired 2-tailed Student's *t*-tests.

Results

1. Isolation and characterization of the cell population from Papanicolaou smear samples

We attempted to establish fetus-derived trophoblast cell lines by collecting the Pap smear sample from pregnant women. The cell samples were collected by centrifugation and seeded into cell culture dishes (Fig. 1A). Clusters of fibroblast-like cells with a homogenous morphology were observed in randomly selected areas after incubation for 72 hours (Fig. 1B). These adherent cells had a high proliferative potential in serum-containing medium. Isolated cells were stably sub-cultured for at least 12 passages (>40 days) (Fig. 1C). These cells became elongated and spindle-shaped upon repeated passages (Fig. 1B). Expression of HLA-G was detected by immunofluorescence staining in AF-MSCs, but not in our cell lines (Fig. 1D). This observation was supported by the FACS analysis, in which HLA-G was detected in AF-MSCs, and negatively in the cell lines isolated by us (Fig. 1D). Furthermore, the G-banding karyotype analysis showed normal female chromosomes (Fig. 1E). These findings indicate that the cells from Pap smear samples were derived from the cervix, which is in agreement with Eiró et al. [6]; trophoblast stem cells derived from the fetus could not be established in the current study.

2. Characterization of human uterine cervical stromal cell lines

We focused on establishment of hUCSC lines from cells derived from Pap smears of pregnant women. Of the 60 samples, only 6 hUCSC lines, including the one (Fig. 1, hUCSC #1) assessed earlier, were established. Similar to hUCSC #1, the two other hUCSC lines (#2, #3) showed high proliferation in serum-containing medium and could be stably subcultured for at least 12 passages (>40 days) (Fig. 2A). HLA-G expression profiles of hUCSC #2 and #3 were similar to those of hUCSC #1 as observed in the immunofluorescence and FACS analysis (Fig. 2B). Karyotype of hUCSC #2 and #3 using G-banding showed normal female chromosomes (Fig. 2C). The immunophenotype of hUCSCs was analyzed by flow cytometry and it showed that AF-MSCs and hUCSC lines were negative for CD31, CD34, and CD45, indicating that they are not endothelial or hematopoietic in origin, but were positive for the MSC-specific markers CD29, CD44, CD73, CD90, and CD120a (Fig. 2D). In addition, both these MSCs abundantly expressed mesenchymal markers (Snail and Slug) and markers of extracellular matrix (fibronectin and syndecan) (Fig. 2E). The clonogenic and proliferation capacities of AF-MSCs and hUCSCs were compared by the colony-forming unit (CFU)-F assay. Colonies with a diameter larger than 5 mm were counted. The number of colonies formed did not significantly differ between hUCSCs and AF-MSCs or

between the hUCSC lines (Fig. 2F).

3. Adipogenic, osteogenic, and chondrogenic differentiation of human uterine cervical stromal cells *in vitro*

For successful and efficient tissue regeneration using MSCs, their multilineage differentiation capacity must be preserved upon *in vitro* expansion. To investigate the adipogenic and osteogenic differentiation potentials of hUCSCs, cells at passage 4–6 were seeded at a density of 3×10^3 cells/cm^2 and cultured in 10% FBS. At confluency, adipogenic and osteogenic differentiation were induced. Expected morphological changes and formation of neutral lipid droplets, as detected by Oil Red O, were observed at 1.5 weeks after induction of adipogenic differentiation (Fig. 3A). Reverse transcription polymerase chain reaction (RT-PCR) analysis demonstrated that adipocyte markers, LPL and aP2, were highly expressed by these cells upon induction of adipogenic differentiation (Fig. 3A). On osteogenic differentiation, von Kossa staining exhibited dark brown or black labeling, indicating mineraliza-

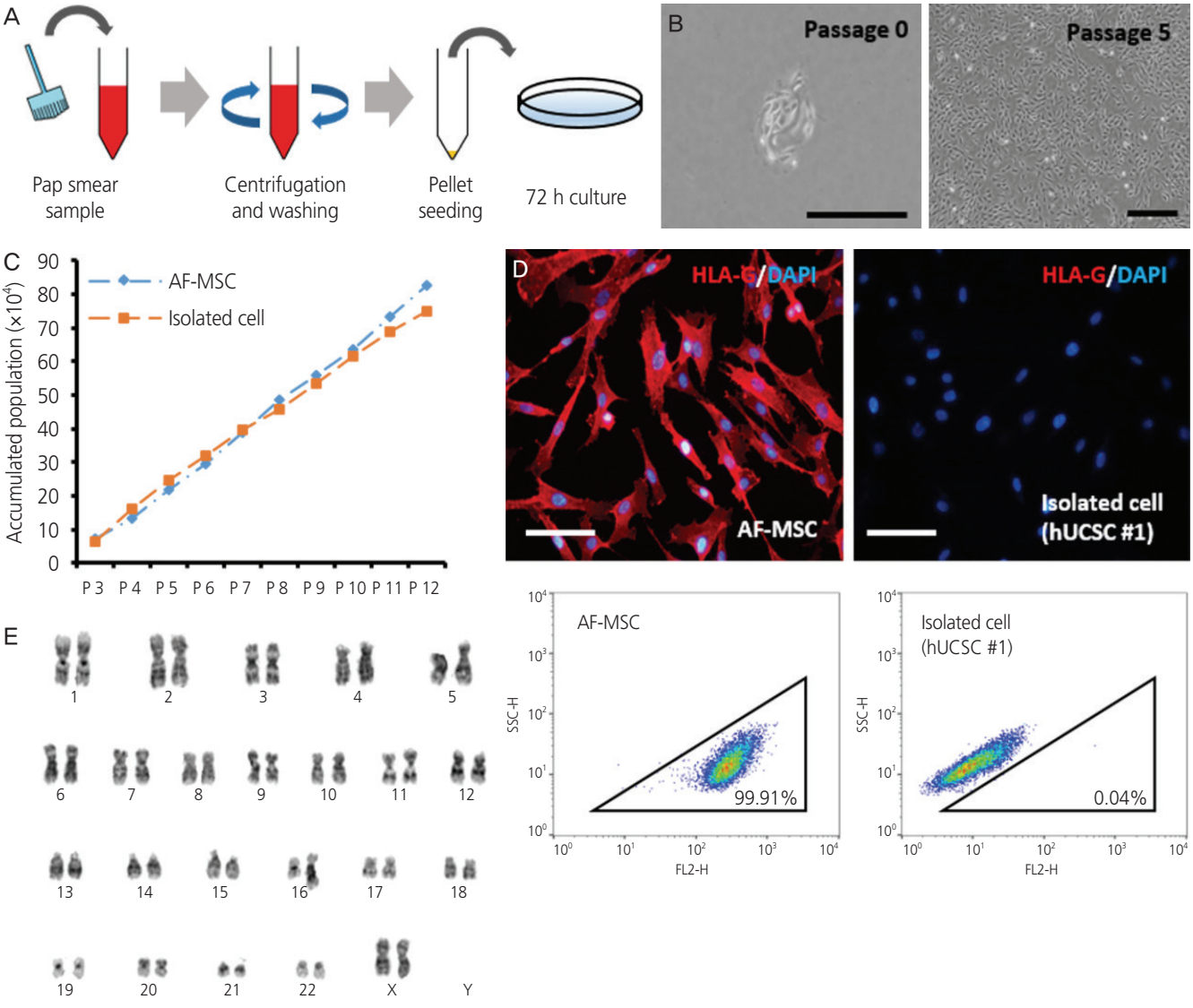

Fig. 1. Isolation of fibroblast-like cells from Papanicolaou (Pap) smear samples. (A) Method used to isolate cells from Pap smear samples. (B) Morphology of isolated cell line at passage 0 and 5 (scale bar=500 μm). (C) Accumulated population comparison with isolated cell line and amniotic fluid-derived mesenchymal stem cell (AF-MSC) upon passaging. (D) Immunofluorescence staining and fluorescence-activated cell sorting (FACS) analysis of human leukocyte antigen (HLA)-G in AF-MSCs and isolated cells (scale bar=200 μm). (E) Karyotype of isolated cell line determined by G-banding.

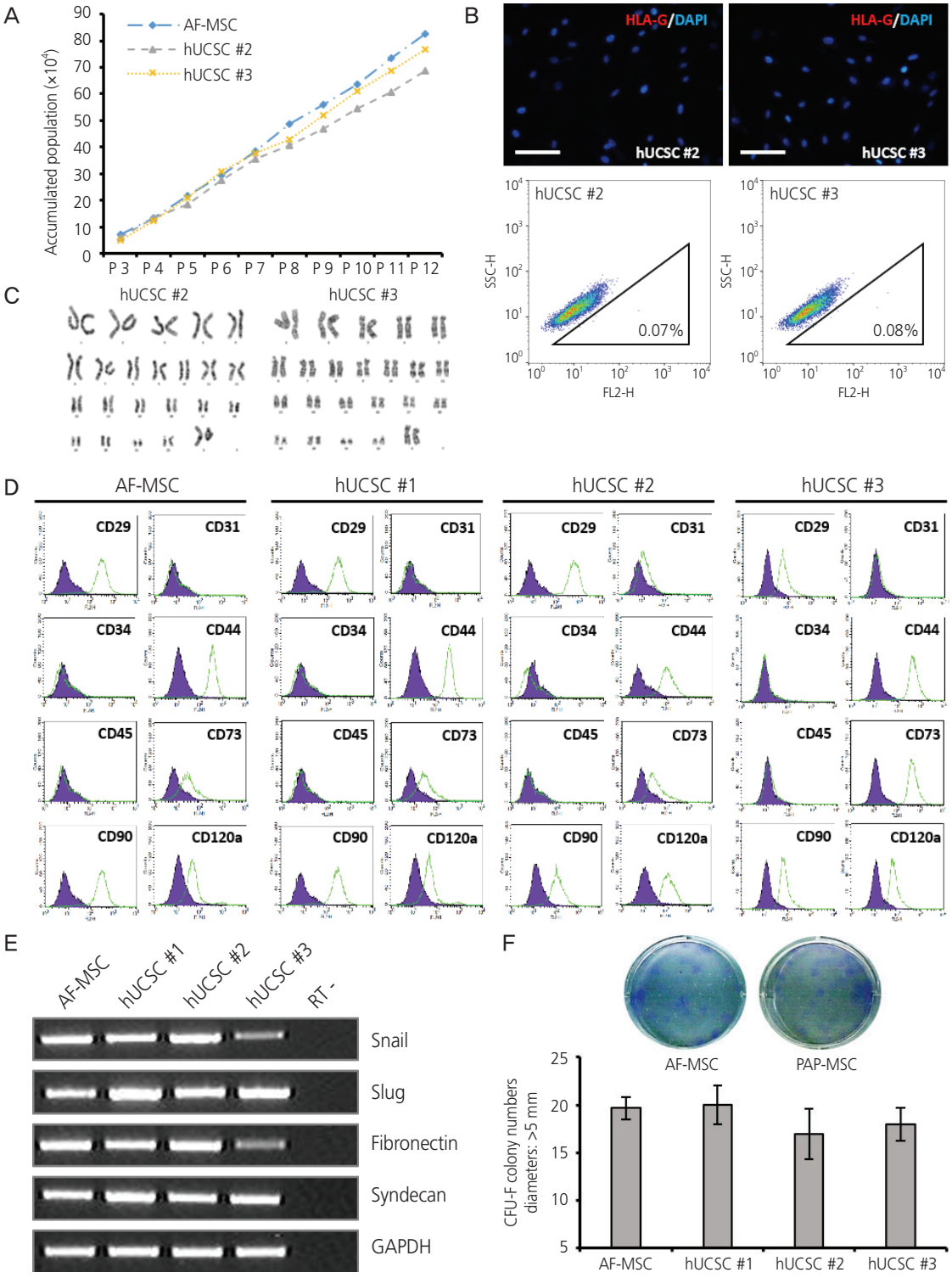

Fig. 2. Characterization of human uterine cervical stromal cell (hUCSC) lines. (A) Accumulated population of hUCSCs and amniotic fluid-derived mesenchymal stem cell (AF-MSC) upon passaging. (B) Immunofluorescence staining of human leukocyte antigen (HLA)-G in hUCSC line #2 and #3 (scale bar=200 μm). Fluorescence-activated cell sorting (FACS) analysis of HLA-G expression in hUCSC line #2 and #3. The percentages of HLA-G-positive cells are shown. (C) Karyotype of hUCSC line #2 and #3 determined by G-banding. (D) FACS analysis of the immunophenotypes of hUCSC lines (green). The isotype control is shown in purple. (E) mRNA expression of human MSC markers in hUCSC lines. (F) Colony-forming unit (CFU) assay investigating the self-renewal capacity of hUCSC lines.

tion of extracellular matrix in these cells (Fig. 3B). These cells expressed the osteocyte markers osteopontin and osteocalcin upon induction of osteogenic differentiation, as seen in RT-PCR (Fig. 3B). The chondrogenic differentiation potential of hUCSCs was evaluated by culturing the cells in chondrogenic differentiation-inducing medium for 3–4 weeks. Glycosaminoglycans were detected by Alcian Blue staining and cells highly expressed the chondrocyte markers collagen type I and aggrecan, according to RT-PCR (Fig. 3C).

4. Efficiency of establishing human uterine cervical stromal cells from donors

As mentioned above, from 60 pregnant women, we were able to establish only 6 hUCSC lines (Table 1). As Pap smear

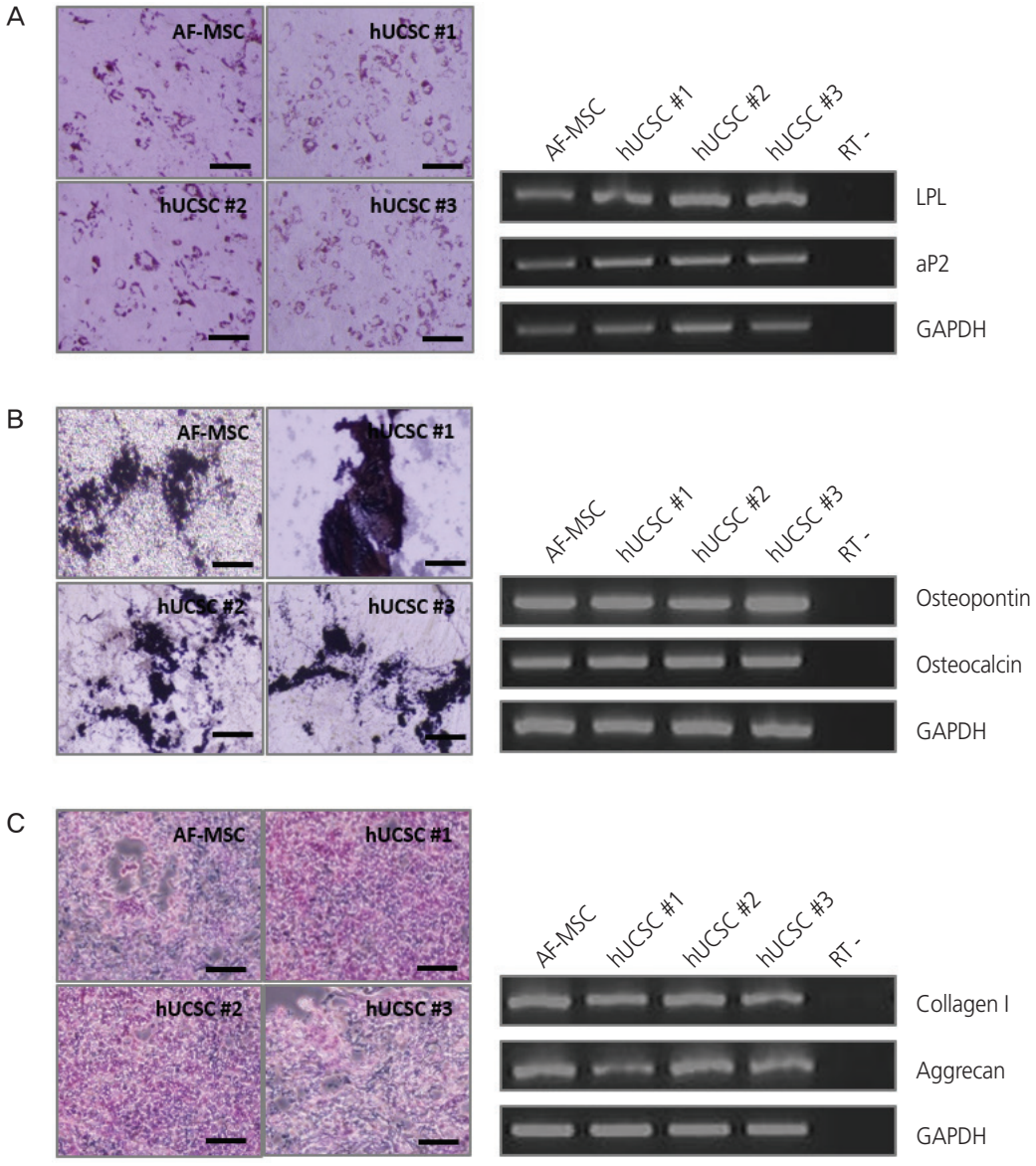

Fig. 3. Differentiation of human uterine cervical stromal cells (hUCSCs). (A) Adipogenic differentiation of hUCSCs at passage 8. Differentiation was evaluated by Oil Red O staining and reverse transcription polymerase chain reaction (RT-PCR) analysis of adipocyte markers (LPL and aP2). (B) Osteogenic differentiation of hUCSCs at passage 8. Differentiation was evaluated by von Kossa staining and RT-PCR analysis of osteocyte markers (osteopontin and osteocalcin). (C) Chondrogenic differentiation of hUCSCs at passage 7. Differentiation was evaluated by Alcian Blue staining and RT-PCR analysis of chondrocyte markers (collagen I and aggrecan). Scale bar=200 μm. AF-MSC, amniotic fluid-derived mesenchymal stem cell; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

samples were randomly selected for this purpose, success or failure in establishment of hUCSCs were independent of donors' age and gestational age. Furthermore, to study how the enzymatic digestion could affect the isolation efficiency, we digested only half of the fresh Pap smear samples with trypsin before starting the culture. Results suggest that the enzymatic digestion of Pap smear sample had no effect on the establishment of cell lines. We found the efficiency of establishing cell lines was 10%, regardless of donors' age, duration of pregnancy and pre-treatment of Pap smear samples.

Discussion

Rapidly advancing MSC-based therapeutic strategies have enormous potential for research and in regenerative medicine. The availability and accessibility of MSCs from various adult tissues and organs facilitate detailed studies of various stem/progenitor cell populations and their metabolic profiles in specific tissues, and promote the clinical applications of these cells. MSCs were first obtained from the stroma of BM, and BM is the most widely used source of stem cells for clinical trials [10]. Although BM-derived MSCs are considered as the gold standard, harvesting them from the BM frequently causes pain and harm to donors and patients [11]. Because of this, attempts were made to obtain MSCs from other sources. In the efforts to discover accessible sources of MSC and standardize the characteristics of these MSC, a variety of different tissue sources, including adipose tissue, the placenta, cord blood, peripheral blood, and amniotic fluid have been explored [12-15]. However, the following problems hinder the application of MSCs: i) donors often object to the harvesting of samples using needles, ii) it is difficult to collect the requisite number of homogeneous MSCs required for transplantation, iii) it is difficult to deliver the required dose of MSCs to the target site within the expected time frame, and iv) the occurrence of graft-versus-host disease is high and the survival rate is low among patients with nonmalignant disorders.

Although the properties of MSCs differ between different tissues and donors, MSCs originating from different tissues share common characteristics and generally meet the accepted criteria for MSCs, such as a self-renewal capacity, a fibroblast-like morphology, a multilineage differentiation potential, expression of typical surface markers such as CD29,

Table 1. Isolation rate of Papanicolaou smear samples

Enzymatic digestion		Gestational weeks	Isolated cell line
	Yes (n=30)	First trimester (n=12)	1
		Second trimester (n=8)	0
		Third trimester (n=10)	1
	No (n=30)	First trimester (n=9)	1
		Second trimester (n=11)	2
		Third trimester (n=10)	1
Total donors	60	60	6

Yield of human uterine cervical stromal cell lines from 60 pregnant donors. Enzymatic digestion was conducted by 0.25% Trypsin/ethylenediaminetetraacetic acid for 30 minutes at 37°C before attachment.

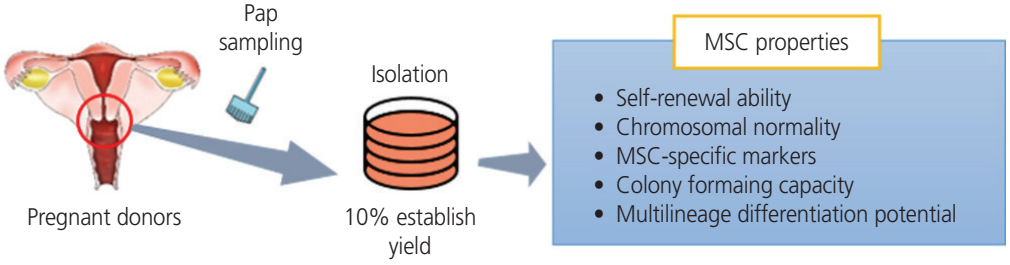

Fig. 4. Establishment of human uterine cervical stromal cells (hUCSCs) from Papanicolaou (Pap) smear samples. MSC, mesenchymal stem cell.

CD44, and CD90, and lack of expression of lineage-specific markers such as CD14, CD34, and CD45 [16]. Wagner et al. [17] showed that the global gene expression patterns of MSCs derived from several tissues significantly differ. MSCs from tissues of mesodermal lineages (e.g., BM-derived MSCs) exhibit a propensity to express predominantly mesoderm-specific transcript homolog protein (MEST). On the other hand, expression of BMP antagonist 1 (also known as CKTSF1B1 and gremlin 1) and connective tissue growth factor (CTGF) are the highest in cord blood-derived MSCs. Expression of Ki-67, CDCA8, and CCNB2 are higher in adipose tissue-derived MSCs than in BM-derived MSCs, implying that the proliferative potentials of the former are higher than those of the latter cells [18]. Distinct expression patterns of DLX5, which is an indicator of the osteogenic potential of stem cells, suggest that the differentiation potential of MSCs depends on the tissue source and the donor [19]. We adopted universally accepted criteria for defining MSCs to characterize fibroblast-like cell lines obtained from Pap smear samples. The proliferative and colony-forming capacities of hUCSCs and AF-MSCs were comparable (Figs. 1C and 2A), allowing extensive *in vitro* expansion of both. Our phenotypic analysis showed that hUCSCs were positive for typical MSC surface markers, such as CD29 (β-integrin), CD44 (hyaluronate receptor), CD71 (transferrin receptor), CD90 (Thy-1), and CD120a (tumor necrosis factor-α1 receptor), but negative for lineage-specific markers, such as CD31 (platelet endothelial cell adhesion molecule-1), CD34 (transmembrane phosphoglycoprotein), and CD45 (protein tyrosine phosphatase, receptor type, C) (Fig. 2D). Furthermore, we demonstrated the multilineage differentiation potential of hUCSCs by culturing them in specific differentiation-inducing conditions. MSCs from cord blood and placenta rarely differentiate into osteocytes, whereas their potentials to differentiate into adipogenic and chondrogenic lineages are comparable to those of BM-MSCs [19]. These findings indicate that differentiation capacity of hUCSCs is comparable with that of BM-derived MSCs, which are accepted as the gold standard, as trilineage differentiation of hUCSCs was successfully achieved (Fig. 3). These results strongly suggest that hUCSCs possess the unique features of MSCs (Fig. 4).

HLA-G, a nonclassical HLA class I molecule, has immunomodulatory actions, which disturb cytolysis, adhesion, and migration of natural killer cells [20]. Related studies revealed that HLA-G expression in MSCs differs between tissues, and that MSCs possess immunosuppressive properties and can inhibit the proliferation and function of major immune cell types, such as natural killer cells [21,22]. In the present study, the hUCSCs we isolated were HLA-G-negative, according to immunofluorescence and FACS analysis (Figs. 1 and 2). Hviid [23] reported that fetal trophoblasts, particularly the extravillous trophoblasts that invade the uterine wall and spiral arteries in the placenta, express HLA-G. By contrast, the maternal cells express HLA-ABC rather than HLA-G, which means that HLA analysis can be used to distinguish between fetal and maternal cells in undefined mixed cell populations. Regardless of the existence of fetal trophoblast cells, cells positive for HLA-G expression would depend on placental disorders, period of gestation, etc. [24-26]. Meanwhile, it is reasonable to assume that HLA-G-negative hUCSCs lack immunosuppressive properties; however, another study demonstrated that HLA expression is unrelated to inhibitory effects on T cell proliferation [19]. Therefore, the immunomodulatory effects of hUCSCs must be studied to resolve this question and to facilitate their broad clinical application.

At the beginning of this study, we aimed to establish the fetus-derived trophoblast stem cells from the Pap smears of pregnant women. However, the cells we isolated originated from maternal uterine cervix, but exhibited the following MSC properties, fibroblast-like morphology, self-renewal capacity *in vitro*, expression of MSC markers, and a multilineage differentiation potential. Our continued efforts resulted in isolation of stem cell with 10% efficiency of establishment, from the Pap smear samples of 60 pregnant women. The three hUCSC lines showed features comparable to those of AF-MSCs, generally considered as typical adult MSCs (Fig. 4). In conclusion, despite a low efficiency of establishment of hUCSCs, and the absence of fetal cells in the Pap smear samples, our results suggest that hUCSCs could be considered as a simple, safe, low-cost, and donor-specific source of cells for MSC-based therapy and regenerative medicine.

Acknowledgements

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ment of Technology (KIAT) (N0002405,2017), and School of Life Sciences and Biotechnology for BK21 PLUS, Korea University.

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 Korea University (KUGH16060-001) 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.

Supplementary material

Supplementary Table 1 associated with this article can be found online at https://doi.org/10.5468/ogs.20073.

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Perspective of the comparative effectiveness of non-pharmacologic managements on postpartum hemorrhage using a network meta-analysis

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Objective
Postpartum hemorrhage (PPH) is the leading cause of maternal mortality worldwide and is both unpredictable and inevitable. While uterotonic drugs are routinely recommended, there is ongoing debate on the ideal intervention to control uterine bleeding. This review aims to compare the use of non-pharmacologic treatments with peripartum hysterectomy in cases of life-threatening uncontrolled obstetric hemorrhage. The review's objective is to use a network meta-analysis to help prevent maternal deaths and rank the treatments according to success rates.

Methods
We searched MEDLINE (PubMed), Embase, and the Cochrane Library, from January 2014 until December 2018. A second search was carried out in April 2019 before the final data analysis. Network meta-analysis allows for the calculation of the effect size between treatment groups through indirect treatment comparison.

Results
We confirmed that balloon-assisted management is the best intervention for uncontrolled postpartum bleeding with pharmacologic treatment. This is followed by uterine artery embolization and surgical procedures, which can help avoid the need for a hysterectomy. The balloon tamponade demonstrated lower failure rate than the surgical procedure with odds ratio (OR) of 0.44 and 95% confidence intervals (CIs) 0.50–30.54. Uterine artery embolization had a lower risk for hysterectomy than the surgical procedure group (OR, 0.74; 95% CI, 0.22–2.50).

Conclusion
For the quick treatment of postpartum bleeding, balloon tamponade is the best method for uncontrolled postpartum bleeding with pharmacologic treatment, followed by uterine artery embolization and surgical procedures.

Keywords: Postpartum hemorrhage; Balloon tamponade; Uterine artery embolization; Network meta-analysis

Introduction

Maternal and perinatal mortalities are surrogate measures of national health status and indicators of social development. In 2015, 303,000 women, around 830 women per day, were estimated to have died due to pregnancy- or childbirth-related complications worldwide. Fortunately, the global maternal mortality rate has been decreasing, with an annual continuous reduction rate of 2.3% [1]. Similarly, the maternal mortality ratio in Korea decreased from 14 deaths per 100,000

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live births in 2005 to 8.4 in 2016 [2].

Worldwide, blood loss after birth contributed to nearly a quarter of all maternal mortality cases, with the most common cause of postpartum hemorrhage (PPH) being uterine atony, the failure of the uterus to contract after birth. Maternal morbidity due to uterine atony is far more common than any other causes of bleeding, such as placental abruption, placenta accreta, placenta previa, or peripartum hysterectomy [1,3-6].

More than one third to half of maternal mortality cases are reported within the first 24 hours after giving birth [1,3]. Furthermore, while the risk factors for severe hemorrhage have been identified, such hemorrhages continue to be unpredictable and inevitable. Active management of the third stage of labor, which involves administration of uterotonic drugs, early clamping of the umbilical cord, and controlled cord traction, has become standardized nationwide [7-9]. Routine administration of uterotonic agents during the third stage of labor is a key intervention and the most effective at preventing PPH due to uncertain causes [7,8].

In 2009, the World Health Organization (WHO) working group defined "maternal near-miss morbidity" as a life-threatening obstetric hemorrhage that requires urgent medical attention to prevent the death of the mother [10]. Near-miss events are used to monitor the quality of maternal health care and provide rapid and useful feedback to improve obstetric care. The non-pharmacological procedure to treat obstetric hemorrhage is stressful and surgically challenging, inevitably causing additional maternal morbidity and, occasionally, infertility.

Even though the risk factors for uterine atony have been identified, up to half of women with uterine atony after cesarean delivery had no risk factors [6]. It has been established that a prolonged third stage of labor increases the frequency of PPH [9]. However, active management with uterotonic agents and controlling the umbilical cord have been shown to decrease PPH by decreasing the duration of the third stage of labor [9]. The active and expectant management of obstetric hemorrhage were updated as part of postpartum management, and despite very low quality evidence, active management has been introduced in low income countries to reduce hemorrhage [8].

In cases of unresponsive bleeding in which uterotonic agents have been administered, non-surgical treatments, such as balloon tamponade or embolization, should be immediately or simultaneously applied [11-14]. If the bleeding worsens, surgical procedures such as uterine compression suture, pelvic vessel ligation, and hysterectomy are performed [15-17]. In clinical practice, however, there is still uncertainty about whether to perform non-surgical and/or surgical procedures after uncontrolled bleeding with pharmacological managements. Due of the lack of certainty, and the small number of studies published, it is essential to assess the impact of these forms of care on both the mother and the baby. Effective prevention of PPH, and appropriate intervention during PPH, are key in decreasing maternal mortality.

Therefore, this study aimed to use a network meta-analysis to compare the effect of non-pharmacologic managements versus peripartum hysterectomy on uncontrolled bleeding in cases of life-threatening obstetric hemorrhage. This review will help prevent maternal deaths by ranking different treatments according to effectiveness.

Materials and methods

This network meta-analysis was conducted according to the guideline of the PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations [18].

1. Search strategy

We searched MEDLINE (PubMed), Embase, and Cochrane Library from January 2014 to April 2019. Only studies in English were retrieved. The search terms used were: "(postpartum hemorrhage OR obstetric hemorrhage) AND (uterine packing OR balloon tamponade OR balloon occlusion OR brace suture OR vessel ligation OR Blakemore tube OR Bakri OR B-lynch OR square suture OR uterine artery ligation OR internal iliac artery ligation OR uterine artery embolization)", using both MeSH terms and text words.

2. Inclusion and exclusion criteria

The following study types were included: randomized controlled trials (RCT), observational studies, and controlled trials (non-RCT/CT), all written in English. We excluded studies with case series or studies that only published an abstract. Participants included women in the third stage of labor who had a vaginal or cesarean birth in a hospital or a community setting. In electronic medical records, eligible patients were

PPH patients with at least 500 mL of blood loss at delivery and/or at least 1,000 mL of blood loss at delivery and received some blood products and/or uterotonic drugs. The use of uterotonic drugs typically includes ergometrine, misoprostol, misoprostol plus oxytocin, carbetocin, ergometrine plus oxytocin and oxytocin on its own. The types of interventions included: 1) trials where they carried out non-pharmacological treatment after failure of administered uterotonic agents (any dosage, route, or regimen) at birth for preventing PPH; and 2) trials evaluating non-pharmacological treatments like uterine packing, balloon tamponade or balloon occlusion, brace suture, vessel ligation, uterine artery ligation, internal iliac artery ligation, Blakemore tube, Bakri® balloon tamponade, or B-lynch or square suture. All non-pharmacologic treatments were divided into 3 groups: Bakri® balloon tamponade, uterine artery embolization and surgical procedures, such as B-lynch, uterine strapping, and compression suture. The compression suture was defined as any method of compression suture, except B-lynch, which is recommended as standard.

3. Outcomes

The main outcome was hysterectomy after birth due to uncontrolled bleeding, calculated as the failure rate of the interventions.

4. Selection and analysis of studies

After the literature search, the reviewers (KJ Lee, K Hong, H Hwang, S Sohn) independently screened the retrieved titles and abstracts. The full text of manuscripts selected for inclusion were examined and the inclusion and exclusion criteria were applied (Fig. 1). Disagreements between reviewers were resolved by consensus or through the participation of a third reviewer.

Reviewers independently extracted the following information from the included studies: author, year and country of publication, number of participants, type of intervention, and outcomes studied (Table 1).

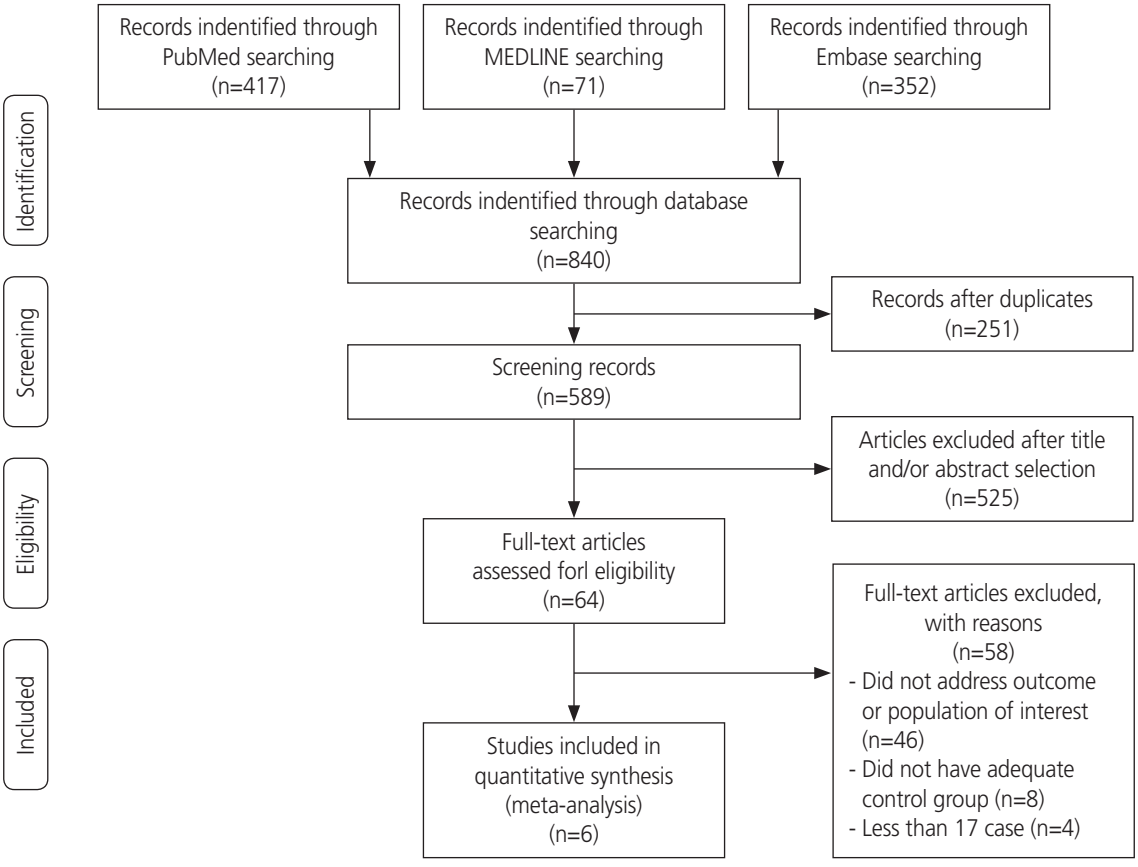

Fig. 1. PRISMA flow chart of study selection process.

Table 1. Characteristics of selected studies

Author	Year of publication	Country	Total number of participants	Type of intervention		Failure rate[a] (95% CI)	
				Treatment A	Treatment B	Risk A	Risk B
Chai and To [19]	2014	Hong Kong	80	Bakri balloon tamponade	General compression suture	0.26 (0.11–0.46)	0.36 (0.20–0.55)
Feng et al. [20]	2016	China	75	Uterine artery embolization	Uterine strapping General compression suture	0.12 (0.01–0.36)	0.14 (0.06–0.25)
Guo et al. [21]	2018	China	205	Bakri + vaginal gauze	Bakri balloon tamponade	0.04 (0.01–0.08)	0.08 (0.04–0.13)
Howard and Grobman [22]	2015	US	420	Bakri balloon tamponade	Uterine artery embolization	0.10 (0.03–0.23)	0.15 (0.03–0.38)
Yan et al. [26]	2014	China	74	Bakri balloon tamponade	B-lynch	0.05 (0.00–0.26)	0.19 (0.06–0.38)
Zhao et al. [27]	2014	China	87	Bakri balloon tamponade	B-lynch	0.00(0.00–0.06)	0.10 (0.02–0.26)

CI, confidence intervals.
[a]Failure rate = number of failures/population.

5. Assessing the consistency and quality of included studies

Consistency was tested using the Wald test, which calculated the linearity of regression coefficients for all models [23]. The Revman v5.3 program was used as the Risk of Bias Assessment tool for Non-randomized Studies [24,25]. We assessed 6 parameters including 1) selection of participants, 2) confounding variables, 3) measurement of exposure, 4) blinding of outcome, 5) incomplete outcome data, and 6) selective outcome reporting. Each parameter was graded as unclear, low risk or high risk of bias. Overall bias was considered as "low risk of bias" if the paper was classified as 'low risk' in all domains, "some concerns" if there was at least one domain with a rating of 'some concern', and "high risk of bias" if there was at least one domain with a 'high risk' or several domains with 'some concerns' that could affect the validity of the results. The overall risk of bias was determined according to the previously reported standards [26]. We investigated publication bias using funnel plots, which were visually assessed for symmetry [27]. Finally, an Egger's regression test

Fig. 2. The summary of each study's risk of bias. Green positive icons indicate low risk of bias and red negative icons indicate high risk of bias.

and Begg's test were performed to analyzed the asymmetry of the funnel plot [28].

6. Statistical analysis of network meta-analysis

A network meta-analysis was used to evaluate the effects of various treatments on PPH. Selected publications were reviewed with this method using the frequentist approach. In our study, 2 networks were constructed: network A was based on treatment group type and network B was based on specific treatments. A network diagram was created to demonstrate how each intervention is connected to others through direct comparisons. Within this network diagram, the line width indicates the proportion of patients on a particular treatment, with direct comparison between nodes. Odds ratios (ORs) were summarized as the effect size of each treatment in forest plots. The directions of each treatment were compared with the reference group who had a hysterectomy. Heterogeneity between studies was represented by I^2. We considered I^2 values of more than 50% as indicators of high heterogeneity, but we used random effect models throughout the study since the network meta-analysis of PPH may have had both between and within group variance. However, since the I^2 was 0 in the models used, there were no difference between the random or fixed effect model. The models were used to calculate OR and 95% confidence intervals (CI). For comparison between treatments, each intervention was ranked by the surface under the cumulative ranking curve (SUCRA), known as a *P*-score, which is a frequentist approach to calculate SUCRA without resampling [29]. SUCRA indicates priority where the larger the SUCRA, the better priority. A value of $P<0.05$ was considered statistically significant. Statistical analysis was performed using the 'netmeta' package in R-Studio 1.2.1335 (R studio, Boston, MA, USA).

Results

A total 589 observational studies were initially identified. After screening titles and abstracts, the full text of 64 studies were reviewed, of which 58 were then eliminated. Six studies were finally selected as part of the analyses. Fig. 1 shows the flow of study selection.

The characteristics of the selected publications are summarized in Table 1. The selected publications included 4 studies in China, and one study each in Hong Kong and the USA. Failure to manage bleeding after one or more non-pharmacological treatments leads to postpartum hysterectomy, which was deemed the failure rate. Comparing the B-lynch operation with the use of Bakri balloon tamponade, Yan et al. [30] Chinese study demonstrated the highest failure rate (0.26 vs. 0.185), while the Zhao et al. [31] study observed

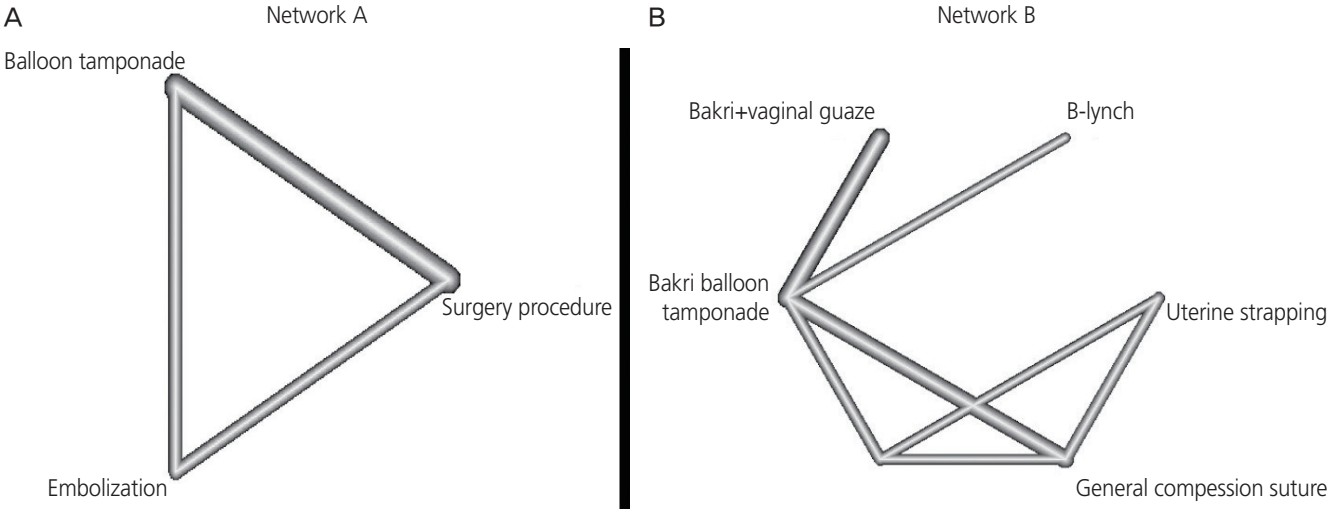

Fig. 3. Network diagrams for postpartum hemorrhage treatments. Nodes represent an intervention and their size is proportional to the number of trials comparing this intervention to any other in the network. The lines connecting each pair of interventions represent an indirect comparison and are line widths are proportional to the number of trials making each indirect comparison. (A) Network A compares 1 type of management, and (B) network B compares complex treatment management.

the lowest failure rate (0 vs. 0.097).

Fig. 2 shows the quality assessment of selected studies. The overall risk of bias was low, except for confounding variables and selective outcome reporting. Feng (2016) and Zhao (2014) did not consider confounding variables. The quality of all studies was reasonable.

Fig. 3 represents direct and indirect relationships among interventions. We also created 2 networks to compare failure rates among studies: the first network grouped by treatment with one management intervention and another network grouped by complex treatments (Fig. 3). The thick line between the balloon tamponade and the surgical procedure represents the most frequent intervention.

Fig. 4 shows indirect and network analysis for PPH treatments. In network A, 3 treatment groups, including balloon tamponade, uterine artery embolization, and surgical procedure, were compared. The surgical procedure was set as the control group, and the balloon tamponade approach was shown to have a 56% lower risk of hysterectomy (OR, 0.44; 95% CI, 0.50–30.54), while uterine artery embolization had 26% lower risk of hysterectomy than the control group (OR, 0.74; 95% CI, 0.22–2.50). In conclusion, non-surgical treatment groups (i.e. Bakri® balloon tamponade, uterine artery embolization) showed a lower failure rate than the surgical group.

In network B, the general compression suture was set as the control group, and compared to all other complex treatments. The B-lynch suture had a risk of hysterectomy that was 4 times higher than general compression suture (OR, 3.91; 95% CI, 0.50–30.54), while uterine artery embolization had 5% lower risk than general compression suture (OR, 0.44; 95% CI, 0.50–30.54). Bakri® balloon tamponade plus vaginal gauze had a failure risk that was 72% lower than general compression suture (OR, 0.28; 95% CI, 0.07–1.18). In conclusion, balloon tamponade and embolization, which were included in the non-surgical treatment group in network A, resulted in a lower failure rate than the control group. On the contrary, B-lynch suture and uterine strapping, which were included in the surgical procedure group in network B, resulted in a higher failure rate than the control group.

The rank of interventions is shown in Table 2. In network A, balloon tamponade ranked higher (0.89 vs. 0.44) than uterine artery embolization. Balloon tamponade had the highest treatment success. In network B, treatments using Bakri (Bakri balloon tamponade plus vaginal gauze and only Bakri balloon tamponade) had the highest ranks (0.95 vs. 0.69 vs.

Table 2. Surface under the cumulative ranking curve (SUCRA): rank of 'network A' treatment groups and 'network B' specific treatments in failure rates

Intervention	SURCA	Rank
Network A		
Balloon tamponade	0.89	1
Uterine artery embolization	0.44	2
Surgery	0.17	3
Network B		
Bakri balloon tamponade + vaginal gauze	0.95	1
Bakri balloon tamponade	0.69	2
Embolization	0.48	3
General compression suture	0.45	4
Uterine strapping	0.34	5
B-lynch	0.09	6

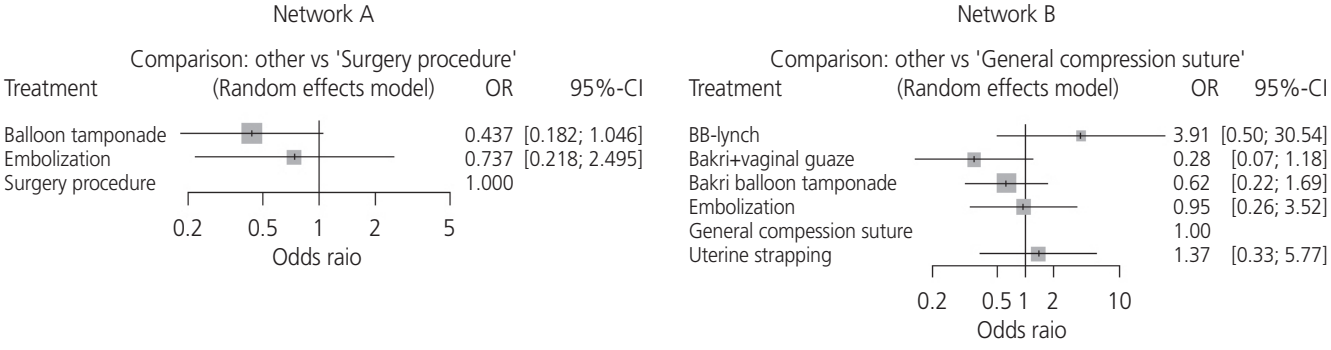

Fig. 4. Forest plot with odd ratios (ORs) and 95% confidence intervals (CIs) from pairwise, indirect and network analysis for postpartum hemorrhage treatments.

0.48) compared with all other treatments.

The consistency test revealed a *P*-value of 0.83 in network A and 0.98 in network B. Thus, our null hypothesis was rejected, indicating that both networks were appropriate. This analysis was repeated for all cases, and no inconsistencies were found. Although the number of included studies was insufficient to determine asymmetry, we found no visual asymmetry in the funnel plots (Fig. 5). Egger's regression test and Begg's test indicated no significant asymmetry, with *P*-value 0.10 and 0.26, respectively.

Discussion

Excessive bleeding after birth is the world's most common cause of death among mothers during childbirth. While most women will have moderate bleeding at birth, others may bleed excessively, which can pose a serious risk to their health and life. To reduce excessive bleeding at birth, the routine use of prophylactic uterotonic drugs has become standard practice worldwide [32].

The use of hysterectomy, the single most dramatically altering procedure, as well as a stressful and surgically challenging procedure, has been a main reason behind low maternal mortality rates in developed countries. The WHO listed peripartum hysterectomy as an identification criterion for "maternal near-miss", which has been introduced as an analytical tool to address health system failures, with the overall goal of improving obstetric care.

In order to guarantee an immediate response and a multidisciplinary team approach, every obstetric practitioner needs to be trained in the management of PPH. Internationally recognized guidelines [33] indicate that one or more second-line measures, including intrauterine (balloon) tamponade, hemostatic brace suturing, ligation of the uterine arteries, and interventional radiology, should be available in hospitals with delivery units and that obstetric practitioners should be familiar with these procedures.

The aim of this study was to provide a new methodology to overcome the limitations of previous systematic reviews and meta-analyses, which only assessed the effect of non-pharmacological treatment, surgical or non-surgical procedures, separately and without considering any prior intervention with pharmacologic treatments. With the network meta-analysis proposed here, these comparisons could be made.

The most recent study on PPH was a systematic review on pharmacological uterotonic agents in prevention of PPH [27]. To our knowledge, this is the first study to conduct a network meta-analysis comparing surgical and non-surgical approaches in the treatment of non-pharmacologic PPH. Considering the effect size of PPH management in each intervention, performing non-surgical treatment prior to surgical treatment helps PPH management. This study is expected to provide further evidence of the effect of non-pharmacological treat-

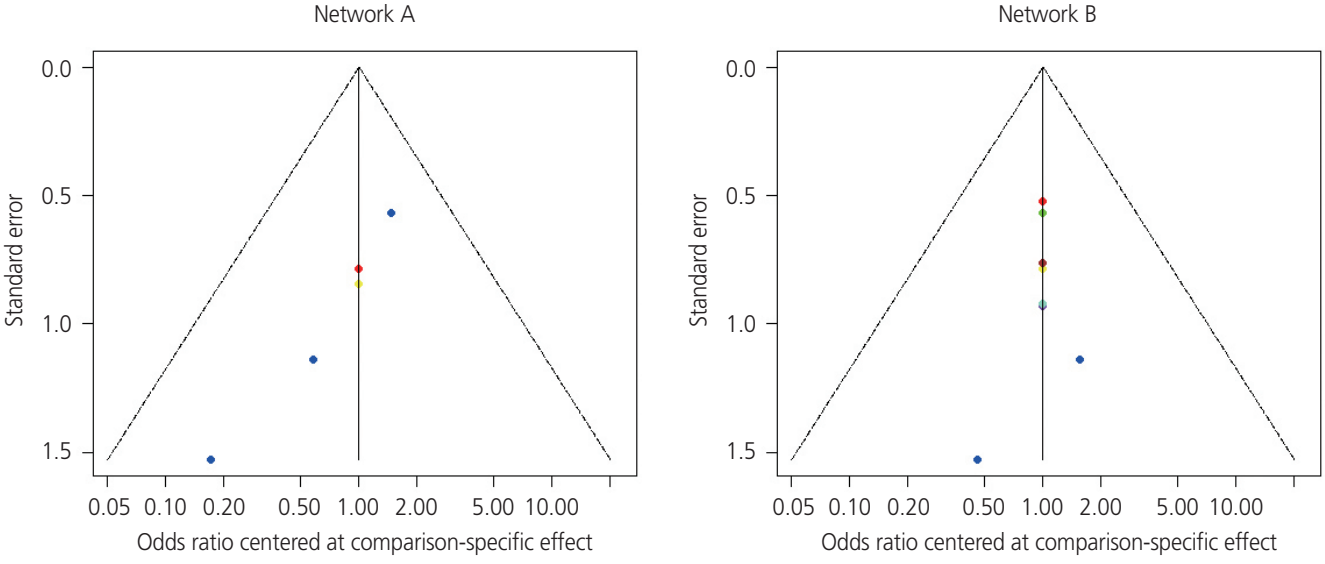

Fig. 5. Funnel plots of selected studies based on treatment grouping.

ment on uncontrolled PPH, and thus help decision making processes carried out by emergent medical professionals and patients. The California Maternal Quality Care Collaborative is actually managed by the OB Hemorrhage Toolkit V20 [33].

Our results are consistent with those of previous studies [34.35]. In PPH, about 80% of patients did not undergo hysterectomy when treated by the Barkley balloon method [34], which suggests that the Barkley balloon method is an appropriate first choice treatment of PPH. On the other hand, uterine artery embolization has treated more than 90% of postpartum bleeding cases, deeming it suitable for the first treatment of postpartum bleeding [35].

This network meta-analysis demonstrates selection bias towards observational studies, due to the difficulty in conducting randomized controlled studies of postpartum bleeding, a life-threatening condition. Consequently, the homogeneity of baseline characteristics of the interventions was not ensured and statistical significance was not achieved, due to the small number of studies. For the same reason, the mode of delivery (i.e. vaginal, cesarean) was not able to be considered as a subgroup. Finally, since there are various reasons for PPH [36] and the effect of treatments may differ, more well-designed studies should be provided to perform a meta-analysis classified by reasons behind why bleeding has occurred.

In conclusion, balloon tamponade is the best method for uncontrolled postpartum bleeding with pharmacologic treatment, followed by embolization and surgical procedures, for the quick treatment of postpartum bleeding.

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Conflict of interest

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

Ethical approval

No institutional approval was required because this was an analysis of publicly available data that were produced by Statistics Korea according to the Bioethics and Safety Act (IRB-2019-0014).

Patient consent

Informed consent was not required because this was an analysis of publicly available data that were produced by Statistics Korea according to the Bioethics and Safety Act (IRB-2019-0014).

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Original Article

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Delayed diagnosis of gestational diabetes mellitus and perinatal outcomes in women with large for gestational age fetuses during the third trimester

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Objective

We evaluated the incidence of newly diagnosed gestational diabetes mellitus (GDM) during the 3rd trimester in women with suspected large for gestational age (LGA) fetuses on ultrasound and assessed their perinatal outcomes.

Methods

A retrospective cohort study was performed. Singleton pregnant women with suspected LGA on the 3rd trimester ultrasound and whose results of GDM screening at midpregnancy had been normal were enrolled. All participants were retested with 100-g oral glucose tolerance test (OGTT) within 2 days after diagnosis of LGA. We compared perinatal outcomes between the newly diagnosed with GDM group and the non-GDM group.

Results

Among 169 pregnant women, 13% (23/169) were newly diagnosed with GDM. The women in the GDM group had a higher HbA1c level at diagnosis (5.8 vs. 5.3, $P<0.01$) and earlier gestational age at delivery (38.0 vs 38.9 weeks of gestation, $P=0.003$) than those in the non-GDM group. The rate of cesarean delivery (CD) was significantly higher in the GDM group than that in the non-GDM group (73.9%, vs. 49.3%, $P=0.028$) with similar proportions for the indications of CD except CD on maternal request (CDMR). The CDMR rate was higher in the GDM group than non-GDM group (41.2% vs. 23.6%) but it did not reach statistical significance. There were no significant differences in the obstetrical and neonatal complications between the two groups.

Conclusion

Among pregnant women with suspected LGA, 13% were newly diagnosed with GDM in late pregnancy. Nonetheless, there were no differences in the perinatal outcomes between women with newly diagnosed GDM and those without GDM. However, concerns over shoulder dystocia appear to increase CD rates in the GDM group.

Keywords: Gestational diabetes mellitus; Large for gestational age; Oral glucose tolerance test

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Introduction

As gestational age progresses and the placenta increases in size, the levels of pregnancy-associated hormones secreted from the placenta such as estrogen, progesterone, cortisol, and placental lactogen also increase. In particular, the secretion of human placenta lactogen (hPL) increases approximately 10-fold in the second half of pregnancy. hPL, which is a potent antagonist of insulin action, induces fasting hypoglycemia, postprandial hyperglycemia and hyperinsulinemia physiologically in pregnant women by promoting lypolysis and increasing free fatty acid in maternal serum to conserve glucose for fetal growth [1]. As the pregnancy progresses, insulin sensitivity begins to decrease, and insulin resistance increases by hormones secreted in the placenta during the pregnancy [2]. As a result, the risk of diabetes increases theoretically in the latter half of pregnancy.

Gestational diabetes mellitus (GDM) is known to induce complications for both mother and fetus. Maternal complications include gestational hypertensive disease, risk of cesarean delivery (CD), future development of type 2 diabetes mellitus (DM), cardiovascular disease and metabolic syndrome [3-7]. If glucose control is poor in women with GDM, the risk of unexplained stillbirth may be similar to that of pregnant women with pregestational DM [8,9]. Neonatal hypoglycemia, hyperinsulinemia, and risk of macrosomia can develop in neonates of women with GDM [10,11].

GDM is known as a leading cause of excessive fetal growth. Large for gestational age (LGA) is defined as birth weight above the 90th percentile for gestational age [12]. Mothers with diabetes, either GDM or pregestational DM, have a 3-fold higher rate of LGA than women with normal range blood glycemic control, with 15–45% of all cases of LGA [13]. A high concentration of glucose in maternal serum is delivered to the fetus through the placenta, which results in fetal hyperglycemia. Then, insulin secretion is increased in the fetus to synthesize lipid and glycogen from blood glucose, resulting in excessive accumulation of fat in the shoulder and middle of the body and increased body weight of fetus. LGA can lead to complications for both mother and infant, it increases the risk of shoulder dystocia, clavicle fracture, brachial plexus injury, and neonatal intensive care unit admission rate. In the mother, it increases the rate of CD, vaginal laceration, and postpartum hemorrhage [10,11].

Most pregnant women undergo an oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation. If they are diagnosed with GDM, blood glucose control will be necessary throughout the pregnancy for prevention of complications. However, LGA is sometimes suspected on the ultrasound in late pregnancy in women with normal routine blood glucose test results at 24–28 weeks of gestation. There are a few studies about maternal glucose intolerance in the 3rd trimester. One prospective study evaluated the incidence of abnormal 75-g OGTT results and their perinatal outcomes in previously normoglycemic pregnant women during the 3rd trimester [14]. It showed that the incidence was 13.5% and there was no increased risk of perinatal outcomes. However, there has been no previous study about delayed diagnosis of GDM in women with LGA fetuses during the 3rd trimester. We focused on pregnant women with suspected LGA on the 3rd trimester ultrasound but whose previous routine glucose testing had been normal between 24 and 28 weeks of gestation. This study was performed to evaluate the incidence of newly diagnosed GDM in the 3rd trimester of pregnancy and the perinatal outcome in these women.

Materials and methods

1. Study design

A retrospective cohort study was performed. Among singleton pregnant women who delivered at Seoul Metropolitan Government-Seoul National University Boramae Medical Center from January 2010 to December 2018, women who were retested for an OGTT because of suspected LGA on ultrasound in the 3rd trimester were enrolled. Their results of GDM screening at 24–28 weeks of gestation had been normal. Women with pre-gestational DM, GDM already diagnosed or no glucose testing during the 2nd trimester, twin pregnancy, or fetal congenital malformations were excluded.

Routine blood glucose testing at 24–28 weeks of gestations was performed using a 2-step process: 50-g oral glucose challenge test (GCT), followed by a 100-g OGTT for abnormal result in the 50-g GCT (>140 mg/dL). Interpretation of the diagnostic 100-g OGTT followed the Carpenter-Coustan criteria. The reference values were as follows: fasting, 95 mg/dL; 1-hour, 180 mg/dL; 2-hour, 155 mg/dL; 3-hour, and 140 mg/dL; 2 or more of the plasma glucose concentrations are met or exceeded for a positive diagnosis [15]. The test was performed in the morning after an overnight fasting

over 8 hours, with the women remaining seated and at rest. Estimated fetal weight (EFW) was calculated by the Hadlock formula using ultrasonography, and LGA was defined as EFW more than 90th percentile for gestational age. We used the reference of percentile distributions of birth weight according to gestational ages in Korea [16]. If an LGA fetus was suspected during the 3rd trimester, the 100-g OGTT was retested same as routine manual within 2 days after diagnosis of LGA on ultrasound. If women were newly diagnosed with GDM, they started diet control and checked blood sugar levels unless delivery was imminent.

We compared the incidence of CD, obstetrical complications (gestational hypertensive disease, preterm birth, and shoulder dystocia), and neonatal complications (neonatal intensive care unit [NICU] admission and neonatal hypoglycemia) between the newly diagnosed with GDM group and the non-GDM group.

This study was approved by the Institutional Review Board of the Seoul Metropolitan Government-Seoul National University Boramae Medical Center (IRB No. 30-2019-111).

2. Statistical analyses

For statistical analysis, comparison of the proportions was performed using Pearson's χ^2 test or Fisher's exact test. Continuous variables were compared using the Mann-Whitney *U* test. Logistic regression analysis was used to evaluate the relationship between the presence or absence of GDM and the pregnancy outcomes of interest after adjustment for gestational age. *P*-value <0.05 was considered statistically significant. International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) statistics software ver. 20.0 (IBM Corp., Armonk, NY, USA) was used for data analysis.

Table 1. Maternal characteristics

Characteristics	100-g OGTT in late pregnancy		*P*-value
	Positive (n=23)	Negative (n=146)	
Maternal age (yr)	34.7 (24–40)	33.1 (22–45)	0.077
Nulliparity	13 (56.5)	83 (56.8)	0.976
Pregestational BMI (kg/m^2)	23.4 (18.1–30.7)	22.9 (17.5–46.5)	0.174
Family history of type 2 DM	3 (13.0)	25 (17.1)	0.625
HbA1c (%)	5.8 (4.9–7.1)	5.3 (4.6–6.4)	0.000

Values are presented as number of patients (%) or median value and range.
OGTT, oral glucose tolerance test; BMI, body mass index; DM, diabetes mellitus.

Table 2. Neonatal characteristics and perinatal outcomes according to the diagnosis of gestational diabetes mellitus in late pregnancy

Characteristics	100-g OGTT in late pregnancy		*P*-value
	Positive (n=23)	Negative (n=146)	
Polyhydramnios	0	3 (2.1)	0.481
Gestational age at delivery (wk)	38.0 (34.0–40.6)	38.9 (34.9–41.3)	0.003
Birth weight (g)	3,512 (2,640–4,480)	3,603 (2,310–4,730)	0.360
LGA at delivery	20 (87.0)	124 (84.9)	0.547
Male fetus	11 (47.8)	84 (57.5)	0.761
Shoulder dystocia	0	4 (2.7)	0.422
NICU admission	1 (1.8)	12 (8.2)	0.512
Neonatal hypoglycemia	0	0	-
Gestational hypertensive disorders	1 (4.3)	3 (2.1)	0.504
Preterm birth	2 (8.7)	5 (3.4)	0.238

Values are presented as number of patients (%) or median value and range.
OGTT, oral glucose tolerance test; LGA, large for gestational age; NICU, neonatal intensive care unit.

Results

Of 169 pregnant women who were retested using the 100-g OGTT, 13% (23/169) were newly diagnosed with GDM. There were no significant differences in the maternal age, parity, pre-pregnancy body mass index, family history of DM, gestational age at OGTT, and fetal sex between the newly diagnosed with GDM group and the non-GDM group. The women in the GDM group had higher level of HbA1c at diagnosis (5.8 vs 5.3, *P*<0.01) and earlier gestational age at delivery than those in the non-GDM group (38.0 vs. 38.9 weeks of gestation, *P*=0.003), but birth weight was not significantly different between the two groups (3,512 g vs. 3,603 g, *P*=0.360) after adjustment for gestational age at delivery (*P*=0.548). There were no significant differences in incidence of obstetrical complications (gestational hypertensive disease, preterm birth, and shoulder dystocia) and neonatal complications (NICU admission and neonatal hypoglycemia) between the two groups. Tables 1 and 2 show maternal, neonatal characteristics, and perinatal outcomes.

The results of routine blood glucose testing at 24–28 weeks of gestation are described in Table 3. The values of the 50-g GCT were similar in both groups. If the result of 50-g GCT was above the cutoff value, the 100-g OGTT was

Table 3. Results of previous routine blood glucose testing between 24 and 28 weeks of gestation according to the subsequent diagnosis of gestational diabetes mellitus in late pregnancy

Characteristics	100-g OGTT in late pregnancy		*P*-value
	Positive (n=23)	Negative (n=146)	
50-g GCT (mg/dL)	127 (80–165)	123 (68–176)	0.374
100-g OGTT	6 (26.1)	28 (19.2)	0.442
Fasting (mg/dL)	83 (75–91)	84 (69–107)	0.791
1-hour (mg/dL)	166 (159–175)	152 (109–199)	0.177
2-hour (mg/dL)	156 (134–169)	128 (90–164)	0.018
3-hour (mg/dL)	128 (119–138)	109 (48–157)	0.044

Values are presented as number of patients (%) or median value and range.
OGTT, oral glucose tolerance test; GCT, glucose challenge test.

Table 4. Results of retested 100-g oral glucose tolerance test (OGTT) after identifying excessive fetal growth during the 3rd trimester ultrasound

Characteristics	100-g OGTT in late pregnancy		*P*-value
	Positive (n=23)	Negative (n=146)	
Estimated fetal weight at 100-g OGTT (g)	2,742 (1,491–3,769)	2,884 (1,290–4,197)	0.268
Gestational age at 100-g OGTT (wk)	34.8 (30.1–38.9)	35.3 (29.0–40.7)	0.270
Values of OGTT (mg/dL)			
Fasting	87 (73–120)	81 (58–108)	0.006
1-hr	188 (159–268)	147 (69–204)	0.000
2-hr	170 (121–260)	123 (87–177)	0.000
3-hr	134 (54–213)	110 (53–155)	0.026
Abnormal OGTT			
Fasting	4 (17.4)	5 (3.4)	0.006
1-hr	17 (73.9)	8 (5.5)	0.000
2-hr	18 (78.3)	8 (5.5)	0.000
3-hr	13 (56.5)	10 (6.8)	0.000

Values are presented as number of patients (%) or median value and range.
GCT, glucose challenge test.

performed. The profiles of the 100-g OGTT results were also similar between the groups, but the 2-hour value was significantly higher in women diagnosed with GDM (156 vs. 128, *P*=0.018).

Table 4 demonstrates the results of retested 100-g OGTT after identifying excessive fetal growth on the 3rd trimester ultrasound. The gestational age and EFW on ultrasound at the time of 100-g OGTT were not significantly different between the two groups. In the GDM group, 17.4%, 73.9%, 78.3% and 56.5% of patients revealed abnormal serum glucose level in fasting, 1-hour, 2-hour, and 3-hour values in 100-g OGTT, respectively.

The characteristics of the delivery mode are shown in Table 5. The rate of trial of labor was significantly lower in the GDM group than that in the non-GDM group (39.1% vs 71.2%, *P*=0.002). The rate of CD in the GDM group was significantly higher than that in the non-GDM group (73.9% vs. 49.3%, *P*=0.028). When we analyzed the indication for CD, the proportions of indications were similar in both groups, except CD on maternal request (CDMR). The rate of CDMR without any obstetrical indication was higher in the GDM than that in the non-GDM group (41.2% vs. 23.6%), but it could not reach statistical significance. The distribution of other indications for CD was similar between the two groups.

After delivery, we conducted a postpartum 75-g OGTT in women diagnosed with GDM. None of the women were diagnosed with DM and only one had impaired glucose tolerance (fasting 112 mg/dL, 2-hour 187 mg/dL).

Discussion

This is the first study to analyze the incidence of newly diagnosed GDM and perinatal outcomes in previously normoglycemic pregnant women with suspected LGA fetuses in the 3rd trimester.

While showing normal results in routine blood glucose test at mid-pregnancy, 13% of pregnant women with suspected LGA fetuses in the 3rd trimester of pregnancy were newly diagnosed with GDM. Nonetheless, there were no differences in the perinatal outcomes between the newly diagnosed GDM group and the non-GDM group. However, concerns over complications of GDM, such as shoulder dystocia, appear to increase CD.

Because GDM is the main cause of fetal excessive growth and insulin resistance increases as gestational age progresses, newly developed GDM can be considered in pregnant woman when EFW is large for gestational age in the 3rd trimester, even if their previous GDM screening at 24–28 weeks of gestation had been normal. After diagnosis of GDM, even

Table 5. Characteristics of delivery mode in both groups

Characteristics	100-g OGTT in late pregnancy		*P*-value
	Positive (n=23)	Negative (n=146)	
Trial of labor	9 (39.1)	104 (71.2)	0.002
Induction of labor	6 (66.7)	52 (50.0)	0.337
Spontaneous labor pain	2 (22.2)	28 (27.9)	0.715
Premature rupture of membrane	1 (11.1)	23 (22.1)	0.439
Operative delivery	4 (17.4)	26 (17.8)	0.961
Cesarean delivery	17 (73.9)	72 (49.3)	0.028
Indications for CD			
Previous CD	5 (29.4)	20 (27.8)	0.893
Failure to progress	3 (17.6)	21 (29.1)	0.336
Malpresentation	1 (5.9)	4 (5.6)	0.958
Fetal deceleration	0	6 (8.3)	0.218
Placenta previa	1 (5.9)	4 (5.6)	0.958
CDMR	7 (41.2)	17 (23.6)	0.142

Values are presented as number of patients (%).
OGTT, oral glucose tolerance test; CD, cesarean delivery; CDMR, cesarean delivery on maternal request.

if in late pregnancy, pregnant women used to control their blood sugar levels to prevent obstetric and perinatal complications associated with GDM. A previous study showed that patients diagnosed with GDM during the 3rd trimester had infants with lower birthweight than those of non-diabetics or those with a single pathological value of GTT. Glucose control in women with late-onset GDM may lead to lower birthweights, and there was no difference in CD rate and neonatal outcomes after adjustment for potential confounders in one study [17].

In our study, about 13% of participants were newly diagnosed with GDM in late pregnancy. Generally, the more advanced the gestational age, the higher the possibility of GDM development because of increasing insulin resistance with the growing placenta volume. Therefore, there were several previous studies to evaluate the relationship between abnormal blood glucose levels during the 3rd trimester and perinatal outcomes in previously normoglycemic pregnant women. In one study, the incidence of newly developed GDM and glucose intolerance, which was diagnosed when satisfying only one abnormal value of four blood glucose levels in 100-g OGTT, was 4.3% and 9.9%, respectively [17]. In another study, 486 high-risk pregnant women were retested for GDM after 32 weeks of gestation, and 4.7% were newly diagnosed with GDM [18]. The authors called it late-onset GDM. There was a study reporting that 6.7% of 404 women were diagnosed with GDM in the 3rd trimester [19]. The incidence of newly diagnosed GDM during the 3rd trimester varied among the previous studies but was lower than that in our study (13%). This is probably because we enrolled only pregnant women with LGA fetuses, who are likely to have GDM. Retesting GDM in pregnant women with LGA fetuses during the 3rd trimester seems to be meaningful because they are more likely to have GDM than normal pregnant women. A previous study showed an incidence of newly diagnosed GDM during the 3rd trimester similar to our result [14]. However, our study differs from the previous study in two aspects, study population and diagnostic method. We enrolled only pregnant women with LGA fetuses in the 3rd trimester, who were at high risk of GDM, and we used a 100-g GTT following Carpenter-Coustan criteria as a diagnostic test, unlike their 75-g OGTT.

Gestational age at delivery was significantly earlier in the GDM group than non-GDM group (38.0 vs. 38.9 weeks of gestation, P=0.003) in our study. This is presumed to be a result of earlier intervention considering maternal and fetal complications of GDM. The median value of birth weight has no statistical difference between the two groups after adjusting for gestational age. After GDM diagnosis, it is expected that the occurrence of GDM-related complications, including LGA, will be reduced because those women will start to control their diet and monitor their blood sugar levels [20]. There were no differences in other perinatal outcomes between the newly diagnosed GDM group and non-GDM group in this study. The intervention performed after the diagnosis of GDM in the GDM group may have affected the perinatal outcomes.

From the aspect of delivery mode, the rate of trial of labor was significantly higher in the non-GDM than in the GDM group. Conversely, CD was more often performed in the GDM group. These results may be due to concerns over complications of GDM, such as shoulder dystocia, caused by LGA in the GDM group. The rate of CDMR was particularly higher in the GDM group, although the difference did not reach statistical significance.

We usually do not test the HbA1c value when a routine blood glucose test is conducted during the 2nd trimester if not indicated. In our study, the HbA1c value measured during the 3rd trimester was significantly higher in the newly diagnosed GDM group than non-GDM group (5.8 vs 5.3, $P \leq 0.05$). HbA1c is a standard method for assessing long-term glucose control over the previous several weeks, about 2–3 months [21]. If HbA1c is requested during the 2nd trimester and screened together at the time of blood glucose testing, it may be useful to predict future development of GDM even if the results did not reach the diagnostic threshold for the GDM at that time of routine blood glucose testing. HbA1c can be a more precise tool than OGTT to evaluate glucose metabolism by reflecting long-term blood glucose control. However, in order to apply this protocol to actual practice, it is required to evaluate the cost-effectiveness of this additional test.

GDM can affect not only the current but also the next pregnancy. History of GDM corresponds to the high-risk group according to the GDM risk assessment by criteria of the Fifth International Workshop-Conference on Gestational Diabetes. It is recommended that these pregnant women take the test for GDM as soon as possible after diagnosis of their next pregnancy [22]. This will enable timely GDM screening at the next pregnancy, and adequate manage-

ment according to the results may improve the pregnancy outcome because women with a history of GDM have risk of developing GDM during early pregnancy, which can be associated with poor pregnancy outcome compatible with pregestational DM. Moreover, there is a 50% likelihood of women with GDM developing overt diabetes within 20 years [23]. Thus, it can provide important information on their future health care.

This study had some limitations. It was a retrospective study, and a number of pregnant women with LGA fetuses could not be enrolled because they had not been retested for GDM during the 3rd trimester. We have plans for prospective studies to enroll more pregnant women with LGA fetuses during the 3rd trimester. In addition, we want to evaluate the relationship between HbA1c at the time of routine blood glucose testing at 24–28 weeks of gestation and future development of LGA fetuses during the 3rd trimester.

In conclusion, the rate of newly diagnosed GDM was 13% in pregnant women with suspicious excessive fetal growth in the 3rd trimester of pregnancy. Nonetheless, there were no differences in the perinatal outcomes between women with newly diagnosed GDM and those without GDM. However, concerns over complications of GDM, such as shoulder dystocia, appear to increase the rate of CD.

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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 the Seoul Metropolitan Government-Seoul National University Boramae Medical Center (IRB no. 30-2019-111).

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Maternal, infant, and perinatal mortality statistics and trends in Korea between 2009 and 2017

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Objective
To provide updates on maternal, infant, and perinatal mortality using the national population data of South Korea between 2009 and 2017 and describe the mortality rate by target groups, timing, or causes of events to provide a basis for detecting vulnerable populations and ensuring timely medical and political interventions.

Methods
Pregnancy-related mortality in women, as well as deaths of infants, in South Korea was identified using population data from Statistics Korea. Records from death certificates, cremation reports on infant and fetal deaths, and the complementary cause-of-death investigation system were reviewed for the 2009–2017 period.

Results
A total of 461 maternal deaths, 11,717 infant deaths, and 12,249 perinatal deaths, including fetal deaths over 28 gestational weeks, were identified from 3,945,159 live births between 2009 and 2017. The maternal mortality ratio was 13.5 deaths per 100,000 live births in 2009 and decreased to 7.8 in 2017. Only the rate of deaths related to hypertensive disorders showed an increasing tendency. Both the infant and perinatal mortality rates improved (from 3.2 deaths per 1,000 live births in 2009 to 2.8 in 2017 and from 3.5 to 2.7, respectively). Among the external causes of infant mortality, assaults including homicides accounted for 25% (n=150), and this proportion was constant throughout the study period.

Conclusion
Overall improvements were observed in all maternal, infant, and perinatal mortality measures. In-depth analysis and interventions with respect to certain causes, such as hypertensive disorders in mothers or assaults in infants, should be considered priority issues.

Keywords: Maternal mortality; Infant mortality; Perinatal mortality; Cause of death; South Korea

Introduction

Maternal, infant, and perinatal mortality are surrogate measures of the national health status and indicators of social development, for which cross-country comparisons are frequently conducted. The maternal mortality ratio and infant mortality ratio in South Korea decreased from 14 deaths per 100,000 live births in 2005 to 8.4 in 2016 and from 4.7 to

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2.8, respectively [1]. Although the changes are meaningful, the maternal mortality ratio is still higher than the average in Organization for Economic Cooperation and Development (OECD) countries, and the indices seem to have been stagnant in recent years. A detailed epidemiological description is necessary to develop and implement proper public health interventions for improving mortality measures. However, few reports are available on this issue thus far [2].

Previously, Statistics Korea reported an overview of infant, maternal, and perinatal mortality statistics in South Korea, with a focus on the number of deaths, the crude death rate, and the ranking of causes of death. We reinterpreted the mortality rate from the point of view of the obstetrician with researchers of the vital statistics division in Statistics Korea because of a rapid diminution in the number of births in South Korea.

The primary purpose of this study was to provide updates on maternal, infant, and perinatal mortality in South Korea. Specifically, we aimed to describe the mortality rates by target groups, timing, or causes of events to provide a basis for detecting vulnerable populations and ensuring timely medical and political interventions.

Materials and methods

A retrospective investigation of mortality events recorded in 2017 was conducted. The data were compared with previous records in the 2009–2016 period.

1. Sources of data

Mortality events were aggregated from 3 different data sources: death certificates, cremation reports on infant and fetal deaths, and the complementary cause-of-death (COD) investigation system (Supplementary Data 1).

The death certificate data, based on the Act on the Registration, etc. of Family Relationships, are ascertained in real time to follow the population dynamics at the national level [3]. When an event occurs, a family member or local community officer submits the death certificate to the population dynamics system. The collected data are forwarded to the county level and province level, sequentially, and finally entered into the Statistics Korea database.

Cremation reports on infant and fetal deaths are described based on the Act on Funeral Services, etc. [4]. The reports first collected at each crematorium are forwarded to the provincial office and finally entered into the Statistics Korea database.

The COD system serves as a retrospective reconfirming process of mortalities detected from death certificates, cremation reports, or the National Health Insurance Service (NHIS). Maternal death that occurred during pregnancy or within 6 months of delivery, infant death within 1 year of birth, and fetal death over 16 weeks of gestation are first screened by Statistics Korea. The health-care institutions where the deceased was born, died, or had any experience of pregnancy- or delivery-related medical care are then requested to complete the complementary investigation on medical history.

2. Data validation

All event records ascertained from different sources are submitted to Statistics Korea, which reviews the completeness of the data and integrates them into a unified national mortality database based on personal identification codes. Multiple data sources not only enable crosschecking for quality assurance but also reduce the burden on respondents. To minimize bias from missing data, substituting values were calculated based on relevant records and variables.

3. Coding principles

The cause of death was defined as (a) the disease or injury that initiated the train of morbid events leading directly to death or (b) the circumstances of the accident or violence that produced the fatal injury [5] and coded according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) [5].

In cases where the cause of death was unclear, additional administrative data from the NHIS, National Cancer Center, National Forensic Service, National Police Agency, or Centers for Disease Control and Prevention were referenced for completion.

The causes of maternal mortality and infant, fetal, and neonatal mortality were classified based on the World Health Organization (WHO) recommendations of the general mortality condensed list and infant and child mortality condensed list, respectively [5].

4. Statistics

Maternal death was defined as "the death of a woman while pregnant or within 42 days of termination of pregnancy, irre-

spective of the duration and the site of the pregnancy, from any cause related to or aggravated by the pregnancy or its management, but not from accidental or incidental causes" [5]. It was subdivided into direct obstetric deaths ("those resulting from obstetric complications of the pregnant state [pregnancy, labor, and puerperium]; from interventions, omissions, or incorrect treatment; or from a chain of events resulting from any of the above") and indirect obstetric deaths ("those resulting from previous existing disease or disease that developed during pregnancy and that was not due to direct obstetric causes, but that was aggravated by physiologic effects of pregnancy") [5]. The maternal mortality ratio and rate were calculated based on these definitions.

The infant mortality rate was calculated from deaths that occurred before 1 year after birth. Among those, neonatal mortality was classified as deaths that occurred before 28 days after birth. Fetal death was defined as the death of the fetus over 16 gestational weeks or "death prior to the complete expulsion or extraction from its mother of a product of conception, indicated by the absence of evidence of life after such separation" [5]. Meanwhile, perinatal death was calculated as the sum of fetal deaths that occurred after 28 gestational weeks and neonatal deaths that occurred before 7 days after birth.

The detailed definitions and equations for the indices used in this study are summarized in the technical note.

5. Technical note

The terms used in this study are based on the ICD-10 by the WHO.

Maternal death: the death of a woman while pregnant or within 42 days of termination of pregnancy, irrespective of the duration and the site of the pregnancy, from any cause related to or aggravated by the pregnancy or its management, but not from accidental or incidental causes.

Direct obstetric death: maternal death resulting from obstetric complications of the pregnant state (pregnancy, labor, and puerperium); from interventions, omissions, or incorrect treatment; or from a chain of events resulting from any of the above.

Indirect obstetric death: maternal death resulting from previous existing disease or disease that developed during pregnancy and that was not due to direct obstetric causes, but that was aggravated by physiologic effects of pregnancy.

Maternal mortality ratio = (yearly number of maternal deaths [direct and indirect]/yearly number of live births) × k

k may be 1,000, 10,000 or 100,000, as preferred and indicated by the country.

Infant death: death of a newborn that occurred <365 days after birth.

Infant mortality rate = (yearly number of infant deaths/yearly number of live births) × 1,000

Neonatal death: death of a newborn that occurred <28 days after birth.

Neonatal mortality rate = (yearly number of neonatal deaths/yearly number of live births) × 1,000

Fetal death: death prior to the complete expulsion or extraction from its mother of a product of conception, indicat-

Table 1. Maternal mortality by year and maternal age: Korea, 2009–2017

Year	No. of maternal deaths	Maternal mortality ratio[a]	Age-specific ratio					No. of births
			Maternal age (yr)					
			≤24	25–29	30–34	35–39	≥40	
2009	60	13.5	18.0	9.6	9.4	26.4	78.3	444,849
2010	74	15.7	14.6	8.2	13.0	29.6	96.9	470,171
2011	81	17.2	0.0	12.4	14.5	33.7	65.8	471,265
2012	48	9.9	7.3	7.9	7.1	20.3	26.0	484,550
2013	50	11.5	0.0	8.8	7.7	23.3	54.4	436,455
2014	48	11.0	4.2	9.4	9.0	19.5	16.9	435,435
2015	38	8.7	0.0	5.3	8.8	14.1	8.0	438,420
2016	34	8.4	9.5	5.9	7.3	11.7	15.7	406,243
2017	28	7.8	0.0	4.1	6.2	14.0	15.9	357,771

[a]Deaths per 100,000 live births.

ed by the absence of evidence of life after such separation.

Perinatal death: fetal death and neonatal death occurring during the perinatal period. This study defines the perinatal period as from the 28th gestational week to before 7 days after birth, which corresponds to the definition of the United Nations and the OECD. Note, however, that the WHO uses the 22nd gestational week as the origin.

Perinatal mortality rate = (yearly number of perinatal deaths/yearly number of births*) × 1,000

*Number of births = number of live births + number of fetal deaths that occurred during the 28th gestational week or later.

Results

A total of 461 maternal, 11,717 infant, and 12,249 perinatal deaths with relevant baseline profile were identified between 2009 and 2017. All mortality indices showed a decreasing trend, although with some fluctuations, during the period.

The maternal mortality ratio decreased from 13.5 deaths per 100,000 live births in 2009 to 7.8 in 2017 (Table 1). In 2009 and 2010, the age-specific ratio showed a J-shaped pattern with a decrease in the age group less than 25 through 30–34 years followed by an increase with age in those older than 35 years. Since 2011, however, the ratios have been proportionated with age in pregnant women aged 25 years and older with no or the lowest records of deaths in those aged less than 25 years, except in 2016.

Of the 28 total maternal deaths in 2017, 79% (n=22) had direct obstetric causes (Table 2). While the mortality ratio from both direct and indirect causes decreased from 10.1 deaths per 100,000 live births and 3.4 in 2009 to 6.1 and 1.7 in 2017, respectively, the ratio of direct to indirect causes increased from 3.0 to 3.7. Specifically, complications predominantly related to the puerperium (e.g., infections or embolisms) were the most common, followed by complications of labor and delivery (e.g., postpartum hemorrhage). The proportion of these 2 entities constituted more than 70% of all direct causes across the years, except in 2009 (68.9%), 2011 (66.4%), and 2016 (64.3%). Meanwhile, the mortality ratio of hypertensive disorders, which was as low as 0.4 in 2009, increased more than 2-fold, at 1.1, in 2017 (Pearson's correlation coefficient, r=0.6), whereas all other causes showed a decrease or at least remained relatively constant

Table 2. Maternal mortality by groups of cause of death: Korea, 2009–2017

Year	Direct causes														Indirect causes	
	Total		Pregnancy with abortive outcome		Hypertensive disorders		Other maternal disorders		Maternal care-related problems		Complications of labor and delivery[a]		Complications related to the puerperium[b]		Other obstetric conditions		Total	
	No. of deaths	MM ratio[c]	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio	No. of deaths	MM ratio
2009	45	10.1	2	0.4	2	0.4	2	0.4	4	0.9	17	3.8	14	3.1	4	0.9	15	3.4
2010	45	9.6	1	0.2	3	0.6	0	-	3	0.6	17	3.6	20	4.3	1	0.2	29	6.2
2011	56	11.9	4	0.8	5	1.1	1	0.2	4	0.8	13	2.8	24	5.1	5	1.1	25	5.3
2012	31	6.4	3	0.6	4	0.8	1	0.2	1	0.2	7	1.4	15	3.1	0	0.0	17	3.5
2013	38	8.7	2	0.5	2	0.5	0	-	6	1.4	11	2.5	16	3.7	0	0.0	12	2.8
2014	39	9.0	2	0.5	4	0.9	2	0.5	1	0.2	17	3.9	13	3.0	0	0.0	9	2.1
2015	36	8.2	0	0.0	3	0.7	0	0.0	3	0.7	12	2.7	17	3.9	1	0.2	2	0.5
2016	28	6.9	2	0.5	4	1.0	0	0.0	4	1.0	4	1.0	14	3.4	0	0.0	6	1.5
2017	22	6.1	0	0.0	4	1.1	1	0.3	1	0.3	8	2.2	8	2.2	0	0.0	6	1.7

MM, maternal mortality.
[a]Including abnormalities of forces of labor (e.g., uterine inertia) and postpartum hemorrhage; [b]Including obstetric embolism; [c]Deaths per 100,000 live births.

(–0.7≤r≤–0.2) during the same period.

The infant mortality rate gradually improved from 3.2 deaths per 1,000 live births yearly in 2009 to 2.8 in 2017 (Table 3). The rate was higher in boys, regardless of year; there was a 23.1% decrease in the mortality rate in girls (3.1 in 2009 and 2.5 in 2017), the decrease remained only 6.8% in boys (3.3 in 2009 to 3.1 in 2017). When the infantile period was specifically analyzed, the mortality rate was higher in newborns younger than 28 days after birth than in those 28 days or older, with the ratio of neonatal to postneonatal deaths ranging between 1.1 and 1.4.

Across the age groups of mothers, the infant mortality rate was the lowest in mothers aged 25–29 years and increased with age thereafter (Fig. 1). Especially, the rate peaked in teenage pregnancies, although there was a decreasing trend in the recent 3 years. However, the mortality rate increased in late labors in mothers older than 40 years of age during the same period and remained similar (6.7 deaths per 1,000 live births yearly in 2009 and 6.5 in 2017).

The risk of both infant mortality and perinatal mortality was overwhelming during the first and second trimesters (Table 4). The mortality rate then decreased with gestational week until the term, followed by a rebound in the post-term period.

Of the 1,000 infant mortality events in 2017, 51.7% (n=517) were due to conditions originating in the perinatal period, followed by congenital malformations, deformations, and chromosomal abnormalities (16.8%, n=168), which were the 2 major categories throughout the observation period (Supplementary Table 1). Infantile respiratory distress was the single most common cause among the perinatal conditions, along with sepsis and asphyxia. In 2017, the mortality rates were 0.3, 0.1, and 0.1 from these 3 causes,

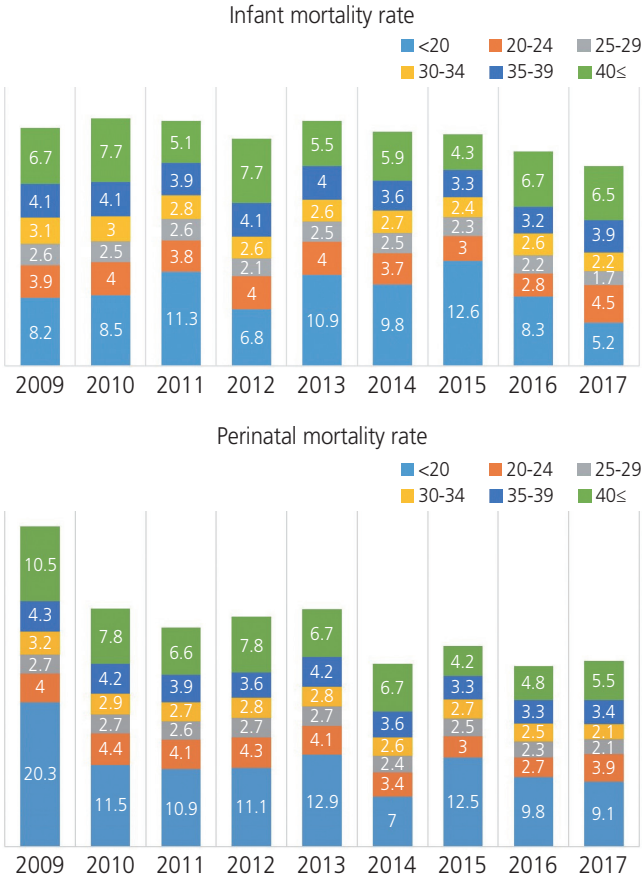

Fig. 1. Infant mortality by condensed list of cause of death: Korea, 2009–2017.

Table 3. Infant mortality rate[a] by year, sex, and period of death: Korea, 2009–2017

Year	No. of infant deaths	Infant mortality rate	Rate by sex		Rate by period	
			Boys	Girls	Neonatal (<28 day)	Postneonatal (≥28 day)
2009	1,415	3.2	3.3	3.1	1.7	1.5
2010	1,508	3.2	3.7	2.7	1.8	1.4
2011	1,435	3.0	3.4	2.7	1.7	1.3
2012	1,405	2.9	3.1	2.7	1.7	1.2
2013	1,305	3.0	3.1	2.9	1.7	1.3
2014	1,305	3.0	3.2	2.8	1.7	1.3
2015	1,190	2.7	2.9	2.5	1.5	1.2
2016	1,154	2.8	3.1	2.6	1.6	1.2
2017	1,000	2.8	3.1	2.5	1.5	1.3

[a]Deaths per 1,000 live births.

Table 4. Infant and perinatal mortality rate[a] by gestational age: Korea, 2009–2017

Year	Infant mortality rate					Perinatal mortality rate				
	Gestational age (wk)					Gestational age (wk)				
	<28	28–31	32–36	37–41	≥42	<28	28–31	32–36	37–41	≥42
2009	464.0	75.7	9.1	1.5	2.4	251.7	180.8	18.4	1.0	3.0
2010	440.0	76.7	9.1	1.4	3.4	242.8	145.3	17.9	1.0	3.4
2011	405.0	65.1	8.5	1.4	3.2	209.0	137.8	16.7	1.0	2.4
2012	411.2	58.6	6.9	1.2	1.7	215.3	137.1	15.1	1.0	2.6
2013	374.0	66.5	7.7	1.3	1.1	195.9	134.7	17.3	1.0	2.3
2014	397.2	63.7	7.5	1.3	-	198.6	144.6	14.4	1.0	4.2
2015	367.5	47.8	6.4	1.1	3.1	190.9	115.2	14.3	0.9	-
2016	443.3	58.7	7.2	1.0	-	200.8	121.2	12.0	0.8	2.0
2017	378.1	55.0	6.2	1.2	2.9	177.7	107.5	11.4	0.8	2.9

[a]Deaths per 1,000 live births.

accounting for 23.2% (n=120), 8.3% (n=43), and 4.4% (n=23) of all deaths due to perinatal conditions (n=517), respectively. In congenital abnormalities, most events were due to heart conditions or Down's syndrome. There were 33.3% (n=56) of deaths due to heart conditions and 17.3% (n=29) of deaths due to other circulatory anomalies, as well as 10.1% (n=17) due to Down's syndrome or other chromosomal disorders in this entity (n=168). Unfortunately, >20% of the external causes were assaults, and this proportion remained this high across the years, ranging between 24.2% (n=15) and 35.6% (n=21), except in 2012 and in 2014 when there were 12 (15.6%) and 6 (11.5%) deaths, respectively.

The perinatal mortality rate, including fetal deaths at 28 gestational weeks or more and neonatal deaths before 7 days after birth, gradually decreased during the past 9 years (Supplementary Table 2). Similar to infant mortality, the perinatal rate was slightly higher in boys than in girls, with the ratio of rates ranging from 1.0 to 1.2. Specifically, the fetal mortality rate was higher than the neonatal mortality rate before 7 days after birth, with 1.7 and 1.0 deaths per 1,000 live births yearly, respectively. However, compared with 2009, the fetal mortality rate decreased by 26.1% in 2017, whereas the decrease was 16.7% in the neonatal mortality rate during the same period. The rate distribution by maternal age and gestational week was similar to that observed in infant mortality (Table 4, Fig.1). The increasing trend of mortality rate in the recent 3 years among those whose mothers were 40 years or older was also comparable.

Discussion

Pregnancy-related mortality and perinatal mortality have gradually improved during the 2009–2017 period. The maternal mortality ratio has dropped by 42.2% to reach 7.8 deaths per 100,000 live births, and it has been continuously decreasing in the recent 3 years. Although the rate of decrease has recently slowed down, both infant mortality and perinatal mortality have improved to 12.5% and 22.9%, respectively.

In South Korea in 2009, the maternal mortality ratio was among the highest in OECD countries, with 13.5 deaths, and was still increasing in contrast to the overall decreasing trend observed in high-income states [6,7]. For this reason, the Ministry of Health and Welfare has initiated a "Supporting Policy for Underserved Area of Obstetric Care" since 2011, especially for underdeveloped small cities and towns, which include administrative and financial support as well as organizing transfer and referral systems [8]. Although a single policy may not fully explain the changes, the improvement in maternal mortality since 1 year after its implementation is, at least partly, attributable to such a societal approach.

It should be noted that maternal deaths due to hypertensive disorders are increasing while those due to all other specific causes are decreasing. Robust risk factors such as advanced maternal age, obesity, or working during pregnancy have been extensively discussed to date [9-11]. Although the prevalence of overweight or preexisting hypertension is low and stable at present in South Korea [12,13], increasing age

at delivery is inevitable in high-income countries, which will eventually lead to relevant maternity characteristics in undesirable directions [14,15]. As chronic morbidities are hardly reversible when symptomatic, preventive measures should not only focus on a specific age group but should also be devised as a lifelong modification.

Complications at delivery or puerperium were decreasing but remain the 2 most significant causes of deaths. As our data only provide grouping variables, it is difficult to identify specific reasons. However, a previous study in South Korea suggested that amniotic fluid embolism (ICD-10: O88.1), obstetric blood-clot embolism (O88.2), uterine inertia (O62.2), and immediate postpartum hemorrhage (O72.1) were common causes in each group, respectively. While such embolic and hemorrhagic events are among the frequent causes of maternal mortality in high-income and aging countries [16,17], it is also suggested that a significant portion of these causes are preventable by establishing a patient referral or proper transfer system [16].

Teenage pregnancy is a continuous threat to infant and, especially, perinatal mortality. However, as the absolute number of mortality events in teenage mothers is relatively small for both infant and perinatal mortality (8 cases [0.8%] and 14 cases [1.5%] in 2017, respectively), this has been less likely to be considered a priority issue. Unlike common beliefs that socioeconomic factors may influence the outcome of teenage pregnancies, studies have shown that merely young age, independent of other factors, is a risk for undesirable results [18]. Thus, an approach should focus on avoiding pregnancy itself in younger age through proper education [19], rather than trying to support teenage mothers.

The fact that both the number of mortality events (22, 22, 20, 12, 15, 6, 21, 15, and 17 cases between 2009 and 2017) as well as the proportion (27.5%, 28.2%, 26.3%, 15.6%, 24.6%, 11.5%, 35.6%, 24.2%, and 30.9%) out of the total external causes of infant deaths are due to child assault including homicide, and that these numbers are actually constant or rather increasing especially in proportion despite the overall decreasing mortality, is not only a tragedy but a disgrace for any developed country. Immediate interventions beginning from scrutinized descriptive epidemiology to surveillance systems and integrated medical, social, and political resolutions are required.

This study evaluated all available data sources with respect to maternal and perinatal deaths in South Korea at present. We sought to ensure the completeness and reliability of the data by incorporating a complementary COD process. However, as the data are originally based on death certificates with diagnosis based on ICD, possible explanatory information such as education level or socioeconomic status is lacking. Nevertheless, our study represents the most up-to-date vital status information at the country level, and we are positive that it can serve as evidence for further scrutiny.

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Conflict of interest

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

Ethical approval

No institutional approval was required because this was an analysis of publicly available data that were produced by Statistics Korea according to the Bioethics and Safety Act (IRB-2019-0270).

Patient consent

Informed consent was not required because this was an analysis of publicly available data.

Supplementary materials

Supplementary materials associated with this article can be found online at https://doi.org/10.5468/ogs.20081.

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Risk factors for type-specific persistence of high-risk human papillomavirus and residual/recurrent cervical intraepithelial neoplasia after surgical treatment

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Objective

This study aimed to investigate the clinicopathologic risk factors for type-specific persistence of high-risk human papillomavirus (hrHPV) and residual/recurrent cervical intraepithelial neoplasia (CIN) after surgical treatment.

Methods

Patients with CIN-2/3 who underwent conization or loop electrosurgical excision procedure (LEEP) at Korea University Hospital were enrolled. All patients underwent hrHPV testing and genotyping before conization or LEEP followed by both hrHPV genotyping and cytology. The significance of associations between patient characteristics and persistence of infection were assessed by multivariate logistic regression analyses.

Results

Among 398 women with pathologically confirmed CIN-2/3, 154 (38.7%) patients showed hrHPV persistence after surgical treatment. In multivariate analysis, high preoperative hrHPV load (P<0.05; odds ratio [OR], 2.063), presence of CIN-2 at treatment (P<0.01; OR, 2.732), and multiple hrHPV infections (P<0.001; OR, 4.752) were associated with hrHPV persistence. HPV 53 was the most likely to persist after treatment (24/43, 55.8%). The risk of residual/recurrent CIN-2/3 was higher in persistent infection with HPV 16 than other types (P<0.05). Menopause (P<0.001; OR, 3.969), preoperative and postoperative hrHPV load (P<0.05; OR, 2.430; P<0.05; OR, 5.351), and infection with multiple hrHPV types (P<0.05; OR, 2.345) were significantly related to residual/recurrent CIN following surgical treatment.

Conclusion

HPV load before treatment and infection with multiple hrHPV types were predictors of postoperative hrHPV persistence. HPV 53 was the type most likely to persist, but HPV 16 was the type that was most closely associated with residual/recurrent CIN-2/3.

Keywords: Cervical intraepithelial neoplasia; Conization; Human papillomavirus; HPV DNA tests

Introduction

Cervical intraepithelial neoplasia (CIN) is caused by persistent infection with high-risk human papillomavirus (hrHPV) and is a precursor of cervical cancer [1]. Persistent infection with hrHPV is the direct cause of the vast majority of CINs and invasive cervical cancers [2]. Age, parity, smoking, sexual

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behavior, and socioeconomic status have been reported as potential factors mediating persistent infection by hrHPV [3].

The standard treatment for CINs, especially high-grade lesions, is conization or the loop electrosurgical excision procedure (LEEP) [4]. Even if the lesion is completely removed, these patients have a higher risk for recurrence of high-grade lesions compared to the general population [5]. Because of this potential risk, close monitoring after surgical treatment for CIN is standard practice. Screening for patients at risk of residual/recurrent high-grade lesions using combined testing for hrHPV with cytology has a negative predictive value of 99% [6,7]. Detection of HPV infection has been highlighted as an objective marker with high sensitivity in screening or follow-up, as there is less interobserver variability compared to cytology testing alone [8]. High-grade squamous intraepithelial lesions (HSILs) with hrHPV infection have been demonstrated to be highly predictive of invasive cervical cancer [7,9].

It is well established that persistent infection by HPV after conization is a precursor for relapse of CINs. Patients tested as HPV- after conization had a 6.5% risk of residual/recurrent high-grade lesions, while those tested as HPV+ had a 60.9% risk [10]. Determining the characteristics and risk factors for HPV persistence after CIN treatment has implications for the early detection and treatment of high-grade lesions and cervical cancer, as well as for our understanding of the natural history of HPV infection. Several studies have analyzed persistent infection by HPV and residual/recurrent CINs after conization or LEEP [3,11-14]. However, specific HPV genotypes have different natural histories, and individual phylogenetic species have different carcinogenicities. In terms of HPV persistence, therefore, a detailed analysis of specific HPV genotypes can help expand our understanding of the nature of HPV infections [15].

In this study, we aimed to analyze genotype-specific persistence of HPV and persistence/recurrence of CIN after surgery in patients that underwent hrHPV genotyping before and after conization or LEEP.

Materials and methods

1. Study population and inclusion criteria

A flowchart of participant enrollment is provided in Fig. 1. A retrospective analysis was performed on 1,029 patients who underwent conization or LEEP at Korea University Guro Hospital and Anam Hospital between January 2014 and September 2018. We included patients for whom information on HPV genotypes before and after surgical treatment was available. Of these 1,029 patients, 504 were excluded because of preoperative negative hrHPV or lack of HPV data. The remaining 525 patients were documented to have hrHPV based on the Anyplex™ II (Seegene, Seoul, Korea) HPV genotyping test before operation. Patients with histologically confirmed CIN of grade 2/3 through punch biopsy were included. Of the remaining 525 patients, 31 and 19 were excluded due to diagnosis of VAIN or CIN-1, and due to invasive cervical cancer respectively. Finally, of the remaining 475 patients, 77 were lost to follow-up for HPV DNA testing after conization or LEEP, resulting in a total enrollment of 398 patients for this study.

Conization was performed using the surgeon's choice of cold-knife or Bovie blade after the application of Lugols' iodine solution to confirm previous cervical lesions. Hemostasis was then achieved with electric coagulation.

Postoperative follow-up was done between 3–6 months after surgical treatment, and follow-up visits occurred every 6–12 months thereafter. Patients underwent cervical inspections, HPV DNA genotyping tests, and cervical cytology. Papanicolaou smears were interpreted by the Bethesda system and histologic diagnoses of excised specimens were based on the World Health Organization classification.

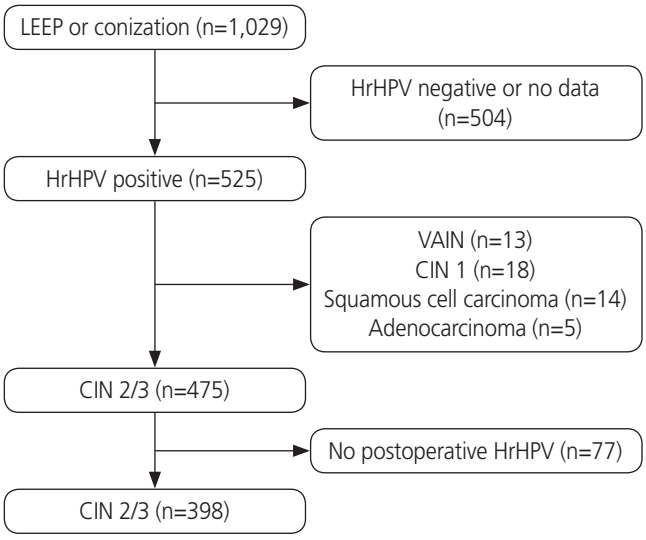

Fig. 1. Composition of enrolled patients' population. LEEP, loop electrosurgical excision procedure; hrHPV, high risk human papillomavirus; VAIN, vaginal intraepithelial neoplasia; CIN, cervical intraepithelial neoplasia.

Table 1. Overview of patient characteristics based on the postoperative human papillomavirus (HPV) status

Characteristics		Postop hrHPV persistence (until 12 mon)		*P*-value
		Negative (n=244, 61.3)	Positive (n=154, 38.7)	
Age (yr)		38.37±10.57	41.96±12.63	<0.005
Parity	0	100 (41.0)	63 (40.9)	0.988
	≥1	144 (59.0)	91 (59.1)	
Menopause	No	212 (86.9)	114 (74.0)	<0.005
	Yes	32 (13.1)	40 (26.0)	
Marital status	No	77 (31.6)	58 (37.7)	0.210
	Yes	167 (68.4)	96 (62.3)	
Previous treatment.	No	239 (98.0)	150 (97.4)	0.739
	Yes	5 (2.0)	4 (2.6)	
Preoperative HPV load[a)]	+	6 (2.5)	2 (1.3)	<0.005
	++	123 (50.4)	49 (31.8)	
	+++	82 (33.6)	77 (50.0)	
	Unknown	33 (13.5)	26 (16.9)	
Multiplicity of HPV	Single	177 (72.5)	59 (38.3)	<0.001
	Multiple	67 (27.5)	95 (61.7)	
Co-infection with low-risk HPV	No	215 (88.1)	108 (70.1)	<0.001
	Yes	29 (11.9)	46 (29.9)	
Cytology	Normal	6 (2.5)	1 (0.7)	0.232
	ASCUS	62 (25.4)	40 (26.1)	
	LSIL	48 (19.7)	44 (28.8)	
	ASC-H	37 (15.2)	17 (11.1)	
	HSIL	87 (35.7)	49 (32.0)	
	AGUS	2 (0.8)	0 (0.0)	
	SCC	2 (0.8)	2 (0.8)	
CIN at treatment	CIN 2	71 (29.1)	79 (51.3)	<0.001
	CIN 3	173 (70.9)	75 (48.7)	
Type of surgery	LEEP	81 (33.3)	62 (40.3)	0.161
	Conization	162 (66.7)	92 (59.7)	
Resection margin	Negative	214 (88.1)	122 (79.2)	<0.05
	Positive	29 (11.9)	32 (20.8)	
Endocervical resection margin	Negative	200 (94.3)	136 (88.3)	<0.05
	Positive	14 (5.7)	18 (11.7)	
Glandular involvement	No	118 (48.4)	82 (53.2)	0.342
	Yes	126 (51.6)	72 (46.8)	
Follow-up cytology	Normal/ASCUS/LSIL	240 (98.4)	133 (86.4)	<0.001
	ASC-H/HSIL	4 (1.6)	21 (13.6)	
CIN persistence/recurrence	No	239 (98.0)	102 (66.2)	<0.001
	Yes	5 (2.0)	52 (33.8)	
CIN 2+ persistence/recurrence	No	244 (100.0)	139 (90.3)	<0.001
	Yes	0 (0.0)	15 (9.7)	

Values are presented as mean±standard deviation or number (%).
hrHPV, high-risk human papillomavirus; ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesion; ASC-H, atypical squamous cells, cannot exclude HSIL; HSIL, high grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; SCC, squamous cell carcinoma; CIN, cervical intraepithelial neoplasia; LEEP, loop electrosurgical excision procedure.
[a)]HPV viral copy number. +, $<10^2$; ++, 10^2–10^5; +++, $>10^5$.

2. Human papillomavirus (HPV) test with Anyplex™ II

We extracted HPV DNA in accordance with the manufacturer's guidelines. Nucleic acids were extracted from 400 μL of sample using the MICROLAB STARlet automated purification system (Hamilton, Reno, NV, USA). HPV detection and genotyping were performed using an Anyplex II HPV28 and CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions [16]. The Anyplex II HPV28 has been shown to perform comparably to the Roche Cobas 4800 HPV assay (Cobas) (Roche Molecular diagnostics, Branchburg, NJ, USA) and the HR Hybrid Capture 2 (Qiagen, Gaithersburg, MD, USA), which are accredited for cervical cancer screening and detection of hrHPV [16,17]. Furthermore, Anyplex II HPV28 is considered a reliable and validated test for detecting hrHPV genotypes [18].

3. Human papillomavirus (HPV) persistence and genotype-specific persistence

Persistent hrHPV infection was defined as the presence of hrHPV at the first follow-up visit after surgery regardless of

Table 2. Risk factors associated with high-risk human papillomavirus (hrHPV) persistence after surgical treatment

Characteristics		Univariate			Multivariate		
		OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age (yr)	≥50	2.553	1.406–4.636	<0.005	2.486	0.652–9.494	0.182
	<50						
Parity	≥1	0.811	0.512–1.284	0.372			
	0						
Menopause	Yes	2.577	1.355–4.899	<0.010	1.085	0.257–4.586	0.912
	No						
Marriage	Yes	0.763	0.500–1.165	0.211			
	No						
Previous treatment	Yes	1.151	0.303–4.374	0.836			
	No						
Preoperative HPV load[a)]	+++	2.231	1.329–3.746	<0.010	2.063	1.139–3.737	<0.050
	+-++						
Multiplicity of HPV	Multiple	4.402	2.713–7.144	<0.001	4.752	2.593–8.710	<0.001
	Single						
Co-infection with low risk HPV	Yes	2.917	1.603–5.307	<0.001	1.584	0.750–3.344	0.228
	No						
Follow-up cytology	ASC-H/HSIL/SCC	0.797	0.505–1.256	0.327			
	Normal/ASCUS/LSIL						
CIN at treatment	CIN 2	2.674	1.656–4.310	<0.001	2.732	1.451–5.128	<0.010
	CIN 3						
Type of surgery	Conization	0.884	0.561–1.395	0.597			
	LEEP						
Resection margin	Positive	1.936	1.117–3.353	<0.05	1.319	0.558–3.121	0.528
	Negative						
Glandular involvement	Yes	0.762	0.484–1.201	0.242			
	No						

OR, odd ratio; CI, confidence interval; HPV, human papillomavirus; ASC-H, atypical squamous cells, cannot exclude HSIL; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; LEEP, loop electrosurgical excision procedure.
[a)]HPV viral copy number. +, $<10^2$; ++, 10^2–10^5; +++, $>10^5$.

HPV type. HPV type-specific persistence was defined as the presence of the same HPV type before surgery and at the first follow-up visit after surgery. Multiple HPV infections in a patient were considered separate units when analyzing the HPV type-specific persistence rate. Patients who were HPV negative at the first follow-up visit, or had cleared all HPV types present before surgery, were defined as being clear of HPV infection.

4. Statistical analysis

The Chi Square (χ^2) and Fisher's exact tests were used to evaluate the significance of differences in variables between the HPV persistent group and the HPV non-persistent group. The risk for HPV persistence was modelled by logistic regression analysis and is presented as relative risks (odds ratios [ORs]) with 95% confidence intervals (CIs). Backward stepwise multivariable logistic regression was applied to identify factors independently predictive of persistent/recurrent CIN1+ and CIN2+.

P-values (from 2-sided tests) less than 0.05 were considered significant. Data were analyzed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 398 patients who tested positive for hrHPV and underwent conization or LEEP met the inclusion criteria. The mean number of follow-up visits was 2.8 (range 1–7) and median follow-up period was 17.3 (range 4–48) months. Overall, the prevalent hrHPV genotypes were HPV 16 (n=128, 32.2%), HPV 52 (n=80, 20.1%), HPV 58 (n=74, 18.6%), and HPV 53 (n=43, 10.8%). Two hundred thirty-six patients (59.3%) were infected by a single hrHPV type. Of the remaining 162 patients infected with multiple types of hrHPV, 118 patients (29.6%) were infected with 2 hrHPV types, 32 patients (8.0%) were infected with 3 hrHPV types, and 12 patients (3.0%) were infected with more than 4 hrHPV types.

Persistent hrHPV infection after conization or LEEP was identified in 154 patients (38.7%) of the 398 patients enrolled. Baseline characteristics of these patients are presented in Table 1. Mean age at diagnosis was 41.96±12.63 years in patients with hrHPV persistence compared to 38.37±10.57 years in those without hrHPV persistence (*P*<0.005). Initial results from cervical cytology were not significantly associated with hrHPV persistence (*P*=0.232). Among patients who did not show hrHPV persistence, 25.4% had ASCUS and 37.3% had ≥HSIL at initial cytology. However, in patients with hrHPV persistence, 26.1% had ASCUS and 32.8% had ≥HSIL. Residual/recurrent CIN2+ occurred in 15 patients (9.7%) with hrHPV persistence, while it did not occur in those without persistent infection. Both patient groups (with and without persistent hrHPV infection) were similar in terms of previous treatment for CIN, type of surgery, glandular involvement, and socioeconomic status including parity and marital status.

Unadjusted and adjusted odds ratios for clinical factors selected for multivariable analysis are presented in Table 2. Age ≥50 years (OR, 2.553; 95% CI, 1.406–4.636; *P*<0.005) and post-menopausal status (OR, 2.577; 95% CI, 1.355–4.899;

Table 3. Human papillomavirus (HPV) genotype-specific rate of persistence and multiple infection[a)]

Types	HPV genotypes before conization or LEEP	Persistence of the same type after conization	Multiple hrHPV infection before conization or LEEP
HPV 16	128 (32.2)	19 (14.8)	52 (40.6)
HPV 18	23 (5.8)	2 (8.7)	17 (73.9)
HPV 31	33 (8.3)	8 (24.2)	17 (51.5)
HPV 33	42 (10.6)	9 (21.4)	23 (54.8)
HPV 52	80 (20.1)	22 (27.5)	43 (53.8)
HPV 53	43 (10.8)	24 (55.8)	36 (83.7)
HPV 58	74 (18.6)	22 (29.7)	46 (62.2)
HPV others	41 (10.3)	6 (14.6)	7 (17.1)

Values are presented as number (%).
hrHPV, high-risk human papillomavirus; LEEP, loop electrosurgical excision procedure.
[a)]Persistence of the same type after conization or LEEP was found in 120 of 398 (30.2%) patients.

P<0.01) were risk factors for hrHPV persistence in univariate analysis; however, these factors were not significant in multivariate analysis (*P*=0.182 and *P*=0.912). HPV was more likely to persist in patients with CIN-2 than in patients with CIN-3 (OR, 2.674; 95% CI, 1.451–5.128; *P*<0.01). HPV viral load at baseline was an independent predictor of persistence. HPV with a viral copy number >10^5 at baseline was associated with an increased rate of persistence at 12 months (OR, 2.063; 95% CI, 1.139–3.737; *P*<0.05). Positive resection margin was not a significant risk factor for hrHPV persistence (OR, 1.319; 95% CI, 0.558–3.121; *P*<0.01), although it was associated with hrHPV persistence in univariate analysis (OR, 1.936; 95% CI, 1.117–3.353; *P*<0.05). Furthermore, there was no significant association between hrHPV persistence and socioeconomic status, history of previous treatment, co-infection with low risk HPV, or glandular involvement.

Type-specific persistence of hrHPV was found in 120 of the 398 patients (30.2%). Table 3 shows the rates and patterns of HPV infection according to HPV genotype. HPV 16 was the most prevalent genotype (128/398, 32.2%), followed by HPV 52 and 58. Among 43 patients with HPV 53, 24 (55.8%) showed persistent infection with the same HPV genotype. HPV 58 showed type-specific persistence after surgical treatment in 22 (29.7%) patients, while HPV 52 showed type-specific persistence in 27.5% (22/80) of patients. In contrast, HPV 16 and 18 showed relatively lower rates of type-specific persistence (19/128, 14.8%, and 2/23, 8.7%, respectively). In addition, among 43 patients infected with HPV 53, 36 (83.7%) were co-infected with other hrHPVs, while 40.6% (52/128) of those infected with HPV 16 were co-infected with other hrHPVs.

Fifty-seven patients (14.3%) showed residual/recurrent CIN during follow-up. Table 4 shows the risk factors for residual/recurrent CIN or CIN-2/3 after conization or LEEP. Post-menopausal status (OR, 3.969; 95% CI, 1.733–9.088; *P*<0.001) and infection with multiple hrHPV types (OR, 2.345;

Table 4. Logistic regression results for predicting the residual/recurrent cervical intraepithelial neoplasia (CIN) or CIN-2/3 after conization or loop electrosurgical excision procedure (LEEP) (n=57)

Variables		Values	Multivariate		
			OR	95% CI	*P*-value
CIN after conization or LEEP (n=57)					
Menopause	Yes	19/72 (26.4)	3.969	1.733–9.088	<0.001
	No	38/326 (11.7)			
Preop HPV load[a]	+++	30/159 (18.9)	2.430	1.135–5.202	<0.050
	+–++	18/178 (10.1)			
	Unknown	9/61 (14.8)			
Postop HPV load[a][b]	+++	18/35 (51.4)	5.351	1.091–26.236	<0.050
	+–++	27/100 (27.0)			
	Negative	12/244 (4.9)			
Multiplicity of HPV	Multiple	31/162 (19.1)	2.345	1.109–4.958	<0.050
	Single	26/236 (11.0)			
CIN 2/3 after conization or LEEP (n=15)					
Menopause	Yes	6/72 (8.3)	4.31	1.154–16.115	<0.050
	No	9/326 (2.8)			
Pathology before conization or LEEP	CIN 3	12/248 (4.8)	10.87	2.146–55.046	<0.010
	CIN 2	3/150 (2.0)			
Postop HPV load[a][b]	+++	7/35 (20.0)	4.24	1.201–14.963	<0.050
	+–++	7/100 (7.0)			

Values are presented as number (%).
HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; CI, confidence interval.
[a]HPV viral copy number. +, <10^2; ++, 10^2–10^5; +++, >10^5; [b]Within 18 months after treatment.

Table 5. Characteristics of women showing residual/recurrent cervical intraepithelial neoplasia (CIN)-2/3 after treatment

No.	Age (yr)	Parity	Menopause	HPV load[a)]	HPV Type	Treatment	Cytology	Pathology	RM	GI	Follow up				Time to diagnosis of residual/recurrent CIN 2/3 (mon)
											HPV	Type change	Cytology	Path	
1	25	0	No	+++	16,18,33	Cone	ASCUS	CIN 2	-	-	16,18,33	-	HSIL	CIN2	11.7
2	27	0	No	+++	33,39	LEEP	ASCUS	CIN 3	+	-	16	+	LSIL	CIN3	8.4
3	27	0	No	++	52	Cone	HSIL	CIN 3	+	+	52	-	ASCUS	CIN3	9.3
4	38	1	No	++	16,18	LEEP	ASCUS	CIN 3	-	+	18,35,53	-	ASCUS	CIN2	23.1
5	40	2	No	++	33	Cone	HSIL	CIN 3	+	+	33	-	HSIL	CIN3	13.3
6	41	2	No	++	33	Cone	HSIL	CIN 3	-	+	35,56	+	ASCUS	CIN2	12.5
7	42	1	No	+++	31	Cone	LSIL	CIN 3	+	+	31,59	-	HSIL	CIN3	7.7
8	45	4	No	+++	16	Cone	HSIL	CIN 3	-	+	16	-	HSIL	CIN3	9.5
9	49	1	No	++	58,68	Cone	HSIL	CIN 3	-	-	58,68	-	HSIL	CIN3	2.1
10	52	2	Yes	++	31	Cone	HSIL	CIN 3	+	+	31	-	ASCUS	CIN3	13.3
11	56	2	Yes	++	53,58	LEEP	HSIL	CIN 2	-	-	53,58	-	LSIL	CIN2	4.4
12	56	2	Yes	+++	16	Cone	HSIL	CIN 2	-	-	16	-	ASCUS	CIN3	6.7
13	57	2	Yes	++	16,35	LEEP	SCC	CIN 3	-	-	16,35	-	HSIL	CIN2	4.3
14	59	2	Yes	++	33,52	LEEP	HSIL	CIN 3	-	-	33,52	-	HSIL	CIN3	5.7
15	63	3	Yes	+	45	LEEP	HSIL	CIN 3	-	+	45	-	HSIL	CIN3	4.7

HPV, human papillomavirus; RM, resection margin; GI, glandular involvement; Path, pathology; Cone, conization; ASCUS, atypical squamous cells of undetermined significance; HSIL, high-grade intraepithelial lesion; LEEP, loop electrosurgical excision procedure; LSIL, low-grade intraepithelial lesion; SCC, squamous cell carcinoma.

[a)]HPV viral copy number. +, $<10^2$; ++, 10^2–10^5; +++, $>10^5$.

95% CI, 1.109–4.958; *P*<0.05) were independent predictors of residual/recurrent CIN. In addition, risk of residual/recurrent CIN that was dependent on viral load was significantly greater in patients with a higher HPV load at baseline (OR, 2.430; 95% CI, 1.135–5.202; *P*<0.001), and in those with a higher HPV load at 18 months of treatment (OR, 5.351; 95% CI, 1.733–9.088; *P*<0.001). Of 15 patients with persistent or recurrent CIN-2/3 after conization or LEEP, CIN-2 lesions were found in 5 patients and CIN-3 lesions in 10 patients. Highly significant predicators of persistent or recurrent CIN-2/3 were menopause (OR, 4.31; 95% CI, 1.154–16.115, *P*<0.05) and higher HPV load after treatment for 18 months (OR, 4.24; 95% CI 1.201–14.963; *P*<0.05). In addition, initial CIN grade was also a predictor of residual/recurrent CIN. Patients with CIN-3 were more likely to have residual/recurrent CIN2+ after conization (OR, 10.87; 95% CI, 2.146–55.046; *P*<0.01). Compared to those with CIN-2 at enrollment, having CIN-3 was a risk factor for persistent or recurrent high-grade cervical precancerous lesions, although CIN-2 but not CIN-3 was a risk factor for persistence of hrHPV.

Detailed characteristics of the 15 patients with residual/recurrent CIN-2/3 are provided in Table 5. The mean age of patients with residual/recurrent CIN-2/3 was 45.1 (25–63) years, which was older than the mean age of all patients (*P*<0.05). Of these 15 patients, 6 (40.0%) were post-menopausal. The most prevalent hrHPV type was HPV 33, detected in 5 (33.3%) patients, followed by HPV 16 in 4 (26.7%), HPV 18 in 2 (13.3%), HPV 31 in 2 (13.3%), and HPV 52 in 2 (13.3%). Most residual/recurrent CIN-2/3 patients had a viral copy number above 10^2, except for 1 patient. Eight patients (53.3%) were infected with a single hrHPV type while the remaining 7 (46.7%) patients were infected with multiple hrHPV types. HPV genotyping revealed that 13 of the 15 patients (86.7%) remained positive for the same hrHPV at follow-up, while 2 (13.3%) patients were positive for a different hrHPV at follow-up from the type detected at enrollment. Persistent infection with HPV 16 was associated with a significantly increased risk for residual/recurrent CIN-2/3 compared to other types of HPV (OR, 3.422; 95% CI, 1.092–10.728; *P*<0.05). Among the 15 patients with residual/recurrent CIN2/3, the histopathologic finding was CIN-2 in 3 (20.0%) and CIN-3 in 12 (80.0%) at enrollment. The mean time to diagnosis of residual/recurrent CIN-2/3 was 9.1 (2.1–23.1) months.

Discussion

We aimed to determine which factors affect hrHPV persistence and residual/recurrent CIN after conization or LEEP. There was a significantly increased risk for persistence of hrHPV in patients with high preoperative HPV load and multiple hrHPV types. In addition, patients diagnosed with CIN-2 were at higher risk for hrHPV persistence than those diagnosed with CIN-3. HPV 16 was the most prevalent hrHPV genotype however; we found relatively higher persistence rates of HPV 53, 58, and 52 than HPV 16 or 18. We also found increased risk for residual/recurrent CIN-2/3 in patients who were post-menopausal or had higher postoperative HPV load. Patients with CIN-3 were at higher risk for residual/recurrent CIN-2/3 than CIN-2. The risk of residual/recurrent CIN-2/3 was higher in patients with persistent infection with HPV 16 than other HPV types. Our data support the usefulness of hrHPV genotyping and viral load testing before and after conization or LEEP to manage patients with CIN.

Consistent with previous studies, we observed that 38.7% of patients were positive for hrHPV after conization or LEEP [19,20]. However, the range of hrHPV persistence rates previously reported (7.8–17.4) were lower than what we observed [11,14,21]. The higher persistence rate in our study compared to other studies may be due to different definitions of hrHPV persistence. We defined hrHPV persistence as the presence of hrHPV at the first follow-up visit after surgery, whereas others had different follow-up intervals or defined persistence as positive hrHPV results at 2 or more consecutive visits. Kim et al. [11] detected persistent hrHPV infections in 45.6% of patients who had undergone LEEP with a negative resection margin at 3 months post-surgery, and 14.3% of patients at 6 months post-surgery. In the present study, 7.6% of all patients (15 of 398) were diagnosed with residual/recurrent CIN-2/3 and 9.7% of patients in the hrHPV persistence group (15 of 154) were diagnosed with residual/recurrent CIN-2/3. The median time to diagnosis of residual/recurrent CIN-2/3 was 9.7 months (2.1–23.1).

In our study, viral load was significantly associated with persistence of hrHPV and residual/recurrent CIN-2/3. Similarly, in a French cohort, patients with high viral load were more likely to have persistent hrHPV infections after conization than patients with lower viral load [22]. In addition, postoperative viral load was a predictor of residual/recurrent CIN-2/3. Previous literature found that higher hrHPV viral load at

the 6-month follow-up visit was a significant risk factor for residual/recurrent CIN after conization or LEEP [23], which corresponds well with our findings. We found that infection with multiple hrHPV types before treatment increased the risk of persistence of hrHPV This corresponds well with previous research that demonstrated that infection with multiple hrHPV types has not only been found to be associated with increased risk of persistence hrHPV but also CIN-2/3 [24]. Individual hrHPV types function independently in CIN lesions and infection with multiple strains should be interpreted as having a cumulative effect, rather than a synergistic effect [25]. This is consistent with our finding that infection with multiple hrHPV types was associated with hrHPV persistence rather than residual/recurrent CIN-2/3.

Unexpectedly, we found that HPV persistence rates after treatment were higher in patients with CIN-2 than those with CIN-3, which is inconsistent with a previous study report [19]. Although the association between the severity of the cervical precancerous lesion and the risk of persistence is still unknown, patients with lower grade cervical lesions have been shown to have a higher viral load than those with CIN-3 [26]. Higher grade cervical lesions have been shown to contain lower viral DNA loads due to the presence of more immature and dysplastic squamous cells than lower grade lesions [26]. In our study, and consistent with previous findings, although patients with CIN-2 were more likely to show persistence of hrHPV after conization or LEEP, CIN-3 patients were at higher risk for recurrence of CIN-3 than CIN-2 [27].

In multivariate analysis, the hrHPV persistence rates were not affected by the status of resection margin (P=0.528), however a positive resection margin was associated with a 1.94-fold higher risk of hrHPV persistence (P<0.05) in univariate analysis. As several previous studies have pointed out [13,20,28,29], a positive resection margin is the most significant risk factor for predicting hrHPV persistence and residual/recurrent CIN-2/3. However, a negative resection margin does not always indicate complete excision due to the possibility of multifocal lesions. Sarian et al. [3] investigated the association between patient characteristics and hrHPV persistence, and demonstrated using multivariate analysis that smoking and patient age above 35 years were associated with persistent HPV, while a positive resection margin was not. They noted that positive endocervical margins were strongly affected by patient age (>35 years) because of the tendency for the squamous-columnar junction to be deeper inside the cervical canal after menopause. This finding indicates that the association between patient age and resection margin should be taken into consideration in statistical analyses of future studies.

We found that patients infected with HPV 53, HPV 58, and HPV 52 were at relatively higher risk for HPV persistence after treatment than those infected with other HPV types. One previous study [30] reported that α6 species of HPV (HPV 53, 56, 66) were less carcinogenic than other species of hrHPV, as well as being a low risk for progression despite persistence. Persistence of HPV 53 can be explained by the infection of tissues outside the precancerous lesion that are not causally related to the lesion. This may indicate that HPV 53 might not be causally involved, but might be a bystander [1,15]. HPV 52 is the most common type found in CIN2, while HPV 16 is the most common type in CIN-3 and cervical cancer [30]. HPV 52 is also the most prevalent type in HIV-infected women [31] and is most frequently detected with HPV 16 [32]. Consistent with the most common persistent hrHPV types in our study, So et al. [33] investigated the prevalence and distribution of HPV genotypes in South Korea and reported that the most common types were HPV 53, followed by HPV 52 and HPV 58. In other words, our findings that the above 3 types of hrHPV were the most frequent types in patients with CIN might be due to their tendency to persist after surgical treatment. In agreement with our findings, Gosvig et al. [15], also found that the persistence rate for HPV 16 was lower than that of other carcinogenic HPV types after excision. Since HPV16 and HPV18 are known to be some of the most oncogenic subtypes, they could cause larger lesions, which would be associated with larger ranges of excision.

In our study, 15 patients were diagnosed with residual/recurrent CIN-2/3 during the follow-up period. Among them, 13 patients had persistent infection with the same hrHPV genotypes that they were originally infected. In addition, persistent infection with HPV 16 was significantly associated with residual/recurrent CIN-2/3 (P<0.05). Kang and Kim [34] found that patients with persistent infection with the same hrHPV types pre- and post-surgery, and HPV 18, were at high risk for recurrent CIN-2/3. Similarly, Söderlund-Strand et al. [35] reported that of 5.1% of patients diagnosed with residual/persistent CIN-2/3, all had the same type-specific hrHPV before and after surgery. In other studies, no recurrence was found among patients infected with newly-detected hrHPV

types [36]. These results suggest that special attention should be paid to persistent infection after conization or LEEP with the same hrHPV type as was present before surgery.

The association between patients' age and HPV persistence is still controversial. While, some previous studies found no correlation between patients' age and HPV persistence or recurrent CIN after LEEP [37,38], one study reported that older patients with CIN lesions were at higher risk for HPV persistence [19]. In our study, menopause was a risk factor for persistent/recurrent CIN or CIN2+ after surgical treatment. The association between menopause and CIN persistence/recurrence may be explained by the fact that the squamous-columnar junction of post-menopausal women is deeper within the cervical canal than that of pre-menopausal women. This location change in post-menopausal women would consequently interfere with complete hrHPV eradication or CIN removal due to limited resection depth. In addition, post-menopausal women may have a decreased immune response to HPV infection compared to pre-menopausal women [39].

The strengths of this study include our ability to perform HPV genotyping of all patients before and after conization or LEEP. Consequently, we could assess the associations between patient characteristics, including hrHPV types, hrHPV persistence, and residual/recurrent CIN-2/3, accurately. The limitations of our study include its retrospective design, relatively small sample size, and short follow-up duration. However, Elfgren et al. [40] reported that clearance of HPV DNA was rapid and usually occurred within 6 months of treatment, with little additional clearance after 6 months, therefore a longer term follow-up may not have altered our findings. In addition, the patient's desire to become pregnant in future, could have influenced the cone depth or width during surgery; however, we could not consider this in our analysis. Finally, other factors affecting hrHPV persistence, or residual/recurrent CIN, such as a history of social behavior including smoking, were not evaluated.

In conclusion, our study contributes to the knowledge of the postoperative progress of hrHPV infection and CIN recurrence. We demonstrated that preoperative viral load and infection with multiple hrHPV types are important predictors of hrHPV persistence. HPV 53 was the most persistent type, whereas infection with HPV 16 was associated with the highest risk for residual/recurrent CIN-2/3. Post-menopausal women need to be monitored closely because of their high risk for residual/recurrent CIN. Longer-term and larger-scale studies are necessary to validate our results.

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 Korea University Guro Hospital (No. 2019GR0123).

Patient consent

Anonymized and de-identified information for participants was used for analysis, so the requirement for informed consent or parental permission was waived.

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Prevalence of tumor *BRCA1* and *BRCA2* dysfunction in unselected patients with ovarian cancer

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Objective

The therapeutic benefits of poly(ADP-ribose) polymerase inhibitors highlight the need to evaluate *BRCA1/2* defects in tubal/ovarian cancer (OC). We sought to determine the pattern and disease characteristics associated with tumor *BRCA1/2* mutations and *BRCA1* methylation in women with OC.

Methods

We obtained 111 OC specimens from 2 university hospitals and assessed *BRCA1/2* mutations and *BRCA1* methylation in tumor DNA. The frequency and pattern of *BRCA1/2* defects were examined. Associations between patient/disease characteristics and *BRCA1/2* defects were ascertained (Fisher's exact test). Platinum-free interval (PFI), progression-free survival (PFS), and overall survival (OS) based on the underlying *BRCA1/2* defect were determined (Kaplan-Meier analysis [log-rank test]).

Results

We observed a *BRCA1/2* dysfunction rate of 40% (28/70) in high-grade serous tubal/ovarian cancer (HGSC), including 14.3% *BRCA1* methylation (n=10), 7.1% *BRCA1* mutation (n=5), and 18.6% *BRCA2* mutation (n=13). Defects in *BRCA1/2* genes were associated with stage III/IV HGSC (*BRCA1* methylation: P=0.005 [stage III/IV] and P=0.004 [HGSC]; *BRCA1/2* mutation: P=0.03 [stage III/IV] and P<0.001 [HGSC]). Patients with *BRCA1/2*-mutated cancers showed improved OS (hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.43–0.99; P=0.045) and a trend toward improved PFI (HR, 0.48; 95% CI, 0.22–1.06; P=0.07) and PFS (HR, 0.72; 95% CI, 0.51–1.03; P=0.07). No survival differences were observed between *BRCA1*-methylated and *BRCA1/2* wild-type non-*BRCA1*-methylated cancers.

Conclusion

We observed a high tumor *BRCA1/2* dysfunction rate in HGSC with a unique predominance of *BRCA2* over *BRCA1* mutations. While *BRCA1/2* mutations conferred survival benefits in OC, no such association was observed with *BRCA1* methylation.

Keywords: Ovarian cancer; *BRCA1* methylation; *BRCA1* mutation; *BRCA2* mutation

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Introduction

Poly (ADP-ribose) polymerase inhibitors (PARPi) exhibit potent activity in germline *BRCA1/2*-mutated platinum-sensitive relapsed high-grade serous tubal/ovarian cancer (HGSC). In phase III clinical trials, maintenance therapy with PARPi was associated with a 73% reduction in the risk for disease progression or death as compared to placebo [1]. PARPi target the homologous recombination DNA repair defect (HRD) conferred by *BRCA1/2* mutations, leading to tumor genomic instability and cell death. While germline *BRCA1/2* mutations are detected in 15% HGSCs, genomic and functional data suggest the presence of HRD in approximately 50% HGSC [2]. The identification and validation of other HRD-associated biomarkers in sporadic tubal/ovarian cancer (OC) (hereafter referred to as OC) are crucial to potentially expand the number of women with OC who could benefit from DNA repair-targeting agents such as PARPi.

Somatic *BRCA1/2* mutations have been identified in 4–6.4% of HGSCs, wherein they account for 14.2% of HRD cases [2,3]. Evidence suggests that the clinical benefit from PARPi in patients with somatic *BRCA1/2*-mutated HGSC is similar to that observed in those with germline *BRCA1/2*-mutated disease. In the phase III NOVA clinical trial, 19.7% (n=40) of *BRCA1/2* mutations were classified as somatic [1]. The median progression-free survival (PFS) associated with niraparib as compared to that with placebo (20.9 vs. 11 months, hazard ratio [HR], 0.27; 95% confidence interval [CI], 0.08–0.9; P=0.02) in this subgroup was consistent with the value reported for patients with germline *BRCA1/2*-mutated disease (21 vs. 5.5 months, HR, 0.27; 95% CI, 0.17–0.41; P<0.001) [1].

BRCA1 promoter methylation has been identified as a potential biomarker of response to the PARPi rucaparib [4]. *BRCA1*-methylated tumors are negative for *BRCA1* gene and protein expression, suggestive of a resultant HRD phenotype [5,6]. In addition, *BRCA1*-mutated and *BRCA1*-methylated OCs display similar gene signatures, as detected using gene expression and copy number analyses [7]. In the phase II open label ARIEL-2 study, 12/19 (63%) relapsed platinum-sensitive *BRCA1*-methylated OC patients responded to rucaparib as compared to an 80% response rate reported in *BRCA1/2*-mutated OC patients [4]. This early data suggest the potential role of *BRCA1* methylation as a biomarker of response to PARPi.

Considering the benefit of PARPi in *BRCA1/2* dysfunctional OC and the ongoing development of other agents targeting DNA repair, the knowledge of the prevalence and pattern of *BRCA1/2* gene aberrations within an OC population is imperative. The use of tumor tissues offers the advantage of identifying additional potential somatic biomarkers of response to PARPi as compared to germline mutation testing alone. This information may serve as a guide to drug approval strategies for novel DNA repair targeting drugs at a national level, as the distribution of *BRCA1/2* mutations varies between populations [8]. In Ireland, the frequency of *BRCA1/2* gene aberrations in OC is yet to be examined. At the time of this study, genetic testing for *BRCA1/2* mutations in OC in Ireland was carried out on the basis of clinical risk algorithms in a clinician-dependent manner.

Here, we sought to assess the *BRCA1/2* gene profile in a cohort of Irish women with OC by determining the frequencies of *BRCA1/2* mutations and *BRCA1* methylation in tumors and their association with clinical characteristics and survival.

Materials and methods

1. Sample and data collection

We selected 111 patients with OC treated at 2 university teaching hospitals (including a national tertiary referral gynecologic oncology unit) between 2005 and 2013. All histological subtypes, stages, and grades were included to allow accurate assessment of *BRCA1/2*-mutated and *BRCA1*-methylated profiles. Borderline tumors were excluded. In total, 100 patients were retrospectively included from a prospective clinically annotated Discovary bioresource (St. James's Hospital) after receiving ethical approval for this study (reference 2009/29/01). Patients provided written informed consents prior to specimen collection. Within this bioresource, all patients with epithelial OC with available and adequate tumor tissues (>30% neoplastic cell content [NCC]) were included. Eleven samples were obtained from the Beaumont Hospital Pathology Department after receiving approval from the hospital's ethics committee (REC reference 12/02). Clinical data for these patients were retrospectively obtained through medical records. Patients recruited through both bioresources presented either to the outpatient department or as direct inpatient referrals. All survival data were updated to February 15, 2017. A pathologist specializing in gynecological cancers

reviewed fresh-frozen paraffin-embedded (FFPE) tumor specimens for histology and NCC (as per the 2014 World Health Organization Classification). All specimens were obtained prior to chemotherapy (either at primary debulking surgery or peritoneal biopsy). Specimens with less than 30% NCC (n=20) were macrodissected prior to DNA extraction. The majority of the specimens had over 60% NCC.

2. Assessment of tumor *BRCA1/2* defects

The DNA was extracted from FFPE tumor samples using the QIAamp DNA FFPE Tissue kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol, and quantified using the dsDNA BR assay kit (Qubit, London, UK) as per the manufacturer's instructions.

BRCA1 methylation status was assessed using the MethylProfiler DNA Methylation polymerase chain reaction (PCR) Array System (SABiosciences, Valencia, CA, USA) following the manufacturer's protocol. In brief, DNA methylation-sensitive and methylation-dependent restriction enzymes were used to selectively digest non-methylated or methylated genomic DNA, respectively. After digestion, DNA samples were subjected to real-time PCR using primers flanking the regions of interest. The relative concentrations of differentially methylated DNA were determined by comparing the amount of each digest with that of a mock digest. A cutoff value of 10% methylation was used to define the methylation status of samples.

BRCA1 and *BRCA2* genes were sequenced using the Tumor BRACAnalysis CDx assay (Myriad Genetics, Munich, Germany and Salt Lake City, UT, USA), as previously described [9]. Only deleterious or suspected deleterious mutations were included in analyses (as per the previously defined criteria [10]). Germline or somatic mutation status was not assessed, owing to the restrictions imposed by patients' informed consent.

3. Statistical analysis

Statistical analysis was performed using SPSS® version 21.0 software. *BRCA1/2* mutations and *BRCA1* methylation were associated to the following variables: patient age, histology, International Federation of Gynecology and Obstetrics (FIGO) stage, degree of surgical cytoreduction, and platinum sensitivity using the Fisher's exact test. Survival analyses were carried out for platinum-free interval (PFI), PFS, and overall survival (OS) to compare patients with *BRCA1/2*-mutated disease or *BRCA1*-methylated disease with patients carrying *BRCA1/2* wild-type non-*BRCA1*-methylated (hereafter referred to as *BRCA1/2*-intact) tumors. PFI was defined as the interval between completion of chemotherapy and disease recurrence (as defined by the CA125/RECIST criteria), death, or date of last follow-up, whichever occurred first. PFS was defined as the interval between first surgical debulking or diagnostic biopsy (for patients receiving adjuvant or neoadjuvant chemotherapy, respectively) and disease recurrence

Table 1. Patient and disease characteristics

Parameter	No. of patients (%)
Age at diagnosis, median (range)	59 (23–86)
FIGO stage	
I	27 (24.8)
II	13 (11.9)
III	56 (51.4)
IV	13 (11.9)
Pathology	
Serous	
High grade	70 (64.2)
Low grade	0
Endometrioid	
Grade 3	3 (2.8)
Grade 2	11 (10.1)
Grade 1	3 (2.8)
Clear cell	17 (15.6)
Mucinous, grade 1	3 (2.8)
Other	2 (1.8)
Cytoreduction	
Microscopic	53 (66.2)
0–1 cm	13 (16.3)
≥1 cm	14 (17.5)
Missing	29
Platinum sensitivity	
Resistant[a)]	25 (23.1)
Partially sensitive[b)]	12 (11.1)
Sensitive[c)]	55 (50.9)
No platinum chemotherapy	16 (14.8)
Missing	1

Percentages reflect percentage of total non-missing data.
FIGO, International Federation of Gynecology and Obstetrics; PFI, platinum-free interval.
[a)]Resistant: PFI less than 6 months; [b)]Partially sensitive: PFI between 6–12 months; [c)]Sensitive: PFI greater than 12 months.

(as defined by the CA125/RECIST criteria), death, or date of last follow-up, whichever occurred first. OS was defined as the interval between first surgical debulking or diagnostic biopsy (for patients receiving adjuvant or neoadjuvant chemotherapy, respectively) and death from any cause or date of last follow-up, whichever occurred first. All survival estimates were determined using Kaplan-Meier analysis (log-rank test). For all tests, a value of $P<0.05$ was considered statistically significant. Univariate and multivariate analyses of PFI, PFS, and OS were performed using the Cox proportional hazard regression model, which estimated HR and 95% CI.

Results

1. Patient and disease characteristics

Patient and disease characteristics are listed in Table 1. Two patients were excluded from the analysis, one owing to insufficient tumor DNA and the other who carried both *BRCA2* mutation and *BRCA1* methylation, leaving a total cohort of 109 evaluable patients. The median age of patients at diagnosis was 59 years, and 63.3% (n=69) presented with advanced stage disease (FIGO stage III/IV). In total, 64.2% patients (n=70) had HGSC; stage III/IV HGSC comprised 53.2% (n=58) of the cohort, and 78.9% (n=86) and 5.5% (n=6) of patients received adjuvant and neo-adjuvant platinum-based therapy, respectively. None of the patients received PARPi therapy during the course of illness. The first PARPi therapy in Ireland was approved after the end of the follow-up period. Reasons for no primary chemotherapy included stage IA/IB disease (6%, n=7), peri-operative death (3%, n=3), age greater than 80 years old (3%, n=3), and other (3%, n=3). Microscopic surgical debulking (R0) was achieved in 66.2% (n=53/80) of patients with available data (data were missing for 26.6% [n=29] patients).

2. Frequency of *BRCA1/2* aberrations

Methylation analysis revealed 10 tumors with at least 10% *BRCA1* promoter methylation (median, 49.86%; range, 18.11–69.23%). All *BRCA1*-methylated tumors were stage III HGSC, totaling a *BRCA1* methylation rate to 14.3% (n=10/70) in HGSC. Tumor *BRCA1/2* gene sequencing re-

Table 2. Details of *BRCA1/2* mutations identified in the Irish cohort

Gene	Age	Stage	Exon	Mutation (HGVS cDNA)	Protein (HGVS protein)	Mutation type
BRCA1	50	3	10	c.1808C>A	p.Ser603*	Nonsense
	37	3	11	c.2418del	p.Ala807Hisfs*8	Frameshift
	40	3	11	c.962G>A	p.Trp321*	Nonsense
	65	3	3	del exon 3		Large genomic rearrangement
	49	4	2	c.68_69del	p.Glu23Valfs*17	Frameshift
BRCA2	57	3	10	c.1310_1313del	p.Lys437Ilefs*22	Frameshift
	57	3	11	c.3570del	p.Lys1191Serfs*6	Frameshift
	55	3	11	c.3717del	p.Lys1239Asnfs*20	Frameshift
	61	3	11	c.4638del	p.Phe1546Leufs*22	Frameshift
	66	2	11	c.4712_4713del	p.Glu1571Glyfs*3	Frameshift
	59	3	11	c.5073dupA	p.Trp1692Metfs*3	Frameshift
	71	1	11	c.5101C>T	p.Gln1701*	Nonsense
	49	1	11	c.6486_6489del	p.Lys2162Asnsf*5	Frameshift
	74	3	11	c.6486_6489del	p.Lys2162Asnsf*5	Frameshift
	53	3	11	c.6486_6489del	p.Lys2162Asnsf*5	Frameshift
	43	3	11	c.6486_6489del	p.Lys2162Asnsf*5	Frameshift
	54	3	2	c.19G>T	p.Glut7*	Nonsense
	55	4	7	c.631+1G>A	Unknown	Splice variant

HGVS, Human Genome Variation Society.

vealed 18 pathogenic mutations (5 *BRCA1* and 13 *BRCA2*) with an overall tumor *BRCA1/2* mutation rate of 16.5% (n=18/109). No individuals were identified to carry more than a single tumor mutation, giving credence to each reported deleterious or suspected deleterious mutation. All mutations were identified in HGSC, and the combined germline and somatic *BRCA1/2* mutation rate in HGSC was 25.7% (18/70). *BRCA1* mutations were only observed in stage III/IV disease, while 3 *BRCA2* mutations occurred in stage I/II cancers. In total, 16 of 18 mutations were classified as pathogenic as per the CLINVAR database [11], and 15 of 18 were curated as per the ENIGMA consortium [12]. *BRCA1* mutations comprised one large genomic rearrangement, 2 frameshift, and 2 nonsense mutations, of which the c.1808C>A mutation has not been previously reported. The common *BRCA1* Ashkenazi Jewish founder mutation c68_69del (also been reported as a separate British founder mutation [13,14]) was identified in one patient of unknown ethnicity. Most *BRCA2*

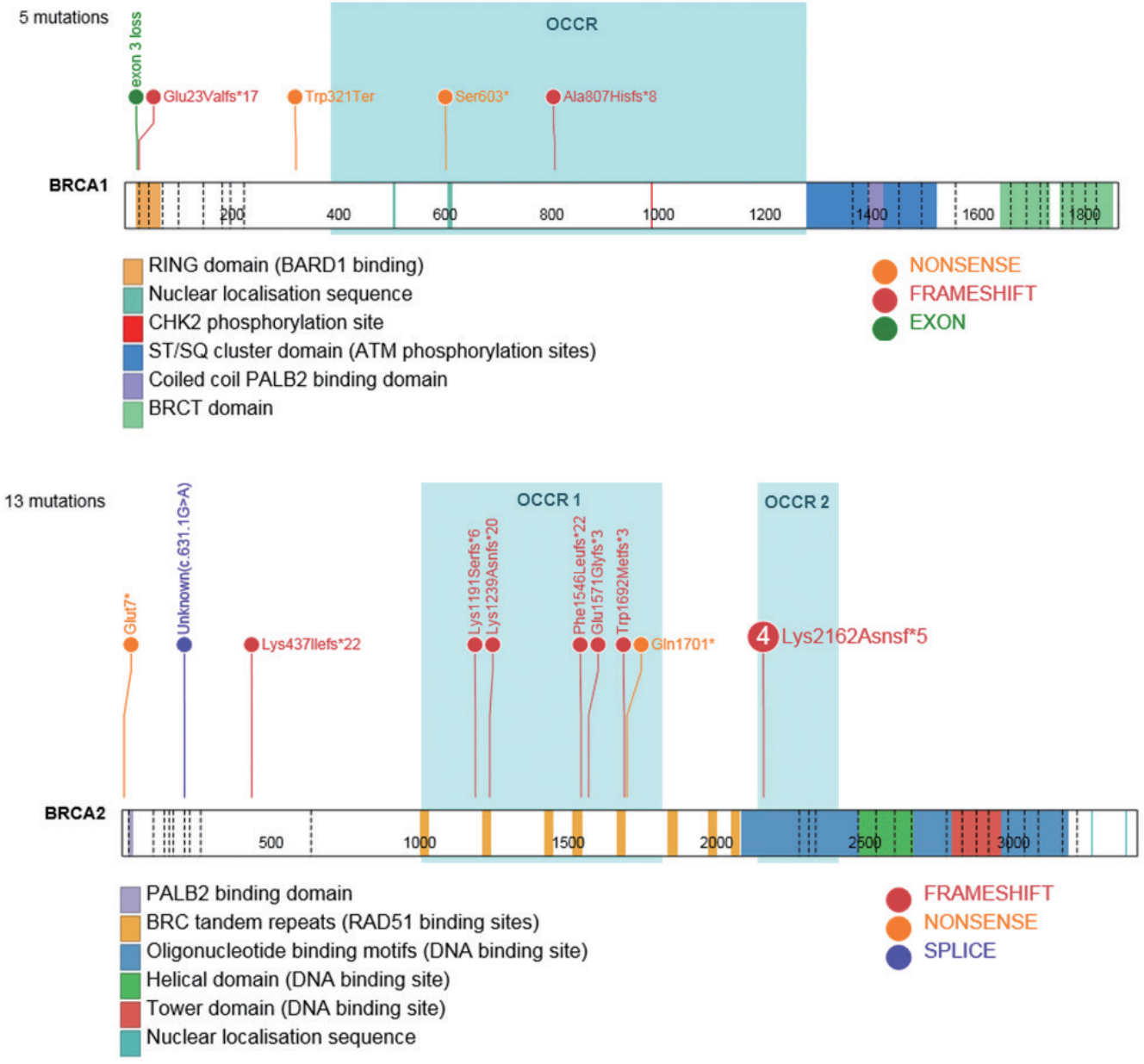

Fig. 1. Localization of the identified *BRCA1/2* mutations in *BRCA1/2* proteins. Numbers on the protein graph correspond to amino acid locations; dashed lines delineate exons. Figure created using ProteinPaint software [16]. OCCR, ovarian cancer cluster region.

mutations were frameshift mutations except for a previously unreported nonsense mutation, c.19G>T, and one splice variant, c.631+1G>A, which were thought to result in abnormal mRNA splicing. Biochemical analysis revealed a similar mutation at this splice donor site that was found to be deleterious by Myriad Genetics laboratories. The *BRCA2* mutation c.6486_6489del was identified in 4 samples from unrelated patients, thereby accounting for 31% of all *BRCA2* mutations. This mutation has been previously reported as a germline variant in hereditary breast OC syndrome and observed in multiple ethnicities. Overall, 76.9% (10/13) of *BRCA2* mutations were located in the RAD51-binding domain (exon 11), which is essential for homologous recombination DNA repair [15]. Three variants of unknown significance (2.7%) were identified in 2 patients (Table 2; Fig. 1 and [16]).

3. Association of patient and disease characteristics with *BRCA1/2* gene aberrations

Using the Fisher's exact test, *BRCA1/2* mutations were found to be significantly associated with stage III/IV disease (P=0.03) and HGSC (P<0.001). We failed to identify any association with younger age or platinum sensitivity. This is potentially owing to the small number and unknown germline/somatic status of *BRCA1/2* mutations in our cohort. *BRCA1* methylation also significantly differed between HGSC and non-HGSC (P=0.004). It was observed in 22.7% of HGSC but not detected among other OC subtypes, which comprised 34% of the entire cohort. Moreover, *BRCA1*-methylated OC was associated with FIGO stage III/IV disease (P=0.005). No significant correlation was identified between *BRCA1* methylation and platinum sensitivity or other clinical variables (Table 3).

4. Survival analyses

BRCA1/2 aberrations were identified in FIGO stage III/IV HGSCs, with the exception of 3 *BRCA2* mutations (FIGO stage I/II disease). Survival analyses were restricted to FIGO stage III/IV HGSC (n=58) to minimize the bias of low stage and grade in the *BRCA1/2* intact arm, thus allowing a more accurate assessment of the survival impact of *BRCA1/2* aberrations. After a median follow-up of 3.8 (range, 0–11.5) years, pa-

Table 3. Correlation between tumour *BRCA1/2* defects and clinico-pathological factors

Parameter	Non mut/meth (n=81)	*BRCA1* meth (n=10)	*BRCA1/2* mut (n=18)	*P*-value	
				BRCA1 meth vs. non mut/meth	*BRCA1/2* mut vs. non mut/meth
Age					
<59	33 (40.7)	6 (60)	12 (66.7)	0.320	0.070
≥59	48 (58.6)	4 (40)	6 (33.3)		
FIGO stage				0.005	0.030
I–II	37 (45.7)	0 (0)	3 (16.7)		
III–IV	44 (54.3)	10 (100)	15 (83.3)		
Pathology				0.004	<0.001
High grade serous	42 (51.9)	10 (100)	18 (100)		
Non-high grade serous	39 (47.6)	0 (0)	0 (0)		
Cytoreduction				1.000	0.440
Macro <1 cm	47 (58)	7 (70)	12 (66.7)		
Macro ≥1 cm	11 (13.6)	2 (20)	1 (5.5)		
Missing	23 (28.4)	1 (10)	5 (27.8)		
Platinum sensitivity				0.150	0.750
PFI <6 mon	17 (21)	5 (50)	3 (16.7)		
PFI ≥6 mon	48 (59.3)	5 (50)	13 (72.2)		
No chemo/missing	16 (19.8)	0 (0)	2 (11.1)		

FIGO, International Federation of Gynecology and Obstetrics; Non mut/meth, *BRCA1/2* wild type non-*BRCA1*-methylated; *BRCA1* meth, *BRCA1*-methylated; *BRCA1/2* mut, *BRCA1/2*-mutated; Macro, macroscopic residual disease; PFI, platinum-free interval.

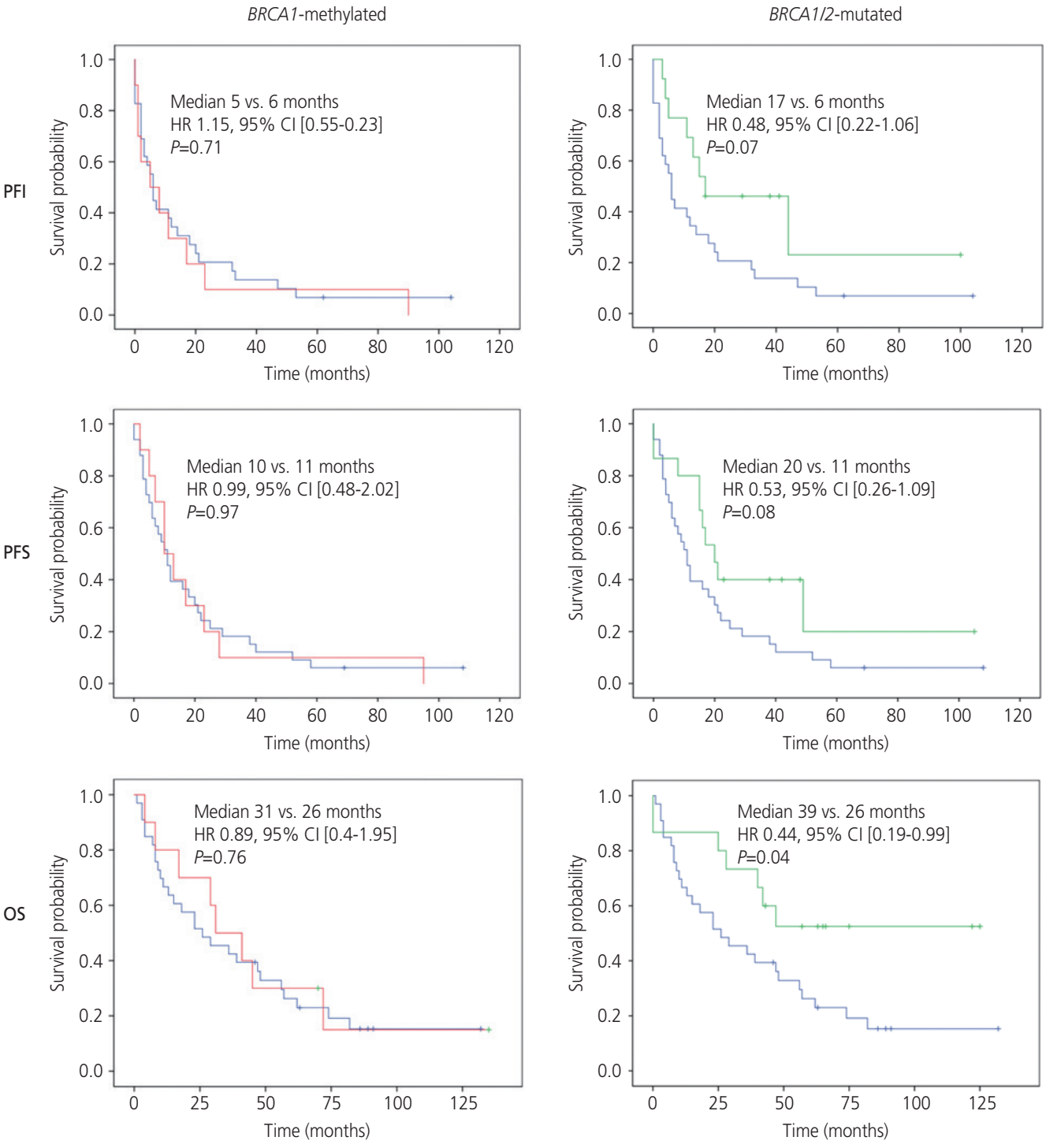

Fig. 2. Survival analyses according to tumor-specific *BRCA1/2* defect. Platinum-free interval (PFI), progression-free survival (PFS), and overall survival (OS) of patients with International Federation of Gynecology and Obstetrics (FIGO) stage III and IV high-grade serous tubal/ovarian cancer (HGSC). Comparison of patients with *BRCA1*-methylated HGSC and those with *BRCA1/2*-mutated HGSC to patients with *BRCA1/2* wild-type non-*BRCA1*-methylated HGSC. In all graphs, blue curves indicate non-*BRCA1*-methylated; red curves indicate *BRCA1*-methylated; and green curves indicate *BRCA1/2*-mutated. HR, hazard ratio; CI, confidence interval.

tients with *BRCA1/2*-mutated tumors showed a trend toward improved PFI and PFS as compared to those with *BRCA1/2*-intact tumors (median survival: 17 vs. 6 months [HR, 0.48; 95% CI, 0.22–1.06; *P*=0.07] and 20 vs. 11 months [HR, 0.53; 95% CI, 0.26–1.09; *P*=0.08], respectively). The lack of expected statistical significance likely relates to the small sample size. OS significantly improved in patients with *BRCA1/2*-mutated tumors (median survival of 39 months) as compared to that in patients with *BRCA1/2*-intact disease (median survival of 26 months) (HR, 0.44; 95% CI, 0.19–0.99; *P*=0.045). No difference in survival was identified between the *BRCA1*-methylated and *BRCA1/2*-intact groups, as evident from the estimated median survivals of 5 vs. 6 months (HR, 1.15; 95% CI, 0.55–2.38; *P*=0.71), 10 vs. 11 months (HR, 0.99; 95% CI, 0.48–2.02; *P*=0.97), and 31 vs. 26 months (HR, 0.89; 95% CI, 0.40–1.95; *P*=0.76) for PFI, PFS, and OS, respectively (Fig. 2). After adjustment for residual disease in the multivariate analysis, *BRCA1/2*-mutated OC lost statistical significance with respect to improved OS (though the trend was similar), while the associations between *BRCA1* methylation and PFI, PFS, and OS failed to show any significant change (Table 4).

Discussion

This is the first study to assess the prevalence of *BRCA1/2* aberrations in Irish patients with OC. We found an overall *BRCA1/2* dysfunction rate of 25.7% (9.2% *BRCA1*-methylated and 16.5% *BRCA1/2*-mutated tumors). All cases were observed in HGSC, which comprised 64.2% of the study population. Within this subgroup, 14.3% of tumors were *BRCA1*-methylated and 25.7% were *BRCA1/2*-mutated, making an overall *BRCA1/2* dysfunction rate of 40% in HGSC. Our findings are in line with those of other large studies, which reported germline/somatic *BRCA1/2* mutation and *BRCA1* methylation rates in the range of 19–27% and 10.5–14%, respectively, in HGSC [2,3,9,17]. Considering the therapeutic benefits of PARPi in *BRCA1/2*-mutated HGSC, and possibly in *BRCA1*-methylated HGSC [4], this degree of *BRCA1/2* dysfunction within the most aggressive and lethal subtype of OC reinforces the crucial need outlined in the recent international guidelines to routinely test germline *BRCA1/2* mutation status in patients with non-mucinous OC [18]. Testing FFPE tumor specimens for *BRCA1/2* mutations using next-generation sequencing (NGS) may allow rapid analysis using low concentrations of DNA samples, making it a cost-effective approach. Tumor DNA sequencing differs from germline DNA sequencing owing to tumor heterogeneity and the risk of nucleic acid degradation during paraffin embedding process. As a result, concerns exist in using tumor *BRCA1/2* mutation testing followed by germline testing of mutation-positive cases to comprehensively detect germline *BRCA1/2* mutations. Our study was restricted in terms of testing the germline/somatic status of the identified mutations from tumor DNA. However, the tumor BRACAnalysis CDx test used in this study has been validated in different cohorts of HGSC FFPE specimens with matched blood samples. Upon application to FFPE specimens corresponding to each blood sample, this test correctly identifies all cases of germline-mutated *BRCA1/2* HGSC in addition to 8.7% cases of so-

Table 4. Univariate and multivariate analyses for platinum-free interval (PFI), progression-free survival (PFS) and overall survival (OS) according to *BRCA1/2* aberrations amongst advanced stage high grade serous ovarian cancers

Variable	PFI		PFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Univariate analyses						
BRCA1/2 mut	0.48 (0.22–1.06)	0.070	0.53 (0.26–1.09)	0.080	0.44 (0.19–0.99)	0.050
BRCA1 meth	1.15 (0.55–2.38)	0.710	0.99 (0.48–2.02)	0.970	0.89 (0.40–1.95)	0.760
Multivariate analyses						
BRCA1/2 mut	0.42 (0.00–0.97)	0.040	0.52 (0.25–1.09)	0.080	0.55 (0.24–1.29)	0.170
BRCA1 meth	1.10 (0.52–2.35)	0.810	1.00 (0.47–2.11)	1.000	0.89 (0.39–2.05)	0.790
Residual disease	2.75 (1.13–6.70)	0.030	2.57 (1.15–5.75)	0.020	4.31 (1.83–10.20)	0.001

The residual disease variable within the model is binary as follows: 0: <1 cm residual disease at surgical cytoreduction, 1: ≥1 cm residual disease at surgical cytoreduction.
BRCA1/2 mut, *BRCA1/2*-mutated; *BRCA1* meth, *BRCA1*-methylated.

matic *BRCA1/2* mutations [19]. Other reports using different *BRCA1/2* panels and NGS platforms, where both germline and tumor tissues were available for analysis, have shown a discordance rate of ≤3% between tumor and blood-based testing for *BRCA1/2* germline mutation [20]. Moreover, upfront tumor *BRCA1/2* mutation followed by reflex germline mutation testing in mutation-positive patients may serve as a more cost-effective strategy than upfront germline mutation testing followed by subsequent tumor mutation testing in germline mutation-negative cases. Finally, the availability of tumor DNA allows *BRCA1* methylation testing, further reinforcing the potential greater utility of tumor tissues in detecting therapeutic targets beyond germline *BRCA1/2* mutations in a single test. However, further studies are warranted to determine the potential of *BRCA1* methylation, in contrast to germline/somatic *BRCA1/2* mutations, as a plausible therapeutic target.

In our study, *BRCA1* methylation, like *BRCA1/2* mutation, was associated with advanced stage HGSC. We failed to observe any association with younger age at diagnosis, contradicting the previous reports [17]. *BRCA1* methylation decreased *BRCA1* mRNA and protein expression in OC [5,6], suggestive of the sensitivity of HRD to platinum chemotherapy and PARPi. *In vitro*, *BRCA1*-methylated breast/OC cell lines demonstrate high sensitivity to cisplatin and olaparib as compared to *BRCA1/2*-intact cell lines [21,22]. We failed to translate these findings in the clinic, consistent with no survival difference between *BRCA1*-methylated OC and *BRCA1/2*-intact OC. Several large studies corroborate our observations [2,17], while others report a negative prognostic effect of *BRCA1* methylation on survival [23]. Nevertheless, a few small studies have reported the superior platinum response and improved PFS amongst *BRCA1*-methylated tumors [24,25]. A larger study involving 213 patients with OC demonstrated similar values of HR for OS in germline *BRCA1*-mutated and *BRCA1*-methylated disease (HR, 0.88; 95% CI, 0.64–1.24 and HR, 0.89; 95% CI, 0.60–1.30, respectively; each group was compared to a *BRCA1/2*-intact population) [26]. Data regarding clinical responses of *BRCA1*-methylated OC to PARPi are limited to the ARIEL-2 study results, which reported a promising response rate of 63% in *BRCA1*-methylated tumors (n=12/19) [4]. These conflicting results are likely related to sample size and heterogeneity within *BRCA1*-methylated OC, as observed with *BRCA1*-mutated OC. While *BRCA2*-mutated OC consistently shows significant survival benefits, some reports revealed no survival difference between *BRCA1*-mutated OC and *BRCA1/2* wild-type OC [27]. The survival benefit conferred by *BRCA1* mutations may be potentially of lesser magnitude or diluted by the heterogeneous effect of different *BRCA1* mutations [28] and mono- or biallelic *BRCA1* mutations [29] on homologous recombination, thereby necessitating large cohorts to confirm this benefit [30]. Further, a significantly larger cohort of *BRCA1*-methylated OC would be necessary to detect survival benefits, if any.

The clinicopathological associations of *BRCA1/2*-mutated disease observed in the present study are similar to those previously reported. *BRCA1/2* mutations were solely detected in HGSC, were associated with improved OS, and showed a trend toward significantly better PFI and PFS. The small sample size of our study limits the strength of survival analyses. A single *BRCA2* mutation, c6486_6489del, accounted for 22% of all mutations detected. This is a known pathogenic germline mutation associated with hereditary breast OC syndrome. No tumor carried the *BRCA1* c.2681_2682delAA variant, a founder mutation originating from Irish/West Scottish Celts [31]. We observed the predominance of *BRCA2* mutations, with a *BRCA2*:*BRCA1* mutation ratio of 2.6:1. In Caucasian HGSC cohorts, germline *BRCA1* mutations were found to be 1 to 3 times more frequent than *BRCA2* mutations [2,3,32]. *BRCA1* mutations have higher penetrance and confer a 36–53% lifetime OC risk as compared to an estimated 11–25% lifetime risk with *BRCA2* mutations [33]. The small sample size of our study may possibly lead to biased results. However, the heterozygote population distribution of *BRCA1/2* mutations varies worldwide. An analysis of the Exome Aggregation Consortium and Exome Variant Server databases demonstrates a high frequency heterozygote *BRCA2* germline mutations in some populations, with some populations having a very low rate of *BRCA1* mutation carriers [8]. A very low frequency of *BRCA1* heterozygote population could potentially explain our findings, though this cannot be verified in the absence of the frequency of *BRCA1/2* mutation in Irish population. Two publications had reported a relatively higher *BRCA2:BRCA1* mutation ratio [34,35], including a 2.7:1 ratio of *BRCA2:BRCA1* mutations amongst 120 patients with non-mucinous OC undergoing routine *BRCA1/2* germline mutation testing. Interestingly, the reports originate from Scotland/Northern Ireland and West Scotland. These populations share a common Celt genetic

ancestry with the Irish population, thereby reinforcing the likelihood that our findings could be representative of the distribution of *BRCA1/2* mutations amongst Irish patients with HGSC. Drawing such conclusions is however limited in our study by ethical constraints to perform germline testing on the identified tumor mutations to determine their germline/somatic status. As these reported mutations occurred singly in individuals and were classified as deleterious or suspected deleterious, a predominance of *BRCA2* mutations has therapeutic and prognostic implications for patients because 76.9% of *BRCA2* mutations identified were located in the RAD51-binding domain of the gene. These mutations are associated with improved PFS and OS in contrast to those located outside this domain [15].

In conclusion, we observed a *BRCA1/2* dysfunction rate of 40% within the Irish HGSC population, owing to *BRCA1/2* mutations and *BRCA1* promoter methylation in tumors; we noted a unique predominance of *BRCA2* over *BRCA1* mutations. This observation reinforces the need for routine *BRCA1/2* germline and somatic testing to facilitate therapy selection (PARPi and other forthcoming DNA repair targeting agents) and cancer prevention for germline mutation carriers and their relatives. A better understanding of the clinical and therapeutic relevance of *BRCA1* methylation in OC is needed, given its potential to expand the therapeutic benefits of DNA repair targeted agents to a larger number of women with OC.

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Conflict of interest

Roshni D. Kalachand has received conference travel fees from Astra Zeneca. Kirsten M. Timms is an employee of and may hold shares in Myriad Genetics Inc. The other authors have no conflicts of interest to declare.

Ethical approval

Samples and patient data obtained from the Discovary bioresource (St James's Hospital) received approval from the hospital's ethics committee (reference 2009/29/01).

Samples and patient data obtained from the Beaumont Hospital Pathology Department receivedapproval from the hospital's ethics committee (REC reference 12/02).

Patient consent

The patients provided written informed consent for the research carried out within this publication.

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Original Article

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Congenital uterovaginal abnormalities, it's embryogenesis, surgical management and clinical implications

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Objective
Congenital Mullerian duct malformations are a challenging group of conditions for surgeons and need surgical experience and skill. Accordingly, the aim of this study is to present the diagnosis, surgical management, and clinical implications of congenital uterovaginal abnormalities.

Methods
Between 1980 and 2015, 8 patients with congenital uterovaginal abnormalities were diagnosed. In one patient a unique case of an unusual horseshoe shaped double uterus communicating via a transverse canal along with agenesis of the cervix and vagina was noted, and utero-vaginal agenesis was diagnosed in 6 patients. Complete androgen insensitivity syndrome with its female phenotype associated with bilateral testicular tissue in the inguinal canal with an accompanying absence of the ovaries, uterus, uterine tubes, vagina, and an imperforate hymen, was diagnosed in one patient. Clinical examination of all the patients revealed well-developed secondary sexual characteristics. A modified McIndoe vaginoplasty procedure was the surgical treatment common to all patients to treat vaginal agenesis. The surgery was performed by a consultant (Dr. K.G. Paul) using the standardized surgical technique.

Results
An unusual Mullerian duct anomaly, uterus bicornisacollis, was successfully corrected by uteroplasty and a new cervix was constructed. Complete vaginal agenesis was corrected by a modified McIndoe vaginoplasty technique. None of the patients had any significant post-operative complications.

Conclusion
Knowledge of congenital uterovaginal abnormalities diagnosed in this study is essential for surgeons, clinical anatomists, radiologists, and morphologists who may increase the success of their diagnostic evaluations and surgical approaches to the region.

Keywords: Androgen insensitivity syndrome; Infertility; Vaginal agenesis; Wolffian duct

Introduction

The reproductive system or genital system is a system of sex organs within an organism involved in producing offspring. In men, the genital system includes the prostate, testes, and penis. In women, it includes the ovaries, fallopian tubes, uterus, and vagina. A basic understanding of the embryology of the reproductive tract is essential to restore reproductive and sexual functioning and normalize genital anatomy whenever possible.

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The vagina is the elastic, muscular part of the female genital tract, which extends from the vulva to the cervix. In humans, the vagina receives sperms during sexual intercourse, is the channel for the birth canal during the birth process, and also functions as an excretory channel for menstrual flow. Congenital vaginal agenesis is a rare malformation estimated to occur among 1 in 4,000–5,000 female births. The McIndoe technique was first described in 1938 by Bainster and McIndoe as a surgical procedure that improves quality of life and sexual satisfaction and provides a functional vagina with minimal complications for most patients with vaginal agenesis. Congenital and acquired genital anomalies in the adolescent population may lead to difficulties in carrying a pregnancy to term, infertility, and recurrent pregnancy loss, which lead to a great amount of social inhibition and psychological distress for the affected individuals. Such abnormalities are a challenging group of conditions for the reproductive surgeon and need surgical experience and skill. They also require a basic knowledge of the embryology of the reproductive tract to increase the success of diagnostic evaluations and surgical approaches to the region. Accordingly, the aim of this study was to evaluate congenital uterovaginal abnormalities, and their embryogenesis, surgical management, and clinical implications.

Materials and methods

Between 1980 and 2015, 8 patients with congenital uterovaginal malformation were diagnosed in our clinics.

1. Case I: congenital uterine malformation with vaginal agenesis

A 20-year-old unmarried woman presenting with primary amenorrhea was admitted to the Gynaecology Clinic of Princess Marine Hospital, Gaborone, Botswana. A diagnostic laparoscopy procedure revealed a double uterus, with communication via a transverse canal, along with agenesis of the cervix, vagina, and external vaginal opening (hymen). The urethral orifice was normal and the patient had well-developed secondary sexual characteristics.

Surgical correction (uteroplasty) of a uterine malformation (Supplementary Fig. 1): a lower abdominal transverse incision was given and the abdomen was exposed; each medial wall of the uterus was cut from above with a downward trajectory until the endometrial cavity was entered. Each uterine cavity was then opened by separating the uterus into anterior and posterior walls. Once the endometrial cavities of both uteri were exposed, the uterine walls were then reapproximated and sutured together with synthetic absorbable sutures, and both uterine cavities were converted into a single full space. The lower end of the uterus was then opened and the surgeons created a cervix. The newly created cervix was supported by a small plastic tube that was removed after 6 weeks during McIndoe vaginoplasty. Six weeks after the uteroplasty, McIndoe vaginoplasty was done to treat vaginal agenesis (Supplementary Fig. 1).

2. Case II: utero-vaginal agenesis

Six unmarried woman (age 19–23 years) presented with primary amenorrhea. Diagnostic laparoscopy procedures revealed the presence of well-developed ovaries with an absence of a vagina, hymen, uterus, and uterine tubes. The urethral orifice was normal with well-developed secondary sexual characteristics. In all 3 patients, McIndoe vaginoplasty was performed to treat vaginal agenesis. Out of 6 patients, one was operated at INHS Asvini, Mumbai between 1980 and 1983, 3 patients were operated at 166 Military Hospital, Jammu between 1993 and 1995, and 2 patients were operated at Command Hospital, Calcutta during between 2000 and 2003(Supplementary Fig. 2).

3. Case III: complete androgen insensitivity syndrome

A 20-year-old unmarried woman presenting with primary amenorrhea was admitted to the Gynaecology Department at the 166 Military Hospital, Jammu. A diagnostic laparoscopy procedure revealed the presence of bilateral testicular tissue (testicular feminization with its female phenotype) in the inguinal canal with an absence of ovaries, uterus, uterine tubes, and vagina, and an imperforate hymen. The urethral orifice and the external genitalia were normal. With the help of a geneticist, the karyotype was mapped and revealed a female phenotype, 46, XY. The testes were removed in order to avoid a risk of malignancy (Supplementary Fig. 3).

4. Modified McIndoe vaginoplasty

To treat vaginal agenesis, 7 patients (caseI and caseII) aged between 19 to 23 years underwent McIndoe vaginoplasty. The patients were operated on under general anaesthesia and placed in a lithotomic position with urinary catheteriza-

tion (Supplementary Fig. 4). The surgery was performed by consultant Dr. K.G. Paul using the standardized surgical technique. In this operation, the labia are retracted with Allis clamps, a transverse incision is made in the epithelium, and blunt finger dissection is then carried out to create a new vaginal space or canal (optimum vaginal length of 10–12 cm) between the rectum and bladder. Two proposed paddle-shaped full-thickness skin grafts approximately 10 cm in length and 6 cm in width over the inguinal ligaments from the anterior superior iliac spine to the pubic tubercles were harvested from the patient (Supplementary Fig. 5). After harvesting the full-thickness skin graft, to close the wound, the skin is stretched and sutured using a synthetic absorbable suture (Supplementary Fig. 5). These full-thickness skin grafts allow for sufficient penetration of the transudation nutrients from the bed of the graft that are necessary for nutrition during the first 72 hours until microcapillary growth has been completed, which reduces postoperative contraction. An artificial mould was created using a dental impression material and a full-thickness skin graft. A mould was shaped using 3M Putty Material and covered with a layer of soft sponge and a condom. Subsequently, the condom was applied, tied on to its open end. The size of the mould corresponding to the neovagina was selected (Supplementary Fig. 4). The full-thickness skin graft was then sewn over this mould with the deep surface of the graft facing outward so as to be in contact with the newly created vaginal wall. The margins were sutured together with synthetic absorbable suture and introduced into the neo-vaginal canal or space (Supplementary Fig. 4). To hold the skin graft covered mould in place for 12 days, the labia were sutured at the midline with interrupted 0-nylon sutures without tension. A mould covered with a layer of soft sponge and a condom was removed on the 12th postoperative day. The new vagina was thoroughly inspected and irrigated with normal saline solution.

Results

An unusual Mullerian duct anomaly, uterus bicornis (double uterus) acollis (absence of cervix), was successfully corrected by uteroplasty and a new cervix was constructed. Complete vaginal agenesis in 7 patients was corrected by a modified McIndoe vaginoplasty technique. Clinical examination performed postoperatively revealed a neovagina of adequate length and caliber. None of the 7 patients had any significant post-operative complications.

Discussion

The genital system is concerned with the maintenance and propagation of the species. In women, the internal genitalia include ovaries, fallopian tubes, the uterus, and the vagina; the external genitalia include mons pubis, labia majora and minora, the clitoris, the vestibule, and the perineum. Gonads initially develop in females (ovaries) and males (testes) from the undifferentiated genital ridge at approximately the 5th week of development, and gonadal differentiation becomes apparent at approximately the 7th week of embryonic life. In embryos of both sexes, the primitive sex ducts are indifferent and consist of 2 paired ducts—mesonephric (Wolffian) and paramesonephric (Mullerian). With the development of the testis from the genital ridge, the mesonephric duct is retained in males as the duct system of the testis and the paramesonephric duct mostly degenerates. In females, however, the paramesonephric duct plays an important role in the development of reproductive organs and the mesonephric duct and its tubules mostly regress.

At first, these paired bilateral Mullerian ducts pass caudally (through the cranial vertical part), lateral to the mesonephric duct. In the pelvis, they cross (through the intermediate horizontal part) ventral to the mesonephric duct and grow medially. During the 8th week, they reach the caudal end of the mesonephric duct and to contact and fuse with their counterparts (caudal vertical part) to form a Y shaped uterovaginal bulb or tubercle that bulges into the dorsal of the urogenital sinus. Caudal vertical parts of both Mullerian ducts fuse in the caudo-cranial direction, which normally occurs between the 6th and 11th weeks of gestation. The partition between them completely disappears and forms a single duct utero-vaginal canal by the end of 3rd month. The cranial part of the utero-vaginal canal forms the entire uterus, and points of fusion of the 2 ducts represent the site of the future fundus. The cranial vertical parts and most of the intermediate horizontal parts of each Mullerian duct form the respective fallopian tubes. Any disruption of Mullerian duct development and fusion during embryogenesis can result in a broad and complex spectrum of congenital abnormalities, termed Mullerian duct anomalies. A uterus is absent in 2% to 7% of pa-

tients with vaginal agenesis [1]. Congenital anomalies of the uterus result from varying degrees of failure of fusion of the Mullerian ducts but may occasionally arise from true duplication of the ducts [2]. The incidence reported in the literature ranges between 0.13–0.4% among the general population [3,4]. Unilateral suppression of Mullerian ducts results in an unicornuate uterus, which is unique among Mullerian abnormalities and is the rarest of the uterine abnormalities, accounting for 0.3–4% of uterine abnormalities [5]. Simón et al. [6] also reported that approximately 3.2 percent of fertile women have a bicornuate uterus. In our study (casel), a unique case of an unusual Mullerian duct anomaly, horseshoe shaped uterus bicornis (double uterus) acollis (absence of cervix) along agenesis of the vagina was noted. This type of abnormality may be due to failure of Mullerian duct fusion in the caudo-cranial direction. To the best of our knowledge, such a unique and unusual Mullerian duct anomaly observed in this study has not been cited in modern literature.

The upper four-fifth of the vagina above the hymen develops from the lower part of the utero-vaginal canal and the lower one fifth develops below the hymen from the ectoderm of the genital folds. The vagina opens into the exterior through the vestibule, which is derived from the ectoderm of genital folds after the rupture of the urogenital membrane. Vaginal agenesis (absence of the vagina) is one of the most significant congenital anomalies of the female reproductive tract from a physical and psychological perspective. Vaginal agenesis is estimated to occur in 1 in 4,000–5,000 live female births. The purpose of vaginal agenesis treatment and appropriate management is not only to create an adequate passageway for penetration but also to facilitate satisfactory sexual intercourse and normalize genital anatomy where possible, and to alleviate her psychological concerns. To treat vaginal agenesis, the McIndoe technique is the preferred method for vaginal reconstruction, and several authors have documented satisfactory sexual relationships using the McIndoe method in over 75% of patients [7]. Mayer-Rokitansky-Kuster Hauser syndrome is a congenital malformation characterized by the failure of the Mullerian duct to develop, resulting vaginal agenesis and absence or hypoplasia of the uterus. Ovaries are normal in the majority of cases, which are more frequently associated with renal and vertebral malformations. Vaginal agenesis is associated with malformations in the urinary tract in 17–56% of patients [8-10]. In our study, urinary tract malformations were absent. Buss and Lee [11] reported an 80–90% graft take in 94% of their patients. Complete graft take 10 days postoperatively was described by Garcia and Jones [12] in 73% of their patients, with an 80–90% graft take in a further 20% of patients. The degree of failure of graft take was not recorded in our present study.

The sex-determining region Y (SRY) gene is located on the short arm of the Y chromosome. During the 6th week of male fetal development, the SRY gene provides instructions for making a protein called the SRY protein (testis determining factor) that influences the development of male phenotypes, such as testes from the genital ridges at the medial posterior abdominal cavity. Complete androgen insensitivity syndrome (testicular feminization) is a syndrome when a male, genetically XY, because of various abnormalities of the X chromosome, is resistant to the actions of androgen hormones, which in turn stops the forming of the male genitalia and gives a female phenotype. This syndrome occurs in one out of 20,000 live births and can be incomplete (various sexual ambiguities) or complete (the person appears to be a woman).Testicular feminization, or androgen insensitivity, is a rare syndrome that is characterized by primary amenorrhea, a 46, XY karyotype, a female phenotype, and the presence of testes rather than ovaries [13-15]. Patients have a congenital resistance to the effects of androgens, which results in an absence of Wolffian duct derivatives and the development of a female phenotype. However, production of the Mullerian inhibitory factor persists, which accounts for the complete regression of the Mullerian duct system. Therefore, patients with testicular feminization have no uterus or fallopian tubes and are without the upper third of the vagina [16]. In approximately one third of these individuals undescended testes have a well-recognized propensity for tumoral proliferation, and the risk of gonadal malignancies increases with age. Hence, it is essential that all women with primary amenorrhea should undergo a complete investigation of the genital tract and endocrine system quickly and accurately to allow clinical and psychologic input.

In conclusion, the findings suggested that a modified McIndoe vaginoplasty technique is a simple, effective procedure for the treatment of vaginal agenesis. We believe that the present study has provided some important data that will contribute to the scientific literature, providing the data of embryogenesis, surgical management, and clinical implications of uterovaginal congenital abnormalities. Awareness of the rare congenital abnormalities noted in our study is essen-

tial for surgeons, clinical anatomists, radiologists, and morphologists to increase the success of reproductive diagnostic evaluation and surgical approaches to the region.

Conflict of interest

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

Ethical approval

This study was reviewed and approved by the appropriate institutional human research ethics committee (reference number: HREC10AUG19).

Patient consent

Each patient's informed consent for the purpose of this study (i.e. publication without disclosure of personal identity) was obtained.

Supplementary materials

Supplementary Figures associated with this article can be found online at https://doi.org/10.5468/ogs.2020.20046.

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Original Article

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Effects of high-frequency, high-intensity transcutaneous electrical nerve stimulation versus intravenous opioids for pain relief after hysteroscopy: a randomized controlled study

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Objective

To compare the time spent in the Post-Anesthesia Care Unit (PACU) and the pain-relieving effects of treatment with high-frequency, high-intensity transcutaneous electrical nerve stimulation (TENS) or intravenous (IV) opioids after hysteroscopy.

Methods

All patients who postoperatively reported a visual analogue scale (VAS) pain score of ≥3 were included in the study. TENS treatment was given with a stimulus intensity between 40 and 60 mA for 1 minute and repeated once if there was insufficient pain relief. In the opioid group, a fractionated dose of 5 mg morphine was administered. If the patient reported insufficient pain relief after the assigned treatment, the patient was reassigned to the other treatment group.

Results

Seventy-four women were randomized to TENS (n=38) or IV opioids (n=36) for treatment. Both groups reported significant pain relief after discharge from the PACU, with a decrease of VAS scores from 5.6 to 1.4 in the TENS group (P<0.001) and 5.1 to 1.3 in the opioid group (P<0.001). There were no significant differences between the groups. When only the responders in both groups, i.e., patients with VAS scores of <3 on respectively assigned treatments, were compared, the TENS responders (n=22) were found to have spent a significantly shorter time in the PACU (91 vs. 69 minutes, P=0.013) compared to the opioid responders (n=20).

Conclusion

Using TENS as first line of pain relief may reduce the need for postoperative opioids. In addition, TENS appears preferable as the first line of treatment due to its association with a shorter time spent in the PACU if the patient responds to the treatment.

Trial Registration

Västra Götalandsregionen Identifier: 211261

Keywords: Hysteroscopy; Pain management; Opioids; TENS

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Introduction

Hysteroscopy is a minimally invasive intervention that can be used to diagnose and treat many intrauterine and endocervical problems. Diagnostic and operative hysteroscopy have become standard in gynecologic practice. In Sweden, approximately 5,000 hysteroscopy operations are performed every year. At Sahlgrenska University Hospital Östra, approximately 160 hysteroscopy operations are performed every year [1].

Pain is sometimes a problem after hysteroscopy [2] and conservative treatment with opioids often gives adequate pain relief [3]. However, opioids have serious side effects, such as sedation, nausea and respiratory depression [4]. Hence, the patient needs surveillance, resulting in a longer time spent in the Post-Anesthesia Care Unit (PACU) after surgery.

Furthermore, there is a serious concern regarding "the opioid epidemic" in the United States [5], where society faces an escalating problem of the use of opioids after surgery, which has led to opioid addiction and sometimes abuse in some patients. Prolonged opioid use after surgery is a common and previously underestimated problem [6]. The risk of previous opioid naive patients to end up in long-term opioid use is estimated at approximately 6% after both major and minor interventions [6], indicating that long-term opioid use might be considered as a common complication after surgery. Hence, there is an increased interest in the use of non-opioid alternatives for the treatment of postoperative pain [7].

Transcutaneous electrical nerve stimulation (TENS) is a pain treatment that delivers an electrical current through the skin. The exact mechanism of action of TENS treatment is still unknown. However, the effects of peripheral nerve stimulation has traditionally been linked to the activation of afferent

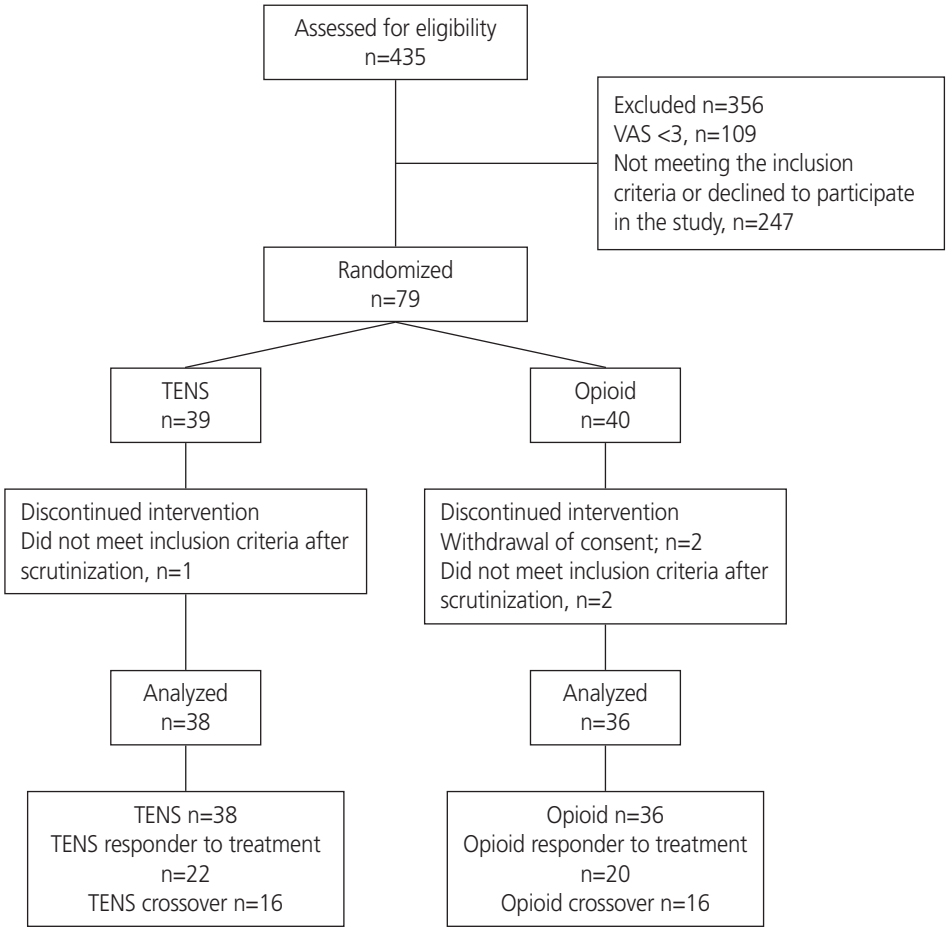

Fig. 1. Patient flowchart diagram. VAS, visual analogue scale; TENS, transcutaneous electrical nerve stimulation.

nerve fibers (Aβ-fibers) modulating Aδ and C-fibers in the spinal cord, which is compatible with the gate control theory of pain [8]. Release of endogenous endorphins following high-frequency TENS application has also been suggested as a mechanism of action [9,10]. Furthermore, the diffuse noxious inhibitory controls theory (DNIC) refers to an endogenous pain modulatory pathway, which has often been described as "pain inhibiting pain through continuous pain" by noxious or intense cutaneous stimulation, such as TENS [11].

TENS has been shown to be effective for treatment of a variety of gynecological conditions such as dysmenorrhea, pain after endometrial biopsy, surgical abortion, and gynecological laparoscopy, as well as labor pain [12-17]. In addition, previous studies from our center indicate that TENS treatment for postoperative pain results in a shorter time in the PACU [16,17].

Studies by De Angelis et al. [18] and Lisón et al. [19] have indicated that TENS treatment is effective for intraoperative pain relief during hysteroscopy. However, to our knowledge, there is limited data concerning the effects of TENS for postoperative pain treatment after hysteroscopy.

Hence, the aim of the present randomized control study was to compare the time spent in the PACU after surgery as well as the postoperative pain relieving effects of high-frequency, high-intensity TENS and pharmacological treatment compared to intravenous (IV) opioids in patients undergoing hysteroscopy.

Our hypothesis was that patients receiving TENS for pain relief after undergoing hysteroscopy would spend a shorter amount of time in the PACU than those treated with pharmacological IV opioids.

Materials and methods

1. Patients

For 31 months, 435 patients admitted to the operating theatre at Sahlgrenska University Hospital/Östra for hysteroscopy were assessed for eligibility. Two hundred forty-seven patients declined to take part in the study, while 109 patients did not meet the inclusion criteria, i.e., pain scored ≥3 on the visual analogue scale (VAS). Seventy-nine patients had VAS pain scores of ≥3 i.e., requiring pain relief (Fig. 1). The exclusion criteria for the study were the following: alcohol or drug dependence, affected sensation over the current dermatome, use of a pacemaker/implantable cardioverter defibrillator (ICD), need for an interpreter, pain according to VAS of ≤3, and an age less than 18 years. The patients were randomized using the closed-envelope technique after reporting a postoperative pain score ≥3 according to the VAS

Table 1. Premedication, pharmacological treatment during surgery and postoperative pharmacological treatment in the Post-Anesthesia Care Unit (PACU) in the transcutaneous electrical nerve stimulation (TENS) group and in the pharmacological treatment intravenous (IV) opioid group

Variables	TENS treatment group (n=38)	IV opioid treatment group (n=36)
Premedication		
NSAID	30 (78.9)	31 (86.1)
Paracetamol	37 (97.4)	35 (97.2)
Pharmacological treatment during surgery		
Total intravenous anesthesia	38 (100.0)	36 (100.0)
Fentanyl peroperatively I	1 (2.6)	0
Postoperative pharmacological treatment		
Morphine IV	16 (42.1)	36 (100.0)
Morphine equivalents per patient[a]	3.1±5.1	5.3±1.9
Other analgesics in the PACU[b]	2 (5.3)	0

All patients received morphine IV perioperatively during surgery. Data are presented as number of patients (%), except morphine equivalents per patient, which is presented as mean±standard deviation.
NSAID, non-steroidal anti-inflammatory drug.
[a]*P*-value <0.001 for comparison between the TENS group and IV opioid group; [b]Only crossover patients received additional analgesic treatment postoperatively.

in the PACU after hysteroscopy. Both patients and investigators knew the randomization allocation. To achieve optimal pain relief, high-intensity, high-frequency TENS was used, generating paresthesia over the treated area. Consequently, it was impractical to blind both the patient and nurse to the treatment allocation.

2. Perioperative procedures

The standard procedure before hysteroscopy was performed according to clinical routine. All patients received a peripheral vein catheter before surgery. Preoperatively, 72 patients were administered paracetamol, while 61 patients received non-steroidal anti-inflammatory drugs (NSAIDs) orally (Table 1). Preoperatively, the patients were administered 5 mg morphine IV (range 2–8 mg) during general propofol-remifentanil anesthesia administration. The hysteroscopy procedure was performed according to the hospital's treatment routines depending on an indication for the surgery.

3. Postoperative procedures

This study uses the same method as previously presented in studies from our center [16,17]. Thus, the description of our methods partly resembles the wording in our earlier publications.

Nurses at the PACU administered both TENS treatment as well as pharmacological treatment and assessed pain intensity according to VAS upon arrival and departure of the patients, as well as at certain time intervals during the patients' PACU stay according to clinical routine. Pain intensity was defined as severe pain with a VAS score of 7–10, moderate pain with a VAS score of 5–6, and mild pain with a VAS score of <5 [20]. In the TENS group, the electrodes were positioned over the area where the patient experienced pain, which was most frequently over dermatome TH12 (Fig. 2), with high-frequency (80 Hz) stimulation (CefarPrimo®, Cefar Medical AB, Lund, Sweden). High-intensity stimulation (strong stimulation) was achieved by increasing the stimulation gradually to achieve a stimulation intensity of 40–60 mA. Five patients found the stimulation unbearable. Thus, for these patients, only a level of 19–38 mA was achieved. The intensity of the pain was assessed directly after TENS-stimulation, i.e., after 60 seconds. If adequate pain relief was not obtained (VAS, <3), the stimulation was repeated. If the patient did not experience any pain relief after 2 TENS stimulation episodes or if the patient was not able to tolerate the intensity of the stimulation, the patient received pharmacological treatment with IV opioids.

In the group given pharmacological treatment with IV opioids, a fractionated dose of an average of 5.3 mg (range, 5–15 mg) morphine was given. The pain intensity was re-estimated after approximately 10 minutes after injection. If the patient reported insufficient pain relief (VAS, >3) after approximately 10 minutes, primary analgesics (i.e., morphine, paracetamol, NSAID, or tramadol) were administrated according to local clinical guidelines. If the patient still reported inadequate pain relief after 30 minutes from the start of pain treatment, TENS was administered.

A nurse assessed all patients for nausea and sedation in the PACU. Nausea was assessed using VAS (0–10; 0=absence of nausea and 10=worst nausea). Sedation was estimated by the Ramsay sedation score [21]. The patients were transported to the ward when they were considered stable with regards to respiration and circulation (according to standard clinical criteria), and when adequate pain relief, defined as a VAS score of <3, had been obtained. The time of the arrival and discharge of the patient in the PACU was registered in the patients' charts. Since it is mandatory that the patients report a VAS score <3 before leaving the PACU, the time to obtain adequate pain relief was of interest. Hence, the time in the PACU is used in the present study as a surrogate measure for the effectiveness of the pain treatment. Other criteria for transport to the ward from the PACU were the presence of stable respiration and circulation, absence of

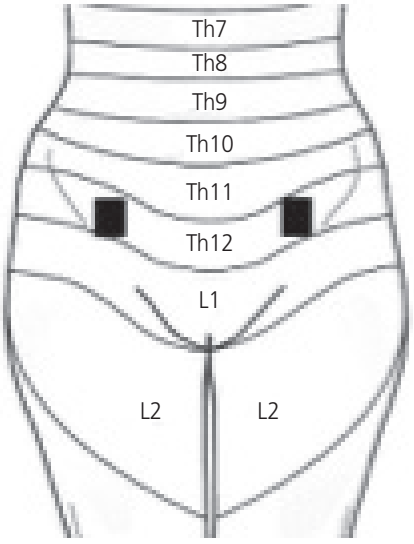

Fig. 2. Electrode placement L1 (front) and Th12 (back).

current bleeding and nausea, and a patient who was awake and oriented to time and room.

4 Statistical analysis

Sample size was calculated for a power of 80% with time spent in the PACU as the primary outcome variable. To demonstrate a difference between the groups with 80% power and a 2-sided significance level of alpha 0.05, a sample size of 50 participants in each group (mean time with standard deviation in the PACU in minutes, 44.3±30.7 vs. 62.1±34.3 in the TENS and pharmacological treatment with IV opioid group, respectively) was required [16]. For the comparison between the 2 groups, Fisher's exact test was used for dichotomous variables. The χ^2 analysis was used for non-ordered categorical variables. The Mann-Whitney *U*-test was used for continuous variables. For the comparison within the groups, a Wilcoxon signed rank test was used for continuous variables. All tests were 2-tailed, and a significance level of 5% was used. The data were analyzed based on an intention-to-treat (ITT) analysis. A subgroup analysis was performed based on "responders to treatment".

Results

1. Patients

The patients were randomized to TENS (n=39) or standard pharmacological treatment (n=40). Three patients did not meet all of the inclusion criteria while two patients withdrew consent to participate in the study. Thus, 38 patients were analyzed in the TENS group and 36 patients were analyzed in the opioid treatment group (Fig. 1).

There were no significant differences concerning health characteristics between the groups (Table 2). The indications for hysteroscopy were resection (i.e., myoma and polyps), diagnostics, extractions and other indications such as removal of placental residues postpartum, searching for intrauterine devices, and sterilization (Table 3). There were no statistically significant differences with regards to indication for surgery, time for surgery, or postoperative pain at the PACU before treatment between the groups (Tables 3 and 4). Furthermore, there were no significant differences between the groups concerning the use of premedication or pharmacological treatment during surgery (Table 1).

Table 2. Health characteristics in the transcutaneous electrical nerve stimulation (TENS) group and the pharmacological treatment intravenous (IV) opioid group

Variables	TENS treatment group (n=38)	IV opioid treatment group (n=36)	*P*-value
Age (yr)	49.8±12.9 (28–76)	51.1±12.6 (31–78)	0.725
Previous abdominal surgery	9 (23.7)	11 (30.6)	0.604
Chronic pain	9 (23.7)	10 (27.8%)	0.792
Analgesics use[a)]	14 (36.8)	8 (22.2)	0.251

Data are presented as number of patients (%), except age, which is presented as mean±standard deviation (range).
[a)]Continuous analgesic use in terms of non-steroidal anti-inflammatory drug and paracetamol. One patient in the opioid group used tramadol regularly due to chronic pain.

Table 3. Indications for gynecologic hysteroscopy surgery and time for surgery in the transcutaneous electrical nerve stimulation (TENS) group and the pharmacological treatment intravenous (IV) opioid group

Variables	TENS treatment group (n=38)	IV opioid treatment group (n=36)	*P*-value
Indication for surgery[a)]			0.967[a)]
Resection	16 (42.1)	14 (38.9)	
Diagnostics	1 (2.6)	1 (2.8)	
Extraction	3 (7.9)	4 (11.1)	
Others	18 (47.4)	17 (47.2)	
Time for surgery (min)	21.2±13.7	19.9±12.5	0.596

Data are presented as number of patients (%), except time for surgery, which is presented as mean±standard deviation.
[a)]Comparison between the TENS group and IV opioid treatment group.

2. Time in the Post-Anesthesia Care Unit

There were no significant differences between the groups with regards to total time spent in the PACU. The TENS group spent 87 minutes in the PACU while the pharmacological treatment with IV opioid group spent 96 minutes (*P*=0.249) (Table 4). When only the responders to the respective treatments (i.e., patients who reported VAS <3 on assigned treatment) were compared (per protocol analysis), the TENS responders reported a significantly shorter time spent in the PACU compared to the responders in the opioid group (Table 5).

3. Pain and pain relief

Pain intensity was measured upon arrival in the PACU according to Serlin et al. [20]. There were no significant differences between the groups in terms of pain intensity. Majority of patients in the study (n=50, 67.6%) reported moderate or severe pain in the PACU. Both groups reported significant pain relief, with a decrease in VAS score from 5.6 at arrival to 1.4 at departure from the PACU in the TENS group (*P*<0.001) and a decrease in VAS score from 5.1 at arrival and 1.3 at departure from the PACU in the opioid group (*P*<0.001). There were no significant differences between the groups. Approximately one third (n=24) of the patients reported complete pain relief (VAS, 0) after treatment (Table 4).

There was no significant difference with regards to numbers of crossovers, i.e., patients who reported a VAS score of ≥3 after initial treatment, between the groups (TENS group n=16 [42%]; opioid group n=16 [44%]) (Fig. 1). Of the 25 crossover patients (72%) who reported moderate or severe pain at arrival in the PACU, there were no significant differences between the crossover groups (TENS vs. opioid) with regards to pain intensity. There were no differences between the crossover group (n=32, i.e., non-responders) and the responders to treatment (n=42) in terms of the number of patients with mild, moderate and severe pain upon arrival in the PACU. In the crossover group, 12 (38%) of the patients reported complete pain relief (VAS, 0) after additional treatment. Nevertheless, postoperative opioid consumption was significantly lower (*P*<0.001) in the TENS group (3.1 morphine equivalents per patients) versus the IV opioid group (5.3 morphine equivalents per patients) despite the presence of crossover patients (Table 1).

4. Chronic pain

Nineteen (26%) of the patients in the study suffered from chronic pain (Table 2), while 8 (25%) of the patients in the crossover group had chronic pain (TENS n=3, opioid n=5).

Table 4. Pain relief and time in the Post-Anesthesia Care Unit (PACU) in the transcutaneous electrical nerve stimulation (TENS) group and in the pharmacological treatment intravenous (IV) opioid group according to visual analogue scale (VAS)

Variables	TENS treatment group (n=38)	IV opioid treatment group (n=36)	*P*-value
VAS before treatment	5.6±1.8	5.1±1.3	0.408
VAS at leave from the PACU	1.4±1.2[a)]	1.3±1.1[a)]	0.698
VAS 0 after treatment	12 (31.6)	12 (33.3)	1.000
Time at the recovery ward (min)	87.3±36.5	96.4±38.5	0.249

Data are presented as mean±standard deviation, except VAS 0 after treatment, which is presented as number (%).
[a)]*P*-value within groups, before treatment and at discharge from the PACU; TENS <0.0001, IV opioid <0.0001.

Table 5. Pain relief and time in the Post-Anesthesia Care Unit (PACU) according to visual analogue scale (VAS) in the patients who responded to transcutaneous electrical nerve stimulation (TENS) treatment and pharmacological treatment intravenous (IV) opioids (VAS, <3), i.e., per protocol analysis

Variables	TENS treatment group (n=22)	IV opioid treatment group (n=20)	*P*-value
VAS before treatment	5.2±1.7	4.8±1.2	0.396
VAS at leave the PACU	1.1±1.2	1.6±0.9	0.082
VAS 0 after treatment	9 (40.9)	3 (15.0)	0.091
Time in the PACU (min)	68.8±20.1	91.0±34.4	0.013

Data are presented as mean±standard deviation, except VAS 0 after treatment, which is presented as number (%).

5. Nausea and sedation

There were no significant differences with regards to sedation, nausea and use of anti-emetics between the groups (data not shown).

Discussion

The results from this randomized controlled study indicate that both treatment with high-intensity, high-frequency stimulation using TENS and pharmacological treatment with IV opioids are effective for postoperative pain relief after gynecologic hysteroscopy. This is consistent with previous studies demonstrating the effectiveness of TENS for postoperative pain relief [16,17]. However, there was no difference between the groups with regards to time spent in the PACU after surgery. When only responders to treatment were compared, both groups reported similar pain relief, but the TENS group spent a shorter time in the PACU.

If the patient responds to the TENS treatment, the patient reports instant pain relief with no need for monitoring of adverse side-effects such as sedation, nausea and respiratory depression after treatment. This is a clinically relevant advantage with the TENS treatment modality, since it only takes two minutes to assess if the patient responds to it or not. In contrast, it takes approximately 20 minutes to evaluate the effects of pharmacological treatment with IV opioid [22]. Thus, pharmacological treatment with IV opioids is delayed by at most a few minutes if the patient does not respond to TENS, making TENS a more preferable choice for initial pain treatment in the PACU.

Chronic pain after surgery in the lower part of the abdomen is a frequent complication, with a study showing that 5–32% of the patients develop chronic pain after hysterectomy [23]. Thus, it is important to achieve optimal postoperative pain treatment to prevent development of chronic pain. More than 2/3 of the patients reported moderate or severe pain upon arrival in the PACU. Thus, obtaining adequate pain relief was difficult for many patients, resulting in a high number of crossovers in more than 40% of the patients (n=32) wherein they received both pharmacological treatment with IV opioid and TENS to obtain sufficient pain relief (VAS, <3). The crossover group did not differ from the responders to treatment with regards to pain intensity or previous chronic pain. Nevertheless, opioid consumption was significantly lower in the TENS group. Thus, using TENS as the first line of pain relief may reduce the need for postoperative opioids.

Previously, other methods for peri- and postoperative pain relief such as para-cervical blockade has been tested after hysteroscopy. However, these have limited effects on postoperative pain after uterus intervention [24]. Furthermore paracervical blockade entails a risk of inducing bradycardia and hypotension, which is likely a result of accidental intravascular injection [25]. Thus, TENS seems to be a more effective method for postoperative pain relief after hysteroscopy with the benefit of being completely reversible within seconds and with limited side effects.

The importance of properly managing postoperative pain treatment is well known partly because of the risk of developing chronic pain after surgery due to insufficient pain treatment [26]. Furthermore, it is important to reduce the suffering of patients and minimize any complications due to inadequate pain treatment, such as impaired respiration and immobilization.

In the pursuit of reducing or even replacing opioids, a less harmful alternative for the patient such as TENS might be considered a suitable choice due to the minimal frequency of side effects associated with short-term TENS treatment. Based on the results of this present study and the possible serious short- and long-term complications of opioid treatment, TENS may to preferable as a first line of pain relief in patients undergoing hysteroscopy reporting a postoperative pain VAS score of ≥3.

In the present study, high-frequency (80 Hz), high-intensity (40–60 mA) TENS stimulation for 60 seconds was used, and if needed, was repeated once. This is the same treatment model used for primary dysmenorrhea, surgical abortion, gynecological laparoscopy and angina pectoris [13,16,17,27]. It is important to achieve a strong stimulation level (at least 40 mA). By gradually increasing stimulation intensity, most patients tolerated the treatment, which is in line with previous studies [16,17]. Stimulation intensity seems to be crucial for the pain-relieving effect. Results from a study by Moran et al. [28] indicate that strong non-painful intensities seem more efficient than mild intensity stimulation. Furthermore, for optimum pain relief, it seems important to adjust the position of the electrodes so that the paresthesia over the abdomen covers the area in which the patient localized the abdominal pain. Before the start of the treatment, skin sensation should be assessed, since TENS should not be used if there

is impaired sensation. There were no reported side effects of TENS treatment in the study except for intolerable intensities of stimulation for some patients. However, the stimulation lasted for only 60 seconds and all the women in the TENS group were informed that this short period of (uncomfortable) high-intensity TENS was needed to obtain postoperative pain relief. To acclimatize the patients, the stimulation intensity was increased gradually to obtain a high-intensity stimulation level. An additional advantage of TENS treatment is that there is no need to monitor the patients after the treatment. This is in contrast to pharmacological treatment with IV opioids, wherein patients need to be assessed due to the risk of respiratory depression associated with opioids. The clinical routine for TENS treatment at our center has been previously described in detail [16,17].

In addition to the pain-relieving effect of TENS and opioids, the cost of treatment needs to be considered. The TENS device used in the present study costs 121 Euros. At our center, costs for consumable materials, i.e., the reusable electrodes and electrode gel, amounted to less than approximately 20 Euros for the study patients, i.e., 0.37 Euro per patient. In comparison, one ampoule of 10 milligrams per milliliter morphine costs 0.31 Euros. In the present study, 1 to 2 ampoules of morphine were used per patient. However, the TENS device can be used for several daily treatments for up to 10 years. Furthermore, assistant nurses can administrate the TENS treatment, whereas morphine can only be administrated by nurses (or physicians) and the patient needs to be monitored for at least 30 minutes due to side-effects, especially respiratory depression. Hence, the nurses' competence and time can be used for other specific tasks when using TENS for postoperative pain treatment. Furthermore, if the patient responded to TENS treatment, their time spent in the PACU was reduced by 22 minutes, which has an impact on the overall cost for the stay in PACU and which enables them to perform more operations per day.

First, the surgical indication for hysteroscopy affects the time and difficulty of the surgery as well as the quantity of distending media, among others, which may in turn affect postoperative pain. In the present study, there was no difference with regards to indication for hysteroscopy and time of surgery between the groups. Hence, the peri-operative conditions seem to not have affected the patients' reported postoperative pain at the PACU before treatment.

Second, it was not possible to have a control group untreated for postoperative pain for ethical reasons. Hence, pharmacological treatment with IV opioids and TENS treatment were compared with each other. It would have been a good addition to have blinded the patients and investigators to their respective treatment allocations. However, due to technical and clinical logistic reasons, it was not possible to blind the investigators, and most importantly the patients, to the treatment allocation. This might have affected the outcome. Nevertheless, it should be emphasized that the patients reported their own experiences pain intensities using the VAS scale (i.e., the nurse did not estimate or influence the patient's report).

Third, the large number of crossovers might have affected the results.

Fourth, according to the power calculation, 100 patients should be included to demonstrate a possible statistical difference between the groups. However, due to reorganization in the hospital resulting in allocation of the patient groups to a different hospital, only 79 patients were randomized for the present study.

In general, results from clinical trials will be stronger with the same results from other independent groups. Hence, further randomized controlled studies are warranted to confirm the pain-relieving effect of TENS on postoperative pain and its effects on opioid consumption.

TENS and short-term pharmacological treatment with IV opioids are both effective treatments for pain relief after gynecologic hysteroscopy, with more than one third of patients reporting complete pain relief. However, many patients seem to need a combination of both treatments to obtain adequate pain relief. Thus, further studies are warranted to further characterize this group of patients.

TENS may be preferable as a first line of treatment since it is associated with a shorter time spent in the PACU and a faster onset of pain relief if the patient responds to the treatment compared to IV opioids. In addition, the effects of TENS treatment can be assessed in a few minutes, which allows for a prompt shift to pharmacological treatment with IV opioids if the patient does not respond to TENS treatment. Thus, TENS as a first-line pain relief method is an option to reduce the need for postoperative opioids.

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Conflict of interest

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

Ethical approval

The study is in accordance with the Helsinki Declaration of 1975 as revised in 2000. The Regional Ethics Review Board in Gothenburg, Sweden approved the study (Dnr 442-13).

Patient consent

All participants received written and verbal information regarding the study before giving their written consents to participate in the study.

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Case Report

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Obstetrics & Gynecology Science

An extremely rare elderly case of proximal epithelioid sarcoma of the vulva: case report with a review of literatures

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We experienced an extremely rare case of proximal epithelioid sarcoma (PES) of the vulva in a 77-year-old woman. After history taking and physical examination, the patient was tentatively diagnosed as having Bartholin's cyst in the right labium. Based on histopathological and immunohistochemical (IHC) findings, however, a final diagnosis of PES of the vulva was made. After receiving CyberKnife treatment, the patient survived but with recurrent episodes and poor prognosis. In conclusion, our case indicates that patients with PES of the vulva should be appropriately managed with radiotherapy after a differential diagnosis based on histopathological and IHC findings.

Keywords: Vulva; Vulvar neoplasms; Sarcoma; Immunohistochemistry; Diagnosis, differential

Introduction

Vulvar cancer is the fourth most common gynecologic malignancy, accounting for 5% of all cancers of the female genital tract [1,2]. Of the diverse histological types of vulvar cancer, squamous cell carcinoma is the most common (95%), followed by melanoma, sarcoma, and basalioma [3]. The incidence of primary sarcoma of the vulva is estimated to be approximately 1.5–5% of all vulvar malignancies. Primary sarcoma of the vulva frequently affects the labia majora, Bartholin gland, clitoris, and labium minus [4].

Epithelioid sarcoma (ES) is a malignant soft tissue tumor that can be classified into proximal and distal types. Proximal PES occurs in the trunk and pubic regions, and distal PES occurs in the upper and lower limbs. Of the cases, proximal epithelioid sarcoma (PES) of the vulva is an extremely rare entity, and its occurrence has been described in a paucity of literature [5-7].

We experienced an extremely rare case of PES of the vulva in a 77-year-old woman. Herein, we report our case with a literature review.

Case report

In July 2016, a 77-year-old woman visited our clinic with a chief complaint of asymptomatic mass over the right vulva. On history taking, the patient had no underlying diseases other than hypertension and diabetes mellitus. She had an obstetrical history of 6-0-8(0/8)-4(3/1). Grossly, she had a mass measuring 3×2 cm in size on the skin over the right lower labia, which was suggestive of Bartholin's cyst in the

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right labium (Fig. 1A). Therefore, she underwent a Bartholin gland cystectomy. An intraoperative frozen biopsy showed positive findings of malignancy. Moreover, histopathological examinations revealed a poorly differentiated malignant neoplasm with a plump cytoplasm and eccentric nuclei, which was suggestive of PES of the vulva (Fig. 1B). In addition, immunohistochemistry (IHC) was positive for CD34, pan-cytokeratin (CK-PAN), and vimentin (VT; Fig. 1C).

After the operation, the patient had an enlargement of the right external iliac and inguinal lymph nodes on magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT). In August 2016, she underwent a reoperation for excision of the right inguinal lymph node and a laparoscopic excision of the right pelvic lymph node. An intraoperative frozen biopsy confirmed the malignancy in the right pelvic and inguinal lymph nodes. On the basis of the histopathological examination finding, the patient was diagnosed as having a proximal metastatic ES of the vulva. Of the seven lymph nodes, two were involved in the metastasis.

After the postoperative recovery period between September 23 and October 31, 2016, the patient received radiotherapy at a dose of 46.8 Gy/26 Fx to the vulva and pelvic and inguinal lymph nodes. During the course of the radiotherapy, she developed severe skin desquamation. Thus, she completed only 26 of the 28 treatment cycles of radiotherapy. In August 2017, she had a recurrence with a bone metastasis on her chest CT scans at a follow-up visit. Therefore, she underwent further examination with MRI and PET/CT, which revealed multiple bone metastases (Fig. 2, upper panel). Therefore, she additionally received palliative radiotherapy to the C2 spine at a dose of 30 Gy/4 Fx and C5-T2 spine at a dose of 30 Gy/10Fx between September 18 and 29, 2017.

In December 2017, the multiple bone metastases showed increases in size and number on the follow-up MRI scans. Therefore, the patient received CyberKnife treatment to the T8 and T12 spinal cord segments at doses of 36 Gy/4 Fx and 36 Gy/4 Fx, respectively, between January 9 and 18,

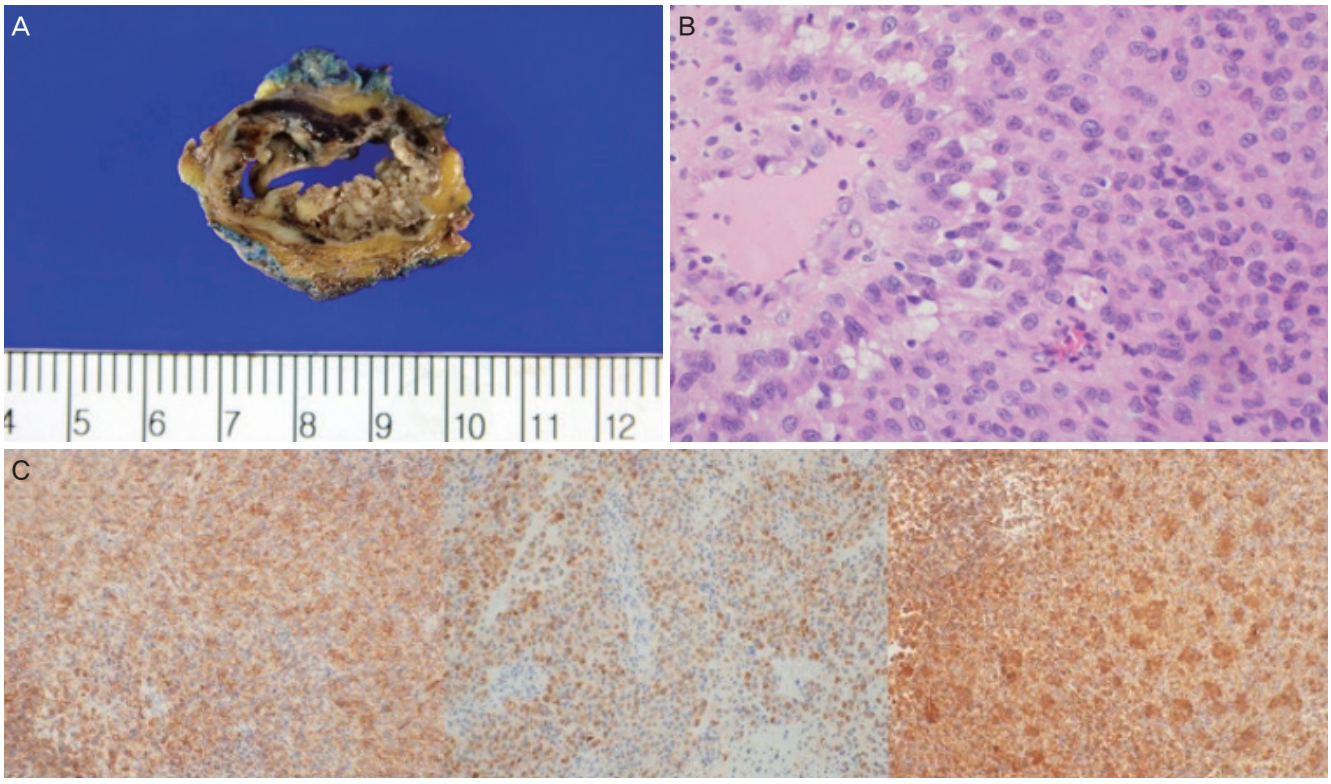

Fig. 1. Gross, histopathological, and immunohistochemical findings. (A) Shown is the gross finding of a mass of 3×2 cm in size in the skin over the right lower labia, which is suggestive of Bartholin's cyst in the right labium. (B) The histopathological examination result shows a poorly differentiated malignant neoplasm with a plump cytoplasm and eccentric nuclei. (C) From left to right are the positive immunohistochemical findings for CD34, pan-cytokeratin, and vimentin.

2018. Thereafter, the patient was followed up using imaging modalities at 3-month intervals. Eight months later, she underwent abdominal and pelvic CT (APCT) and chest CT, which revealed findings suggestive of multiple peritoneal carcinomatosis (Fig. 2, middle panel). In addition, she presented with lymphadenopathy and caval lymph node metastasis (Fig. 2, lower panel). Therefore, she received CyberKnife treatment to the pericaval lymph node at a dose of 4,800 Gy/4 Fx between October 10 and 26, 2018. Meanwhile, she complained of dyspnea, for which she underwent chest posteroanterior radiography, which revealed a small amount of pleural effusion in both lungs. The fluid cytology findings were negative for malignancy, so the patient received only conservative management with chest tube insertion for chest pleural effusion drainage. Subsequently, on APCT and chest CT, the peritoneal carcinomatosis and lymphadenopathy had aggravated, accompanied by a new bone metastasis. The patient was therefore recommended to undergo chemotherapy, but she and her caregivers refused the treatment because of her old age and poor systemic condition. For >3 years after disease onset, she survived while receiving supportive care. The patient was followed up until May 23, 2019.

Discussion

ES is a soft tissue malignancy. The first case of PES of the vulva was described by Pier et al. in 1972 [8]. It often affects the labia majora of young women, and its differential diagnoses include Bartholin's cyst, lipoma, and genital warts [7,9]. Its benign appearance as an asymptomatic subcutaneous nodule poses diagnostic and therapeutic dilemmas for clinicians [8-10]. On histopathological examination, PES of the vulva can be observed as plump spindle cells or large polygonal cells with a deeply acidophilic cytoplasm, which resemble epithelioid or squamous cells. Therefore, it can be diagnosed using IHC [6]. Our case showed high IHC positivity rates for CD34, CK-PAN, and VT, which is in agreement with the results of the previous studies in this series [11,12].

No treatment guidelines have been established for PES of the vulva [5]. However, surgical excision with a wide margin of >2 cm remains the mainstay of treatment. A strong corre-

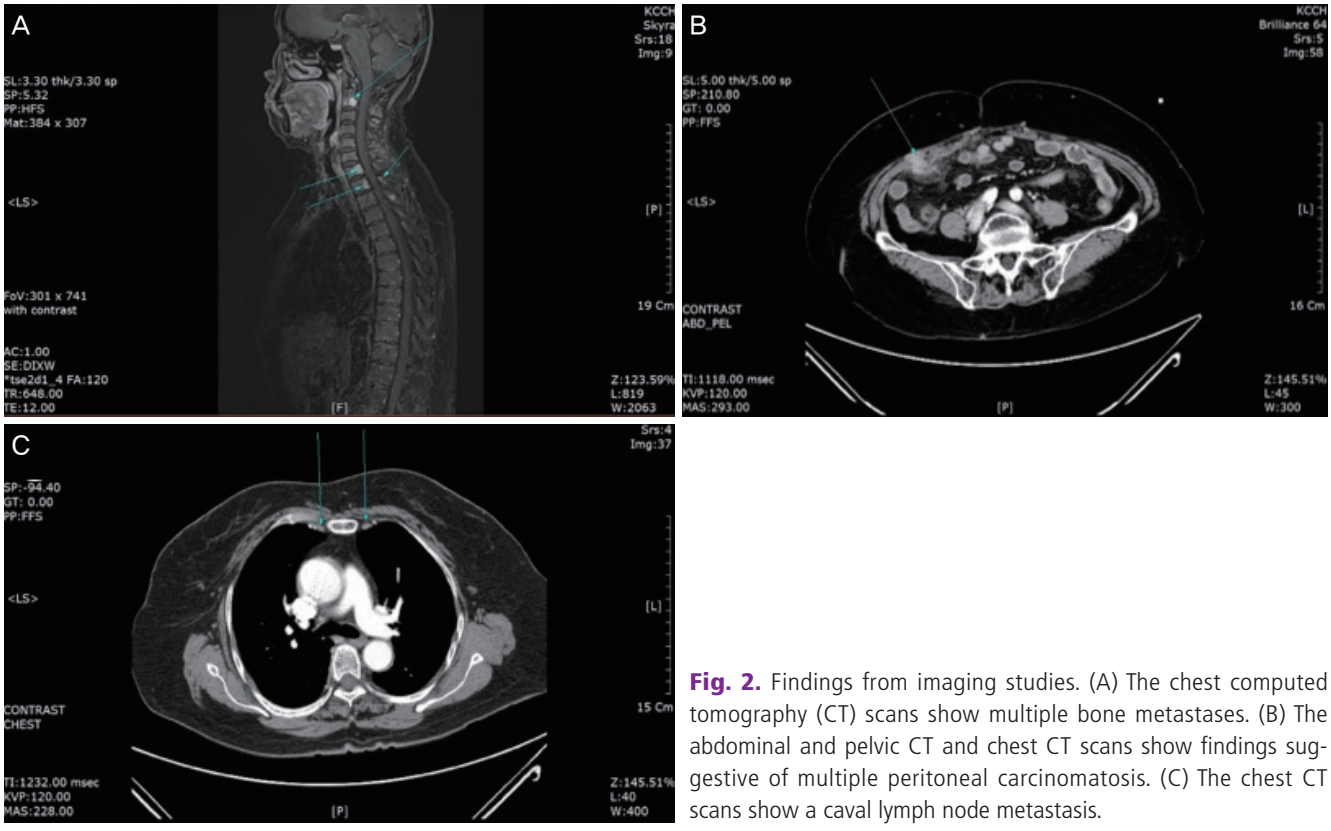

Fig. 2. Findings from imaging studies. (A) The chest computed tomography (CT) scans show multiple bone metastases. (B) The abdominal and pelvic CT and chest CT scans show findings suggestive of multiple peritoneal carcinomatosis. (C) The chest CT scans show a caval lymph node metastasis.

lation was found between inadequate margins and increased risk of local recurrence. Poor prognosis is associated with the presence of lymphatic metastasis. Adjuvant radiotherapy is recommended for high-grade tumors or cases with inadequate surgical margins [13]. We did not perform chemotherapy for our case, in accordance with previous studies that showed that the treatment effects of postoperative chemotherapy are still controversial [7,11-14]. Argenta et al. [13] performed adjuvant chemotherapy for 7 of 31 patients with vulva ES or malignant rhabdoid tumor and reported that of the 7 patients, 3 were disease-free at 8, 11, and 21 months but the remaining 4 died of disseminated disease within 8 months of diagnosis.

PES of the vulva is an extremely rare entity, with only 29 cases described in the English literature. According to the previous studies in this series, the median age of onset was 36.4 years; most of the cases occurred during the early postmenopausal period except, while some cases occurred in young women [7].

In the present case, active radiotherapy using the CyberKnife treatment for the metastatic site was effective in prolonging survival. A poor prognosis is indicated in PES of the vulva. Patients with PES of the vulva are vulnerable to local recurrence despite the presence of negative surgical margins, and up to 60% of them experience a distant metastasis [15-18]. According to Hasegawa et al. [16], the tumor-related mortality rate was 65% in 20 cases of PES of the vulva, which was lower than the metastasis rate (75%). Moreover, Ulbright et al. [19] reported 100% mortality due to distant metastasis in patients with local recurrence.

In conclusion, our case indicates that patients with PES of the vulva should be appropriately managed with radiotherapy after a differential diagnosis on the basis of histopathological and IHC findings.

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 the Korea Institute of Radiological and Medical Sciences (KIRAMS 2020-02-006) and conducted in accordance with the principles of the Declaration of Helsinki.

Patient consent

The institutional review board waived the need for patient 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

Complications associated with intravesical migration of an intrauterine device

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The intrauterine device (IUD) is the most common method of reversible contraception in women. However, IUD can perforate the uterus and also migrate into pelvic or abdominal organs. A 43-year-old woman with a 5-year history of IUD placement and without specific symptoms, decided to remove her IUD and undergo tubal ligation. Radiological assessment, including a pelvic X-ray and ultrasonography, revealed no copper IUD within the uterus. Retrieval attempts with cystoscopy were unsuccessful. The IUD was found embedded in the fundal part of the bladder wall and was subsequently removed through a laparotomy incision. Although there are cases in the literature that were successfully managed with cystoscopy, in chronic cases, the formation of granulation tissue may preclude retrieval of an IUD using this intervention.

Keywords: Intrauterine devices; Contraception; Migration; Bladder

Introduction

Intrauterine devices (IUDs) are effective, safe, and widely used birth control methods, accounting for 16.5% of birth control used in undeveloped countries and 9.4% of birth control used in developed countries [1]. The incidence of uterine perforation by IUD is reported to be between 1.3 and 1.6 per 1,000 insertions [2], indicating perforation is a relatively infrequent but potentially serious complication. Perforations may occur either immediately, by improper insertion, or years after insertion by device migration. We report a case of an IUD that penetrated the bladder wall and became symptomatic 5 years after insertion.

The IUD is used by more than 150 million women around the world, making it the most widely used reversible method of contraception [1]. Although IUDs are commonly considered to be safe, it also has some serious complications. Uterine perforation due to an IUD is seen in 0.05 to 13 cases out of 1,000 IUD placements [2]. Following the uterine rupture, an IUD may potentially migrate to the pelvic or intra-abdominal cavity, causing several complications. A literature review of the 18 years until 1999 showed 165 reported cases of migrated IUD, which shows that migration to the bladder is uncommon and has been reported in only 31 cases [3]. The United Kingdom Selected Practice Recommendations recommends a follow-up visit after the first menses, or 3–6 weeks after insertion, to exclude infection, perforation, or expulsion [4].

Case report

A 43-year-old woman—gravid 7, live 7—was referred to the Jahrom University of Medical Science Gynecologic Clinic with complaints of unspecified lower abdominal pain and dysuria. These symptoms had persisted for three months, despite repeated treatments for urinary tract infections by several gynecologists. She had a history of a copper-T IUD insertion 5 years prior to presentation. The patient's documents were

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reviewed, and included tubal ligation consent, pelvic ultrasound, and pelvic X-ray. On pelvic X-ray, her IUD appeared to be upside down (in the reverse position). Further, in a report of an ultrasound performed two months before surgery, the radiologist noted that the IUD was unable to be visualized in the endometrial cavity. There was a linear echogenic structure in the myometrium of the anterior fundal part of the bladder, measuring about 22×2 mm, which might have been a migrated IUD. A T-shape IUD was inserted by a midwife in the patient's village 5 years ago, but the patient did not present for routine follow-ups after insertion prior to her decision to pursue tubal ligation. During the years following insertion, she was asymptomatic; however, for the past 2 to 3 months she experienced pyuria and leukocytes in her urine, though all urine cultures were normal during this period.

During an abdominal-pelvic examination, mild tenderness was found in the suprapubic area during deep palpation. The IUD threads were visible during inspection of the vaginal canal, so the gynecologist tried to remove the IUD but was unsuccessful. The patient opted to have the IUD removed in the operating room, followed by tubal ligation. Preoperative laboratory findings were normal with the exception of urinalysis, which showed pyuria and leukocytes. Her urine culture was normal, so she started broad-spectrum antibiotics and was transferred to the operating room. During laparotomy, the gynecologist observed severe adhesions between the large intestine, the posterior part of the fundal uterus, and bladder. Tubal ligation was done despite difficulty visualizing both tubes due to adhesions and the abdominal wall was closed. Then, the gynecologist tried to remove the IUD through the vaginal canal, but was unsuccessful. The gynecologist then consulted with an expert professor of gynecology who tried to remove the IUD through the vaginal canal but was unsuccessful. The bulging point of the left anterior vesical wall was visible and was ultimately determined to be the ramus of the IUD following uterine perforation. An immediate consultation was made with a urologist. The IUD and the stones caused by the IUD were visualized by cystoscopy; however, these were not able to be removed through cystoscopy.

The IUD and stones were ultimately removed by the urologist through an incision on the anterior vesical wall. After IUD removal and bladder repair, a posterior uterine wall perforation was repaired. This perforation was 1×2 cm in size and was likely caused by the failed attempts to remove the

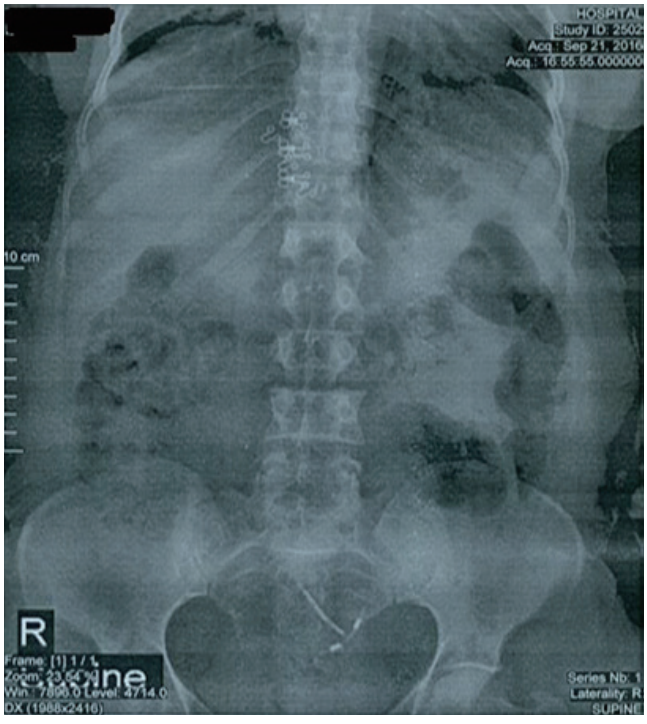

Fig. 1. Migrated intrauterine device and stone seen on abdominal plain X-ray. In abdominal plain X-ray, intrauterine device (IUD) looks upside down (in reverse position) in uterine cavity, it's also left leaning instead of longitudinal position along the middle line of the uterus. A few stones has been formed on the right branch of the IUD which shows passage of the time.

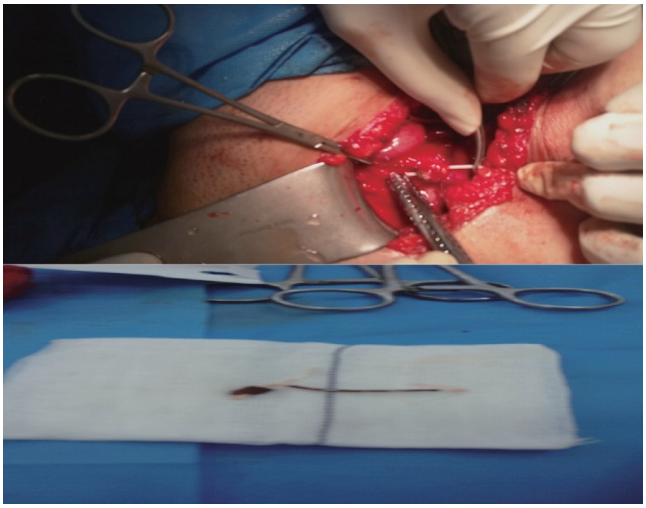

Fig. 2. Removal of the intrauterine device (IUD) during cystostomy. During laparotomy, left branch of the IUD was seen as a bulging point in the left side of fundus of bladder, so through a transverse incision on the bladder and despite the severe adhesion, the IUD was removed and the incision site was repaired.

IUD through the vaginal canal.

The patient was discharged on hospital day seven and experienced an uneventful postoperative period (Fig. 1).

A plain frontal supine abdominal X-ray showed a T-shaped IUD on the left side of the pelvis with a radio-opaque stone (Fig. 2).

Vaginal ultrasound showed a linear echogenic structure in the myometrium of the anterior fundal area of the bladder, measuring about 22×2 mm, which may have been the migrated IUD.

Discussion

The IUD is a popular contraceptive method. Although IUDs are commonly considered safe, they are occasionally associated with serious complications such as pelvic pain, bleeding, spotting, increased risk of pelvic inflammatory disease, and unexpected pregnancies [3]. Uterine perforation is an uncommon IUD complication. Known risk factors for uterine perforation include inadequate training of family planning providers, insertion during the early puerperal period when the uterus is soft and bulky, past history of perforation, and an anatomically highly flexed uterus [5]. The overall reported incidence of IUD perforation is about 0.87 per 1,000 insertions [6]. IUD migration into the peritoneal cavity and uterine structures is another rare complication of this contraception method.

Because of the rarity of bladder perforation by an IUD, it may be misdiagnosed. While some patients experience hematuria, lower abdominal pain, and irritative urinary symptoms, others may experienced a mild complications. Complete migration of an IUD into the bladder cavity can also lead to stone formation. To date, half of the cases with IUD migration to the bladder presented with stones that varied in size from 1–10 cm. Foreign bodies in the bladder cavity may act as a nidus for stone formation, and infections may also serve as predisposing factors. The presence of urinary tract symptoms and a history of IUD insertion with failure to locate the threads may indicate device migration [7]. The present case presented with repeated episodes of cystitis, which were cured following administration of antispasmodic and antibiotic therapies.

To determine the location of the migrated IUD, different imaging modalities have been used. The transvaginal and transabdominal-ultrasonography approaches are useful methods for detecting IUD migration [8]. Abdominal X-ray is the preliminary modality for investigating IUD migration, especially for the detection of stones caused by IUDs. In some cases, computed tomography is needed for diagnosis [7]. Cystoscopy is another means of visualizing the intravesical IUD and may assist with removal [9]. The accepted treatment for IUD-associated perforations is abdominal surgery. Initially, this was accomplished via laparotomy; however, as surgical techniques have developed, laparoscopy is often used [10].

It is not known when the IUD migrated to the bladder: during insertion, during intercourse, due to hard work, or because of unknown causes. So, for earlier detection of IUD migration and preventing its complications, regular follow-ups are highly recommended. The cause of IUD migration in this patient was unknown and she has not returned for follow-up.

For women with IUDs who have bladder stones and recurrent urinary tract infections, migration of the IUD into the bladder must be considered as a differential diagnosis. Furthermore, a simple abdominal X-ray and—if needed—cystoscopy can be very useful imaging modalities for patients who complain of unexplained urinary symptoms or pelvic pain.

We would like to address some important points about IUD migration which were ignored during the treatment of our case. Even when the IUD threads are visible in the vagina, removal may not be easy. Consequently, radiologic images are essential. This is particularly true when the IUD is not visible in the uterine cavity on ultrasonography reports. Another important point is that attempts to remove an IUD during laparotomy should occur prior to closing the abdomen wall to prevent another laparotomy.

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Conflict of interest

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

Ethical approval

We followed all the ethical guidelines according to the protocol of the ethics committee. Process of the study was completely explained to the patient and a written consent was obtained before beginning the study but we do not have any ethical code due to the type of study (case report) .The consent form of the patient is attached.

Patient consent

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

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Video Article

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Obstetrics & Gynecology Science

Minimally invasive search for a missing vibrator

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Objective

To report a unique surgical procedure that was utilized to locate a missing vibrator in the pelvis of a patient. Emergency room admissions and surgery secondary to the malfunctioning of devices intended for sexual stimulation are extremely common. Emergency room staff of hospitals in the United States usually are skilled in the detection and removal of these devices. Occasionally, surgical intervention is warranted if the device enters a cavity that cannot safely be explored in the emergency room setting. We report a case of a vibrator that was lost during sexual activity. A flat plate X-ray showed it to be in the abdominal cavity. Careful questioning of the patient revealed that the device had an unusually small diameter. Surgical intervention showed that the device ultimately ended up in the bladder without causing traumatic injury.

Methods

We created a narrated video to demonstrate the surgical procedure (Canadian Task Force Classification III).

Results

Laparoscopy and cystoscopy were used to visualize and successfully remove the device. The patient recovered uneventfully.

Conclusion

Following laparoscopic confirmation of the location of the device, it was removed via cystoscopy. This case demonstrates how background information, such as the size of the missing device in this case, can be critical to providing high quality patient care.

Keywords: Cystoscopy; Laparoscopy; Foreign bodies; Vibrator

In the United States, injuries from the use of sex-related instruments are a very common reason for emergency room visits [1,2]. Insertion of the instrument and its penetration through the vaginal wall regularly result in the object penetrating the abdominal cavity [3,4], leading to possible bowel damage. It is rare for large objects to enter and become trapped in the bladder [5].

A 29-year-old G1 Po Ab1 Caucasian woman visited the emergency room at about 1 A.M. and reported that her vibrator had become lost during sexual activity. The patient said that her partner suddenly started vaginal intercourse after using the vibrator to stimulate the clitoris directly. The patient was uncertain about the location of the vibrator and

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felt discomfort for a while. She thought the vibrator was in the vagina as sexual intercourse progressed because she could still feel the vibration. After intercourse, the patient could not find the vibrator in the vagina but still felt the sensation of vibration within the pelvis. When the patient was unable to find the vibrator, she presented to the emergency room. She stated that the vibration lasted about 30 minutes, approximately the battery life of the device. An X-ray of the pelvis showed that the vibrator was approximately at the level of the intrauterine device of the patient within pelvis and in the horizontal position.

Careful questioning of the patient revealed that the vibrator was an unusual type called Vesper™ (Crave Corp., Bloomington, MN, USA), which has an unusually small diameter of approximately 1.2 cm. The device is approximately 10 cm long and on a chain to be worn like a necklace, maximizing the ease of use of the vibrator. The chain can be removed to use the vibrator for sexual activity. The patient confirmed that she removed the chain from the device before use.

Repeated vaginal and rectal examinations by the emergency room staff and gynecologists did not show evidence of the device in either the vagina or the rectum, so it was assumed that the device passed through the vaginal wall and entered the abdominal cavity. The patient had a body mass index of 22.5, so it was believed that her weight did not affect the examinations. It was assumed that the colon had not ruptured because of the limited softness found on examination. However, before laparoscopic exploration, the patient agreed to undergo a colostomy and repair of the colon if found necessary. Laparoscopic exploration showed that the device was in the bladder. Its location was also demonstrated by moving a sponge stick placed in the vagina and by gently manipulating the poly bulb. The vibrator was visualized and removed with a cystoscope.

To safely remove the vibrator through the urethra, it was necessary to fill the bladder with about 1 L of normal saline to change the orientation of the vibrator from horizontal to vertical, resulting in no morbidity from removal. The patient recovered without incident immediately after removal of the foreign body and was discharged from the hospital.

To provide the highest level of care when treating a patient with a foreign body, it is important to understand, as much as possible, the object in question [6-8]. Most gynecologists are familiar with sex-related injuries and associated morbidities. In situations similar to that of our patient, most gynecologists generally assume that the vibrator will be too large to fit through the urethra [9-12]. In our case, the vibrator was measured to have an maximum diameter of about 1.2 cm, which is about the same as the diameter of a 36 French catheter. Thus, the device was able to enter the urethra and occupy the bladder without damaging it [13,14].

Another interesting aspect of this case was that the device was assumed to be in the pelvic cavity because it appeared on the X-ray to be approximately at the level of the intrauterine device. Retrospectively, we believe that if a computed tomography scan had been performed or a lateral X-ray taken, the radiologist might have been able to accurately locate the device. Thus, the patient could have been spared the laparoscopy because the device could have been removed with a simple cystoscopy.

Conflict of interest

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

Ethical approval

The Institutional Review Board (IRB) committee at the Marchand Institute for Minimally Invasive Surgery reviewed this study and determined that it was exempt from IRB approval.

Video clip

Video can be found with this article online at https://doi.org/10.5468/ogs.20121.

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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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- Korean Society of Maternal Fetal Medicine
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Readership

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:

- Researchers can get the cases for research projects and rationale of their researches;
- 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.

Editorial Office

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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.

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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).

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Evidence-Based Medicine

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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

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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.

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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

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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.

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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.

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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.
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- 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).
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Obstetrics & Gynecology Science

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Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

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All necessary files have been uploaded, and contain:

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Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'.
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- 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.

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Obstetrics & Gynecology Science

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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.

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Use more forms if needed to add names.

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