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LETTER FROM THE PRESIDENT

Dear TJOD (Turkish Society of Gynecology and Obstetrics) family

As the Turkish Society of Gynecology and Obstetrics, we are delighted to meet you in 2024 through the TJOG family, a scientific platform that has achieved significant success. The most unique and scientifically valuable publications in Obstetrics and Gynecology are carefully reviewed and presented to you, our esteemed physicians. In this issue, we have eight research articles with high scientific content.

Distinguished members, as it is known, we will hold the Turkish Gynecology and Obstetrics Congress in 2024, a tradition that continues every year. We have been preparing for a long time for our National Congress to be held in Cyprus between May 15-19, 2024. I would like to invite all my colleagues to our congress, one of the most well-attended congresses in Turkey, in the atmosphere of a scientific feast.

I present my best greetings and regards, wishing to see you at our 21st National Congress.

Bulent Tiras, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We welcome you back in 2024 with the first issue of the year. I am grateful to our referees and editorial board for their hard work on our March issue. In this issue, our publications are mainly on reproductive endocrinology and oncology. I believe that the effect of intrauterine insemination on endometrial damage and the evaluation of endometrial receptivity in recurrent pregnancy loss will be of interest to you. I also believe that our novel article on the prediction of lymphovascular invasion in early-stage endometrial cancer in oncology will be enthusiastically read.

I would also like to state that we have been working intensively to enhance the international prestige of our journal. Since the publication of our June 2024 issue, we have been planning to apply for inclusion in the DOAJ index, a prestigious and reputable index. We have organized and developed our entire infrastructure for this matter.

We are confident that the March issue will be read with great enthusiasm by esteemed obstetricians and gynecologists, and we have already started working on the June issue. With respects and regards.

Ercan Yilmaz, Prof. MD
Fatih Sendag, Prof. MD

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2024;21:1-6

Effect of endometrial injury on pregnancy outcomes in infertile women undergoing intrauterine insemination

İntrauterin inseminasyon uygulanan infertil kadınlarda endometrial hasarın gebelik sonuçlarına etkisi

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Abstract

Objective: One of the most common treatments for infertility is intrauterine insemination (II). The objective of this study was to determine the effect of endometrial injury on pregnancy outcomes in unexplained infertility in women undergoing II.

Materials and Methods: In this randomized clinical trial, 122 women with unexplained infertility who were referred to Shariati Hospital from 2018 to 2020 were enrolled. They underwent ovulation induction using letrozole and gonadotropins. On day 9 of stimulation, they were randomly assigned to two similar groups of the same size. The first group underwent endometrial local injury by pipelle endometrial sampling, and the second group (control group) received no intervention. Only 1 II cycle was performed for each patient. Patients with negative pregnancy outcomes were followed up for 3 months. Endometrial thickness, dominant follicle count, chemical and clinical pregnancy rate, miscarriage rate, and spontaneous pregnancy rate after the II cycle were compared between the two groups.

Results: Endometrial thickness, dominant follicle count, chemical and clinical pregnancy rate, and miscarriage rate in the same II cycle were not different between the two groups (p>0.05). However, the spontaneous pregnancy rate after the II cycle was significantly higher in the endometrial injury group (p=0.02).

Conclusion: Endometrial injury increases pregnancy rates in later cycles but not in the same II cycle.

Keywords: Endometrium, infertility, intrauterine insemination, pregnancy outcome

Öz

Amaç: İnfertilitenin en yaygın tedavi yöntemlerinden biri intrauterin inseminasyondur (İİ). Bu çalışmanın amacı, İİ uygulanan kadınlarda açıklanamayan infertilitede endometrial hasarın gebelik sonuçlarına etkisini belirlemektir.

Gereç ve Yöntemler: Bu randomize klinik çalışmaya, 2018'den 2020'ye kadar Shariati Hastanesi'ne sevk edilen, açıklanamayan infertilitesi olan 122 kadın dahil edildi. Letrozol ve gonadotropinler kullanılarak ovülasyon indüksiyonu uygulandı. Hastalar stimülasyonun 9. gününde, aynı büyüklükteki iki benzer gruba rastgele atandılar. Birinci grupta pipelle endometrial örnekleme yoluyla endometriyal lokal hasar oluşturuldu, ikinci gruba (kontrol grubu) ise herhangi bir müdahale yapılmadı. Her hastaya sadece 1 İİ siklusu uygulandı. Gebelik sonuçları olumsuz olan hastalar 3 ay süreyle takip edildi. İki grup arasında endometrial kalınlık, baskın folikül sayısı, kimyasal ve klinik gebelik oranı, düşük oranı İİ siklusu sonrası spontan gebelik oranı karşılaştırıldı.

Bulgular: Endometrial kalınlık, baskın folikül sayısı, kimyasal ve klinik gebelik oranı ve aynı İİ siklusunda düşük oranı açısından iki grup arasında fark yoktu (p>0,05). Ancak İİ siklusu sonrası spontan gebelik oranı endometriyal hasarlanma grubunda anlamlı olarak daha yüksekti (p=0,02).

Sonuç: Endometriyal hasarlanma sonraki sikluslarda gebelik oranlarını artırmaktadır ancak aynı İİ siklusunda bu durum söz konusu değildir.

Anahtar Kelimeler: Endometriyum, infertilite, intrauterin inseminasyon, gebelik sonucu

PRECIS: Endometrial injury increases spontaneous pregnancy rates after the first intrauterine insemination cycle.

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Introduction

Infertility is defined as not attaining pregnancy for a year without using contraceptive methods. Infertility treatment is performed according to the cause of infertility. Of course, sometimes the cause of infertility is unclear. One of the possible treatments is intrauterine insemination (IUI)[1,2].

IUI is a common method for achieving pregnancy in infertile women and is performed multiple times. Each IUI cycle begins with the female patient receiving letrozole and gonadotropins to stimulate ovulation. Follicular development is monitored by sonography, and human chorionic gonadotropin (hCG) is injected to induce the egg, after it reaches 18 mm in diameter. At 24-36 h after hCG injection, semen is placed into the uterus with an intrauterine catheter. If the first IUI cycle is not successful, another cycle is repeated after 1 month. The number of cycles is usually 3 to 6. The IUI success rate is 15-17% per cycle, which increases with each repetitive cycle[1,2].

For patients who could not get pregnant with IUI treatment, in vitro fertilization (IVF) or microinjection is considered to be the next strategy, which is costly and has more complications. Therefore, increasing the success of IUI can be effective in reducing the cost and infertility problems in infertile couples[1,2].

In previous studies, the effect of endometrial injury on different methods of treatment has been examined. Some studies have shown that the use of endometrial injury increases pregnancy rates; however, other studies did not show an increase in pregnancy rates. Our focus in this study is the effect of endometrial scraping on IUI, which requires more research according to prior studies. The goal of this study was to examine the effect of local endometrial injury by pipelle endometrial sampling on pregnancy rates in infertile women who received IUI. This study helps increase the effectiveness of IUI; therefore, the use of costly and time-consuming methods, such as IVF, is reduced.

Materials and Methods

This study is a prospective randomized clinical trial and was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.REC.1394.1569). This study was registered in the Iranian Registry of Clinical Trials (no: IRCT20160224026750N2). All participants provided informed consent before entering the study. Each patient was assigned a specific code to keep the data private and the patient's information confidential. Allocation concealment was performed accordingly, and all the stages of this study complied with the Declaration of Helsinki.

Study samples were infertile women aged 18-35 years undergoing IUI at Shariati Hospital from 2018 to 2020. Medical information was collected through interviews, examinations, and test results. Inclusion criteria were unexplained infertility, infertility duration of 2 to 6 years, body mass index (BMI) of 30 kg/m^2, regular menses, normal hysterosalpingography, normal pap smear, normal uterine cavity, follicle stimulating hormone (FSH) level of 10 mIU/mL on the 3rd day of the cycle, normal thyroid-stimulating hormone and prolactin levels, and anti-Mullerian hormone level of more than 1 µg/L.

Exclusion criteria were age over 35 years, uterine myoma, ovarian cyst, uterine cavity abnormalities, endometrial polyp, blockage of fallopian tube, infertility due to male factor, receiving other treatments other than IUI in the last 3 months, and history of diabetes and heart or pulmonary disease.

All male individuals had normal parameters for semen analyses (sperm concentration of more than 15x10^6 per mL, normal morphology of more than 4%, and progressive motility of more than 32%) based on the World Health Organization[3].

Sample size analysis was performed based on Abdelhamid[4] (α=0.05, β=0.2). The sample size was measured to be 122, and they all met the criteria. None of the patients dropped out for any reason. Transvaginal ultrasound was performed on the 3rd day of menstruation for all women. For ovarian stimulation, letrozole oral intake (Iranian hormone, Iran, 2.5 mg per day) was started on the 3rd day of the cycle for 5 days for all patients. In addition, recombinant FSH (Gonal-F, Merck Serno, Switzerland, 75 IU) was injected on the 4th and 6th day of the menstrual cycle for all patients.

Endometrial thickness and follicular development were monitored using transvaginal ultrasound on day 9 of the menstrual cycle. On the 9th day of the cycle, patients were randomly divided into two groups of 61 patients. Patients in the first group underwent local endometrial injury in the posterior uterine wall by pipelle endometrial sampling on day 9 of the cycle. In the second group (control group), no intervention was performed.

When at least one follicle reached 18 mm in each patient, 5000 IU of hCG (Choragon, Ferring, Swiss) was injected for ovulation induction in all patients. After 24 h, 0.5 mL of prepared sperm was placed into the uterus using an intrauterine catheter for all patients. After IUI, all patients took 400 mg progesterone suppositories (Cyclogest, Actover, Britain) daily for 2 weeks to support the luteal phase.

Two weeks after IUI, β-hCG levels was measured to evaluate chemical pregnancy. If chemical pregnancy was positive, patients continued taking progesterone until 8th week of pregnancy. If β-hCG test was negative, patients were followed up for 3 months to determine if they naturally got pregnant or not. Clinical pregnancy test was performed 2 weeks after positive chemical pregnancy test by observing pregnancy sac in transvaginal ultrasound. In this study, the IUI cycle was performed only once for each patient.

In both groups, the patients were similar in terms of age (Figure 1), BMI, infertility duration (Figure 2), FSH in 3rd day of cycle, and polycystic ovary ($p>0.05$) and it is shown in Table 1. Data regarding the two groups, including endometrial thickness, dominant follicle count, chemical pregnancy rate, clinical pregnancy rate, spontaneous pregnancy rate, and abortion rate, were gathered and compared.

Statistical Analysis

Comparison of the groups was performed using independent sample t-test, Fisher's Exact test, and chi-square test. Data analysis was performed using IBM SPSS 13 and significance level was less than 0.05.

Results

The ovulation induction outcomes are shown in Table 2. After ovulation induction, there were no statistically significant differences in endometrial thickness (Figure 3) and the number of dominant follicles between the two groups (p>0.05).

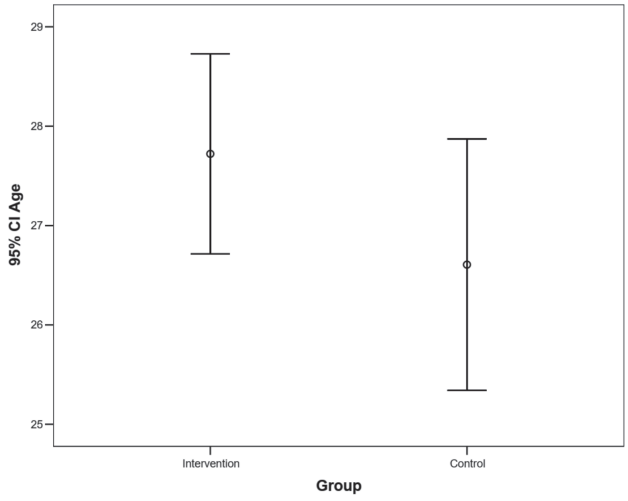

Figure 1. Age of infertile women who received IUI in two groups of endometrial injury and control

IUI: Intrauterine insemination, CI: Confidence interval

The pregnancy outcomes are shown in Table 3. The chemical pregnancy rate, clinical pregnancy rate, and abortion rate were not significantly different between the two groups (p>0.05). However, the spontaneous pregnancy rate after the IUI cycle in the endometrial injury group was significantly higher than that in the control group (p=0.02). The total pregnancy rate (clinical pregnancy plus spontaneous pregnancy) in the endometrial injury group was also significantly higher than that in the control group (p<0.01) due to higher spontaneous pregnancy rate in that group.

It is worth mentioning that multiple pregnancies did not occur in any patient.

Discussion

IUI is a non-invasive and cheap method compared with other fertility assistance methods, and it is widely used in infertile couples. The main obstacle in infertility treatments is the failure of embryo implantation during treatment cycles. The percentage of embryo implantation in these cycles is 15%. During the process of healing the endometrial injury, cytokines and growth factors are secreted from immune cells, which improve embryo implantation. Endometrial injury by biopsy increases endometrial blood flow and strengthens the endometrial immune system. Therefore, it can affect endometrial tissue thickness and future pregnancy outcomes[1,2].

Some studies suggest that the effect of endometrial injury persists for a long time, even up to 6 months[5,6]. These findings may explain the high spontaneous pregnancy rate for 3 months after endometrial injury in our study. Due to the author's personal difficulties and lack of time, we could not follow-up the patients for more than 3 months.

Table 1. Demographic characteristics of infertile women who received IUI in two groups of endometrial injury and control

	Endometrial injury group (61 women)		Control group (61 women)		p-value
	Mean	SD	Mean	SD	
Age (year)	27.72	3.93	26.61	4.94	0.17
BMI (kg/m^2)	24.17	2.97	25.15	2.69	0.06
Infertility duration (year)	2.84	2.16	3.44	2.37	0.14
Patients with polycystic ovary	7	0	8	0	0.8
FSH index in day 3 of IUI cycle (mIU/mL)	5.46	2.41	6.31	3.14	0.4

BMI: Body mass index, FSH: Follicle stimulating hormone, IUI: Intrauterine insemination, SD: Standard deviation

Table 2. Ovulation induction outcomes of infertile women who received IUI in two groups of endometrial injury and control

	Endometrial injury group (61 women)		Control group (61 women)		p-value
	Mean	SD	Mean	SD	
Endometrial thickness (mm)	7.93	1.13	7.59	1.3	0.12
Dominant follicle count	1.69	0.62	1.92	0.74	0.15

IUI: Intrauterine insemination, SD: Standard deviation

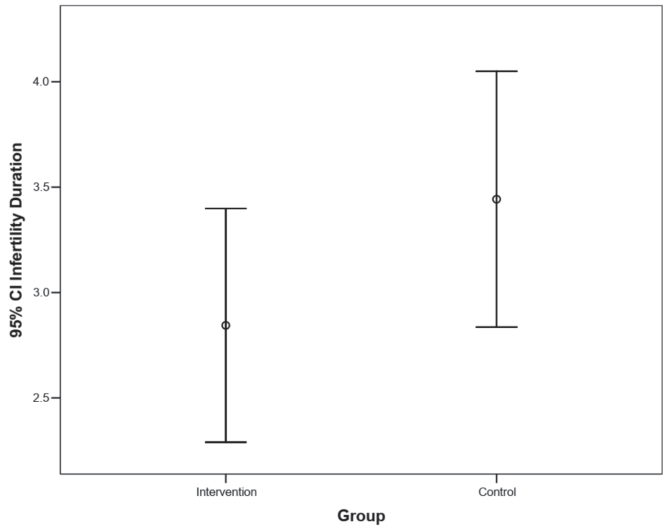

Figure 2. Infertility duration of infertile women who received IUI in two groups of endometrial injury and control

IUI: Intrauterine insemination, CI: Confidence interval

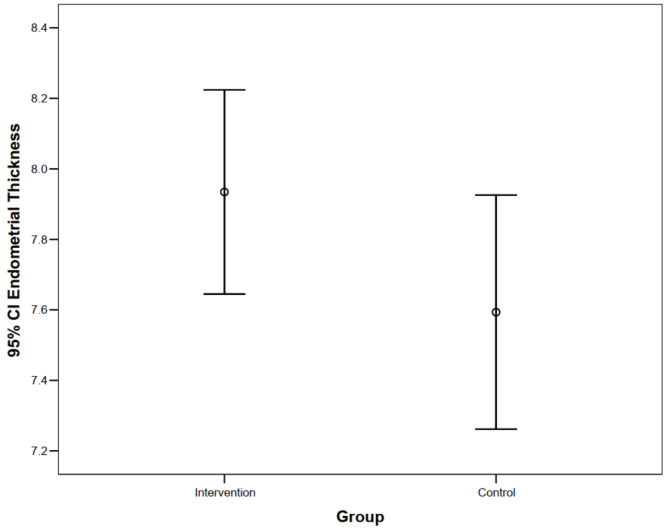

Figure 3. Endometrial thickness of infertile women who received IUI in two groups of endometrial injury and control

IUI: Intrauterine insemination, CI: Confidence interval

Many studies have evaluated the effect of local endometrial injury on pregnancy rates using different treatments. Concerning unexplained infertility, studies have shown that endometrial injury has a positive effect on pregnancy rates in unexplained infertile women[6,7].

Effect of Endometrial Injury on the Same IUI Cycle

According to previous studies by Abdelhamid[4], Bahaa Eldin et al.[8], Wadhwa et al.[9] and Gupta et al.[10] endometrial sampling performed on the same IUI cycle significantly increases pregnancy rates for that cycle. However, other studies by Maged et al.[11], Ashrafi et al.[12] and Ghuman et al.[13] did not show a significant increase in pregnancy rates. Our findings suggest that endometrial sampling during the same IUI cycle does not increase pregnancy rates during that cycle.

Effect of Endometrial Injury After the First IUI Cycle

According to a past study by Maged et al.[11] endometrial sampling performed on the first IUI cycle significantly increases pregnancy rates after the first cycle. However, other studies by Wadhwa et al.[9] and Ghuman et al.[13] showed no increase in pregnancy rates after the first cycle. Our findings suggest that endometrial sampling increases pregnancy rates after the first cycle.

Studies Conducted on Past Literature

In systematic reviews by Sar-Shalom Nahshon et al.[14] and Bui et al.[15], they concluded that concerning the effect of endometrial injury on IUI, reaching conclusions and comparing the results is difficult due to varied types of enrolled patients, type and time of intervention, and the number of IUI cycles in different studies. Almog et al.[16] conducted a literature search on endometrial injury and found that endometrial injury has a positive impact on implantation and improves the pregnancy rate. However, more research needs to be conducted on patient selection, timing, technique, and number of endometrial biopsies. Their results support our study results. In a systematic review by Vitagliano et al.[17] they concluded that endometrial injury during the follicular phase of the same cycle of IUI improves clinical pregnancy and ongoing pregnancy rates in IUI cycles.

Table 3. Pregnancy outcomes of infertile women who received IUI in two groups of endometrial injury and control

	Endometrial injury group (61 women)		Control group (61 women)		p-value
	Quantity	Percentage	Quantity	Percentage	
Chemical pregnancy rate	15	24.6%	9	14.8%	0.31
Clinical pregnancy rate	14	23%	8	13.1%	0.4
Spontaneous pregnancy rate after IUI cycle	6	9.8%	0	0%	0.02
Miscarriage rate	1	1.6%	1	1.6%	1
Total pregnancy rate (clinical and spontaneous pregnancy)	20	32.8%	8	13.1%	<0.01

IUI: Intrauterine insemination

In some studies, endometrial thickness and dominant follicle count were not different between the control and endometrial injury groups[9,18,19]. However, another study showed higher endometrial thickness and dominant follicle count in the endometrial injury group[10].

In general, some studies show that endometrial injury done by sampling has a positive effect on IUI pregnancy rates[8-11,14,15,17,19,20], whereas other studies found no change at all[10,12,13,18,21,22]. The designs of past studies differ from one another. Timing of endometrial sampling seems to be important in terms of pregnancy results. In conclusion, more studies with larger sample sizes and more variables need to be conducted on this matter.

Study Limitations

Due to lack of time, the number of IUI cycles performed was a limitation of this study. It is recommended that future studies examine pregnancy rates in multiple consecutive IUI cycles.

Conclusion

Endometrial thickness and dominant follicle count were not significantly different between the control and endometrial injury groups. In the first IUI cycle, endometrial injury during the follicular phase did not change pregnancy rates compared with the control group. However, after the first cycle, endometrial injury increased the spontaneous pregnancy rate compared with the control group. Therefore, endometrial injury does not immediately affect pregnancy in the same cycle; however, it does have a positive effect on pregnancy rates in later cycles. In conclusion, performing endometrial sampling during the follicular phase of the IUI cycle is a simple, practical, and cost-effective procedure that increases the chances of later pregnancy.

Ethics

Ethics Committee Approval: This study is a prospective randomized clinical trial and was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.REC.1394.1569).

Informed Consent: All participants provided informed consent before entering the study.

Authorship Contributions

Surgical and Medical Practices: S.H., Concept: S.H., S.M., R.P., Design: S.H., S.M., M.T., Data Collection or Processing: S.H., S.M., R.P., Analysis or Interpretation: S.H., M.T., Literature Search: S.M., R.P., Writing: S.H., S.M., R.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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Clinical Investigation / Araştırma

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Is there a difference in the clinical profile and outcome of women using levonorgestrel IUD for abnormal uterine bleeding and those using it for contraception?: A comparative cross-sectional study

Anormal uterin kanama için levonorgestrelli RİA kullanan kadınlar ile bunu kontrasepsiyon amacıyla kullanan kadınların klinik profili ve sonlanımları arasında fark var mı?: Karşılaştırmalı kesitsel bir çalışma

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Abstract

Objective: The most common indications for Levonorgestrel intrauterine device (LNG-IUD) are contraception and management of abnormal uterine bleeding (AUB). This study was conducted with the aim of exploring the differences in the clinical profile and outcome of women using LNG-IUD for contraception and AUB.

Materials and Methods: This was a retrospective comparative cross-sectional study of women who underwent LNG-IUD (52 mg) between 2012 and 2017. Their electronic health records were reviewed until the last documented follow-up or until December 2021.

Results: A total of 235 women had LNG-IUD with an age range of 21 to 62 years and a mean of (37.98 years±6.76). Of these women, 153/235 (65.1%) had it for contraception and 82/235 (34.89%) had it for AUB. The follow-up was 1-94 months with (mean ± SEM) follow-up for the AUB group of (21.48±2.31) months and for contraception group was (20.74±1.76) months (p-value of 0.80). There was a significant difference between the two groups in the age and body mass index (BMI), where women who had LNG-IUD for AUB were older (mean of 42.54±6.49 years, p-value <0.001) and had higher BMI (31.88±7.52 kg/m^2, p-value =0.011). All LNG-IUDs that were indicated for contraception were inserted in an outpatient setting. However, 68.3% in the AUB, the insertion was in the operating theater in conjunction with hysteroscopy. After combining both expulsion and removal of LNG-IUD during the follow-up period, there was no significant difference between the 2 groups in the overall retention rate during the follow-up (p-value =0.998).

Conclusion: this study shows that women using LNG-IUD for the management of AUB are older and have a higher BMI compared with those using it for contraception. AUB women experienced more expulsion compared with the contraception group, but there was no difference between the 2 groups in the overall survival/retention of LNG-IUD.

Keywords: Levonorgestrel intrauterine device, abnormal uterine bleeding, contraception, expulsion, medicated intrauterine device

PRECIS: In this study women using LNG-IUD for abnormal uterine bleeding or for contraception had similar long-term retention rate when followed for up to 94 months.

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Öz

Amaç: Levonorgestrelli rahim içi cihazın (LNG-RİA) en sık endikasyonu kontrasepsiyon ve anormal uterin kanamanın (AUK) tedavisidir. Bu çalışma, kontrasepsiyon ve AUK için LNG-RİA kullanan kadınların klinik profili ve sonlanımlarındaki farklılıkları araştırmak amacıyla yapılmıştır.

Gereç ve Yöntemler: Bu çalışma 2012 ve 2017 yılları arasında LNG-RİA (52 mg) uygulanan kadınlar üzerinde yapılan retrospektif, karşılaştırmalı, kesitsel bir çalışma idi. Elektronik sağlık kayıtları, belgelenen son takiplere veya Aralık 2021'e kadar incelenmiştir.

Bulgular: Yaşları 21 ile 62 arasında değişen ve ortalama yaşı 37,98±6,76 yıl olan toplam 235 LNG-RİA takılmış kadın hasta dahil edildi. Bu kadınların 153/235'inde (%65,1) doğum kontrolü için, 82/235'inde (%34,89) AUK için LNG-RİA takılmıştı. Takip süresi 1-94 ay olup, (ortalama ± SEM) AUK grubu için 21,48±2,31 ay, kontrasepsiyon grubu için 20,74±1,76 ay (p-değeri 0,80) idi. Her iki grup arasında yaş ve vücut kitle indeksi (VKİ) açısından anlamlı fark vardı; AUK grubundaki LNG-RİA takılan kadınların daha yaşlı (ortalama 42,54±6,49 yıl, p-değeri <0,001) ve daha yüksek VKİ'ye sahip oldukları görüldü (31,88±7,52 kg/m², p-değeri=0,011). Doğum kontrolü için kullanılan tüm LNG-RİA'lar ayakta tedavi ortamında yerleştirildi. Ancak AUK endikasyonu ile yerleştirilen LNG-RİA'ların %68,3'ü histeroskopi ile birlikte ameliyathanede yerleştirildi. Takip süresi boyunca LNG-RİA'nın düşmesi ve çıkarılması durumları birleştirildikten sonra, takip sırasında genel olarak RİA'nın yerinde kalması oranında 2 grup arasında anlamlı bir fark yoktu (p-değeri =0,998).

Sonuç: Bu çalışma, AUK tedavisi için LNG-RİA takılan kadınların kontrasepsiyon amacıyla kullananlara göre daha yaşlı ve VKİ'lerinin daha yüksek olduğunu göstermektedir. AUK grubu kontrasepsiyon grubuyla karşılaştırıldığında daha fazla RİA'nın düşmesi deneyimi yaşadı, ancak LNG-RİA'nın genel olarak yerinde kalması oranı açısından 2 grup arasında fark yoktu.

Anahtar Kelimeler: Levonorgestrelli rahim içi araç, anormal rahim kanaması, doğum kontrolü, RİA'nın düşmesi, ilaçlı rahim içi araç

Introduction

The Levonorgestrel intrauterine device (LNG-IUD) consists of a T-shaped body that has a reservoir for the synthetic progestinlevonorgestrel. It was first introduced in 1990 as a contraceptive method as Mirena® Bayer Schering Pharma, AG, Berlin, Germany, containing 52 mg and releasing 20 mcg per day[1]. Initially, it was approved for 5 years. Soon, it was introduced for the management of heavy menstrual bleeding. It was approved for this indication by the US Food and Drug Administration (FDA) in 2015. In 2020, the FDA approved it for 6 years of use[1], and in August 2021, it was approved for 7 years of use[2]. Although there are other versions of the device that contain lower doses of levonorgestrel and for shorter durations, Mirena® LNG-IUD remains the most widely used version of medicated IUDs[1]. In Oman; Mirena® is the only available version of a medicated IUD.

Besides these two common indications, LNG-IUD is also used to decrease dysmenorrhea in all age groups, including teenage girls. Moreover, it is used for endometrial protection in women on postmenopausal estrogen therapy and for the treatment of endometrial hyperplasia[3]. There might be other evolving uses in any condition that might benefit from thickened cervical mucus, induced morphological changes of decidualization of the stroma, and atrophy of the endometrial glandular epithelium[4]. Most studies reporting on LNG-IUD abbreviate it as LNG-IUS, intrauterine system[5]. In our experience in Oman and other countries, hardly any gynecologist uses IUS as an abbreviation, so we opted to use what is commonly and practically used LNG-IUD rather than LNG-IUS. This choice does not compromise the literature search as almost all search engines provide options for searching for both abbreviations.

Although there is a plethora of literature on LNG-IUD evaluating each of its indications on its own, there are not many studies on comparing the demographic and clinical outcomes between different indications, especially in a population similar to ours in Oman. Awareness and understanding of the differences in women using LNG-IUD for different indications is important for gynecologists to best select the appropriate patients, modify the counseling, the insertion process, and the post-insertion management and follow-up to optimize the outcomes.

Some studies focused on comparing women with heavy menstrual bleeding and contraception in terms of the expulsion rate of LNG-IUD and clinical characteristics that might be related[6]. Considering that abnormal uterine bleeding (AUB) and contraception are the most common indications for LNG-IUD and understanding the impact of cultural factors on women's lives, we conducted this retrospective comparative study with the aim of exploring the differences in the clinical profile and outcome of women using LNG-IUD for contraception and for AUB.

Materials and Methods

This is a retrospective cross-sectional study to compare the clinical profile and outcome of women who had LNG-IUD for contraception and those who had it for the management of AUB with normal endometrial histopathology in a tertiary level hospital in Oman. Figure 1 shows the study flowchart. The included women were those who had LNG-IUD inserted in the period from January 2012 to December 2017 and were followed up in the same hospital until December 2021. The inclusion criteria were premenopausal women who opted for LNG-IUD as a management option for AUB or contraception. Those women who had a prescription for LNG-IUD in the electronic hospital information system were checked, and then the individual patient records were reviewed to solicit only those where the prescription was translated into actual insertion of the IUD. Exclusion criteria were women who had malignant and pre-malignant endometrial or cervical conditions on endometrial biopsy and women who had LNG-IUD for other indications, such as endometriosis, without AUB. Women who had LNG-IUD with a cavity distorted by submucous myomas or endometrial polyps were also excluded.

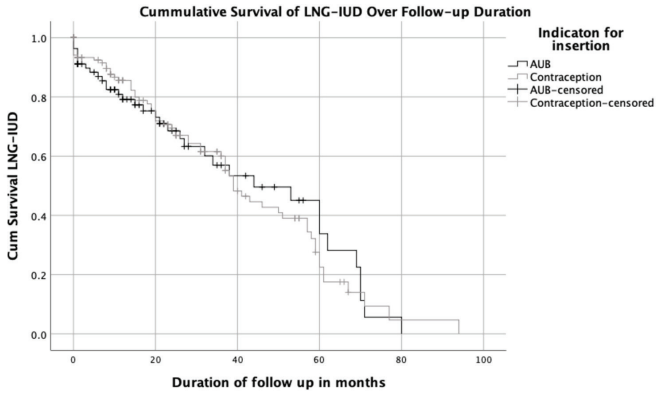

Figure 1. Study flowchart

LNG-IUD: Levonorgestrel intrauterine device, AUB: Abnormal uterine bleeding

The collected data include demographics such as age, number and type of deliveries, body mass index (BMI), and medical history. When the indication for LNG-IUD was not recorded as a diagnosis explicitly by the treating gynecologist, the clinical notes were reviewed for details. The presence or absence of significant medical disorders was recorded. The medical disorders were categorized into cardiovascular disorders, such as hypertension, valvular heart disease, coronary heart disease, and heart failure; metabolic and endocrine disorders, such as diabetes and thyroid disorders; neurological disorders, such as epilepsy, multiple sclerosis, and myasthenia gravis; and immunological and connective tissue disorders, such as systemic lupus erythematosus and Sjogren's syndrome.

Syndrome, rheumatoid arthritis, respiratory disorders such as asthma and bronchiectasis, hematologic disorders such as sickle cell disease, thalassemia, bleeding disorders, renal disorders including any cause of chronic kidney diseases, psychiatric disorders, and infective disorders such as retroviral infection or hepatitis B or C infections. The notes of the insertion procedure were reviewed for the facility where the insertion occurred in the outpatient department set-up (OPD) or in the operating theater in conjunction with hysteroscopy. The uterocervical length in centimeters with uterine sound was recorded. As internationally recommended, the department protocol for the management of AUB in pre-menopausal women mandates an endometrial histological evaluation with or without hysteroscopy if the woman is above 40 years of age or if she is younger than 40 years of age with risk factors of high BMI or chronic anovulation. Information was also collected on the occurrence of side effects, complications of expulsion, malposition, or uterine perforation, and contraception failure or failure to control bleeding. Expulsion was diagnosed by the absence of the IUD string on vaginal speculum examination. Pelvic ultrasound confirmed the absence of the IUD from the uterus, and abdominal X-ray confirmed its absence from the abdomen. If the LNG-IUD was removed, information was collected for the date of removal, did it require hysteroscopy for removal, and what was the reason for removal.

This study was approved by the Ethics and Research Committee of the College of Medicine and Health Sciences MERC#1731.

Statistical Analysis

Data were collected and analyzed using IBM-SPSS version 23 software. The two groups were compared for clinical features, duration of follow-up, and outcome.

For continuous variables such as age and BMI, descriptive statistics were reported as mean, and standard deviations. An independent t-test was used to test the difference in the means. Leven's test was used for continuous variables to test the difference in the mean when there was a significant difference in the size of the groups and they did not have a normal distribution. A p-value of <0.5 was considered for significance.

Most of the categorical variables are dichotomous variables (yes/no) like the presence or absence of complications and side effects. Other categorical variables include grouping variables such as the order of parity (deliveries) and the number of cesarean section groups. Categorical variables were described by frequencies and percentages. Chi-square and Fisher's Exact tests were used to compare frequencies between the groups. The Mann-Whitney U test was used to assess the difference between the 2 groups when there was a continuous variable that was not normally distributed, such as BMI and duration of follow-up.

Results

In the period between January 2012 and December 2017, 235 women met the inclusion criteria having LNG-IUD with the age range of 21 to 62 years and a (mean of 37.98 years +/- 6.76). Of these women, 82/235 (34.89%) and 153/235 (65.1%) had it for contraception. The 2 groups were compared in terms of their demographics, as shown in Table 1.

There was a significant difference between the two groups in age and BMI, where women who had LNG-IUD for AUB were older (mean of 42.54±6.49 years, p-value <0.001) and had higher BMI (31.88±7.52 kg/m^2, 0.011). Women in the AUB group were less likely to have a medical condition compared with women in the contraception group, with 33 (14.0%) versus 54 (23.00%). There was a difference between the two groups in cesarean deliveries, where the AUB group had more cesareans in general, especially higher order cesareans of 3 or more.

All LNG-IUDs that were inserted for contraception were inserted in the OPD as expected. None of the patients required insertion in the operating theater or under anesthesia. However, 68.3% in the AUB, the IUD insertion was performed in the operating theater in conjunction with hysteroscopy. In the overall cohort, the complication rate was as follows: 16/235 (6.8%) experienced expulsion, malposition was detected by ultrasound in 1/235 (0.4%), and it occurred in the contraception group. There was one case of uterine perforation 1/235 (0.4%) and it was also in the contraception group. Other complications included pelvic infection (20/235;8.5%) and pregnancy (2/235;0.8%). When comparing the two groups in those complication rate per group

as shown in Table 2, expulsion occurred more in women with AUB compared to the contraception group 10 (12.20%) vs. (6, 3.9%) with of p-value 0.027). The difference between the 2 groups in pelvic infection and pregnancy was statistically not significant.

When comparing the reported side effects of LNG-IUD, women in the AUB group reported more AUB 33 (40.24%) as a side effect compared to women in the contraception group 31 (20.26%) with a p-value of 0.002. No women in the AUB group reported mood changes, as reported by one woman in the contraception group. Both groups reported weight gain while on the LNG-IUD, but it was not significantly different between both groups, as shown in Table 3. On follow-up ultrasound, bilateral simple ovarian cysts were found in similar proportions in both groups of 9/83 (11.0%) and 16/153 (10.5%) of the AUB group and the contraception group, respectively, with a p-value of 0.90. Amenorrhea occurred in 7.3% of the AUB group and in 9.8% of the contraception group, which was not statistically different with a p-value of 0.524.

Women were followed up for a range of 1-94 months. The mean (mean ± SEM) follow-up for the AUB group was (21.48±2.31) months and for the contraception group was (20.74±1.76) months p-value of 0.80. Cumulative Kaplan-Meier rates of LNG-IUD survival (for removal or expulsions) is used. No significant difference was observed between the 2 groups in the overall rate of removal or expulsion during the follow-up

Table 1. The demographic and clinical characteristics of the LNG-IUD in AUB group and contraception group, Oman, 2012-2021

Variable	AUB group	Contraception group	p-value	95% CI
	n=82	n=153		
	Mean ± SD	Mean ± SD		
Age	42.54±6.49	35.54±5.54	<0.001	5.32-8.58
BMI	31.88±7.52	29.29±6.84	0.011	0.60-4.58
Parity no. (%)				
Nulliparous	5 (6.1%)	1 (0.7%)	0.012	
Parous	77 (93.0)	152 (99.3%)		
Parity categories	no. (%)	no. (%)		
0	5 (6.10)	1 (0.65)	0.017	
1-2	14 (17.07)	40 (26.14)		
3 or more	63 (76.83)	112 (73.20)		
Cesarean sections n (%)				
0	57 (69.51)	115 (75.16)	0.028	
1-2	16 (19.51)	34 (22.22)		
3 or more	9 (10.98)	4 (2.7%)		
Medical disorders no. (%)				
Yes	33 (14.00)	54 (23.00)	0.48	

AUB: Abnormal uterine bleeding, BMI: Body mass index, CI: Confidence interval, LNG-IUD: Levonorgestrel intrauterine device, SD: Standard deviation

Table 2. Complications of LNG-IUD in the AUB and contraception groups, Oman 2012-2021

Variable	AUB group	Contraception group	p-value
	n=82	n=153	
Pregnancy	1 (1.2%)	1 (0.70)	0.65
Expulsion	10 (12.20)	6 (3.9%)	0.027
Malposition	0	1 (0.7%)	0.49
Uterine perforation	0	1 (0.7%)	0.49
PID	7 (8.5%)	13 (8.5%)	0.99
Hysteroscopic removal	4 (4.9%)	1 (0.7%)	0.032

LNG-IUD: Levonorgestrel intrauterine device, AUB: Abnormal uterine bleeding

(p-value of 0.998) as shown in Table 4. At 36 months, AUB continuation was 0.53±0.077 and for contraception group 0.600±0.53. At 60 months, the continuation was 0.338±0.094, and 0.225±0.059 for the contraception group. During the follow-up period, the number of women retaining the LNG-IUD continued to decrease in both groups at similar rates. The decreased numbers during the follow-up occurred because the LNG-IUD was expelled or removed. Removal occurred at the elapse of 5 years or before. Removal before the end of the efficacy period was due to desire of pregnancy, occurrence of side effects such as AUB, or complications such as pregnancy or PID and these reasons with their frequency is shown in Table 5. Of the overall cohort of women with LNG-IUD, 28/235 had serial insertion of IUD to continue the primary indication. 16/82 and 12/153 had sequential immediate insertion of LNG-IUD when expelled or removed with a p-value of 0.346.

Discussion

This study was carried out in a tertiary hospital in Oman, a country that has contraception services provided by primary

Table 3. Side effects of LNG-IUD in the AUB and contraception groups in Oman 2012-2021

Variable	AUB group	Contraception group	p-value
	n=82	n=153	
AUB	33 (40.24)	31 (20.26)	0.002
Amenorrhea	6 (7.3)	15 (9.8)	0.524
Mood changes	0	1	
Weight gain	2 (0.90)	4 (1.7)	0.94
Ovarian cyst	9 (11.0)	16 (10.5)	0.90

LNG-IUD: Levonorgestrel intrauterine device, AUB: Abnormal uterine bleeding

Table 4. Expulsion rate of LNG-IUD in the AUB and contraception groups at different time intervals in Oman 2012-2021

Expulsion at	AUB group (n=82)	Contraception group (n=153)	p-value
3 months	4 (22.2)	1 (2.0)	0.016
6 months	2 (50.0)	0 (0)	0.429
12 months	1 (6.7)	1 (4.2)	1.000
36 months	2 (6.9)	3 (7.7)	1.000
60 months	0 (0)	1 (3.6)	1.000
72 months	1 (16.7)	0 (0)	0.400
Overall	10 (12.2)	6 (3.9)	0.027

LNG-IUD: Levonorgestrel intrauterine device, AUB: Abnormal uterine bleeding

Table 5. Comparison of reasons for removal of LNG-IUD between the AUB and contraception groups in Oman 2012-2021

Reason for removal	AUB group 25/82 (30.5%)	Contraception group 42/153 (27.5%)	p-value
AUB	13 (52.0%)	8 (19.0%)	1.00
Malposition	6 (24%)	7 (16.7)	0.462
Weight gain	1 (4.0%)	2 (4.8%)	-
Pelvic pain/cramping	0	6 (14.3%)	-
Pelvic Infection	1 (4.0%)	4 (9.5%)	-
Divorce	0	1 (2.4%)	-
Desire for pregnancy	1 (4.0%)	6 (14.3%)	-
Unexplained	3 (12%)	8 (19.0%)	
Total	25	42	

LNG-IUD: Levonorgestrel intrauterine device, AUB: Abnormal uterine bleeding

health care. Still, 65% of women who had LNG-IUD in this tertiary hospital used it for contraception. This can be explained by the fact that many of these women (23%) had a medical disorder for which they were already being followed up in the same hospital. This makes it logistically easier for women to attend for care. Also, the availability of medical records and multidisciplinary communication regarding what is suitable and what is not for the patient when all aspects of care are available in the same institution. A third reason is that these women might have been referred to the hospital specifically for LNG-IUD because it is not available in primary health care in Oman[7]. As we are aware, there are no studies comparing the insertion setup between different levels of health care institutions. Most studies compare the types of health care professionals inserting IUDs for contraception, including nursing staff, general practitioners/family physicians, and obstetricians[8,9].

There were 2 main significant differences between women who had LNG-IUD for the management of AUB and those who had it for contraception. These differences were in age and BMI. Women in the AUB group were older with a mean age of 42.54±6.49 years compared to 35.54±5.54 years in the contraception group. Women in the AUB group had a higher BMI mean of 31.88±7.52 kg/m^2. These findings are supported by the known pathophysiology of AUB. The relationship between age and AUB can be explained in relation to the different etiologies of AUB. A significant subset of AUB is caused by ovulatory ovarian dysfunction, which is more common in women aged 41-50 years[10,11]. Adenomyosis, another etiology of AUB, is more common in middle-aged parous women[12]. Leiomyoma of the uterus is also a cause of AUB, where the literature supports a relationship between the age of the women and delayed menopause as risk factors for the development of uterine leiomyomas[13,14]. Likewise, the finding that women with AUB have a higher BMI than those using LNG-IUD for contraception is not surprising. Women with high BMI have more than double the risk of AUB compared with those with normal BMI[15,16]. High BMI has been considered a significant risk factor for abnormal endometrial histology in women with AUB[17,18]. Also AUB is also more prevalent in women with high BMI, and high BMI is a stronger predictor of abnormal endometrial pathology in women with AUB[16,19]. This strong relationship between BMI and AUB has resulted in the debate of whether it should replace age as a stronger indication for endometrial biopsy[16,19]. This relationship between obesity and AUB can be explained by recent advances in the neuroendocrine physiology of the role of leptin and adiponectin from the adipose tissue in blunting the level of kisspeptin, which modulates GnRH and LH pulsatility, resulting in anovulation or oligo-ovulation[20,21].

Of the list of complications of LNG-IUD in Table 2, cumulative expulsion occurred in 6.8% of the total study group and more in women with AUB than in the contraception group with 12.2% and 3.9%, respectively, and a p-value of 0.027. Literature reported several expulsion rates in different groups of women between 3.7% and 22%[22,23]. Our overall expulsion rate and expulsion rate per group is less than that reported by Harris et al.[23], who reported an overall 22% cumulative expulsion rate in women using LNG-LUD for non-contraceptive purposes[6]. Our expulsion rate in women using LNG-IUD for contraception is similar to that reported by Gemzell-Danielsson et al.[22] in a similar group with a reported expulsion rate of 3.7%. The higher expulsion rate in women using LNG-IUD for AUB or non-contraceptive use compared with those using it for contraception could be explained by different reasons. Many women with AUB have a pathology causing an enlarged or distorted uterine cavity, such as adenomyosis and uterine leiomyomas. A second explanation possible is that heavy menstrual bleeding is accompanied by more uterine contractions, causing menstrual cramps and pushing the IUD toward the cervical canal. A third possibility is that the presence of menstrual blood and clots in the uterine cavity is likely to facilitate IUD malpositioning or expulsion[24]. Factors other than heavy menstrual bleeding increase the risk of expulsion, including multiparity, previous cesarean delivery, obesity, and the expertise of the health care provider inserting the IUD[6,25]. Our center is a tertiary care and training center, and many LNG-IUD insertions are performed by trainees.

The removal of LNG-IUD is another cause of its loss in survival. As shown in this study and as reported in the literature, AUB is the main reason women request removal of the LNG-IUD[26,27]. Women using LNG-IUD for the management of AUB might request removal because it fails to provide symptom control or develop a new pattern of bleeding that they donot like[24]. Many of these women who discontinue IUD revert to surgical options such as hysterectomy. In women using LNG-IUD for contraception, discontinuation due to AUB reached up to 27% of the overall discontinuation[28].

Although our overall LNG-IUD continuation in this study is similar to other studies, we did feel in clinical practice that women in our culture might be less tolerant to AUB patterns compared with women in other cultures. This study somehow supports that impression where more than 50% of the discontinuation in the AUB group is due to AUB where women are unsatisfied with the vaginal bleeding pattern they have. This proportion is higher than any reported proportion that we came across in the literature. The reason might be that prolonged and unpredictable vaginal bleeding, even if not heavy, causes inconvenience to women of Muslim faith as it is closely tied to some of the religious duties that Muslim women have to perform[29]. Another possible reason is that prolonged vaginal bleeding may cause husband dissatisfaction, as Muslims are advised to abstain from menstrual bleeding in women[30].

Most studies comparing LNG-IUD use for the treatment of AUB and contraception focused on the expulsion rate rather than reasons for removal and so not much reports on discontinuation due to cramping in comparative studies. In a

review by Kaunitz and Inki[24], discontinuation due to LNG-IUD-related cramping was reported to be around 18% in some of the studies he included in his review. In the population of women using LNG-IUD for contraception, discontinuation due to cramping and pain is reported to occur in 13% of those who discontinued[31]. In our study, no women in the AUB group reported cramping as a reason for discontinuation, whereas 14.3% in the contraception group requested removal due to cramping. This might be inaccurate due to recall bias, but in some women, more than one reason contributes to her dissatisfaction, resulting in her request for removal. However, gynecologists tend to minimize the documentation for only one reason.

Study Limitations

The limitations of this study include its retrospective nature, resulting in the loss of adequate information on accurate side effects and reasons for IUD removal. The strength of this study is that it includes women from the most 2 common indications for LNG-IUD, AUB, and contraception. It also provides long-term follow-up data up to 94 months. Also, because women in Oman share common cultural, social, and religious values with other women in the Arabian Peninsula and North Africa, we believe it is safe to assume that these findings are generalizable to the populations in those countries.

Conclusion

This study shows that women using LNG-IUD for the management of AUB are older and have a higher BMI than those using it for contraception. AUB women experienced more expulsion than the contraception group, but there was no difference between the 2 groups in the overall survival/retention of LNG-IUD or in the rate of reported complications. The AUB group reported more abnormal bleeding patterns after the insertion of the LNG-IUD, and this reason was the most common reason to request LNG-IUD removal.

Ethics

Ethics Committee Approval: This study was approved by the Ethics and Research Committee of the College of Medicine and Health Sciences MERC#1731 (Sultan Qaboos University Ethics Committee - date: 14.08.2018, approval number: SQU-EC/108/18).
Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.A.S., M.M., V.G., Concept: M.A.S., Design: M.A.S., Data Collection or Processing: M.A.S., M.S., M.M., Analysis or Interpretation: M.A.S., M.S., A.N., V.G., Literature Search: M.A.S., Writing: M.A.S., V.G.
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Predictive effect of thiol/disulfide homeostasis dynamics on early pregnancy viability: A case-control study

Erken gebelik viabilitesi üzerinde tiyol/disülfit homeostazı: Bir olgu-kontrol çalışması

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Abstract

Objective: The main aim of this study was to investigate the differences in maternal serum thiol/disulfide homeostasis among women with abortion imminens (AI), missed abortion (MA), and healthy pregnancies during the first trimester.

Materials and Methods: This was a prospective case-control study. This study was conducted on pregnant women who visited the Obstetrics Clinic at University of Health Sciences Turkey, Etlik Zübeyde Hanım Gynecology Training and Research Hospital and were diagnosed with either AI or MA during the 6th to 14th weeks of pregnancy. The participants had a normal pregnancy follow-up, no chronic illnesses, and did not take any multivitamin or antioxidant supplements except for folic acid. The study incorporated 33 pregnant women with AI, 36 with MA, and 40 with normal pregnancies. Age, and body mass index were matched across the three groups. This study used a recently developed automated spectrophotometric technique to quantify thiol/disulfide concentrations.

Results: The AI group had considerably elevated levels of total thiol and native thiol (SH) compared with the MA group. Nevertheless, there was no notable disparity observed between the group of healthy pregnancies and the other two groups. Serum disulfide (SS) levels did not exhibit any significant variations among the three groups. Similarly, the ratios of SS/SH, SS/total thiol, and SH/total thiol did not show any significant differences between the groups ($p>0.05$).

Conclusion: Patients with MA had decreased levels of total thiol and SH, which possess antioxidant capabilities, compared to the AI group. A decrease in antioxidant levels in the body may contribute to the etiology of MA. When considering our findings alongside existing literature, it remains inconclusive whether the serum thiol-disulfide ratio can predict a healthy pregnancy or MA following AI. Therefore, it is not yet seen as a promising diagnostic tool for assessing pregnancy viability. Additional investigation is required to establish the influence of dynamic thiol/disulfide homeostasis on early pregnancy loss.

Keywords: Early pregnancy loss, antioxidants, disulfide, oxidative stress, thiol

Öz

Amaç: Bu çalışmanın temel amacı, ilk trimester abortus imminens (AI), düşük ve sağlıklı gebeliği olan kadınlarda anne serumu tiyol/disülfit dengesindeki farklılıkları incelemektir.

Gereç ve Yöntemler: Bu çalışma, Sağlık Bilimleri Üniversitesi, Etlik Zübeyde Hanım Kadın Hastalıkları Eğitim ve Araştırma Hastanesi Kadın Doğum Kliniği'ne başvuran, 6 ile 14. haftalar arasında AI veya düşük tanısı almış, normal gebelik süreci geçiren, kronik hastalığı bulunmayan ve folik asit dışında

PRECIS: This study investigates the role of thiol/disulfide homeostasis in early pregnancy outcomes, suggesting potential new biochemical markers for predicting pregnancy viability.

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herhangi bir multivitamin veya antioksidan takviyesi kullanmayan hamile kadınları içeren prospektif bir çalışmadır. Çalışma, 33 AI, 36 düşük tanısı konmuş ve 40 normal gebelik olgusunu kapsamaktadır. Katılımcıların yaş, ve vücut kitle indeksi her üç grup arasında eşitlenmiştir. Tiyol/disülfit seviyeleri, yeni geliştirilmiş bir otomatik spektrofotometrik yöntemle ölçülmüştür.

Bulgular: AI grubunda, düşük grubuna göre anlamlı derecede yüksek olan toplam tiyol ve doğal tiyol (SH) seviyeleri saptanmıştır. Ancak, sağlıklı gebelik gösteren grup ile diğer iki grup arasında belirgin bir fark bulunmamıştır. Serum disülfit (SS) düzeyleri üç grup arasında istatistiksel olarak anlamlı bir farklılık göstermemiştir. Ayrıca, SS/SH, SS/toplam tiyol ve SH/toplam tiyol oranları gruplar arasında önemli bir fark göstermemiştir (p>0,05).

Sonuç: Düşük olgularında AI grubuna kıyasla düşük toplam tiyol ve SH seviyeleri gözlemlenmiştir; her ikisi de antioksidan özelliklere sahiptir. Vücuttaki antioksidan seviyelerindeki azalma, düşüğün etiyolojisine katkıda bulunabilir. Bulgularımızı mevcut literatürle birleştirdiğimizde, serum tiyol-disülfit oranının AI sonrasında sağlıklı bir gebeliği ya da düşüğü öngörüp öngöremeyeceği konusunda kesin bir sonuca varılamamıştır. Dolayısıyla, gebeliğin sağlıklı ilerleyip ilerlemeyeceğini değerlendirmede umut verici bir tanı aracı olarak görülmemektedir. Erken gebelik kaybında dinamik tiyol/disülfit homeostazının etkisini göstermek için ek araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Erken gebelik kaybı, antioksidanlar, disülfit, oksidatif stres, tiyol

Introduction

In the first half of pregnancy, the threat of abortion imminens (AI) is characterized by the presence of fetal heartbeat within the intrauterine cavity, without rupture of fetal tissue or membrane, accompanied by vaginal bleeding, cramping, or pain. The situation where non-viable pregnancy products are found in the uterus while the cervix is closed is called missed abortion (MA)[1]. MA occurs in situations where, despite the absence of a fetal heartbeat, the pregnancy does not result in bleeding or miscarriage[2]. AI is observed in approximately 20% of all pregnancies[3]. The diagnosis must be confirmed by ultrasonographic imaging of the gestational sac (GS) and the embryo or fetus with a beating heart[4]. The diagnosis and occurrence of MA are gradually increasing (3.89-14.1%) because of the progress in ultrasound imaging technologies[5]. Although certain fetomaternal factors play a role in the etiology, the pathophysiology remains largely unclear. Nevertheless, it is accepted as a multifactorial phenomenon[6]. Spontaneous abortions are most frequently observed before the 8th week of pregnancy, whereas bleeding episodes during pregnancy typically occur between the 8th and 10th weeks of pregnancy[7]. Oxidative stress (OS) is characterized by an imbalance in the excessive production of free radicals and their neutralization, leading to the accumulation of oxidative damage. Human cells are equipped with both enzymatic and non-enzymatic antioxidant defense systems to maintain vital redox balance[8]. Studies examining the role of OS in initiating various diseases and syndromes highlight the significance of this subject[9]. Thiols, organic molecules containing a sulfhydryl group, play a crucial role in redox homeostasis through their oxidation and reduction. Major plasma thiols, including albumin, cysteine, glutathione, thioredoxin, and homocysteine, can undergo reversible oxidation in the presence of oxygen to form disulfide (SS) bonds, thus maintaining a balance between reduced and oxidized states[10]. Under antioxidant protection, the dynamic conversion between SH (thiol) and SS groups establishes a thiol-disulfide equilibrium, playing a role in cellular signaling, enzyme activities, detoxification, and apoptosis[11]. Previous studies have linked abnormal thiol/disulfide homeostasis (TDH) to hypoxia and reperfusion injury, various liver, heart, and neurological diseases, diabetes, cancer, aging, and complications in pregnancy[12].

The successful development of the embryo heavily relies on the implantation of trophoblast cells into the maternal decidua during the first trimester. A mildly hypoxic environment is essential for the proliferation and differentiation of trophoblasts in the early stages of pregnancy, facilitating the formation of a healthy maternofetal circulation[13]. Although less pronounced than in the initial stages, the continuation of pregnancy involves an ongoing hypoxia-reperfusion state and endothelial dysfunction in the maternofetal bed[14]. Previous studies have associated prolonged OS and diminished protective mechanisms with adverse pregnancy outcomes[15]. In the past, the identification of the SH component in TDH could only be accomplished using complex techniques. Nevertheless, a recently uncovered approach developed by Erel and Neselioglu[16] enables the feasible determination of both constituents of this equilibrium. TDH demonstrates the qualities of being dynamic, reversible, and bidirectional[16]. This study aimed to establish the correlation between different groups by analyzing serum TDH levels in women with AI, MA, and healthy pregnancies.

Materials and Methods

This study adhered to the Helsinki Declaration on Human Subject Research and was authorized by the Ethics Committee of Ankara Yıldırım Beyazıt University (date: 15/06/2016, approval no: 169). A total of 109 women aged over 18 years who were hospitalized in the early pregnancy unit or attending the outpatient clinic of University of Health Sciences Turkey, Etlik Zübeyde Hanım Gynecology Training and Research Hospital between 2017 and 2018 were enrolled. Each participant provided their signature on a written informed consent document. The study comprised 109 patients, including 33 with AI diagnosis (group 1), 36 with MA (group 2), and 40 in the healthy pregnancy control group (group 3). AI was diagnosed in those showing a GS, embryo, or fetus on ultrasonography, exhibiting vaginal bleeding without cervical dilation, and without gynecological pathologies such as cervical polyps and cervicitis that could cause bleeding. Pain and hematoma identified by ultrasound were not considered diagnostic criteria.

The defining features of MA in the first trimester are the GS is unhealthy and there is no fetal heartbeat, the cervical os is closed, and there is little or no vaginal bleeding. Forty randomly selected patients, attending routine checkups in our antenatal clinic during the same period and with no artificial insemination history in the ongoing pregnancy, formed a control group representing healthy pregnancies. Demographic details and test results of each expectant mother were prospectively collected. Excluded from the study were those with endocrine disorders (such as diabetes and thyroid disease), viral diseases, and other immunological, rheumatological, or thrombophilic conditions (such as antiphospholipid syndrome). Included were pregnant women between the ages of 18 and 43, in singleton pregnancies of 6 to $13^{6/7}$ weeks, non-smokers, not on any antioxidant or multivitamin supplements except folic acid. Pelvic examinations of the cases were performed and age, birth and personal history, relevant laboratory values and body mass indexes (BMI) were documented. Pregnancy weeks were calculated according to the last menstrual period. Initially, all cases underwent biometric measurements with a transvaginal transducer (7.5 MHz) ultrasound machine in the lithotomy position, assessing pregnancy viability, GS shape, yolk sac, and crown-rump length. Patients diagnosed by ultrasonography underwent blood sample collection. Venous blood samples of 10 cm^3 were drawn into tubes containing ethylenediaminetetraacetic acid immediately post-ultrasonographic diagnosis. Plasma samples were separated from cells by centrifugation at 1500 × g for 10 min and stored at -80 °C until the day of analysis.

Thiol Analysis Method: Plasma SH, total thiol, and SS levels were determined using an innovative and fully automated technique developed by Erel and Neselioglu[16]. This method relies on the conversion of dynamic SS bonds into functional SH groups using sodium borohydride ($NaBH_4$). In their research, they described this method as practical, cost-effective, straightforward, rapid (with an average processing time of about 10 minutes), and completely automated. To prevent excessive reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the SS bond formed during the DTNB reaction, all residual $NaBH_4$ was removed using formaldehyde. The total thiol content of the samples was measured using a modified Ellman's method. The measured total thiol content was then divided by the SH content, and the resultant difference was halved.

Statistical Analysis

The collected data were analyzed using the 23rd version of SPSS developed by IBM Corp. The Kolmogorov-Smirnov test was applied to determine the distribution pattern of continuous variables. In cases where the numerical variables in the study groups exhibited a normal distribution, statistically significant differences were identified using One-Way ANOVA, followed by Tukey's test. For non-normally distributed data, the Kruskal-Wallis test was employed. Descriptive statistics are presented as mean and standard deviations or medians and interquartile ranges for continuous variables and as frequencies and percentages for categorical variables. A threshold of $p<0.05$ was set for statistical significance.

Results

In this study, 109 women were enrolled, comprising 33 with AI, 36 with MA, and 40 with normal pregnancies. No significant differences were observed in age and BMI among the groups. Normal pregnant women differed significantly from those in the AI and MA groups in terms of number of pregnancies, number of births, and duration of pregnancy ($p<0.05$ for each) (Table 1). Spontaneous abortion occurred in 3 women from the AI group and 2 women from the normal pregnancy group, with other pregnancies continuing until at least the 20th week. Ongoing pregnancy rates were calculated to be 90.9% in the AI group and 95% in the normal pregnancy group.

No significant differences were found between the groups in terms of white blood cell count, neutrophil, lymphocyte, hemoglobin, hematocrit, red cell distribution width, platelet count, total protein, and albumin levels. However, the mean corpuscular volume and mean platelet volume were found to be higher in the normal pregnancy group than in the AI and MA groups ($p=0.004$ and $p=0.003$, respectively) (Table 2).

Total thiol levels and SH values in the AI group were significantly higher than those in the MA group ($p=0.001$ for both). However, no significant difference was observed between the healthy group and the other two groups. In addition, there were no notable variations observed between the groups in relation to SS, SS/SH, SS/total thiol, and SH/total thiol ratios (Table 3).

Discussion

The objective of this study was to evaluate the viability of early pregnancy in the first trimester by measuring the TDH levels of the patients. Our results indicated significantly reduced levels of serum SH and total thiol, both known for their antioxidant properties, in patients with MA compared with those receiving AI treatment. No notable differences were found between the three groups regarding SS levels, SS/SH ratio, SS/total thiol

Table 1. Demographic characteristics of the groups are illustrated

Variables	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)
Age (year)	25.0 (7)	27.0 (11)	28.0 (10)
BMI (kg/m^2)	24.24 (6.02)	24.20 (2.92)	24.14 (5.31)
Gravida	1.00 (1)	1.00 (2)	2.00 (2)*
Parity	0.00 (1)	0.00 (2)	1.00 (0)*
Gestational age (week)	8.4 (4.1)	8.2 (3.0)	10.7 (4.7)♯

BMI: Body mass index
Statistical values are expressed as medians and interquartile ranges.
*: $p<0.05$ as compared to abortus imminens
♯: $p<0.05$ as compared to missed abortus.

Table 2. Comparison of the laboratory parameters of the groups

Parameters	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)	P1	P2	P3	P4
WBC (X 10^3/μL) (mean ± SD)[a]	8.38±2.31	7.71±1.86	7.82±1.86	0.334	0.463	0.968	0.348
Neutrophil, (X 10^3/μL) [median (IQR)][k]	5.76 (2.76)	5.36 (1.88)	4.87 (1.48)	0.305	0.258	0.892	0.114
Lymphocyte (X 10^3/μL) [median (IQR)][k]	1.55 (0.98)	1.70 (0.65)	1.51 (0.56)	0.310	0.458	0.114	0.548
MCV (fL) [median (IQR)][k]	85.20 (6.30)	82.65 (6.73)	86.80 (5.75)	**0.004**	**0.009**	**0.003**	0.475
Hb (g/dL) [median (IQR)][k]	12.80 (1.65)	12.20 (1.10)	12.55 (1.40)	0.126	0.833	0.091	0.068
Hct (%) [median (IQR)][k]	37.50 (4.25)	36.70 (1.85)	37.35 (4.90)	0.306	0.731	0.123	0.319
RDW (%) [median (IQR)][k]	14.50 (0.90)	14.45 (1.13)	14.15 (0.98)	0.300	0.107	0.364	0.678
Platelet, (X 10^3/μL) (mean ± SD)[a]	245.78±58.36	229.02±40.50	245.35±65.38	0.354	0.999	0.417	0.432
MPV (fL) [median (IQR)][k]	7.40 (0.95)	7.60 (0.77)	8.05 (1.37)	**0.003**	**0.002**	**0.011**	0.364
Total protein (g/dL) [median (IQR)][k]	6.80 (0.55)	6.90 (0.40)	6.95 (0.58)	0.525	0.283	0.594	0.477
Albumin (g/dL) [median (IQR)][k]	4.30 (0.40)	4.30 (0.40)	4.10 (0.50)	0.305	0.152	0.247	0.754

[a]: One-Way ANOVA, Post-hoc test: Tukey HSD
[k]: Kruskal-Wallis test and Mann-Whitney U test
p values in bold are statistically significant (p<0.05).
IQR: Interquartile range, Hb: Hemoglobin, Hct: Hematocrit, WBC: White blood cell, MCV: Mean corpuscular volume, RDW: Red cell distrubution width, MPV: Mean platelet volume
P1: Comparison of all three groups, P2: Comparison of AI with healthy pregnant, P3: Comparison of MA with healthy pregnant, P4: Comparison of AI with MA, SD: Standard deviation

Table 3. Dynamic thiol/disulphide homeostasis parameters of the patients

Parameters	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)	P1	P2	P3	P4
Total thiol (μmol/L) (mean ± SD)[a]	416.76±54.18	372.91±52.81	392.77±37.48	**0.001**	0.091	0.177	**0.001**
Native thiol (μmol/L) (mean ± SD)[a]	373.75±52.31	332.73±53.07	353.56±35.32	**0.002**	0.167	0.136	**0.001**
Native thiol/Total thiolx100 [median (IQR)][k]	90.55 (1.24)	90.37 (2.35)	90.36 (1.37)	0.882	0.626	0.954	0.701
Disulphide/Total thiolx100 [median (IQR)][k]	4.73 (0.62)	4.81 (1.17)	4.81 (0.68)	0.880	0.622	0.950	0.701
Disulphide (μmol/L) [median (IQR)][k]	20.49 (3.64)	18.23 (6.44)	18.26 (5.24)	0.222	0.175	0.938	0.094
Disulphide/Native thiolx100 [median (IQR)][k]	5.22 (0.76)	5.32 (1.45)	5.33 (0.85)	0.885	0.626	0.954	0.710

[a]: One-Way ANOVA, Post-hoc test: Tukey HSD
[k]: Kruskal-Wallis test and Mann-Whitney U test
p values in bold are statistically significant (p<0.05).
IQR: Interquartile range
P1: Comparison of all three groups, P2: Comparison of AI with healthy pregnant, P3: Comparison of MA with healthy pregnant, P4: Comparison of AI with MA, SD: Standard deviation

ratio, and SH/total thiol ratio, which are markers of OS. The diminished levels of SH and total thiol in the MA group suggest a potential role of these antioxidants in the pathophysiology of miscarriage. Studies focusing on serum TDH in AI, MA, and healthy pregnancies are limited.

The precise etiology of MA remains elusive in about half of the cases, underscoring the necessity for additional research to understand the underlying causes and to prevent this condition. Several studies hypothesize that an imbalance in the antioxidant/oxidant equilibrium may contribute to the etiology of miscarriage. Although pregnancy naturally involves an elevated level of OS, the development of systems that defend against oxidative damage typically offers protection against such complications. However, our analysis of serum TDH in MA patients indicated a significant increase in these levels, suggesting an excessive rise in OS beyond the capacity of antioxidant defenses as a potential significant etiological component contributing to the development of MA[17]. An examination is conducted on the initial phases of the pathophysiology of spontaneous abortion, and novel viewpoints are introduced[18]. Due to the intervillous circulation in the pregnant woman, the oxygen level increases in the placenta[19]. OS is likely to have a significant impact on the process of distinguishing the placenta. A study conducted by Sebire et al.[20] provided evidence that pregnancy losses that occur between the 7th and 12th weeks of pregnancy may be linked to heightened blood circulation between the villi

during this period. Additionally, Hempstock et al.[21] studied placental OS in early pregnancy loss, assessing placental tissues visually for tissue damage and immunohistochemically for OS. Their findings indicated that patients who experienced MA had substantially higher levels of OS and tissue damage. As observed in our study, patients with MA demonstrated a notable decrease in antioxidant serum SH and total thiol levels.

In organisms, dynamic TDH plays a pivotal role as a fundamental component of antioxidant protection, detoxification, regulation of enzymatic activities, and cellular signaling processes. The maintenance of cellular balance is dependent on alterations in the SH-SS equilibrium. It is possible for cells to produce free oxygen radicals during metabolism or as a reaction to the body's defense systems. It is possible for free radicals to damage cells if there is an imbalance of antioxidants. The controlled production of free radicals under suitable conditions is essential for maintaining cellular homeostasis[22].

Patients experiencing recurrent abortions have been observed to lose antioxidant defenses because of increased consumption. An imbalance between oxidants and antioxidants is believed to be associated with pregnancy loss[23].

Oxygen free radicals are naturally produced during cellular metabolism or as a part of the body's defense requirements. However, without a proper antioxidant equilibrium, uncontrolled generation of these radicals can result in pathogenic changes in cells. The controlled production of free radicals is crucial for sustaining cellular homeostasis[22]. Additionally, patients with recurrent abortions have reported a decrease in antioxidant defenses due to their heightened consumption. An imbalance between oxidants and antioxidants has been suggested to be associated with pregnancy loss[23]. Low levels of plasma ascorbic acid, a-tocopherol, total thiols, and erythrocyte reduced glutathione in individuals with unexplained recurrent pregnancy loss or autoimmune or luteal phase insufficiency suggest an increase in OS[24]. Reduced antioxidant levels may exacerbate pro-oxidant damage to endothelial cells, leading to an imbalance between prostacyclin and thromboxane, potentially resulting in pre-eclampsia or miscarriage[24]. A study by Korkmaz et al.[25] looked at how severe pre-eclampsia affected TDH and found a strong link between the level of TDH decline and the severity of pre-eclampsia. In our study, we supported with statistical analysis that there is a significant reduction in antioxidant levels in individuals with MA compared to those with AI.

During the first trimester of pregnancy, vaginal bleeding is frequently observed and is frequently interpreted as an early indication of placental malfunction. It has been proposed that OS has a significant role in the development of pregnancy problems, such as abortion, and contributes to endothelial dysfunction during aberrant placentation[26]. OS significantly affects the physiology and development of pregnancy. Inadequate trophoblast invasion in the placenta may lead to various conditions, such as early- and late-onset preeclampsia, MA, and miscarriages[27].

In our study, the MA group consisted of pregnant women unclassified as high risk. We utilized this innovative methodology to more quickly and easily confirm the diagnosis and accelerate the follow-up process. In our study, which we planned considering this situation, we attempted to understand the importance of whether it makes any contribution to the early diagnosis or treatment of this disease by measuring serum TDH values in patients with suspected MA.

Dalle-Donne et al.[28] reported an increase in lipid peroxidation in the placenta during pregnancy; however, in healthy pregnancies, there was a simultaneous increase in the antioxidant defense mechanism in response to this OS. Gubaljević and Čaušević[29] measured serum 8-isoprostane levels as a potential indicator of OS during pregnancy. They found that healthy pregnant women had higher levels of 8-isoprostane compared to non-pregnant women. When comparing these levels between the two trimesters, second-trimester pregnant women exhibited significantly higher 8-isoprostane levels than those in the first trimester. In another study[30], a small group of pregnant women experiencing vaginal bleeding before the 10th week of gestation and a control group exhibiting comparable features in healthy pregnancies were evaluated for various OS markers to investigate the role of OS in vaginal bleeding during the first trimester of pregnancy. The specific causes disrupting the oxidant/antioxidant balance in first-trimester vaginal hemorrhage remain unclear. In our current study, no significant differences were observed in serum SS levels, a marker of OS, among the groups of AI, MA, and healthy pregnant women. However, there was a decrease in levels of SS and total thiol, both known for their antioxidant properties, particularly in the MA group. This could indicate a reduction in antioxidant levels in cases of MA, although the absence of significant differences in disulfide and other OS markers might be due to the early gestational weeks of the participants. The cross-sectional nature of the study limits the establishment of a causal relationship. Furthermore, the serum levels of OS markers may not directly reflect the levels in tissues, and future molecular or immunohistochemical studies could provide additional insights.

Study Limitations

There are several limitations to this study. First, the sample size is limited, necessitating more comprehensive prospective studies. Second, due to cost-effectiveness issues, genetic analysis was not performed in the abortion group, which might have led to the overlooking of genetic anomalies. The strength of this study lies in being one of the few prospective studies investigating the impact of oxidation on AI and MA.

Conclusion

The etiology of first-trimester vaginal bleeding remains largely undetermined, necessitating further research to elucidate its causes and develop effective treatment approaches.

Several studies suggest that an imbalance in antioxidant/oxidant equilibrium may play a role in the etiology of miscarriages. Although an increase in OS is expected during pregnancy, the development of antioxidant defense systems usually helps prevent potential complications. We noticed a notable reduction in antioxidant levels during serum TDH assessment in patients with MA. The insufficiency of antioxidant defenses in response to physiological OS during pregnancy may be a significant etiological factor in the development of MA. Consequently, additional prospective studies are required to explore the potential role of antioxidant therapy as a preventive approach in both AI and MA cases.

In conclusion, based on the findings of our study and existing literature, it appears that the serum SH/SS ratio cannot yet be considered a reliable diagnostic tool to predict a healthy pregnancy or MA following AI, nor can it be effectively used as a test tool for assessing pregnancy viability.

Ethics

Ethics Committee Approval: This study adhered to the Helsinki Declaration on Human Subject Research and was authorized by the Ethics Committee of Ankara Yıldırım Beyazıt University (date: 15/06/2016, approval no: 169).

Informed Consent: Each participant provided their signature on a written informed consent document.

Authorship Contributions

Surgical and Medical Practices: B.D.Ç., G.D., M.M.A., Concept: S.Ş.A., M.M.A., Design: S.Ş.A., Ö.E., Data Collection or Processing: B.D.Ç., Analysis or Interpretation: G.D., M.A., Ö.E., Literature Search: B.D.Ç., S.Ş.A., M.A., M.M.A., Ö.E., Writing: B.D.Ç., S.Ş.A., G.D.

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Evaluation of endometrial receptivity in recurrent pregnancy loss and recurrent implantation failure

Tekrarlayan gebelik kaybı ve tekrarlayan implantasyon başarısızlığında endometrial reseptivitenin değerlendirilmesi

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Abstract

Objective: The cause of implantation defects in patients with recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) has not been clearly established. We aimed to evaluate the immunohistochemical changes in HOXA-11, β1 integrin, focal adhesion kinase (FAK), cluster of differentiation 44 (CD44), and extracellular matrix protein 1 (ECM1) molecules during the receptive endometrial period in patients with RIF and RPL.

Materials and Methods: This study was retrospectively conducted at a university hospital. After the exclusion of cases with pathology that may cause a change in the level of receptors in the endometrium, biopsies performed during the receptive period were selected, and the patients were categorized into RPL (n=15), RIF (n=16), control (n=16) groups. All preparations were immunohistochemically stained for HOXA-11, β1 integrin, FAK, CD44, and ECM1.

Results: HOXA-11 and β1 Integrin expression changes were similar between the RIF and control groups. However, FAK expression was significantly increased in the RIF group ($p<0.01$). Additionally, ECM1 and CD44 expressions were significantly decreased in the RIF group compared with the control group ($p<0.01$). There was no significant difference in the endometrial staining of HOXA-11, FAK, and ECM1 in patients with a history of RPL. However, β1 Integrin and CD44 levels were significantly decreased in the RPL group compared with the control group ($p<0.05$).

Conclusion: Implantation is a complex process, and altered adhesion mechanisms involved in endometrial receptivity may be related to defective implantation in patients with RIF and RPL. Among the adhesion molecules, the expression of CD44, β1 integrin, FAK, and ECM1 molecules varies in inappropriate implantation compared with the normal population.

Keywords: Adhesion molecules, implantation, endometrial receptivity, recurrent implantation failure, recurrent pregnancy loss

Öz

Amaç: Tekrarlayan implantasyon başarısızlığı (RIF) ve tekrarlayan gebelik kaybı (RPL) olan hastalarda implantasyon başarısızlığının nedeni net olarak belirlenememiştir. Bu çalışmada, RIF ve RPL hastalarında, endometriumun reseptif döneminde, HOXA-11, β1 integrin, fokal adezyon kinaz (FAK), farklılaşma kümesi 44 (CD44) ve ekstraselüler matris proteini 1 (ECM1) moleküllerinin immünohistokimyasal değişikliklerini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Bu çalışma bir üniversite hastanesinde retrospektif olarak yapıldı. Endometriumda, reseptör seviyesinde değişikliğe neden olabilecek patolojisi olan olgular dışlandıktan sonra, endometriumun reseptif döneminde yapılan biyopsiler seçildi. Hastalar RPL (n=15), RIF (n=16), ve kontrol (n=16) grupları olarak 3 gruba ayrıldı. Tüm preparatlar HOXA-11, β1 integrin, FAK, CD44 ve ECM1 için immünohistokimyasal olarak boyandı. Boyanma özellikleri değerlendirildi.

Bulgular: HOXA-11 ve β1 integrin ekspresyon değişiklikleri, RIF ve kontrol grupları arasında benzerdi. Ancak RIF grubunda FAK ekspresyonu anlamlı düzeyde artmıştı ($p<0,01$). Ayrıca RIF grubunda ECM1 ve CD44 ekspresyonlarının kontrol grubuna göre anlamlı düzeyde azaldığı görüldü ($p<0,01$).

PRECIS: We evaluated endometrial receptivity during the implantation window of the endometrium in patients with RIF and RPL using immunohistochemistry.

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RPL öyküsü olan hastalarda HOXA-11, FAK ve ECM1'in endometriyal boyanmasında anlamlı fark yoktu. Ancak RPL grubunda β1 integrin ve CD44 düzeylerinin kontrol grubuna göre anlamlı düzeyde düşük olduğu belirlendi (p<0,05).

Sonuç: İmplantasyon karmaşık bir süreçtir ve endometrial reseptivitede rol oynayan adezyon mekanizmalarındaki değişimler, RIF ve RPL'li hastalarda defektif implantasyonla ilişkili olabilir. Adezyon molekülleri arasında CD44, β1 integrin, FAK ve ECM1 moleküllerinin ekspresyonu, defektif implantasyon durumunda, normal popülasyona göre değişkenlik gösterir.

Anahtar Kelimeler: Adezyon molekülleri, implantasyon, endometrial reseptivite, tekrarlayan implantasyon başarısızlığı, tekrarlayan gebelik kaybı

Introduction

The human endometrium undergoes dynamic changes during the secretory and proliferative phases of the menstrual cycle, ultimately becoming receptive to embryo implantation within a brief timeframe referred to as the "implantation window"[1]. Successful implantation requires precise timing of a live blastocyst's arrival at this receptive endometrium[2]. Despite this well-established knowledge, in vitro fertilization (IVF) procedures still face significant challenges, with implantation failure accounting for approximately 50-75% of pregnancy losses[3]. While half of early pregnancy losses can be attributed to abnormal embryo karyotypes, the remaining 50% are linked to inadequate interactions between the embryo and the endometrium[4].

Despite treating organic endometrial conditions, such as chronic endometritis and endometrial polyps, some patients still experience recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). Recently, studies on endometrial receptivity have gained momentum in understanding the immunological mechanisms underlying implantation and the concept of the implantation window[5]. Numerous immunohistochemical (IHC) markers that may influence endometrial receptivity have been identified, and their expression levels could vary in different uterine pathologies[6]. However, a comprehensive understanding of all these markers is still needed, and the elucidation of specific markers remains indefinite.

The implantation process involves intricate interactions among growth factors, cell adhesion molecules, extracellular matrix proteins, and cytokines. Many of these factors have been previously identified in the receptive endometrium during the implantation window. Limited data for HOXA-11, focal adhesion kinase (FAK), cluster of differentiation 44 (CD44), β1 integrin, and extracellular matrix protein 1 (ECM1) among these markers are available. While some real-time polymerase chain reaction (PCR) studies have presented changes in the expression of HOXA-11, a transcription factor in the homeobox gene family, during the implantation window, IHC studies on this marker are limited[7]. β1 integrin and FAK are molecules involved in cell adhesion processes and have previously been implicated in the ectopic implantation of the endometrium in endometriotic implants[8,9]. Although their expression during the implantation process has been demonstrated[10], their alterations in the context of RIF and RPL have yet to be characterized. Similarly, studies examining the expression of cell adhesion molecules CD44 and ECM1, which are believed to affect the implantation process in infertile patients, are limited in the context of RIF[11].

Materials and Methods

This retrospective case-control study was conducted at the Department of Obstetrics and Gynecology, Gazi University Faculty of Medicine. The study received ethical approval from the Gazi University Ethics Committee of Clinical Studies (approval no: 2019-129, date: 20.05.2019). All experiments were conducted in compliance with applicable guidelines and regulations. Patient data were reviewed from the hospital's medical records. The ethics committee determined that obtaining informed consent from the patients was unnecessary because of the retrospective nature of the study.

Data Collection and Patient Selection

Patients who applied to the obstetrics and gynaecology clinic at our institution between January 2019 and January 2020 and underwent endometrial biopsy for any reason at ages ranging from 21 to 40 years were examined for this study (n=349). Of these, three groups were formed based on their obstetrical history: the RPL group (group 1), the RIF group (group 2), and the control group (group 3). RIF was defined as the absence of pregnancy despite transferring at least four high-quality embryos in at least three fresh or frozen-thawed embryo transfer cycles[12]. RPL was defined as experiencing three or more miscarriages of unknown cause before 20 weeks of gestation[13]. All patients with RIF or RPL who met the abovementioned criteria were included in the study. The control group comprised patients with at least one child who underwent endometrial biopsy for reasons other than infertility, and no endometrial pathology was reported in any of the control group patients.

Patients with endometrial pathology, including endometrial polyps, chronic endometritis, or submucous leiomyoma, were excluded. In addition, patients whose endometrial biopsies were performed outside the receptive period of the endometrium (between the 21st and 24th days of the menstrual cycle was accepted as the receptive period) were excluded. We also excluded patients with systemic diseases affecting endometrial receptivity, such as diabetes mellitus, gynecological malignancies, or any malignancies associated with estrogen or progesterone receptors. Patients with endometrial biopsy results compatible with ectopic pregnancy and infertile patients with conditions such as endometriosis or hydrosalpinx were also excluded. A flowchart of the study and patient selection is shown in Figure 1.

Endometrial biopsy was performed in the midluteal phase (cycle days 21-24) using a pipeline catheter (Plasti-Med, İstanbul, Turkey) or a 3-mm Novak curette.

Interpretation of Morphology

An experienced pathologist examined the hematoxylin and eosin-stained slides and re-examined them for endometrial dating before immunohistochemistry. Endometrial dating was performed according to Noyes criteria[14].

Immunohistochemistry

Paraffin-embedded blocks were sectioned at a four μm thickness, deparaffinize in xylene, and inserted into the Ventana-XT (Roche, US) automated staining device.

The antibodies used were polyclonal rabbit anti-human against HOXA-11 (1:500 dilutions, Thermo Fischer Scientific, US), monoclonal rabbit anti-human against β1 integrin (clone: EPR16895, 1:1000 dilutions, Abcam, US), monoclonal rabbit anti-human against FAK (clone: EP69Y, 1:250 dilutions, Abcam, US), monoclonal rabbit anti-human against CD44 (clone: EPR1013Y, 1:100 dilutions, Abcam, US), and monoclonal rabbit anti-human against Extracellular Matrix Protein-1 (clone: EPR6701, 1:250 dilutions, Abcam, US). Positive controls included endometrial biopsies, colon, spleen, hepatocellular carcinoma, tonsil, and kidney.

Evaluation of Staining

IHC staining was independently evaluated by two authors without knowledge of the clinicopathological information. The immunoreactive scores of the markers were independently noted in the endometrial stroma and epithelium. The first statistical analysis was performed between the positive and negative cases. Cases that showed staining but were statistically insignificant were re-evaluated and compared again for immunoreactivity according to their extent and intensity. The staining intensity method was as follows: If the staining area was less than 10% or the staining intensity was low, it was called mild staining; if the staining area was 11-100% or the staining intensity was high, it was considered intense staining.

Outcome Measures

The primary outcome measure was whether there was a significant difference in immunoreactive scores of the endometrial receptivity-related markers, showing positive and negative staining in patients with RIF and RPL compared with the control group. The secondary outcome measure involved assessing the differences in staining intensities.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 21.0, Statistics,

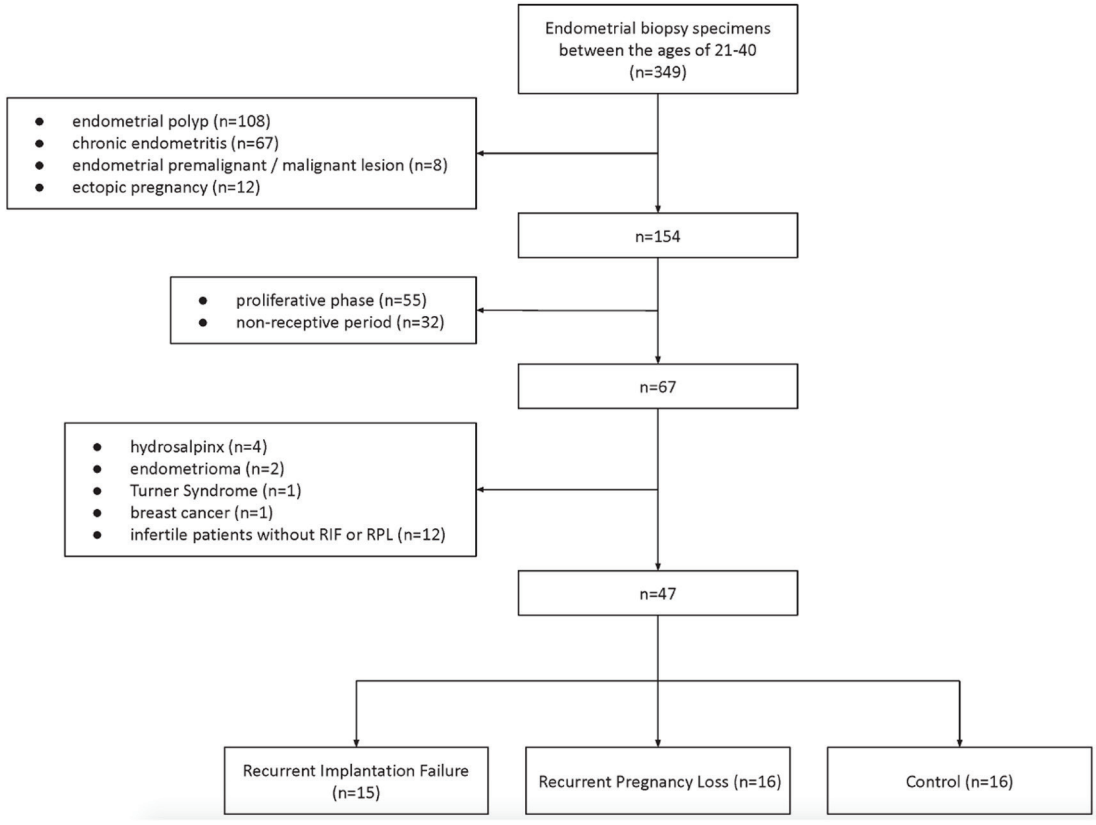

Figure 1. Patient selection

RIF: Recurrent implantation failure, RPL: Recurrent pregnancy loss

2013, Chicago, IBM, USA). The compliance of the variables with normal distribution was examined using graphical (histograms, probability plots) and analytical (Shapiro-Wilk test) methods. One-Way ANOVA was performed to analyze demographic characteristics using the Bonferroni post-hoc test. For categorical data, either the chi-square test or Fisher's exact test was used. Data are presented as mean ± standard deviation or percentages. Statistical significance was defined as $p<0.05$.

Results

The demographic characteristics and obstetric history of the women enrolled in the study are summarized in Table 1.

Endometrial stromal and glandular staining changes of the IHC markers in the RPL and RIF groups compared with the control group are shown in Table 2. Glandular ECM1 staining was decreased and FAK staining was increased in patients with RIF compared with control patients. The results of our study on integrin immunostaining in the RIF group showed a non-significant mild increase compared with controls (100% in the RIF group and 94% in the control group). In patients with RPL, there was a significant decrease in the endometrial glandular staining of CD44 compared with controls (Figure 2). HOXA-11 did not show glandular staining in any patient group. No significant difference was found regarding the other molecules analyzed.

For endometrial stromal staining, there was a decrease in CD44 staining in both the RIF and RPL groups. In addition, a decrease in endometrial stromal β1 integrin staining was observed in RPL patients (Figure 2). HOXA-11 had nearly complete stromal staining in all groups. No statistically significant difference was found in the endometrial stromal staining for the other molecules in both patient groups (Table 2).

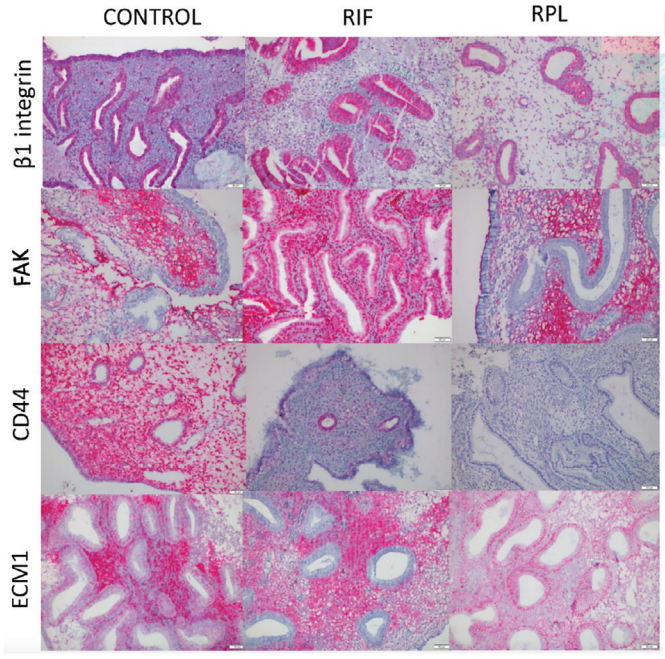

Figure 2. Endometrial staining in all groups

When the pictures were examined in order, β1 Integrin had intense glandular staining in all groups. Although stromal staining was strong in the control and RIF groups, stromal staining was not observed in the RPL group. What is remarkable for FAK is the absence of glandular staining in the RIF group. CD44 did not show stromal staining in the RIF group; in the RPL group, neither stromal nor glandular staining was observed. The absence of glandular ECM1 staining was noted in the RIF group.

RPL: Recurrent pregnancy loss, RIF: Recurrent implantation failure, FAK: Focal adhesion kinase, ECM1: Extracellular matrix protein 1 CD44: Cluster of differentiation 44

Table 1. Demographic features and the obstetric history of the patients

Variable	RPL (n=16)	RIF (n=15)	Control (n=16)	p-value
Age (year)	34.2±3.6	35.1±2.4	36.7±3.1	0.07
BMI (kg/m^2)	24.3±3.7	23.9±2.7	26.3±3.2	0.21
Previous live birth	0.13±0.34	0.13±0.35	2.13±0.71	*
Previous abortion	3.00±0.81	0.07±0.25	0.13±0.34	**

Data are noted as mean value ± standard deviation. RPL: Recurrent pregnancy loss, RIF: Recurrent implantation failure, BMI: Body mass index
*: Control between RPL: p<0.01 Control between RIF: p<0.01 RIF between RPL: p=1.00
**: Control between RPL: p<0.01 Control between RIF: p=1.00 RPL between RIF: p<0.01

Table 2. Staining changes of the endometrial β1 integrin, FAK, HOXA-11, CD44, and ECM1 in the RPL and RIF groups compared to the control group

Molecule	RPL	RIF
Stromal β1 integrin	Decreased (p=0.03)	No difference (p=0.43)
Epithelial β1 integrin	No difference (p=0.50)	No difference (p=0.52)
Stromal FAK	No difference (p=0.11)	No difference (p=0.52)
Epithelial FAK	No difference (p=0.11)	Increased (p<0.01)
Stromal HOXA-11	(p=0.50)	(p*)
Stromal CD44	Decreased (p<0.01)	Decreased (p=0.02)
Epithelial CD44	Decreased (p=0.04)	No difference (p=0.55)
Stromal ECM1	No difference (p=0.30)	No difference (p=0.12)
Epithelial ECM1	No difference (p=0.14)	Decreased (p<0.01)

RPL: Recurrent pregnancy loss, RIF: Recurrent implantation failure, FAK: Focal adhesion kinase, ECM1: Extracellular matrix protein 1, CD44: Cluster of differentiation 44
*: Analysis was not possible as both groups showed 100% staining.

A staining intensity method mentioned in the evaluation of the staining section was also used to compare groups in which there was no difference in the staining properties of the molecules studied. No difference was detected. Stainings in the endometrium are shown in Figure 2.

Discussion

In this study, endometrial receptivity-related IHC markers, including HOXA-11, β1 integrin, FAK, CD44, and ECM, were evaluated in patients with RIF and RPL and their expression changes in endometrial biopsy staining were compared with those in control patients. We found a significant decrease in endometrial glandular ECM1 and stromal CD44 staining in patients with RIF. However, glandular FAK staining increased in these patients. In addition, there was a significant decrease in the endometrial stromal β1 integrin staining and endometrial glandular and stromal staining of CD44 in patients with RPL.

With the increasing use of IVF technologies, endometrial receptivity studies have gained more importance because of RIF in some patients. Studies that provide insight into the molecular mechanisms of endometrial receptivity in patients with RIF and RPL will allow an understanding of the etiology and increase treatment options in both patient groups. It has been emphasized that endometrial receptivity is a complex process involving hormonal, biochemical, and molecular mechanisms, and molecular studies play a role in understanding receptivity and achieving successful implantation[15].

Integrins mediate cell-cell and cell-extracellular matrix adhesion, allowing uterine epithelial cells to bind more tightly. After the binding of integrins to cytoskeletal proteins, FAK, a tyrosine kinase at focal adhesion sites, is activated. Our study on integrin immunostaining in the RIF group showed a nonsignificant mild increase compared with controls. In addition, the epithelial FAK level was higher in the RIF group. These findings suggest that there may be an increase in the barrier function of the endometrium in cases of RIF, which supports the results of a previous study on rats. This study showed that increased focal adhesions could act as a barrier to implantation by making uterine endometrial cells more compact to the blastocyst[10].

RPL is hypothetically considered to occur because of decreased selectivity in the endometrium or superfertilization[16]. In our study, a statistically significant decrease in β1 integrin levels in the endometrial stroma of patients with RPL supports this hypothesis. Decreased expression of β1 integrin in stromal cells may prevent endometrial cells from tightly bonding, thus reducing endometrial selection for the embryo. In this case, it may have resulted in an increased rate of early pregnancy loss. On the other hand, there was no difference in FAK levels in questioning the role of the FAK system. This result may be due to the small sample size and the method used in this study (i.e., we used IHC instead of molecular methods).

Studies using molecular methods have identified a strong relationship between HOXA-11 and implantation failure[17]. HOXA-11 also modulates cell-to-cell and cell-to-extracellular matrix adhesion[18]. In our study, the change in HOXA-11 expression could not be demonstrated immunohistochemically in the endometrium. Similarly, in research conducted with unexplained infertile patients with endometrioma, although an increase in HOXA-11 expression in the ectopic endometrium was detected by PCR, IHC staining for HOXA-11 protein level was not different[19].

The general literature shows that CD44 might play a role in implantation, and its expression physiologically increases in the secretory phase, including the implantation window period in the endometrium[20,21]. A decrease in the expression of CD44 in the mid-secretory phase of patients with RIF has recently been reported[22]. In our study, we similarly found decreased expression of CD44 in the endometrial stroma of patients with RIF, which may confirm the effect of CD44 on implantation. It was shown that CD44 also plays a role in unexplained miscarriages[23], and we found significantly decreased expression of CD44 in the endometrium of our patients with RPL. This finding suggests a defect in vascular invasion and placental angiogenesis in RPL cases, which may be a possible pathophysiological mechanism for increased abortion.

We observed decreased ECM1 staining in the endometrial glandular tissue in patients with RIF. Our data is the first to show that ECM1 was studied in the receptive period of the endometrium in patients with RIF. ECM1 is an extracellular matrix glycoprotein[24], and it was found that maternal-fetal surface ECM1 expression changed in first-trimester curettage materials, and ECM1 expression increased at the implantation site[25]. A previous study reported a decrease in ECM1 expression by PCR in the uterine lavage fluid of unexplained infertile patients; however, IHC staining did not show this decrease[11]. Endometrial samples in this study were performed in the proliferative phase and did not coincide with the implantation window. However, in our study, endometrial biopsies of patients with RIF were performed during the receptive midsecretory phase of the endometrium. Our study is also the first ECM1 study in patients with RPL. Although not statistically significant, a decrease in the endometrial glandular ECM1 level was detected in patients with RPL, which could impact the early pregnancy loss process. However, more studies are required to elucidate this result.

Defective implantation in RIF and RPL may also be related to altered adhesion mechanisms involved in endometrial receptivity. It is noteworthy that changes in the expression of these endometrial receptivity-related molecules may progress with implantation defects during the implantation window period. More new studies on adhesion molecules in these patient groups, whose pathophysiology is not fully understood, will contribute to a better understanding of the underlying mechanism.

Study Limitations

Our study's limitations include the small number of samples and the need for more use of additional IHC methods. Genetic analysis studies, including pregnancy outcomes with more patients in these patient groups, will contribute to the literature.

Conclusion

In conclusion, our findings strongly support the association between adhesion formation and pregnancy failure. Changes in the expression of adhesion-related CD44, ECM1, and FAK molecules have rarely been studied, and their effects have yet to be elucidated in patients with RIF and RPL. We detected changes in CD44, ECM1, and FAK molecule expression in these patient groups. Further research into these changes will help better understand the etiology of RIF and RPL.

Ethics

Ethics Committee Approval: The study received ethical approval from the Gazi University Ethics Committee of Clinical Studies (approval no: 2019-129, date: 20.05.2019).
Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: S.C., A.E., M.E., Concept: S.C., A.E., M.E., Design: S.C., A.E., Ö.E., M.E., Data Collection or Processing: S.C., M.A.İ., A.E., M.İ.G., Ö.E., M.E., Analysis or Interpretation: S.C., M.A.İ., A.E., E.D., M.İ.G., Ö.E., M.E., Literature Search: S.C., Writing: S.C., M.A.İ., A.E., E.D., M.E.
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Clinical significance of initial symptoms in endometriosis-associated ovarian cancer

Endometriozis ile ilişkili over kanserinde başlangıç semptomlarının klinik önemi

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Abstract

Objective: Endometriosis is associated with various symptoms, but their severity varies from case to case. In this study, we investigated the reality of symptoms presented by patients with clinically early-stage endometriosis-associated ovarian cancer (EAOC) and explored the relationship between symptoms and laboratory/imaging findings, pathological findings, and prognosis.

Materials and Methods: This was a retrospective case-control study of patients who received initial surgical treatment and were diagnosed with clinically early-stage EAOC, including ovarian endometrioid carcinoma (OEC), ovarian clear cell carcinoma (OCCC), and seromucinous borderline tumor (SMBT). Patients with OEC/OCCC diagnosed between 2006 and 2016 and those with SMBT diagnosed between 2006 and 2020 were included. Chi-square and Kaplan-Meier estimates were used for statistical analyses.

Results: One hundred-seven patients (OEC, n=31; OCCC, n=39; SMBT, n=37) were included. Fifty-nine (55.1%) patients presented with symptoms, and the proportion of patients with OEC who presented with symptoms was significantly higher than that of others (OEC, 77.4%; OCCC, 43.6%; SMBT, 48.6%). The details of symptoms differed significantly among the pathological types (lower abdominal pain/abdominal discomfort/abnormal bleeding, OEC: 11/8/9; OCCC: 6/12/1; SMBT: 15/5/3). Only in the OEC group did symptomatic patients show significantly higher white blood cell (WBC) count and neutrophil/lymphocyte (N/L) ratio (symptomatic vs. asymptomatic, median: WBC count: 7250 vs. 5000, p=0.008; N/L ratio: 4.6 vs. 1.7, p=0.013). None of the asymptomatic patients showed recurrence during follow-up.

Conclusion: Patients with EAOC show varying symptoms depending on the histological type of the tumor. Laboratory findings underlying symptoms also vary by histopathological type, which may reflect differences in the carcinogenesis process.

Keywords: Adenocarcinoma, clear cell/carcinoma, endometrioid/carcinoma, ovarian epithelial/endometriosis, inflammation, signs and symptoms

Öz

Amaç: Endometriozis çeşitli semptomlarla ilişkilidir ancak bunların şiddeti hastadan hastaya değişir. Bu çalışmada, klinik olarak erken evre endometriozis ile ilişkili yumurtalık kanseri (EAOC) olan hastaların semptomlarının gerçekliğini araştırdık ve semptomlar ile laboratuvar/görüntüleme bulguları, patolojik bulgular ve prognoz arasındaki ilişkiyi araştırdık.

Gereç ve Yöntemler: Bu çalışma, başlangıçta cerrahi tedavi alan ve yumurtalık endometrioid karsinomu (OEC), yumurtalık berrak hücreli karsinomu (OCCC) ve seromüsinöz borderline tümör (SMBT) dahil olmak üzere klinik olarak erken evre EAOC tanısı konan hastaları içeren retrospektif bir olgu kontrol çalışmasıydı. Bu çalışmaya 2006-2016 yılları arasında OEC/OCCC tanısı konulan hastalar ve 2006-2020 yılları arasında SMBT tanısı konulan hastalar dahil edildi. İstatistiksel analizlerde ki-kare ve Kaplan-Meier tahminleri kullanıldı.

Bulgular: Yüz yedi hasta (OEC, n=31; OCCC, n=39; SMBT, n=37) dahil edildi. Elli dokuz (%55,1) hasta semptomla başvurdu ve semptomla başvuran OEC'li hastaların oranı diğerlerine göre anlamlı derecede yüksekti (OEC, %77,4; OCCC, %43,6; SMBT, %48,6). Semptomların ayrıntıları patolojik tipler

PRECIS: Initial symptoms differ according to several histological types in endometriosis-associated ovarian cancer. A detailed elucidation of clinical symptoms may lead to a better understanding of individual cancer biology.

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arasında anlamlı farklılık gösteriyordu (karın alt kısmında ağrı/karın rahatsızlığı/anormal kanama, OEC: 11/8/9; OCCC: 6/12/1; SMBT: 15/5/3). Yalnızca OEC grubunda semptomatik hastalarda anlamlı derecede yüksek beyaz kan hücresi (WBC) sayısı ve nötrofil/lenfosit (N/L) oranı görüldü (semptomatik vs. asemptomatik, medyan: WBC sayısı: 7250 vs. 5000, p=0,008; N/ L oranı: 4,6'ya karşı 1,7, p=0,013). Asemptomatik hastaların hiçbirinde takip sırasında nüks görülmedi.

Sonuç: Erken evre endometriozis ile ilişkili yumurtalık kanserli hastalar tümörün histolojik tipine bağlı olarak değişen semptomlar göstermektedir. Semptomların altında yatan laboratuvar bulguları da histopatolojik tipe göre değişiklik gösterir ve bu da karsinogenez sürecindeki farklılıkları yansıtabilir.

Anahtar Kelimeler: Adenokarsinom, berrak hücreli/karsinom, endometrioid/karsinom, yumurtalık epitelyal/endometriozis, enflamasyon, belirti ve semptomlar

Introduction

Endometriosis is a common gynecological disorder. In addition, it is a known precursor of malignant tumors. Endometriosis-associated ovarian cancer (EAOC) is a particularly distinct subtype of ovarian cancerderived from ovarian endometriosis[1-3]. A large proportion of EAOC cases are clinically International Federation of Gynecology and Obstetrics (FIGO) stage I cases, in which the lesions are clinically confined to the ovaries[4-6]. In general, FIGO stage I ovarian cancer is not considered to have a poor prognosis[7]; however, there are some differences among different histopathological types. Ovarian clear cell carcinoma (OCCC) and ovarian endometrioid carcinoma (OEC) are representative histological subtypes of EAOC[8,9]. Seromucinous borderline tumor (SMBT), which is not strictly a cancer but a borderline malignancy, is also known to occasionally arise from endometriosis[10].

Because SMBT is considered a borderline malignancy, its prognosis is good[11]. On the other hand, OEC and OCCC are more malignant than SMBT. Nevertheless, the omission of postoperative chemotherapy is being considered for some FIGO stage I OEC cases[12]. OCCC is particularly known to have a poor prognosis that is associated with platinum resistance[9]. Aggressive surgical procedures, such as combined resection of the tumor with other organs, may be considered for complete pathological resection of the tumor. from the same endometriosis, a variety of tumors can arise that differ greatly in their phenotype.

Recently, several reports have examined the differences in terms of genetic alteration among various histologic types of EAOC. However, to our knowledge, few studies have focused on the differences in clinical symptoms due to differences in histology. Ovarian cancer has long been generally believed to not present symptoms until advanced stages[13]. However, a recent report indicated that 72% of patients with high-risk early-stage ovarian cancer show physical symptoms at the time of initial presentation[14]. Originally, endometriosis was associated with various symptoms, such as dysmenorrhea, which sometimes reduces daily quality of life[15]. However, the severity of symptoms varies widely from case to case, and asymptomatic cases do arise[16]. Therefore, we hypothesized that symptoms of patients with EAOC can also vary from patient to patient, which may be reflected in histological differences. Although several epidemiological studies have indicated that a history of severe menstrual pain[17,18], suggesting the presence of endometriosis, may increase the risk of ovarian cancer, few studies have investigated the initial symptoms of patients with EAOC in detail.

Thus, the aim of this study was to investigate the clinical significance of the initial symptoms of OEC, OCCC, and SMBT, which are frequently encountered EAOC subtypes, in FIGO stage I cases with lesions confined to the ovaries. In addition, we sought to identify the symptoms that were present at the time of the initial examination and explored the relationship between these symptoms and laboratory, imaging, and pathological findings and prognosis.

Materials and Methods

Patients

This case-control study included patients with OEC, OCCC, and SMBT who underwent initial treatment at our institution. For OEC and OCCC, we included patients clinically diagnosed with FIGO[19] stage I disease who underwent initial surgery between 2006 and 2016. For SMBT, we included patients clinically diagnosed with FIGO stage I disease who underwent initial surgery between 2006 and 2020. Patients whose postoperative clinical course could not be followed for more than one year were excluded; however, no such patients were found. We then compared several factors among the three groups.

Ethics Approval and Consent to Participate

This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (approval number: G531, date: 29.09.2023) and conforms to the Declaration of Helsinki. Informed consent was obtained from all participants via an opt-in approach (wherein participants signed a printed informed consent document) or an opt-out approach (wherein participants were informed about the study through the website).

Evaluation of Clinical Symptoms

Clinical data were extracted from patients' medical records and collected through in-person interview questionnaires. First, we investigated whether the patients reported symptoms or not. If they did, we investigated the details of the symptoms reported and categorized them into several groups according to frequency. A detailed review of patients who showed symptoms revealed that the symptoms could be divided into three categories: abdominal pain, abdominal discomfort (fullness or increased

abdominal girth), and abnormal bleeding. As it is difficult to assume the degree of these symptoms, we qualitatively assessed their presence or absence. Thereafter, we examined the correlation between the symptoms, clinical course of the disease, and laboratory and imaging findings of the patients. This study first examined each of the three histological types of EAOC. A subgroup analysis was also conducted separately for premenopausal and post-menopausal status.

Assessment of Clinical and Laboratory Findings

Information on clinical, laboratory, and imaging findings were extracted from the patients' medical records, including age at the time of initial treatment, white blood cell (WBC) count in the peripheral blood immediately before initial treatment, neutrophil/lymphocyte ratio (N/L-R), serum CA125 level, and maximum tumor diameter. The CA125 values were log-transformed (log-CA125) and used for analysis. The maximum tumor diameter was measured using imaging findings. All patients underwent surgical treatment, and the FIGO stage, assigned based on intraoperative and pathological findings, was evaluated as well. Tumor progression was classified into two groups: the capsuled group (C group; FIGO stages IA, IB, and IC1), which comprised patients whose tumors had not reached the ovarian serosa at the start of surgery, and the uncapsuled group (un-C group; FIGO stages IC2, IC3, and IIIA1), which included patients whose tumors had progressed beyond the ovarian surface. Data regarding recurrence and death from the primary disease were also extracted and analyzed.

Statistical Analysis

Differences in continuous and categorical variables between the two groups were compared using the unpaired t-test and Fisher's Exact test or chi-square test, respectively. Differences in continuous and categorical variables among more than three groups were compared using one-way analysis of variance and Fisher's Exact test, respectively. When significant differences were observed between the groups, post hoc pairwise comparisons were performed using the t-test with Bonferroni correction. $P<0.05$ was considered statistically significant in each analysis, except for the variables analyzed using Bonferroni correction. Kaplan-Meier survival plots based on presenting symptoms were calculated and compared using the log-rank test. All statistical analyses were performed using PRISM version 9.0 (GraphPad Software, San Diego, CA, USA).

Results

Symptoms of Stage I EAOC

One hundred-seven patients with EAOC were included in this study. Of these, 31 patients (29.0%) had OEC, 39 (36.4%) had OCCC, and 37 (34.6%) had SMBT. The age distribution of the patients according to histological type, FIGO classification based on pathological findings, pathological presence of endometriosis, and presence of endometrial disorders are shown in Table 1. Among the 107 patients, 59 (55.1%) experienced at least one symptom. Abdominal pain was the most common symptom (32 patients, 29.9%), followed by abdominal discomfort (25 patients, 23.3%) and abnormal bleeding (13 patients, 12.1%). Of the 59 symptomatic patients, 48 (81.3%) had only one of these symptoms, whereas 11 (18.6%) experienced multiple symptoms (Table 2A).

Symptoms were not statistically associated with menopausal status; however, they were associated with histological subtype. The presentation of symptoms was most common in the OEC group, with 24 (77.4%) of the 31 patients presenting with any of the three above-mentioned symptoms. Eighteen (48.6%) of the 37 patients in the SMBT group presented with symptoms, whereas 17 (43.6%) of the 39 patients in the

Table 1. Demographic and pathological characteristics of participants

	OEC	OCCC	SMBT
Number	31	39	37
Age	50.0 (41.0-58.0)	52.0 (44.0-64.0)	42.0 (34.5-52.5)
FIGO-stage			
IA	8 (25.8%)	15 (38.5%)	30 (81.1%)
IB	1 (3.2%)	0 (0.0%)	2 (5.4%)
IC1	13 (41.9%)	13 (33.3%)	5 (13.5%)
IC2	5 (16.1%)	6 (15.4%)	0 (0.0%)
IC3	4 (12.9%)	4 (10.3%)	0 (0.0%)
IIIA1(i)	0 (0.0%)	1 (2.6%)	0 (0.0%)
Presence of endometriosis (pathologically)	27 (87.1%)	30 (76.9%)	28 (75.7%)
Neoplasm in endometrium	10 (32.3%)	0 (0.0%)	0 (0.0%)

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor, FIGO: Federation of Gynecology and Obstetrics

OCCC group showed symptoms (Table 2B, p=0.011). Analysis of the symptoms showed that lower abdominal pain was the most common symptom in the OEC group (11 patients, 35.5%). However, nine patients (29.0%) in the OEC group also presented with abnormal bleeding, whereas eight patients (25.8%) reported lower abdominal discomfort, indicating that the frequency of each symptom in the OEC group was almost equal. In the OCCC group, the most common symptom was lower abdominal discomfort (12 patients, 30.8%), followed by lower abdominal pain (six patients, 15.4%) and abnormal bleeding (one patient, 2.6%). In the SMBT group, the most common symptom was lower abdominal pain (15 patients, 40.5%), followed by lower abdominal discomfort (five patients, 13.5%) and abdominal bleeding (three patients, 8.1%). There was a significant difference in the distribution of symptoms among the three groups (Table 2C). The OEC group showed an even distribution of symptoms. In contrast, the OCCC group showed a tendency toward lower abdominal discomfort, whereas the SMBT group showed a tendency toward lower abdominal pain.

Differences in Physical Symptoms Among Patients with Stage I OEC, OCCC, and SMBT

We then divided the patients into two groups, pre- and post-menopausal status and conducted a subgroup analysis (Figure 1). The results showed that in the OEC group, 12 of the

Table 2. The analyses about the frequency of symptoms and their background
A. The frequency of the presence of symptoms and its details

	Num	Distribution (%)	95% CI*
No. of symptoms			
0 (No symptoms)	48	44.9	35-55
1 (1 symptom)	48	44.9	35-55
More than 1 (multiple symptoms)	11	10.3	5-18
Symptom description			
Abdominal pain	32	29.9	21-40
Abdominal discomfort	25	23.4	16-33
Abnormal bleeding	13	12.1	7-20

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor, *Calculated by Clopper-Pearson exact method, CI: Confidence interval

Table 2. The analyses about the frequency of symptoms and their background
B. Comparison among age groups and histologies

	No symptoms	With symptoms	p-value
Menopause status			
Premenopausal status	27	31 (53.4%)	0.130
Postmenopausal status	30	19 (38.8%)	
Histology			
OEC	7	24 (77.4%)	0.011
OCCC	22	17 (43.6%)	
SMBT	19	18(48.6%)	

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor

Table 2. The analyses about the frequency of symptoms and their background
C. Comparison among histologies as for the details of symptoms

	OEC	OCCC	SMBT	p-value
Bleeding	9 (29.0%)	1 (2.6%)	3 (8/1%)	
Abdominal discomfort	8 (25.8%)	12 (30.8%)	5 (13.5%)	
Abdominal pain	11 (35.5%)	6 (15.4%)	15 (40.5%)	0.0089

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor

16 patients (75.0%) with premenopausal status and 12 of the 15 patients (80.0%) with postmenopausal status experienced some symptoms. There was no difference in the frequency of symptoms; however, there were differences in the symptom distribution between the two age groups. Patients with premenopausal status mainly reported a single symptom, with lower abdominal pain being the most common symptom (eight patients, 50.0%), followed by lower abdominal discomfort (four patients, 25.0%) and abnormal bleeding (three patients, 18.8%). On the other hand, postmenopausal patients most frequently reported abnormal bleeding (six patients, 40.0%), followed by lower abdominal discomfort (four patients, 26.7%) and abdominal pain (three patients, 20.0%). In the OCCC group, lower abdominal discomfort was the most common complaint in both the premenopausal and postmenopausal status groups. However, the frequency of symptoms significantly differed between the two groups. Only 3 of 15 patients (20.0%) in the premenopausal status presented symptoms, while 14 of 24 (58.3%) patients in the postmenopausal status presented with symptoms. The SMBT group showed the highest frequency of complaints of lower abdominal pain for those in both premenopausal and postmenopausal status. However, 16 of the 27 patients (59.3%) in the premenopausal status presented with symptoms, whereas only two of 10 patients (20.0%) in the postmenopausal status showed symptoms. The OEC, OCCC, and SMBT groups differed in terms of the frequency of symptoms, menopausal status, and distribution of symptoms.

Clinical Significance of Physical Symptoms of OEC, OCCC, and SMBT

The correlation between the presence of symptoms and laboratory, imaging, and pathological findings for each histological subtype of EAOC was analyzed. The results are presented in Table 3. In the OEC group, symptomatic patients showed significantly higher WBC count, N/L-R, and log-CA125 than asymptomatic patients [symptomatic vs. asymptomatic (median): WBC count: 7.25 vs. 5.00, p=0.008; N/L-R: 4.6 vs. 1.7, p=0.013; log-CA125: 8.8 vs. 5.8, p=0.0014, respectively, Table 3A]. Symptomatic patients tended to show slightly larger maximum tumor diameter than asymptomatic patients; however, the difference was not significant (median: 11.0 cm vs. 6.4 cm for symptomatic and asymptomatic patients, respectively; p=0.15, Table 3A). Regarding tumor progression, more symptomatic patients (eight of 24) than asymptomatic patients (one of seven) were in the un-C group; however, this difference was not significant (p=0.64). The symptomatic patients were divided into an abnormal bleeding group and a non-abnormal bleeding group (lower abdominal pain or lower abdominal discomfort) for further analysis (Supplementary Table 1). The results showed that there was no difference between the abnormal and non-abnormal bleeding groups. Regarding the frequency of endometrial disorders, 4 of 9 patients (44.4%) in the abnormal bleeding group and in five of 15 patients (33.3%) in the non-abnormal bleeding group had endometrial disorders.

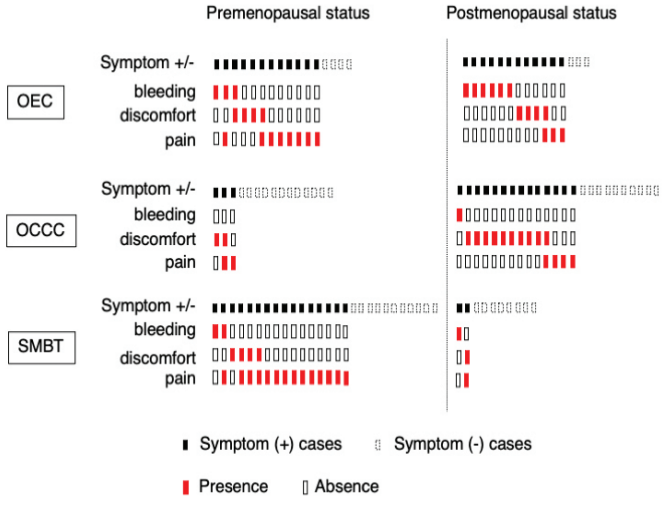

Figure 1. Distribution of clinical symptoms in each case

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor

In the OCCC group, there were no differences in WBC count or N/L-R between symptomatic and asymptomatic patients [symptomatic vs. asymptomatic (median): WBC count: 6.80 vs. 5.88, p=0.17; N/L-R: 2.5 vs. 2.4, p=0.23, respectively, Table 3B]. However, the symptomatic patients showed significantly higher log-CA125 and larger maximum tumor diameter than the asymptomatic patients [symptomatic vs. asymptomatic (median): log-CA125: 5.3 vs. 4.3, p=0.033; maximum tumor diameter: 15.0 vs. 7.9, p=0.0013; respectively, Table 3B]. Regarding tumor progression, nine of 17 symptomatic patients (52.9%) and two of 22 (9.1%) asymptomatic patients were in the un-C group. The difference in the frequency of tumor progression between symptomatic and asymptomatic patients was significant (p=0.0098, Table 3B).

In the SMBT group, the only significant difference between symptomatic and asymptomatic patients was the maximum tumor diameter [symptomatic vs. asymptomatic (median): 9.7 vs. 4.9, p=0.0011, Table 3C]. As all patients in the SMBT group had stage IC1 disease or lower, the presence or absence of intraoperative tumor rupture was examined; however, there was no significant difference in the presence or absence of intraoperative tumor rupture between the symptomatic and asymptomatic patients (1 of 18 symptomatic patients, 5.6% vs. 4 of 19 asymptomatic patients, 21.1%; p=0.17; Table 3C).

We then examined whether the presence or absence of symptoms was a predictor of poor prognosis in OEC and OCCC. The results showed that all asymptomatic patients in both the OEC and OCCC groups survived without recurrence (Supplementary Figure 1). Three of 24 symptomatic patients (12.5%) with OEC and four of 17 symptomatic patients (23.5%) with OCCC died of the disease after recurrence. Most of the deceased cases had uncapsule status; however, one of

the deceased cases in the OCCC group was pathologically confirmed to have stage IA disease after systematic lymph node dissection was performed (Supplementary Figure 1).

Discussion

In this study, we examined the presence and details of physical symptoms at the time of initial diagnosis of EAOC, which is often diagnosed in patients with diseases confined to the ovary. The results showed that among patients with EAOCs, those with OEC presented with symptoms most frequently (77%). In particular, abnormal bleeding was the most common initial symptom in postmenopausal patients with OEC. Postmenopausal patients may be more likely to notice even small amounts of abnormal bleeding, which may have resulted in a higher incidence of initial symptoms in patients with OEC. The fact that abnormal bleeding was particularly common among patients with OEC in this study is a prominent finding. Lurie et al.[20]. reported that patients with endometrioid carcinoma were three times more likely to present abnormal bleeding compared with patients with serous carcinoma. Lurie et al.[21] also reported that patients with localized endometrioid carcinoma were more likely to present with abnormal bleeding compared with patients with localized clear cell carcinoma. In cases of suspected ovarian malignancy with a background of endometriosis, OEC should be considered if the patient presents with abnormal bleeding. Several studies have demonstrated

Table 3. Comparison of clinic-pathological factors between symptom+ and symptom-
A. Analyses in patients with OEC

	Symptom (+)	Symptom (-)	p-value
WBC (x10^3/uL)	7.25 (5.70-10.2)	5.00 (3.30-8.50)	0.0078
N/L ratio	4.6 (2.4-7.1)	1.7 (1.0-6.5)	0.013
CA125 (log-scale)	8.8 (6.4-9.7)	5.8 (3.6-7.0)	0.014
Size (cm)	11 (7.2-13.0)	6.4 (4.5-16.5)	0.15
Capsuled group	16	6	
Un-capsuled group	8	1	0.64

OEC: Ovarian endometrioid carcinoma, WBC: White blood cell, N/L: Neutrophil/lymphocyte

Table 3. Comparison of clinic-pathological factors between symptom+ and symptom-
B. Analyses in patients with OCCC

	Symptom (+)	Symptom (-)	p-value
WBC (x10^3/uL)	6.80 (4.46-8.91)	5.88 (4.67-7.18)	0.17
N/L ratio	2.5 (1.8-4.9)	2.4 (2.1-3.6)	0.23
CA125 (log-scale)	5.3 (4.7-7.6)	4.3 (4.0-5.0)	0.033
Size (cm)	15.0 (12.0-16.0)	7.9 (5.5-10.7)	0.0013
Capsuled group	8	20	
Un-capsuled group	9	2	0.0098

OEC: Ovarian endometrioid carcinoma, WBC: White blood cell, N/L: Neutrophil/lymphocyte

Table 3. Comparison of clinic-pathological factors between symptom+ and symptom-
C. Analyses in patients with SMBT

	Symptom (+)	Symptom (-)	p-value
WBC (x10^3/uL)	5.72 (5.27-7.00)	5.59 (4.95-6.44)	0.37
N/L ratio	2.3 (1.7-3.5)	2.4 (1.5-3.6)	0.78
CA125 (log-scale)	6.1 (4.9-7.6)	5.1 (4.0-6.5)	0.32
Size (cm)	9.7 (7.0-11.2)	4.9 (4.0-6.6)	0.0011
p-stage IA/B	17	15	
p-stage IC	1	4	0.17

OEC: Ovarian endometrioid carcinoma, WBC: White blood cell, N/L: Neutrophil/lymphocyte

that when endometriosis is present, synchronous tumors often develop in the endometrium and ovaries, with endometrioid carcinoma being the most common histological type[1,22]. In the present study, endometrial disorders were detected in 10 of 31 patients with OEC but not in patients with OCCC and SMBT, which may be a cause of abnormal bleeding in patients with OEC. However, not all cases of abnormal bleeding had endometrial disorders, suggesting the possibility of other reasons.

In this study, we further analyzed the details of the symptoms presented by patients with OEC, OCCC, and SMBT. We found that symptomatic patients with OCCC and SMBT had significantly larger tumors than asymptomatic patients, whereas there was no significant difference in tumor size between symptomatic and asymptomatic patients with OEC. Chan et al.[14] reported that approximately 72% of patients with early-stage high-grade tumors, including OCCC but not OEC, confined to the ovary were symptomatic, and their symptoms were associated with tumor size. The results of the present study are comparable with these findings. For OEC, however, the correlation between the presence of symptoms and tumor size was not as strong as that for OCCC or SMBT. Instead, WBC count and N/L-R were strongly associated with the absence of any symptoms, including abnormal bleeding, in patients with OEC. The elevation of both WBC count and N/L-R in patients with OEC suggests a correlation between inflammation and a variety of physical symptoms, including abdominal pain, abdominal discomfort, and abnormal bleeding. We speculate that these differences in the symptom background may reflect differences in the nature of the tumors.

Recent research has rapidly progressed in identifying genetic alterations in cancer cells, and several genetic pathways that are characteristic of SMBT, OCCC, and OEC have been reported. OEC is associated with many genetic changes that are strongly correlated with inflammation[23,24]. Considering the strong correlation between elevated WBC count and N/L-R and the presence of physical symptoms in patients with OEC, it is possible that a persistent inflammatory state is closely related to the development of OEC and the presence of symptoms. The various physical symptoms and high frequencies associated with OEC may reflect a persistent inflammatory response between the ectopic endometrial tissue and the surrounding tissue, which is also a factor in carcinogenesis. Regarding OCCC, several studies have demonstrated that the tumor is associated with specific oncogenic alterations, such as strong involvement of HNF1B and the SWI/SNF complex, including ARID1A[25]. For SMBT, KRAS, a known oncogene, is involved in almost all cases of SMBT[26]. Considering that in the present study, the frequency of symptoms among patients with OCCC and SMBT increased with increasing tumor size, it is likely that patients with OCCC and SMBT are symptomatic only when malignant tumors grow sufficiently because of signal changes, rather than being symptomatic because of interactions in the microenvironment. However, this retrospective observational study has limitations. In addition to the limited number of cases, the inability to ask detailed questions about symptoms systematically is another limitation of this study. Therefore, it was not possible for us to assess the degree of symptoms. To further examine the speculation presented in this study, it is recommended that a systematic interview be conducted prospectively.

Evaluation of the prognostic value of the presence of symptoms in the present study revealed that all asymptomatic patients with OEC and OCCC survived without recurrence, whereas all patients who showed recurrence and died of the disease presented with symptoms. Some studies have reported that prediagnosis high inflammation is associated with decreased ovarian cancer survival, which may be compatible with our results[27,28]. However, as this is a study of some cases, further studies are needed to confirm whether the presence of symptoms is a prognostic factor for OCCC/OEC.

Conclusion

In conclusion, approximately 55% of the patients with early-stage EAOC in this study presented with physical symptoms. The frequency and characteristics of these symptoms varied widely according to the histological type of the tumor, which may reflect the different carcinogenesis mechanisms of OEC, OCCC, and SMBT. Although few studies have focused on understanding the symptoms of ovarian cancer, the importance of patient-reported outcomes has received much attention in recent years, and a proper interview is fundamental to the clinician's work. This study may help to reaffirm the importance of a detailed examination of patients' symptoms to understand their pathophysiology precisely.

Ethics

Ethics Committee Approval: This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (approval number: G531, date: 29.09.2023) and conforms to the Declaration of Helsinki.

Informed Consent: Informed consent was obtained from all participants via an opt-in approach (wherein participants signed a printed informed consent document) or an opt-out approach (wherein participants were informed about the study through the website).

Authorship Contributions

Surgical and Medical Practices: M.O., M.F., K.Y., M.S., S.K., M.T., A.H., K.Y., J.H., M.M., Concept: M.O., K.Y., Design: M.O., K.Y., Data Collection or Processing: M.O., M.F., K.Y., Analysis or Interpretation: M.O., M.F., K.Y., Literature Search: M.O., M.F., K.Y., Writing: M.O., K.Y.

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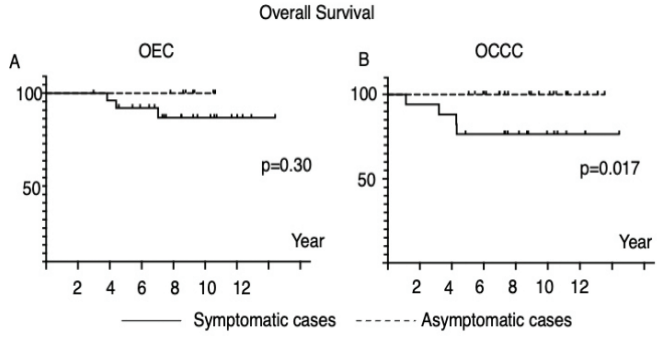
Supplementary Table 1. Comparison of clinical and pathological findings among different symptoms in OEC

	Bleeding	Abdominal disconfort/pain	Symptom (-)	p-value
	n=9	n=15	n=7	
WBC (x10^3/uL)	7.9 (5.60-10.4)	6.3 (5.6-10.7)	5.00 (3.30-8.50)	0.10
N/L ratio	4.6 (2.5-7.7)	4.7 (1.9-8.0)	1.7 (1.0-6.5)	0.094
CA125 (log-scale)	9.0 (5.1-10.0)	8.1 (4.8-9.7)	5.8 (3.6-7.0)	0.023
Size (cm)	12.0 (10.0-15.0)	8.0 (7.0-13.0)	6.4 (4.5-16.5)	0.061
Neoplasm in endometrium	4	5	1	0.44
Capsuled group	6	10	6	
Un-capsuled group	3	5	1	0.62

As for continuous variables, median and interquartile range were described.
As for categorical variables, number was described.
OEC: Ovarian endometrioid carcinoma, WBC: White blood cell, N/L: Neutrophil/lymphocyte



C

Case	Symptom	pStage
1	Bleeding	IC3
2	Bleeding	IC3
3	Pain	IC3

D

Case	Symptom	pStage
1	Discomfort	IC2
2	Discomfort	IC2
3	Discomfort	IA
4	Bleeding	IIIA1(i)

Supplementary Figure 1. Analysis of overall survival from the viewpoint of presence of symptoms

A. Differences in survival based on presence of symptoms in OEC

B. Differences in survival based on presence of symptoms in OCCC

C. Cases of died of disease in OEC were listed. Their symptoms and FIGO stage are as shown

D. Cases of died of disease in OCCC were listed. Their symptoms and FIGO stage are as shown

OEC: Ovarian endometrioid carcinoma, OCCC: Ovarian clear cell carcinoma, SMBT: Seromucinous borderline tumor, FIGO: Federation of Gynecology and Obstetrics

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A novel predictive model of lymphovascular space invasion in early-stage endometrial cancer

Erken evre endometrium kanserinde lenfovasküler alan invazyonunu tahmin edebilmek için oluşturulan yeni bir model

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Abstract

Objective: To predict lymphovascular space invasion (LVSI) positivity in early-stage (stage 1-2) endometrial cancer (EC) using a predictive model with prognostic factors of EC.

Materials and Methods: We included 461 patients who underwent total hysterectomy and bilateral salpingo-oophorectomy with pelvic-paraaortic lymphadenectomy as the primary treatment for presumed early-stage EC at our clinic between 2010 and 2020. Moreover, all surgical specimens were examined histopathologically for the positivity or negativity of LVSI, and the patients were divided into two groups based on these pathologic outcomes. Age, menopausal status, histological type (type 1-2), histological grade (grades 1-2-3), depth of myometrial invasion, and peritoneal cytology results were recorded and analyzed as clinicopathological and demographic characteristics of the patients. The Loess algorithm determined the relationship between the observed and predicted outcomes. The distinction between the algorithms was evaluated by calculating the C-index.

Results: LVSI positivity was significantly associated with advanced age, menopause, type 2 EC, advanced histological grade, malignant peritoneal cytology, cervical involvement, and a tumor exceeding 50% of the myometrial depth ($p<0.001$, respectively). Remarkably, LVSI was most strongly associated with three explanatory variables: 1- More than 50% myometrial invasion [odds ratio (OR): 3.78; 95% confidence interval (CI): 1.80-7.60], 2- Advanced histological grade [OR=1.98 (1.20-3.20) 95% CI], 3- Malignant peritoneal cytology [OR= 3.06 (1.40-6.30) 95% CI]. The penalized maximum likelihood estimation model correctly classified 87% of the included patients (C-index: 0.876).

Conclusion: Our predictive model may help predict LVSI based on different prognostic factors. The prognostic factors included in the nomogram were significantly associated with LVSI, particularly myometrial invasion depth of more than 50%, advanced histological grade, and malignant peritoneal cytology.

Keywords: Endometrial cancer, lymph node metastasis, lymphovascular space invasion, predictive model

Öz

Amaç: Bu çalışmanın amacı erken evre (evre 1-2) endometriyal kanserde (EK) lenfovasküler alan invazyonu (LVAI) pozitifliğini, EK'nin prognostik faktörlerini içeren bir prediktif model kullanarak tahmin etmektir.

Gereç ve Yöntemler: Bu çalışmaya 2010-2020 yılları arasında kliniğimizde erken evre EK tanısıyla primer tedavi olarak pelvik-paraaortik lenfadenektomi ile birlikte total histerektomi ve iki taraflı salpingo-ooferektomi uygulanan veya pelvik-paraaortik lenfadenektomi yapılan 461 hasta dahil edildi. Ayrıca tüm cerrahi örnekler LVAI pozitifliği veya negatifliği açısından histopatolojik olarak incelendi ve hastalar bu patolojik sonuçlara göre iki gruba ayrıldı. Hastaların klinikopatolojik ve demografik özellikleri olarak yaş, menopozal durum, histolojik tip (tip 1-2), histolojik derece (grade 1-2-3), miyometrial invazyon derinliği ve peritoneal sitoloji sonuçları kaydedilerek analiz edildi. Gözlemlenen ve tahmin edilen sonuçlar arasındaki ilişkiyi belirlemek için Loess algoritması kullanıldı. Algoritmalar arasındaki ayrım C-endeksi hesaplanarak değerlendirildi.

PRECIS: Predicting lymphovascular space invasion in early-stage endometrial cancer.

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Bulgular: LVAI pozitifliği ileri yaş, menopoz, tip 2 EK, ileri histolojik derece, malign peritoneal sitoloji, servikal tutulum ve miyometrial derinliğin %50'sini aşan tümör ile anlamlı düzeyde ilişkiliydi. Dikkat çekici bir şekilde LVAI, üç açıklayıcı değişkenle en güçlü şekilde ilişkiliydi: 1- %50'den fazla miyometrial invazyon [odds ratio (OR): 3,78; %95 confidence interval (CI): 1,80-7,60], 2- İleri histolojik derece [OR=1,98 (1,20-3,20) %95 Cl], 3- Malign peritoneal sitoloji [OR=3,06 (1,40-6,30) %95 Cl]. Cezalandırılmış maksimum olasılık tahmini modeli, dahil edilen hastaların %87'sini doğru şekilde sınıflandırmıştır (C-endeksi: 0,876).

Sonuç: Tahmin modelimiz, farklı prognostik faktörlere dayanarak LVAI'nın tahmin edilmesine yardımcı olabilir. Nomogramda yer alan prognostik faktörler, özellikle %50'den fazla miyometrial invazyon derinliği, ileri histolojik derece ve malign peritoneal sitoloji olmak üzere LVAI ile anlamlı düzeyde ilişkiliydi.

Anahtar Kelimeler: Endometrial kanser, lenf nodu metastazı, lenfovasküler alan invazyonu, tahmin modeli

Introduction

Endometrial cancer (EC) is the most common malignancy of the female genital tract in developed countries, and it is the second most common malignancy after cervical cancer in developing countries[1]. In some patients with EC, advanced age is mainly associated with higher treatment failure rates and poor survival rates[2]. Patients with EC are classically present with postmenopausal bleeding, defined as bleeding that occurs at least a year after the natural cessation of menstrual cycles[3]. The current literature offers strong evidence regarding endometrial sampling performed using Pipelle biopsy or conventional dilation and curettage as the most accurate method for EC diagnosis[4]. Staging and treatment planning for EC are generally based on the International Federation of Gynecology and Obstetrics (FIGO) guidelines. Treatment is primarily surgical, and total hysterectomy and bilateral salpingo-oophorectomy are the standard surgical approach[5]; nevertheless, the decision to perform pelvic or additional paraaortic lymphadenectomy for all patients remains questionable. Factors such as advanced age, advanced surgical stage, high-grade tumor, myometrial invasion, lymphovascular space invasion (LVSI), large tumor size, and malignant peritoneal cytology have been reported as poor prognostic factors for EC[6]. EC classification follows the FIGO guidelines, which use a three-step process to categorize the mucinous and endometrioid types according to their histological grade. However, clear, serous, squamous, undifferentiated, and small-cell ECs are not graded or considered high grade (grade 3)[7]. As the depth of invasion into the myometrium increases, so does the risk of lymphatic system invasion, extrauterine involvement, and recurrence, all of which can negatively impact prognosis[8]. LVSI is an independent risk factor for lymph node metastasis and disease recurrence[9]. EC staging now includes LVSI as an essential factor[10]. Adjuvant therapy should be considered for stage I EC patients with lymph node evaluation because of the significant prediction of LVSI for nodal recurrence and poorer overall survival[11].

In cases of EC, it has been reported that approximately 4.4% of individuals who undergo surgical staging characterize malignant peritoneal cytology[12]. However, peritoneal cytology is excluded from the current FIGO guidelines[10].

This study aimed to develop a new predictive model incorporating various prognostic factors to determine the likelihood of LVSI positivity in patients with early-stage EC. Moreover, the aim of this study is to provide clinical risk assessment counseling by using a nomogram with parameters that predict LVSI.

Materials and Methods

The Gaziantep University Faculty of Medicine Clinical Research and Ethics Committee approved this study (ethical approval no: 2022/258, date: 31.08.2022). In addition, this retrospective study was designed to follow the current guidelines of the World Medical Association's Declaration of Helsinki.

Patient Selection and Data Collection

The High-Security Hospital Data System identified 601 patients who underwent surgery for EC between 2010 and 2020 in the Department of Gynecology and Obstetrics of Gaziantep University Faculty of Medicine. Age, premenopausal or postmenopausal status, histopathological EC type (type 1 or 2), histological grade of EC (grade 1, 2, and 3), and myometrial invasion positivity (whether the tumor exceeded ½ of the myometrium) of the included patients were recorded. Further, pathology results regarding cervical involvement of EC, malignant peritoneal cytology positivity, and pelvic-paraaortic lymph node positivity were documented, and these outcomes were recorded. Patients with missing recorded data for inclusion criteria and whose final pathology result was reported as advanced-stage EC were excluded from the study. According to the final pathology results that specified early-stage (stage 1-2) EC diagnosis[10], 461 patients were included in the study.

Biostatistics

In this study, numerical variables were presented using the median (interquartile range), while categorical variables were presented as "n, (%)". Continuous variables were compared using Student's t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were analyzed using the chi-square test (χ^2). The data were summarized with descriptive statistics, including frequency, percentage (%), and mean ± standard deviation. A significance level of p-value 0.05 was considered in this study.

Candidate Predictors and Statistical Modeling

This study examined several factors as potential predictors of LVSI positivity in early-stage EC. This predictive model was designed with candidate predictors that met the necessary conditions and were selected based on previous research[13]. The seven candidate predictors for the final predictive model

included age, menopause status, histological type of EC, degree of myometrial invasion, malignant peritoneal cytology positivity, cervical involvement of EC, and histological grade of EC. No variables with very low or very high frequencies were included in the model.

This model's predicted outcome was prescribed as LVSI positivity in early-stage EC. The Loess algorithm examined the correlation between observed and predicted values. Model discrimination was estimated by calculating the C-index. A 40-bootstrap replication was used for internal validation of the expected model. Predictive model validation consisted of discrimination (Harrell's C-index) and calibration (calibration plots) using the validation set. In line with previous research[14], this study assumed that a >0.75 C-index value represented relatively good discrimination. On the basis of this predictive model, a nomogram was created. All statistical analyses were performed using R version 3.5.1 (R Statistical Software, Institute for Statistics and Mathematics, Vienna, Austria).

Results

The baseline clinical characteristics of the study groups and the comparison of all prognostic factors in patients with and without LVSI are detailed in "Table 1". LVSI positivity was not detected in 396 of 461 patients in the study. However, LVSI was positive in the pathology results of 65 patients. LVSI positivity was strongly associated with a myometrium exceeding 1/2, advanced histological grade, and malignant peritoneal cytology. Of the 65 patients with LVSI positivity, 18 (27.7%) had tumor invasion not exceeding ½ myometrium. Similarly, in 47 (72.3%) of LVSI positivity patients, the tumor did not invade beyond ½ myometrial depth. Tumor invasion did not exceed ½ myometrium in 323 (81.6%) of 396 patients without LVSI positivity. In the remaining 73 (18.4%) of patients without LVSI, the tumor extended beyond ½ myometrial depth. The results showed a significant association between patients with myometrial depth exceeding ½ and patients with LVSI positivity [odds ratio (OR): 3.78 (1.80-7.60) 95% confidence interval (CI)]. Of the 65 patients with LVSI, 7 (10.8%) had grade 1, 18 (27.7%) had grade 2, and 40 (61.5%) had grade 3. In comparison, the findings for the 396 patients without LVSI were as follows: 156 (39.4%) had grade 1, 188 (47.5%) had grade 2, and 52 (13.1%) had grade 3. These results suggest that histological grading is an important prognostic indicator for patients with LVSI positivity [1.98 (1.20-3.20) 95% CI].

Table 1. Demographic, histopathological, and prognostic factors

	LVSI positive n=65	LVSI negative n=396	p
Age [years, (mean ± SD)]	61.89±8.5	58.23±10.24	**0.006**
Menopause status, n (%)			
No	6 (9.2)	80 (20.2)	**0.03**
Yes	59 (90.8)	316 (79.8)	
Histological type of EC, n (%)			
Type 1	46 (70.8)	360 (90.9)	**<0.0001**
Type 2	19 (29.2)	36 (9.1)	
Grade, n (%)			
1	7 (10.8)	156 (39.4)	**<0.0001**
2	18 (27.7)	188 (47.5)	
3	40 (61.5)	52 (13.1)	
Degree of MI, n (%)			
<1/2	18 (27.7)	323 (81.6)	**<0.0001**
≥1/2	47 (72.3)	73 (18.4)	
MPC, n (%)			
Negative	27 (41.5)	351 (88.6)	**<0.0001**
Positive	38 (58.5)	45 (11.4)	
Cervical involvement of EC, n (%)			
Negative	42 (64.6)	357 (90.2)	**<0.0001**
Positive	23 (35.4)	39 (9.8)	

LVSI: Lymphovascular space invasion, EC: Endometrial cancer, MI: Myometrial invasion, MPC: Malign peritoneal cytology, SD: Standard deviation. Values are presented as n(%). Bold values represent p<0.05.

Upon analyzing the peritoneal cytology results of 65 patients with LVSI positivity, it was found that 27 (41.5%) had benign results and 38 (58.5%) had malignant results. In contrast, when the peritoneal cytology results of 396 patients without LVSI were examined, 351 (88.6%) had benign results and 45 (11.4%) had malignant results. Furthermore, a significant correlation was found between patients with malignant peritoneal cytology results and LVSI positivity (OR: 3.06 [1.40-6.30] 95% CI). "Table 2" presents and summarizes predictive model's ORs and CIs. A predictive model based on these candidate variables was created, with a penalized maximum likelihood estimation model correctly classifying 87% of participants. The calibration between the predicted and observed results was acceptable. A plot of the observed and expected results for LVSI positivity in early-stage EC is illustrated in "Figure 1", and a nomogram based on the final refined predictive with the candidate variables is shown in "Figure 2".

Discussion

In this study, a predictive model was created to predict the presence of LVSI in early-stage EC. Accordingly, LVSI positivity was strongly associated with myometrial invasion of more than ½, advanced histological grade, and malignant peritoneal cytology. In addition, LVSI positivity was related to the non-endometrioid type of EC, cervical stromal involvement, and post-menopausal period, according to the predictive model.

There is a discussion regarding the validity of advanced age as an independent prognostic factor; however, it is common for patients over 65 years of age to experience deep myometrial invasion, higher tumor grade, and advanced stage[15]. In the meta-analysis research by Clarke et al.[16], it was reported that early diagnosis strategies focusing on women with postmenopausal bleeding can detect 90% of EC; however, it was concluded that several women with postmenopausal bleeding may not be diagnosed with EC, remarkably. The results of the

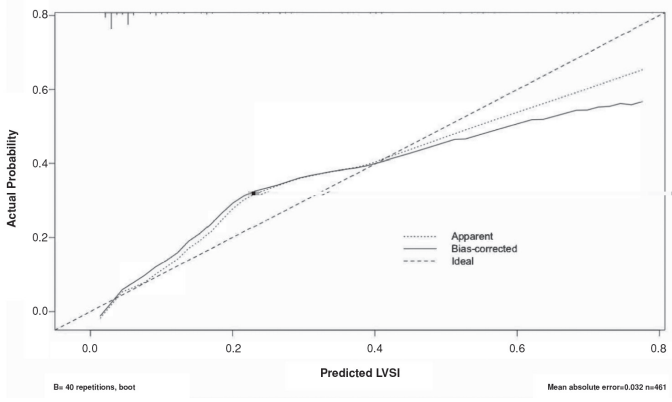

Figure 1. Calibration plot of observed results and predicted outcome to predict LVSI with prognostic factors

LVSI: Lymphovascular space invasion

Table 2. Predicting LVSI risk with prognostic factors

Variables	β-value	OR (95% CI)	p_{adj}
Age [years, (mean ± SD)]	0.009	1 (0.97-1.05)	0.63
Menopause status			
No	0.394	Reference=1	0.53
Yes		1.40 (0.43-5.1)	
Histological type of EC			
Type1	0.542	Reference=1	0.17
Type2		1.72 (0.78-3.78)	
High grade of EC	0.686	1.98 (1.20-3.20)	**0.006**
Degree of MI			
<1/2	1.33	Reference=1	**0.0002**
≥1/2		3.78 (1.80-7.60)	
MPC			
Negative	1.121	Reference=1	**0.0025**
Positive		3.06 (1.40-6.30)	
Cervical involvement of EC			
Negative	0.579	Reference=1	0.12
Positive		1.78 (0.85-3.71)	

LVSI: Lymphovascular space invasion, EC: Endometrial cancer, MI: Myometrial invasion, MPC: Malign peritoneal cytology, SD: Standard deviation, OR: Odds ratio, CI: Confidence interval, $_{Padj}$: Penalized and adjusted p value. Bold values represent p<0.05.

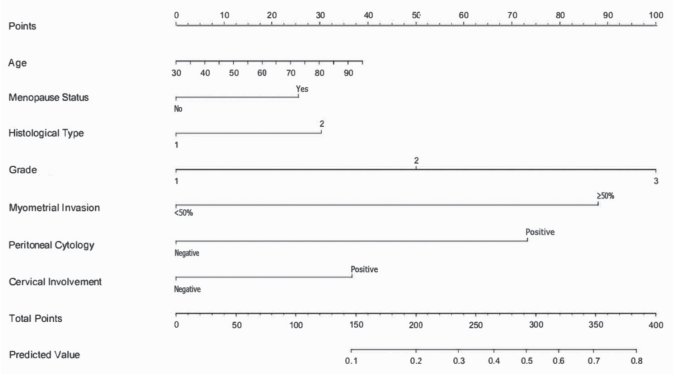

Figure 2. A nomogram based on the final refined estimates with candidate variables of early-stage EC to predict LVSI positivity

LVSI: Lymphovascular space invasion, EC: Endometrial cancer, Peritoneal Cytology Positive: Malign Peritoneal Cytology

present study indicate that LVSI positivity is more prevalent in menopausal patients who have early-stage EC, signifying a robust correlation with advanced age (p=0.006); nevertheless, there was a low level of association between age and LVSI positivity [OR: 1 (0.97-1.05) 95% CI].

Ayhan et al.[17] analyzed 912 low-risk EC patients, and LVSI positivity was detected in 53 (5.8%) patients. Their study reported that LVSI positivity was significantly associated with grade 2 EC (p<0.001), the presence of deep myometrial invasion (p=0.003), and large tumor size (p=0.005), according to the postoperative pathology results. Therefore, the grade of the tumor as a prediction factor was included in our predictive model. In a study by Doghri et al.[18], 62 patients with EC were analyzed to determine the significance of depth myometrial invasion as a prognostic factor. Consequently, their study showed that advanced invasion of the myometrium was associated with a risk of pelvic and para-aortic lymph node metastasis, which is indicated by high-grade EC. In parallel with the previous studies, the present study found a strong and significant correlation between LVSI positivity and exceeding 1-2 myometrium [OR: 3.78 (1.80-7.60) 95% CI].

In the study by Malik et al.[19], the researchers reported that approximately 75-80% of 180 patients had type 1 EC and highlighted that many patients in the early stages showed a favorable prognosis; moreover, they noted that the 5-year survival rate in patients without lymph node involvement was 96%, compared with 67% for those with involvement. In the present study, in which we compared the prognostic factors determined in early-stage EC cases, the histological type of EC showed a significant difference in LVSI positivity [OR: 1.72 (0.78-3.78) 95% CI].

Histological grade has been identified as an independent predictor of recurrence in EC[20]. Additionally, in the PORTEC 1 trial, the results of multivariate analysis confirmed the significance of grade 3 disease for locoregional recurrence[21]. The histological high grade (grade 3) and LVSI positivity were associated with a high statistical significance in the present study [OR: 1.98 (1.20-3.20) 95% CI].

In the staging system for EC, malignant peritoneal cytology is no longer sufficient to upstage the T stage, according to FIGO[10]. In a study by Scott et al.[22], malignant peritoneal cytology was not significantly associated with cure and overall survival in low- and intermediate-risk EC patients. In EC, malignant peritoneal cytology refers to tumor cells in a peritoneal fluid sample taken during surgery. This was once a component of the EC staging criteria but is no longer included in the current revision. However, many associations and organizations still recommend cytological sampling in EC surgery[23]. This study found a significant correlation between malignant peritoneal cytology and LVSI positivity [OR: 3.06 (1.40-6.30) 95% CI].

In stage 2 early-stage EC, the tumor involves the cervix but not beyond the serosa, and there is no endocervical gland involvement[10]. In a study by Toprak et al.[24], a primary tumor diameter of at least 3 cm and an LVSI of at least 50% were determined to be independent markers of cervical involvement in women with EC. Therefore, tumor diameter was not included in our predictive model; additionally, there was no endocervical gland involvement in the patients included, and the tumor did not exceed the serosa. In the present study concerning patients with early-stage EC, a noteworthy association was determined between cervical stromal involvement of EC and LVSI positivity [OR: 1.78 (0.85-3.71) 95% CI].

Study Limitations

It is essential to acknowledge that the predictive model we developed has some limitations that need to be considered. Specifically, the study was conducted retrospectively, and multiple pathologists were involved in evaluating the pathology results. Additionally, the research was conducted in a single-center setting, limiting the generalizability of our findings.

Conclusion

Age, menopausal status, histological type, tumor grade, degree of myometrial invasion, peritoneal cytology, and cervical involvement may predict LVSI positivity in early-stage EC. Clinical consultancy services can be provided by evaluating risk factors using nomograms based on predictions.

Ethics

Ethics Committee Approval: The Gaziantep University Faculty of Medicine Clinical Research and Ethics Committee approved this study (ethical approval no: 2022/258, date: 31.08.2022).

Informed Consent: All patients who participated in the study signed a consent form.

Authorship Contributions

Surgical and Medical Practices: İ.T., M.H.B., F.Ç., S.S., E.Y., Ö.B., Concept: İ.T., M.H.B., E.Y., Ö.B., Design: İ.T., M.H.B.,

H.Ö., Data Collection or Processing: İ.T., S.S., H.Ö., E.U., Analysis or Interpretation: İ.T., E.Y., Ö.B., H.Ö., E.U., Literature Search: İ.T., H.Ö., E.U., Writing: İ.T., M.H.B., F.Ç., E.U.

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Evaluation of the diagnostic utility of MCAM-1 (CD146) in a group of common gynecological cancers: A case-control study

Bir grup yaygın jinekolojik kanserde MCAM-1'in (CD146) tanısal faydasının değerlendirilmesi: Bir olgu kontrol çalışması

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Abstract

Objective: MCAM-1 (CD146) is an endothelial cell adhesion molecule belonging to the immunoglobulin superfamily. Recent studies have identified CD146 expression as a critical marker for tumor progression, migration, and metastasis in various malignancies. This study aimed to evaluate CD146 immunohistochemical expression in various gynecological cancers.

Materials and Methods: This study was conducted in a tertiary medical center in central India. A total of 49 gynecological cancer cases and 16 site-matched controls were included. The cases comprised 27 cervical, 10 endometrial, 10 ovarian, and two miscellaneous cancers. CD146 immunohistochemistry was performed and assessed for immunoreactivity score (IRS), microvascular density (MVD), and microvascular caliber (MVC). An IRS of 5 or more was considered CD146 positive.

Results: The p-values for CD146 positivity for cases vs. control were 0.0531, 0.0580, and 0.007 for cervical, endometrial, and ovarian sites, respectively. The mean MVD was found to be significantly higher in cases compared with benign tissues (p-value <0.00001), and the mean MVC of cases was found to be smaller when compared with the controls (p-value <0.0001).

Conclusion: MVD by CD146 was found to be higher in gynecological malignancies, highlighting its role in cancer neo-angiogenesis and its potential therapeutic role. CD146 epithelial expression was also significantly higher in ovarian cancers. Further studies with a larger sample size are required to confirm that this protein may be a potential theognostic target in gynecological cancers.

Keywords: CD146 antigens, cervical cancer, melanoma cell adhesion molecule, microvascular density, ovarian cancer

Öz

Amaç: MCAM-1 (CD146), immünoglobulin süper ailesine ait bir endotelyal hücre adezyon molekülüdür. Son çalışmalarda, CD146 ekspresyonunun çeşitli malignitelerde tümör progresyonu, migrasyonu ve metastazı açısından kritik bir belirteç olduğu gösterilmiştir. Bu çalışmada çeşitli jinekolojik kanserlerde CD146 immünohistokimyasal ekspresyonunun değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu çalışma Hindistan'ın merkezinde üçüncü basamak bir tıp merkezinde gerçekleştirildi. Toplam 49 jinekolojik kanserli hasta ve 16 bölge açısından eşleştirilmiş kontrol dahil edildi. Hastaların 27'sinde rahim ağzı kanseri, 10'unda endometriyal kanser, 10'unda over kanseri ve 2'sinde çeşitli kanserler mevcuttu. CD146 immünohistokimyasal incelemesi yapıldı ve immünoreaktivite skoru (IRS), mikrovasküler yoğunluk (MVD) ve mikrovasküler kalibre (MVC) açısından değerlendirildi. Beş veya daha fazla IRS, CD146 pozitif olarak kabul edildi.

PRECIS: This study is evaluating CD146 expression on a set of gynecological cancers and site-specific controls. CD146 IHC immunoreactivity score, microvessel density and microvessel caliber are evaluated.

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Bulgular: Kontroller ile kıyaslandığında hastalarda CD146 pozitifliğinin p-değerleri servikal, endometrial ve over bölgeleri için sırasıyla 0,0531, 0,0580 ve 0,007 idi. Ortalama MVD hasta dokularda benign dokulara göre anlamlı derecede yüksek (p-değeri <0,00001) bulunurken, hastaların ortalama MVC'si ise kontrollere göre daha küçük bulundu (p-değeri <0,0001).

Sonuç: CD146 ile MVD'nin jinekolojik malignitelerde daha yüksek olduğu bulundu, bu da onun kanser neo-anjiyogenezindeki rolünü ve potansiyel terapötik rolünü vurgulamaktadır. Yumurtalık kanserlerinde CD146 epitel ekspresyonu da anlamlı derecede yüksekti. Bu proteinin jinekolojik kanserlerde potansiyel bir tanısal hedef olabileceğini doğrulamak için daha büyük örneklem büyüklüğüne sahip çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: CD146 antijenleri, rahim ağzı kanseri, melanom hücre yapışma molekülü, mikrovasküler yoğunluk, yumurtalık kanseri

Introduction

Gynecological cancers (cervix, endometrium, and ovaries) are a significant global burden, with 13.9 lakh new cases and 6.7 lakh deaths reported in 2020[1]. Diagnosis of gynecological cancers involves clinical examination findings, radiological imaging, and histopathological examination[2,3]. The prognosis depends on the site of gynecological cancer, its staging, histological subtype, grade, etc. Biomarkers are measurable biological substances for screening and monitoring of occult gynecological tumors, such as CA-125, CA 19.9, CEA, and HE-4, which have clinical utility in ovarian and endometrial cancers[4].

Melanoma cell adhesion molecule, also known as cluster of differentiation (differentiation), is an endothelial cell adhesion molecule belonging to an immunoglobulin superfamily[5,6]. Lehmann et al.[4] first studied it in malignant melanomas in 1987. It is a membrane glycoprotein expressed by endothelial cells, T-cells, Schwann cells, etc. and plays a pivotal role in vessel formation, immune response, and nerve regeneration[7,8]. Recent studies have found CD146 expression to be a key marker for tumor angiogenesis, progression, migration, epithelial-mesenchymal transition, and metastasis in various malignancies such as melanoma, breast, malignant pleural mesothelioma, prostate, lung, gastric, and gallbladder cancers[5-9]. Increased CD146 expression is strongly correlated with poor prognosis[6]. CD146 is considered a superior marker for disease progression compared with sentinel lymph node analysis. The expression of this protein has been assessed by various methods such as immunohistochemistry (IHC), immunofluorescence, western blotting, and quantitative polymerase chain reaction[5,10,11]. CD146 immunohistochemical expression has been observed in 43% of cervical cancers, 67% of endometrial cancers, and approximately 50% of epithelial ovarian cancers[5,11,12].

This research aims to assess CD146 expression in gynecological cancers compared with benign controls and determine its association with tumor subtypes, biological features, and clinical characteristics. Microvascular density (MVD) and mean vascular caliber (MVC) on CD146 IHC are also explored.

Materials and Methods

This case-control study was conducted in the Department of Pathology and Laboratory Medicine after obtaining ethical clearance from the Institutional Ethics Committee (LOP No IHEC-PGR/2022/STS-ICMR/10, date: 20.06.2022). Waiver of consent was granted because patient identifiers were not used and confidentiality was assured. The study was conducted in accordance with the ethical standards described in the Declaration of Helsinki.

Records, forms, slides, and blocks of diagnosed gynecological cancer cases were retrieved from the archives. The inclusion criteria were cases diagnosed as carcinoma cervix, carcinoma endometrium, and epithelial ovarian malignancies. Suboptimal quality blocks were excluded; cases that had received prior chemotherapy and radiotherapy were also excluded. The sample population included 49 cases and 16 site-matched benign controls: Twenty-seven cases of cervical cancer, 10 cases each of endometrial and ovarian cancers, and 2 cases of miscellaneous cancers. Five cases of chronic cervicitis, 5 benign ovarian tissues, 5 benign endometrial tissues, and one case of tubectomy specimen formed respective benign tissue controls.

CD146 expression, MVD, and MVC were evaluated in these malignancies and compared with site-matched benign controls.

IHC Staining: Representative blocks were selected and stained. Staining was compared with known positive and negative controls. Human placental tissue and melanoma cases were used as positive controls, and omission of primary antibody was used as negative controls. CD146 IHC was performed on tissue blocks with antigen retrieval using Tri sodium citrate buffer. Primary antibody CD146 [Biogenex, Anti Human CD146 (clone EP54)] was applied, followed by washing and incubation with Polyexcel target binder and Polyexcel PolyHRP (horse radish Peroxidase). DAB solution (1 mL of stunt DAB buffer with one drop of Stunn DAB chromogen) was added, counterstaining with hematoxylin, dehydration, and mounting with DPX.

CD146 Expression Criteria: Each biopsy was evaluated for immunoreactivity score (IRS) by assessing staining intensity (0=no staining, 1=faint staining, 2=moderate staining, 3=intense staining) and estimated percentage of all positive cells (0=non reacting cells, 1=1-10% reacting cells, 2=11-25% reacting cells, 3=26-50% reacting cells, 4≥50% reacting cells)[11].

An IRS of 5 or more was considered positive. The cellular localization of CD146 on the cell membrane, nuclear membrane, and nucleoli was also recorded.

MVD and MVC: MVD was analyzed using a CILIKA microscope with morphometric analysis. CD146-immunostained sections were captured, and vessel counting was performed using appropriate reference criteria[12-14]. Three hotspots with the highest microvessel count were determined at low power. These areas were then observed at high magnification (x400) and the average value of 3 hotspot areas was taken as the representative

value of tumor MVD. The high magnification (x400) has a field diameter of 450 µm (0.45 mm) and a field area of approximately 160.000 µm^2 (0.16 mm^2). The MVC was also measured at high magnification.

CD146 in Relation to Diagnosis and Prognosis: We also looked for its relationship with tumor depth in cervical and endometrial variants, along with any correlation with International Federation of Gynaecology and Obstetrics (FIGO) or TNM staging.

Statistical Analysis

We analyzed data using SPSS software. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), diagnostic accuracy (DA), and likelihood ratios were calculated using standard formulas. The significant differences between the groups and the association between CD146 expression and different clinicopathological parameters were evaluated using the Fisher's Exact test. The Kruskal-Wallis test was used to compare the different grades of cancer with their benign counterparts, and an unpaired t-test was used to evaluate the association of MVC. A p-value of <0.05 was considered statistically significant.

Observations and Results

In our study, we analyzed 49 cases and 16 controls, evaluating CD146 expression, MVD, and MVC in cervical cancer (27), endometrial cancer (10), ovarian cancer (10) and one case each of serous tubal intraepithelial carcinoma (STIC) (1) and vulval cancer (1).

Cervical Cancer

All patients with cervical cancer included in our study underwent cervical biopsy, and the biopsy tissue was used for IHC staining. The age of the patients in the cervical cancer group ranged from 37 to 82 years, with an average of 54.51 years. The maximum number of patients was observed in the fourth and fifth decade of life.

Of the 27 cases, 25 were squamous cell carcinoma (SCC) and 2 were adenocarcinoma. In SCC, 17 (68%) cases were moderately differentiated (MDSCC), 5 (20%) cases were poorly differentiated SCC (PDSCC), and 3 (12%) cases were well differentiated SCC (WDSCC).

Nineteen (70%) cases showed CD146 expression, and eight (30%) cases were negative (Figure 1a-1d). In the cervical benign cases, 1 (20%) was positive and the remaining 4 (80%) were negative. The p-value was 0.0531 and was non-significant (Fisher's Exact test) (Table 1). If we consider CD146 positivity of WDSCC in comparison with the rest of SCC, it was 33% in the former and 68% in the latter.

Among all SCCs, the MVD ranged from 4 to 36 in different hotspots with a mean of 14.316±6.64 per 0.16 mm^2, whereas in benign tissues, MVD ranged from 3 to 12 with a mean of 5.53±2.02 per 0.16 mm^2 as explained in Table 1. The p-value was <0.00001 and was significant (Kruskal-Wallis test).

In WDSCC, the MVD ranged from 7 to 16 per 0.16 mm^2 (mean 10.77±0.69). In MDSCC, MVD ranged from 5 to 36 per 0.16 mm^2 (mean 14.505±8.8). In PDSCC, the MVD ranged from 4 to 25 per 0.16 mm^2 (mean 10.13±5.24). Median MVD values were 33.31, 68.75, 87.5, 81.25 per mm^2 for benign cervical tissue, WDSCC, MDSCC, and PDSCC, respectively (Figure 2). MVD was highest in MDSCC, followed by PDSCC and WDSCC, possibly due to increased necrosis and hemorrhage in PDSCC.

The mean MVC in cervical cancers was 9.824±4.873 µm, while in benign tissue it was 31.8±15.380 µm (Table 1). The difference was statistically significant (p<0.0001) based on an unpaired t-test.

Most cervical cancer types were SCC, followed by adenocarcinoma. Among the 11 cases with available FIGO staging, 9 (77%) in the early stage (I-II) and 2 (100%) in the advanced stage (III-IV) showed positive CD146 expression.

Endometrial Carcinoma

Ten random cases of endometrial carcinoma, endometrial tissue used for IHC was of the 6 patients who had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy, and 4 were endometrial curettage.

The age of patients in the endometrial cancers group ranged from 47 to 76 years, with a mean age of 58 years. Maximum number of patients presented in the 5th decade. In carcinoma of the endometrium, 7 (70%) cases were low-grade endometrial carcinoma (LGEC), 2 (20%) cases were high-grade endometrial carcinoma (HGEC), and 1 (10%) was mucinous endometrial adenocarcinoma.

Five (50%) cases showed CD146 expression, whereas the remaining 50% were negative (Figure 1e-1h). Among the benign group, 20% were positive and 80% were negative. The Fisher's Exact test statistic value was 0.5804, indicating a non-significant result (Table 1). Among the 5 positive cases, 2 were HGEC and 3 were LGEC.

In all cases of endometrial carcinomas, MVD ranged from 9 to 32 (mean 17.83±6.07) per 0.16 mm^2. In benign tissues, MVD ranged from 8 to 22 (mean 12.25±3.88) per 0.16 mm^2 (Table 1, Figure 2). The p-value obtained from the Kruskal-Wallis test was 0.001, indicating a significant difference (p<0.05) between the two groups.

In HGEC, the MVD ranged from 14 to 28 (mean 20.83±4.002) per 0.16 mm^2. In LGEC, MVD ranged from 11 to 38 (mean 17.75±6.64) per 0.16 mm^2. In mucinous adenocarcinoma, MVD ranged from 10 to 16 (mean 13.33±3.05) per 0.16 mm^2. In the normal endometrium, MVD ranged from 8 to 22 (mean 12.24±3.89) per 0.16 mm^2. The median MVD values per mm^2 were 70.62, 87.5, and 130.185 for benign, LGEC, and HGEC, respectively (Figure 2). Median MVD showed an increase with higher histological grade, with the highest in HGEC, compared with benign tissue.

The mean MVC in endometrial cancers was 6.52±2.311 µm, while in benign tissue it was 22.23±10.150 µmm. The difference was extremely statistically significant (p<0.0001) based on an unpaired t-test.

The majority of endometrial carcinomas in the study were endometrioid adenocarcinomas. Among the six cases with available FIGO staging, two (50%) were in stage I (early stage; FIGO I-II) and four (75%) were in advanced stage (III-IV) with positive CD146 expression. CD146 expression increased with the stage of endometrial carcinoma.

Ovarian Carcinoma

Ten cases meeting the inclusion criteria and suitable blocks were selected. Samples included were from the following surgical procedures: total abdominal hysterectomy with bilateral salpingo-oophorectomy (7), right oophorectomy (1), ovarian cystectomy (1), and excised ovarian mass (1).

Eight were serous and two mucinous. Two cases were atypical proliferative serous tumors (APST), one was a case of low-grade serous carcinoma (LGSC), and five were high-grade serous carcinoma (HGSC). In the mucinous group, both were mucinous ovarian carcinoma (MOC). All epithelial ovarian tumors are referred to as ovarian cancers in this study. Patients' age ranged from 19 to 65 years (mean: 39.4 years). There were 1, 3, 1, 2, 2 & 1 cases within the age group of 11 to 20 years, 21 to 30 years, 31 to 40 years, 41 to 50 years, 51-60 years, and more than 60 years.

In the evaluated epithelial ovarian tumors, 80% of cases were CD146 positive (Figure 1j-1l), whereas 20% were negative. Among the controls, all were negative. Out of the ten cases, five were HGSC with 80% positive (4) and 20% negative (1). Both cases of MOC were positive (100%), one LGSC was positive (100%), and two APST cases had one positive (50%) and one negative (50%). DA was 86.67%, sensitivity was 80%, specificity was 100%, PPV was 100%, NPV was 71.43%, and likelihood ratio was 0.20 (Table 1). The Fisher's Exact test statistic value is 0.007. The result is significant at $p < 0.05$.

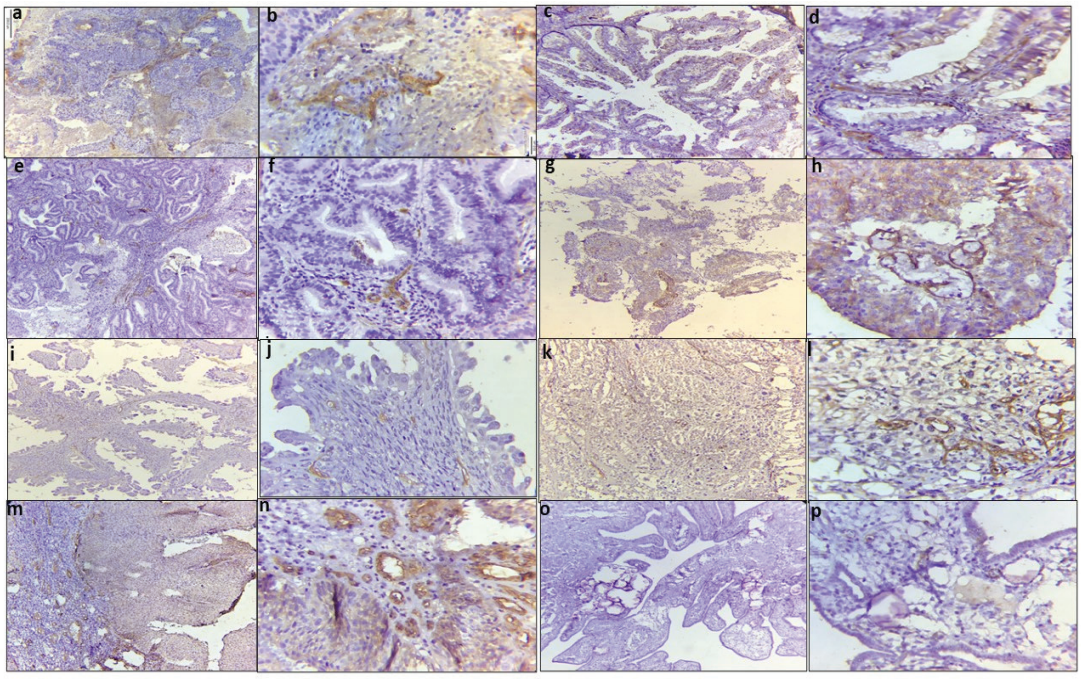

Figure 1. Photomicrographs 'a' (at low power) & 'b' (at high power with a mean MVD of 62.5 per mm^2 per mm^2) show a case of WDSCC, with an IRS of 5 i.e., Positive for CD146 expression; whereas 'c' (at low power) & 'd' (at high power with a mean MVD of 133.312 per mm^2) show a case of endocervical adenocarcinoma, with an IRS of 6 i.e., Positive for CD146 expression

Photomicrographs 'e' (at low power) & 'f' (at high power with mean MVD of 95.81 per mm^2) showing a case of LGEC with an IRS of 3 i.e., negative for MCAM expression whereas 'g' (at low power) & 'h' (at high power with a mean MVD of 147.87 per mm^2) show a case of HGEC with an IRS of 6 i.e., positive for CD146 expression.

Photomicrographs 'i' (at low power) & 'j' (at high power with a mean MVD of 45.81 per mm^2) showing a case of APST with an IRS of 4 i.e., negative for CD146 expression; whereas 'k' (at low power) & 'l' (at high power with a mean MVD of 197.87 per mm^2) showing a case of MOC with an IRS of 6 i.e., positive for CD146 expression.

Photomicrographs 'm' (at low power) & 'n' (at high power with a mean MVD of 231.25 per mm^2) show a case of squamous carcinoma of the vulva with an IRS of 6 i.e., is positive for CD146 expression.

Photomicrograph 'o' (at low power) & 'p'(at high power with a mean MVD of 133.312 per mm^2) showing a case of STIC with an IRS of 3 i.e. Negative for CD146 expression

MOC: Mucinous ovarian carcinoma, MVD: Microvascular density, IRS: Immunoreactivity score, HGEC: High grade endometrial carcinoma, APST: Atypical proliferative serous tumour, STIC: Serous tubal intraepithelial carcinoma

Table 1. Distribution of CD146 expression, MVD, MVC in gynecological cancers and benign controls of the indexed study

Site	Cervical		Endometrial		Ovarian	
Variables	Cancers (n=27)	Benign (n=5)	Cancers (n=10)	Benign (n=5)	Cancers (n=10)	Benign (n=5)
CD 146^{+}	19	1	5	1	8	0
CD 146^{-}	8	4	5	4	2	5
p-value	0.0531		0.5804		0.007	
DA	71.8		50		86.67	
Sensitivity	70		50		80	
Specificity	80		80		100	
PPV	95		83.3		100	
NPV	33.33		44.4		71.43	
LR+	3.52		2.5		-	
LR-	0.37		0.62		0.20	
Mean MVD	14.316±6.64	5.53±2.02	17.83±6.07	12.25±3.88	18.53±8.65	6.73±1.090
p-value	<0.00001		0.0010		0.0001	
Mean MVC (µm)	9.824±4.873	31.8±15.380	6.52±2.311	22.2±10.150	7.1±2.682	13.06±2.300
p-value	0.0001		0.0001		0.0001	

DA: Diagnostic accuracy, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, MVD: Microvascular density, MVC: Microvascular caliber

In ovarian cancers, MVD ranged (Table 1, Figure 2) from 6 to 42 (mean: 18.53±8.653 per 0.16 mm^2), whereas in benign tissue, it ranged from 4 to 12 (mean: 6.73±1.090 per 0.16 mm^2). The p-value (0.0001) indicates significance (p<0.05) based on the Kruskal-Wallis test.

In HGSC, the MVD ranged from 8 to 27 (mean: 18.13±4.793 per 0.16 mm^2). In APST, MVD ranged from 6 to 13 (mean: 9.16±2.595 per 0.16 mm^2). In MOC, MVD ranged from 13 to 42 (mean: 25.66±8.485 per 0.16 mm^2). In LGSC, the MVD ranged from 26 to 35 (mean: 29.66 per 0.16 mm^2). Median MVD per mm^2 were 39.56, 55.78, 185.37, 113.32, and 160.37 for benign, APST, LGSC, HGSC, and MOC, respectively (Figure 2). Among ovarian carcinomas, MVD was lowest in APST and highest in LGSC.

In ovarian cancers, the mean MVC was found to be 7.1±2.682 µm whereas in benign tissue, it was 13.06±2.300 µm as given in Table 1. The two-tailed p-value is less than 0.0001, making the difference extremely statistically significant (calculated by using an unpaired t-test).

Most ovarian cancers were epithelial. Among the ten cases, four early-stage (I-II) and six advanced-stage (III-IV) cases showed positive CD146 expression. CD146 expression increased with higher stages of ovarian carcinoma. MVD was higher in ovarian carcinomas than in benign controls and borderline cases.

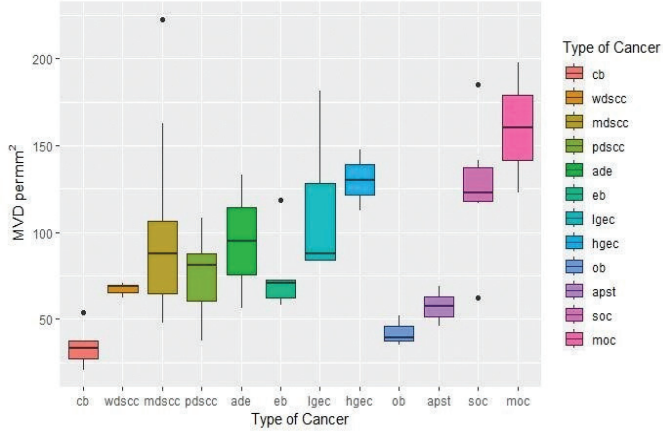

Figure 2. Box and whisker plot showing mean microvascular density in cases and controls of the cervical cancer, endometrial cancer, ovarian cancer

cb: Cervical benign tissue, wdscc: Well differentiated squamous cell carcinoma, mdscc: Moderately differentiated squamous cell carcinoma, pdscc: Poorly differentiated squamous cell carcinoma, ade: Adenocarcinoma cervix, eb: Endometrial benign tissue, lgec: Low grade endometrial carcinoma, hgec: High grade endometrial carcinoma, ob: Ovarian benign tissue, apst: Atypical proliferative serous tumour, soc: Serous ovarian carcinoma, moc: Mucinous ovarian carcinoma

Table 2. A comparison of MVD of common gynecological cancers as depicted in literature along with this indexed study

Author	Study location	Tissue	IHC Marker	Sample Size	Mean MVD	Magnification (Field area)
Index article	India	Cervical cancer	CD146	27	14.316± 6.64	400X (0.16 mm^2)
Liu et al. (2013)[16]	China	Cervical cancer	CD34	56	WDSCC: 30.83±2.98 MDSCC: 43.86±3.92 PDSCC: 54.19±5.36	200X (NA)
Triratanachat et al.[18] (2006)	Thailand	Cervical cancer (SCC)	CD31	60	54.87±25.5	400X (0.085 mm^2)
Index study	India	Endometrial cancer	CD146	10	17.83±6.07	400X (0.16 mm^2)
Kluz et al.[20] (2020)	Poland	Endometrial cancer	CD34	117	19 (Median)	200X
Haldorsen et al.[21] (2013)	Norway	Endometrial cancer	Factor VIII	54	49	250X (0.424 mm^2)
Index study	India	Ovarian cancer	CD146	10	18.53 ± 8.653 per 0.16 mm^2	400X (0.16 mm^2)
Onisim et al.[12] (2019)	Romania	Epithelial ovarian cancer	CD146 & CD34	101	44.73±21.44 by CD146 46.72±22.29 by CD34	400X
Sopo et al.[13] (2020)	Finland	Epithelial ovarian cancers	CD34, CD105 and D2-40	86	340.69±18.5 per mm^2 by CD34 93.94±6.12 Per mm^2 by CD105	200X

WDSCC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma; PDSCC: Poorly differentiated squamous cell carcinoma, MVD: Microvascular density

Miscellaneous

Two cases were included: one vulval carcinoma and one STIC. The vulval carcinoma was CD146 positive (IRS 6) with a mean MVD of 37 vessels per 0.16 mm^2 (Figure 1m-1n). The STIC case was CD146 negative (IRS 4) with a mean MVD of 21.33 vessels per 0.16 mm^2 (Figure 1o-1p), whereas the normal fallopian tube had an IRS of 3 and a mean MVD of 11.66 vessels per 0.16 mm^2.

Discussion

In our study, we observed that epithelial expression of the CD146 marker is a reliable tool for differentiating ovarian cancers from benign controls, exhibiting a sensitivity, specificity, and DA of 100% each. However, in the case of endometrial and cervical cancers, CD146 does not serve as a suitable marker for diagnostic utility. However, MVD on CD146 was significantly different in all forms of gynecological cancers compared with benign controls.

Our study found CD146 expression in 70% of cervical cancers, which is slightly higher than a report of 49% in a study by Zhang et al.[5] In addition, a study on assessment of multiple markers in trophoblastic tumors and cervical cancers reported CD146 expression in 20% cervical carcinoma cases[15]. CD146 expression was higher in cervical cancers but was not statistically significant. Among cervical cancer subtypes, MDSCC showed the highest expression, followed by PDSCC and WDSCC. Liu et al.[16] performed MVD on CD34 and VEGF and observed that expression in SCC progressively increased with the grade of cancer (highest in PDSCC & lowest in WDSCC) and it was found to be statistically significant. Whereas we found that the mean of MVD was highest for MDSCC followed by WDSCC and PDSCC; this difference may be due to the abundance of hemorrhage and necrosis in PDSCC and because we have taken cervical biopsy specimens. Higher vascular density was associated with poorer prognosis in one study, whereas there was no statistical difference in outcomes based on MVD[17,18]. Hu et al.[19] conducted a meta-analysis and suggested that a high count of MVD is associated with poor survival among cervical cancers. MVC was significantly less in cervical carcinoma cases than in benign biopsies.

Our study found CD146 expression in 50% of endometrial cancers, and it has been previously reported as 67%[5]. CD146 expression in endometrial carcinomas was higher in endometrial adenocarcinoma and mucinous compared with that in benign tissues. CD146 was highly expressed in advanced stages (FIGO III-IV), in line with Zhang et al.[5] Mean MVD was higher for endometrial carcinoma compared with that in benign tissue, and within the carcinomas, higher mean MVD was found in HGEC compared with that in LGEC. Similar trends were found in the study by Kluz et al.[20], who also found no relation between FIGO stage of EC and MVD. Haldorsen et al.[21] found that high MVD was significantly associated with reduced recurrence-

free. We found a decreasing trend of MVC in malignant when compared to borderline and benign, and it was significant with a p-value <0.05 but as of our knowledge not much studies have been conducted on MVC in endometrial cancers.

CD146 was significantly more expressed in EOC and MOC than in benign tissue, with a positivity of 80%. Advanced stages of ovarian carcinomas showed increased CD146 expression, but it was not statically significant, in line with Onisim et al.[12] They reported nearly 50% positivity and observed that CD146 can be used as an independent prognostic marker indicating a disease requiring intensive management and increased chances of chemotherapy resistance. They also mentioned that CD146 may be regarded as a novel biomarker of tumor neovasculature and found it to be as reliable as CD34 for MVD estimation. Another study conducted on 133 cases of epithelial ovarian carcinoma found CD146 expression in 65 cases (48.8%). They found that the expression of this molecule was significantly associated with advanced stage (p=0.0001), serous and undifferentiated phenotype (p=0.0004), accumulated p53 (p=0.0002) and residual tumour(p=0.0015)[11]. Our study found a significantly higher CD146 positivity of 80%. High CD146 positivity may suggest a more aggressive form of the disease, which could influence treatment strategies and the need for more intensive management.

Sopo et al.[13] assessed MVD on endoglin and CD34 by IHC and found that MVD was significantly higher in ovarian malignancy and borderline tumors as compared to benign. They also found that high MVD assessed by CD34 had a longer progression-free interval, and this trend was opposite with CD105[13]. In a meta-analysis of 22 studies done on MVD in ovarian cancer, they found that the OS and PFS with high MVD were significantly poorer than with low MVD in ovarian cancer patients[22]. However, high MVD detected by CD34 seems to be more associated with survival in patients without pre-chemotherapy. MVC assessed by CD34 was significantly different between benign and borderline/malignant tumors. We found a decreasing trend of MVC in malignant tumors when compared with borderline and benign tumors, and it was significant with a p-value <0.05. As tubal, vulval, and vaginal are rare gynecological cancers, accounting for less than 5% of all gynecological cancers[1,2], only one case for tubal or vulval cancer could be retrieved. CD146 positivity with high MVD was observed in squamous vulval carcinoma. STIC showed CD146 negativity and high MVD.

Angiogenesis plays a vital role in the growth, metastasis, and progression of invasive carcinomas. Tumors >1 mm require angiogenesis for further growth, leading to metastasis[19,23]. High MVD promotes local tumor propagation and hematogenous spread, worsening prognosis[17]. CD146 demonstrates potential utility as a preoperative marker in biopsy samples[19]. A comparison of MVD of common gynecological cancers as depicted in literature along with this indexed study is depicted in Table 2[12,13,16,18,20,21]. High MVD detected with CD146 can inform surgical decisions, facilitating pelvic node resection in addition to tumor removal. This approach may enhance the precision and effectiveness of surgical interventions in gynecological malignancies. MVD was significantly higher in malignant tissues of cervical, endometrial, and ovarian origin, supporting the role of neo-angiogenesis in malignancy and metastatic potential and eventual poorer survival[24-26]. Vessel size in gynecological cancers of the study was significantly smaller compared with benign controls, regardless of cancer type or grade, aligning with previous studies on complex and intricate vasculature in malignancies[14,16].

CD146 positivity was extensively evidenced in the tumor cell membrane in some cases and in the endothelial cell cytoplasm of microvessels in the tumor stroma. This expression in the tumor cells and stroma opens multiple perspectives regarding the possible implications of this cell adhesion molecule in tumor development, growth, epithelial-mesenchymal transition, and progression. CD146 has also been associated with poor survival in human solid tumors, establishing its role as a prognostic predictive biomarker, but its role as a potential therapeutic target in human solid tumors needs to be further studied[25,26].

Study Limitations

Although the spectrum of gynecological cancers taken was more, the indexed study had a limitation of a small sample size for endometrial and ovarian cancers. CD146 epithelial expression did not differ significantly between benign and malignant tissues among cervical and endometrial carcinomas, whereas it was significant in ovarian carcinomas and borderline tumors as compared to benign tissues.

Future scopes include enhancing CD146-specific detection methods for cervical and endometrial cancers, exploring anti-CD146 therapy for ovarian cancer treatment, and investigating anti-CD146 immunotherapy in combination with traditional treatments as a promising anticancer approach in gynecological cancers[23,27]. This also implies that a combined treatment strategy of anti-CD146 immunotherapy with other traditional chemo- or radiotherapy treatments may be a promising anticancer technique.

Conclusion

To summarize, CD146 epithelial expression was significant in ovarian cancers, and malignant tissues exhibited higher MVD when assessed with CD146 staining, suggesting increased neoangiogenesis. Furthermore, we observed that the vessel caliber is narrower in gynecological malignancies. Overall, CD146 is a promising and reliable marker for assessing MVD in gynecological malignancies. The presence of increased MVD has the potential to enhance clinical management decisions when diagnosing gynecological malignancies. This information could aid in refining treatment strategies and improving patient outcomes.

Ethics

Ethics Committee Approval: This case-control study was conducted in the Department of Pathology and Laboratory Medicine after obtaining ethical clearance from the Institutional Ethics Committee (LOP No IHEC-PGR/2022/STS-ICMR/10, date: 20.06.2022 - All India Institute of Medical Sciences, BHOPAL).

Informed Consent: Waiver of consent was granted because patient identifiers were not used and confidentiality was assured.

Authorship Contributions

Surgical and Medical Practices: U.K., A.H., Concept: A.K., U.K., R.C., N.K., Design: A.K., U.K., R.C., Data Collection or Processing: A.K., U.K., A.H., Analysis or Interpretation: A.K., U.K., N.K., Literature Search: A.K., U.K., R.C., A.H., N.K., Writing: A.K., U.K., R.C., A.H., N.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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A global study on knowledge and perception of HPV and HPV vaccination among young obstetricians and gynecologists

Uluslararası kadın hastalıkları ve doğum asistan hekimleri ile genç uzmanların HPV ve HPV aşısı hakkındaki bilgisi ve algısı

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Abstract

Objective: Our goal is to improve the understanding of human papillomavirus (HPV) and its vaccination among obstetrics and gynecology trainees and young specialists worldwide.

Materials and Methods: This cross-sectional study was conducted through an online survey consisting of 28 questions by the World Association of Trainees in Obstetrics and Gynecology between February and August 2023. The questionnaire collected demographic data of the study participants and assessed the respondents' knowledge and perception of HPV, HPV vaccines, and vaccine dosing schedule.

Results: Two hundred five Ob/Gyn trainees and young Ob/Gyns from 52 countries completed the survey. The majority of respondents were trainees (158, 77.1%). Most trainees and young Ob/Gyns learned about HPV for the first time during medical school (149, 72.6%). Almost all (204, 99.5%) Ob/Gyns responded that HPV was sexually transmitted. More than half of the respondents had not received HPV vaccination (110, 53.7%). The vaccine was recommended for respondents mostly by their Ob/Gyn senior colleagues (110, 53.7%). Most of the respondents knew how to manage HPV-positive patients (179, 87.3%).

Conclusion: This study suggests that even though knowledge on HPV and its vaccination is satisfactory among trainees and young Ob/Gyns, HPV vaccination remains deficient. There is a need to understand, educate, and address the potential problem that lies underneath.

Keywords: Human papillomavirus, vaccination, obstetrics, gynecology, residency

Öz

Amaç: Amacımız, dünya çapında kadın hastalıkları ve doğum asistan hekimleri ve genç uzmanları arasında insan papilloma virüsü (HPV) ve aşılama bilgisi ve anlayışını araştırmaktır.

Gereç ve Yöntemler: Bu kesitsel çalışma, Dünya Kadın Hastalıkları ve Doğum Asistanları ve Genç Uzmanları Birliği tarafından Şubat 2023 ve Ağustos 2023 tarihleri arasında 28 sorudan oluşan bir anket aracılığıyla gerçekleştirildi. Anket ile çalışma katılımcılarının demografik verileri, HPV ve HPV aşılarına dair bilgileri ve anlayışları elde edildi.

Bulgular: Anketi 52 ülkeden 205 kadın hastalıkları ve doğum asistanı ve genç uzmanı tamamladı. Ankete katılanların çoğunluğunu asistan hekimler oluşturdu (158, %77,1). Asistan ve genç uzman hekimlerin büyük bir kısmı HPV'yi ilk kez tıp fakültesi sırasında öğrenmiş (149, %72,6). Katılımcıların neredeyse tamamı (204, %99,5) HPV'nin cinsel yolla bulaştığını yanıtladı. Ankete katılanların yarısından fazlası HPV aşısı yaptırmadığını belirtti (110,

PRECIS: Using a 28-point questionnaire, we have evaluated the human papillomavirus (the most common sexually transmitted infection) and its vaccination knowledge amongst the Ob/Gyn trainees and young specialists globally.

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%53,7). Aşı, katılımcılara çoğunlukla kıdemli meslektaşları tarafından önerilmiş (110, %53,7). Ankete katılanların çoğu HPV pozitif hastaların nasıl yönetileceğini bildiğini belirtti (179, %87,3).

Sonuç: Bu çalışma, kadın hastalıkları ve doğum asistan hekimleri ve genç uzmanları arasında HPV ve HPV aşısı hakkındaki bilgilerinin tatmin edici olmasına rağmen, HPV'ye karşı aşılanma miktarının hala yetersiz olduğunu göstermektedir. Altında yatan potansiyel sorunu anlamaya ve çözmeye ihtiyaç vardır.

Anahtar Kelimeler: İnsan papilloma virüsü, aşılama, kadın hastalıkları, jinekoloji, ihtisas

Introduction

Human papillomavirus (HPV), a double-stranded DNA virus, is the most common sexually transmitted infection worldwide[1]. Over 200 HPV types exist, with types 6, 11, 16, and 18 being the four major types that colonize the human genital tract[2]. Low-risk, non-oncogenic types 6 and 11 are associated with anogenital warts and laryngeal papillomatosis, whereas high-risk, oncogenic HPV types 16 and 18 cause cervical and anal cancers[1-3]. More than 99% of cervical cancers (CC) are attributable to HPV infection[4].

CC is the most common gynecological malignancy in developing countries and the fourth most common cancer in women worldwide, after breast, colorectal, and lung cancers[5,6]. Globally, over 600,000 new cases of CC and more than 340,000 related deaths are reported annually[7]. Approximately 85% of these new cases and 90% of CC deaths occur in low- and middle-income countries (LMICs)[6]. Given the causal relationship between HPV and CC, an effective primary preventive modality against CC is HPV vaccination of pre-adolescent girls before their sexual debut[8]. Three types of HPV vaccines are available: A bivalent Cervarix vaccine that protects against HPV types 16 and 18, a quadrivalent Gardasil vaccine that protects against HPV types 6, 11, 16, and 18, and a nonavalent vaccine that is effective against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58[9].

The World Health Organization currently recommends a single dose of HPV vaccine in girls aged 9-20 years and two doses for women aged 21 years and above[10]. This single dose regimen is comparable to efficacy to the previous two to three dose schedules. The new single-dose regimen is a cost-effective strategy for improving access to HPV vaccines and increasing global HPV vaccine rates, which had only a 13% two-dose completion rate in 2020[10]. This is especially so because cost remains a significant impediment against HPV vaccine uptake, especially in LMICs, where HPV vaccination is yet to be included in national immunization schedules or school vaccination programs, and is thus not freely available to all pre-adolescents in these countries[11]. Poor knowledge and awareness of HPV infections and HPV vaccines have also been identified as a barrier to the uptake of HPV vaccination[8]. Obstetricians and gynecologists (Ob/Gyns) play critical roles in improving awareness and uptake of HPV vaccines among the general female population. Their knowledge of HPV infections and HPV vaccination influences their willingness to recommend HPV vaccination to their clients. Moreover, Ob/Gyns are also at risk of HPV infection and its adverse sequela. Therefore, it is important to assess their knowledge of HPV infections and HPV vaccination. Therefore, this study sought to assess the knowledge of HPV and its vaccination among a global cohort of Ob/Gyn trainees and young specialists.

Materials and Methods

Study Design

This cross-sectional survey investigated the knowledge and perception of HPV and HPV vaccination among Ob/Gyn trainees and young specialists globally. The study was conducted over six months, from February 7, 2023, to August 8, 2023, by the World Association of Trainees in Obstetrics and Gynecology (WATOG). WATOG is an international organization with 85 member countries, representing all Ob/Gyn trainees and young Ob/Gyn specialists who are within 10 years of the start of their residency training. We aimed to receive at least one response from each member country of the WATOG.

Study Instrument

A study-specific questionnaire designed using Google Forms was used in this study. The questionnaire consisted of 28 questions, including multiple-choice, multi-answer, "Yes" and "No", and open-ended questions. The first section of the questionnaire collected demographic data of the study participants, including age, country of origin, country of residency, and training information, including the commencement and completion dates of residency training and specialization. Other sections assessed the respondents' knowledge and perception of HPV, HPV vaccines, and vaccine dosing schedule. The questionnaire was electronically distributed to the study participants through the WATOG Country/National and Regional Representatives.

Statistical Analysis

The data obtained were analyzed using Google Forms.

Ethical Considerations

In line with the Declaration of Helsinki, participation in this study was voluntary. All respondents consented to participate in the study before completing the questionnaire. Collected data were completely anonymized. Ethical approval was not sought for this study because it was a completely anonymous online survey with no patient-related data.

Results

Demographic Characteristics of Participants

Two hundred and five Ob/Gyn trainees and young Ob/Gyns from 52 countries completed the survey. The 52 countries included Albania, Armenia, Australia, Belgium, Bolivia,

Botswana, Brazil, Burundi, Colombia, Democratic Republic of Congo, Dominican Republic, Croatia, Egypt, France, Germany, Greece, India, Iraq, Ireland, Italy, Kazakhstan, Kenya, Kyrgyzstan, Latvia, Malawi, Malaysia, Mexico, Namibia, Nepal, New Zealand, Nigeria, North Macedonia, Pakistan, Peru, Philippines, Poland, Portugal, Romania, Rwanda, Serbia, Sierra Leone, Slovenia, South Africa, Sudan, The Netherlands, Turkey, United Kingdom, Uruguay, United States of America, Venezuela, Zambia, and Zimbabwe.

Respondents were aged between 22 and 43 years, with a mean age of 31.5±3.9 years. Female respondents were 153 (74.6%), while 52 (25.4%) were males. The majority (192, 93.7%) of the respondents had their Ob/Gyn training in their countries of origin. Almost half of the respondents were in their 3rd-4th year of training (91, 44.4%). This was followed by those in their 1st-2nd year of training (50, 24.4%), while others were in their 5th-6th year (46, 22.4%), and 7th-10th year of training (18, 8.8%). The majority of respondents were trainees (158, 77.1%), while young specialists constituted the remaining study participants (47, 22.9%).

HPV and HPV Vaccine Knowledge of the Study Participants

Most trainees and young Ob/Gyns learned about HPV for the first time during medical school (149, 72.6%), whereas more than one-tenth learned about the virus during their Ob/Gyn residency (24, 11.7%). The majority of respondents had learned about HPV during their residency training (176, 85.9%) (Figure 1). More than one-third of those who reported that they had not learned about HPV during residency were first- and second- year residents (10/29, 34.5%). Only two young Ob/Gyn specialists-one from Pakistan and another from Peru- reported that they did not receive lectures on HPV during their Ob/Gyn residency training. One respondent learned about HPV at the International Federation of Gynecology and Obstetrics Vaccination Workshop, which was held in 2022 in Prague, Czech Republic. More than one-tenth (33, 16.0%) of the respondents learned about HPV in high school, elementary school, from family (parents were either Ob/Gyn or urology specialists), a family doctor, social media, the internet, or a friend. One trainee from New Zealand learned about HPV for the first time while being vaccinated in primary school as part of his country's national vaccination program.

Almost all (204, 99.5%) Ob/Gyns responded that HPV was sexually transmitted; only one respondent thought it was transmissible via blood. The reported clinical features of HPV infection wereas follows: Asymptomatic (92, 44.8%), genital warts (86, 41.9%), postcoital bleeding (17, 8.2%), vaginal discharge (17, 8.2%), irregular vaginal bleeding (13, 6.3%), dyspareunia (6, 2.9%), vulvar itching (5, 2.4%), advanced disease/cancer (4, 1.9%), abnormal Pap smear (3, 1.4%), cervicitis (2, 0.9%), genital rash (1, 0.4%), and herpes ulcer (1, 0.4%).

More than half (144, 70.2%) of the respondents learned about HPV vaccination at the same time they learned about HPV. Only four (2.0%) respondents- one trainee each from Belgium, Portugal, Turkey, and the United States of America- learned about HPV vaccination before learning about HPV. They had learned about HPV vaccination while in high school from their general practitioners and pediatricians and received the vaccination before learning more about the virus later on. Most of the respondents reported that the ideal age to initiate HPV vaccination was between 9 and 14 years (126, 61.5%) or before sexual debut (33, 16.1%). The majority (168, 82.0%) responded that even though a person had previously contracted HPV, they could still be vaccinated. Three-fourth (155, 75.6%) of the respondents opined that the vaccine had no adverse effects (Figure 2), while 63, 30.7% believed that the vaccine had no contraindication (Figure 3).

HPV Vaccination Status of the Study Participants

More than half of the respondents had not received HPV vaccination (110, 53.7%) (Figure 4). Those who had been vaccinated (95, 46.3%) had received varying doses of the vaccine, from single to full doses, of either a quadrivalent or nonvalent vaccine, initiated between the ages of 10 and 30 years. Of those who were not fully vaccinated, three started their vaccinations in 2023. Two of these were from Turkey, where the nonavalent vaccine became available in 2023, and one from Romania, where the HPV vaccine is excluded from the national

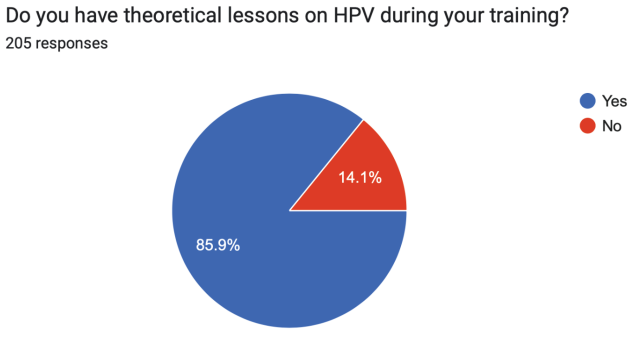


Figure 1. Theoretical lessons on HPV during residency

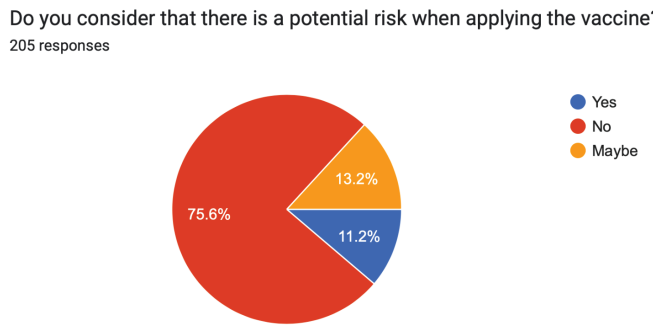


Figure 2. Risks of HPV vaccination

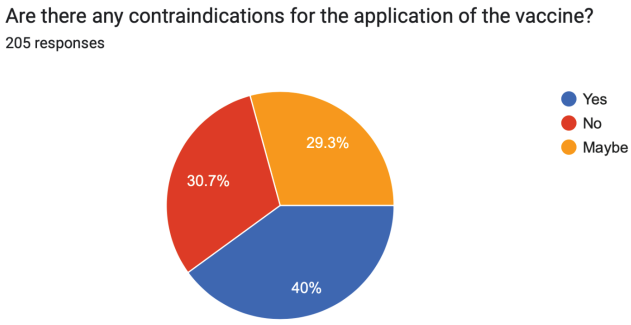


Figure 3. Contraindications to HPV vaccination

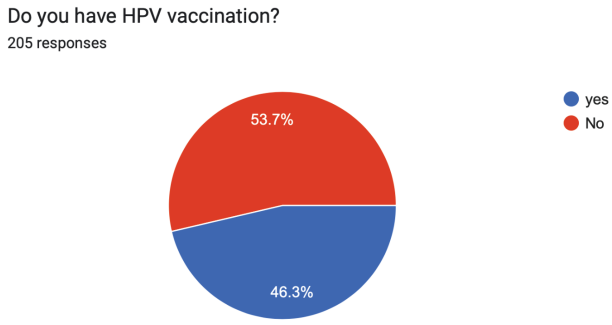


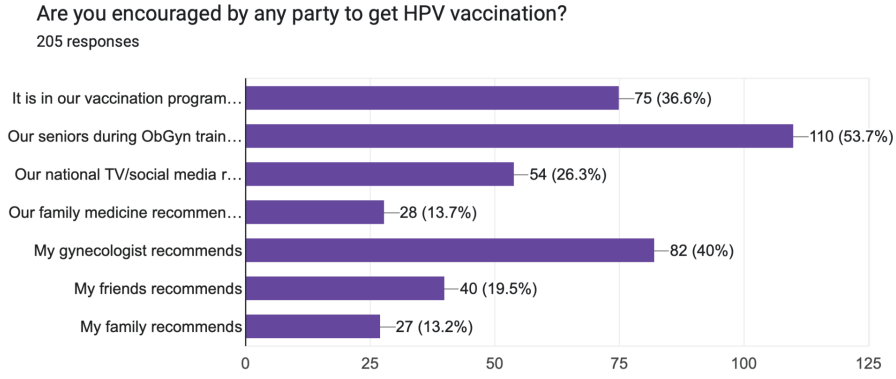
Figure 4. Rate of HPV vaccination among respondents

vaccination programs of both Turkey and Romania. Four other respondents who were not fully vaccinated were from Albania, Kenya, the Philippines, and Turkey. They had each received only a single dose of the vaccine at the ages of 27, 36, 28, and 26 years, respectively. The majority of the respondents (39/95, 41%) received the vaccine in their third decade of life, whereas 31 (32.6%) received it in their second decade of life and 15 (15.7%) in their fourth decade of life. Ten (10.5%) respondents did not remember when they took the vaccine. All respondents volunteered that they had been vaccinated following the recommendation. The vaccine was recommended for respondents mostly by their Ob/Gyn senior colleagues (110, 53.7%), while 82, 40.0% received recommendations from their gynecologists. Others were vaccinated because the vaccine was recommended by their national immunization guidelines (75, 36.6%), family and friends (67, 32.7%), mass/social media (54, 26.3%), or family medicine doctors (28, 13.7%) (Graphic 1). Only eight (3.9%s) respondents did not know where HPV vaccination could be obtained in their countries of residence. A few had never tested for HPV (6, 2.9%), whereas 13 (6.3%) had previously tested positive for a type of HPV.

Prevalence of HPV Risk Factors Among the Study Participants, Screening, and Treatment

Most (186, 90.7%) respondents were non-smokers. The majority of them had multiple (two or more) sexual partners (120, 58.5%), with the number of sexual partners ranging from zero to 20.

A few (9, 4.3%) respondents reported that their countries had no routine Pap smear screening programs (no program in Albania, Kyrgyzstan, Malawi, Pakistan and Sudan; annual program but primary health care services are not sufficient in Venezuela; no regular screening in Austria but a respondent reported screening every 2-3 years in Austria), while in most countries (113, 55.1%) Pap smear is done every three years if not done with HPV screening. Three of six respondents from South Africa replied that routine Pap smear testing in their country is performed every 10 years. The majority of respondents reported that HPV screening was routinely performed in their countries every three to five years (124, 60.4%), while in some, it was not routinely performed (53, 25.8%). Most of the respondents knew how to manage HPV-positive patients (179, 87.3%).



Graphic 1. HPV vaccination encouraging parties

Discussion

Ob/Gyns generally have good knowledge of HPV and its vaccine, having learned about these in medical school and during their Ob/Gyn residency training[12-14], as seen in our study. Ob/Gyns' level of knowledge of HPV and HPV vaccination influences their willingness to recommend the vaccine to clients[12], and recommendation by a healthcare provider (HCP) has consistently been identified as a significant driver of HPV vaccine uptake[15,16]. This was also demonstrated in our study, where all the respondents volunteered that they had received the vaccine following recommendation, mostly by an Ob/Gyn senior colleague, their gynecologist, or family physician. In addition to recommending the vaccine, Ob/Gyns play crucial roles in educating, informing, and answering clients' questions about the vaccine, as well as correcting the misconceptions and misinformation that fuel vaccine hesitancy[16,17]. For these to be effectively performed, Ob/Gyns must have very sound knowledge of the virus and the vaccine. Adequate education and training of HCPs, including the Ob/Gyns, on HPV, the vaccine, and its importance is an important step toward eliminating CC. Medical school curricula must include modules on HPV and the HPV vaccine, its immunology, benefits, and side effects[17]. This is even more so because more than 70% of our study participants learned about HPV for the first time during medical school.

Despite the widespread knowledge of HPV and HPV immunization among the Ob/Gyns in our study, more than 50% of them had not been vaccinated. This was although the majority of them had multiple sexual partners, a prominent risk factor for HPV infection[11]. Similar poor attitudes, perceptions, and uptake of HPV vaccine among Ob/Gyns have been reported by other authors[18,19]. Many unvaccinated Ob/Gyns express concerns over the safety and efficacy of the vaccine, and these concerns constitute a barrier to their recommending the vaccine to their patients[20,21]. This is despite the proven efficacy and safety of the HPV vaccine[22,23]. This further emphasizes the need for targeted educational interventions and further research to understand and address these unfounded concerns, beliefs, and misconceptions held by some Ob/Gyns. This becomes even more important as most of the respondents in our study reported that they took the vaccine following the recommendation of their Ob/Gyn colleagues. Brennan et al.[20] reported that Ob/Gyns were more likely to recommend the HPV vaccine to their patients if they believed/perceived that their other Ob/Gyns were also recommending the vaccine. Tackling the negative perceptions of Ob/Gyns about the HPV vaccine will, therefore, potentially increase the coverage and uptake of the vaccine, both among ob/gyns at individual and collective levels, as well as among the general population.

The majority of our study respondents agreed that the ideal age for HPV vaccination was before becoming sexually active, even though most of them received the vaccine in their third decade of life. The primary target population for HPV vaccination is girls aged 9-13 years before their sexual debut[23]. If administered in this age group, the vaccine is more than 99% effective for preventing HPV infection, and achieving a vaccine coverage rate of >80% in girls reduces the risk of HPV infection in boys[22,23]. Therefore, unvaccinated women up to the age of 45 years should receive the vaccine irrespective of sexual activity or prior exposure to HPV[22]. Most of the Ob/Gyns in our study agreed with this recommendation. Our study also found that most of the participants had yet to complete their vaccine doses. The low completion rate of the multiple HPV dosing schedule prompted the current single dose recommendation to increase global HPV vaccine uptake[10].

Study Limitations

This study is limited by its descriptive nature and convenience sampling technique, which predisposes the subjects to selection bias. The small sample size may also make generalization of the study findings difficult. The study did not explore the reasons for the high non-vaccination rate reported, and details of the concerns of the unvaccinated respondents. These limitations aside, to the best of our knowledge, this is the first study investigating the understanding and awareness of HPV and its vaccination among Ob/Gyn trainees and young specialists worldwide. This study provides crucial data to inform interventions and policies aimed at enhancing global understanding of HPV, and increasing HPV vaccine coverage and uptake rates. Further larger-scale research can build on the valuable groundwork that our study provides.

Conclusion

HPV vaccine uptake among Ob/Gyns globally remains poor despite their good knowledge of HPV and HPV vaccination. There is a need for targeted educational interventions and further research to better understand and address the concerns of unvaccinated Ob/Gyns. Addressing these concerns will potentially improve vaccine recommendation by these ob/gyns, an important driver of vaccine uptake, and increase global HPV vaccine coverage and uptake rates.

Ethics

Ethics Committee Approval: Ethical approval was not sought for this study because it was a completely anonymous online survey with no patient-related data.

Informed Consent: All respondents consented to participate in the study before completing the questionnaire.

Authorship Contributions

Surgical and Medical Practices: E.G.T., Concept: E.G.T., Design: E.G.T., A.E.U., P.R., F.R., Data Collection or Processing: E.G.T., Analysis or Interpretation: E.G.T., A.E.U., P.R., F.R., Literature Search: E.G.T., A.E.U., P.R., F.R., Writing: E.G.T., A.E.U., P.R., F.R.

Conflict of Interest: No conflict of interest was declared by the authors.

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