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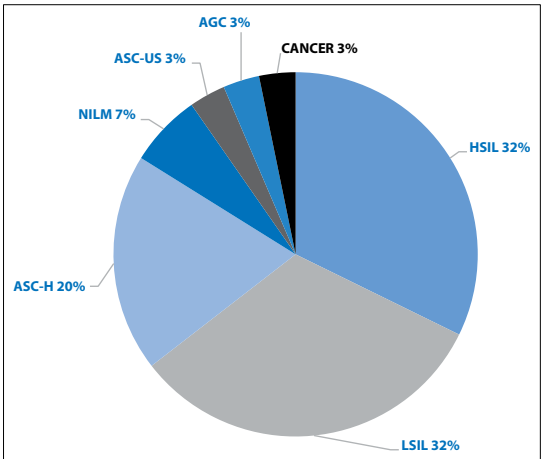

LSIL — low-grade squamous intraepithelial lesion; HSIL — high-grade squamous intraepithelial lesion; AGC — atypical glandular cells; ASC-US — atypical squamous cells of undetermined significance; ASC-H — atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; NILM — negative for intraepithelial lesion or malignancy

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Polskie Towarzystwo Ginekologów i Położników oraz Grupa Organizacyjna Sekcji Diagnostyki Prenatalnej Polskiego Towarzystwa Ginekologów i Położników

zawiadamiają o zwołaniu

Walnego Zgromadzenia Sekcji Diagnostyki Prenatalnej
Polskiego Towarzystwa Ginekologów i Położników

które odbędzie się w dniu **25.03.2022 (piątek)**

o godzinie 13.50 (I TERMIN) lub
o godz. 14.00 (II TERMIN)

w Vienna House Easy Katowice, Sokolska 24, 40-086 Katowice

Zakończenie Walnego Zgromadzenia
Sekcji Diagnostyki Prenatalnej Polskiego Towarzystwa Ginekologów i Położników planowane jest na godz. **15.00**

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Triple negative phenomenon in endometrial cancer: recognition criteria and impact on survival

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ABSTRACT

Objectives: Endometrial cancer is the most common malignant cancer of female reproductive organs. The number of diagnosed cases of endometrial cancer is increasing from year to year. Endometrial cancer is a neoplasm with a good survival rate. However, there are also cases with a fast, aggressive course. In recent years, the triple negative phenomenon (TNP) has been identified as one of the factors determining shorter survival in patients with endometrial cancer.

Material and methods: The study covered 265 patients with histopathologically confirmed endometrial cancer. Patients were divided into two groups: 1) patients with endometrial cancer with TNP; 2) patients with endometrial cancer without TNP.

Tissue microarrays (TMA) were examined with immunohistochemistry to evaluate the expression of estrogen, progesterone and HER2 receptors. In several cases FISH method was used to assess HER2. The expression was evaluated by computer image analysis using the Nuclear Image Analysis virtual microscopy system. The evaluation of HER2 expression was performed manually. The criterion for TNC diagnosis was H-Score < 50 or < 75 and Allred score < 4.

Results: Depending on the scoring system used, TNP was found in from 10.19% to 15.09% of cases. Regardless of the criteria employed in endometrial cancer, the presence of TNP was neither a factor increasing the risk of death nor it affected the patients' survival.

Conclusions: The proportion of TNP diagnosed in endometrial cancer depends on the examined population and the diagnostic criteria. The incidence of TNP did not affect the survival of patients.

Key words: endometrial cancer; triple negative endometrial cancer; triple negative cancer; H-Score; Allred Score; triple negative phenomenon

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INTRODUCTION

Endometrial cancer is the most common female genital cancer in the developed countries [1]. According to the WHO (World Health Organization) report, the number of reported new cases of endometrial cancer was 327,259 in 2018, an increase of 7,659 new cases compared to the previous report published in 2012 [2].

Similarly, to the estrogen receptor (ER), the positive expression of progesterone receptor (PR) is associated with increased survival in endometrial carcinoma [3, 4]. In 2013, a multicentre study was conducted which showed reduced survival in women with endometrial cancer in the absence of PR and ER expression [5]. The lack of PR expression is also associated with a higher risk of relapse of the neoplastic process [6]. The degree of ER expression is considered as an independent prognostic factor. Reduced survival in endometrial cancer patients occurred in the absence of ERα expression [7]. Patients showing no ERα expression were diagnosed with higher neoplastic grading and higher stage of neoplastic progression [8].

The amplification of the HER2 gene, which results in an increased amount of HER2 protein, is characteristic of the second type of endometrial cancer, the so called non-estrogen-dependent endometrial cancer. This concerns about 17–30% of cases [9]. It was found that endometrial cancers with positive expression of HER2 receptor have a more aggressive clinical course [10]. Although HER2 gene amplification is characteristic for type II endometrial cancers, some authors confirm the fact that HER2 positive expression is a prognostic factor for type I endometrial cancers [11, 12].

The triple negative phenomenon (TNP) was first described in association with breast cancer. TNP means the lack of ER, PR and HER2 expressions in neoplastic tissue. As regards breast cancer, the triple negative phenomenon

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has been thoroughly investigated. Triple negative cancers (TNCs) represent about 10–20% of all breast cancers and lead to worse outcomes. The authors of the study cited above suggest a better prognostic value of HER-2 overexpression than that of PTEN [13].

It was not until 2010 that TNP was described in reference to a cancer different than breast cancer. It was found that the percentage rate of endometrial TNCs was 26%. They were at a more severe clinical stage at the time of diagnosis and were accompanied by the presence of lymph node metastases, deeper uterine infiltration and an unfavourable histopathological type, i.e., clear-cell or serous carcinoma. There is a limited number of reports of triple negative endometrial cancer [14–19].

Objectives

The purpose of this study was to assess the percentage of endometrial carcinomas with TNP and the survival odds for patients with endometrial cancer with TNP.

MATERIAL AND METHODS

The study group consisted of 265 women with histopathologically confirmed diagnosis of endometrial cancer who underwent surgery between 2004 and 2016.

The patients were divided into two groups:

1. Patients with endometrial cancer with TNP
2. Patients with endometrial cancer without TNP

The observations were terminated on 9 October 2018. On the last day of the observation, the total of 53 patients was reported to have died. In 26 cases it was not possible to obtain information concerning the exact date of death. Data on patients' deaths were collected from the register of deaths run by Department of State Systems of the Polish Ministry of Digitization.

For the purpose of prognosis assessment, the overall survival (OS) was defined as the time from surgery to the end of observation or to the patient's death.

The research project received a favourable opinion by the Bioethical Commission of the Pomeranian Medical University in Szczecin no. KB-0012/01/01/2015 of 07 January 2015.

The description of the study group is shown in Table 1.

The study was conducted on tissue microarrays (TMA) made of paraffin blocks including material retrieved for the purpose of routine histopathological tests from post-surgery preparations fixed in 10% formalin solution and embedded in paraffin. TMA is a multi-stage technique. First, the representative locations were selected from the original histopathological specimen stained with haematoxylin and eosin. There were three representative locations of tumour metastasis. In the second stage cylindrical tissue samples of 0.6 mm in diameter were extracted from the places in the donor paraffin block that correspond to the places marked in the histopathological preparation and placed in the pre-drilled recipient block. Subsequently, the recipient block was embedded in paraffin. In the final stage, the block was cut into 4-µm-thin segments that are subject to immunohistochemical examination or to fluorescent in situ hybridization (FISH) [20, 21]. A large number of fragments of various preparations in one recipient block formed a map representing a position of each preparation. The array of these fragments in the recipient block needed to be precisely spaced so that each sample can be unambiguously identifiable.

In this study the immunohistochemical protocol was used to assess the expression of ER and PR. The process of immunohistochemical staining by means of the EnVision™ FLEX Dako set consisted of several consecutive stages. First, the slides obtained from the paraffin blocks with the microarrayed tissue was deparaffinised for 60 minutes in a dry oven at 58°C. Then the antigen was deparaffinized by hydrothermal pressure at 120°C with the retrieval buffer En Vision™Flex Target Retrieval Solution, high pH. Subsequently, the endogenous peroxidase was blocked for five minutes by means of En Vision™Flex Peroxidase-Blocking Reagent. For another five minutes the slides were immersed in the (En Vision™Flex Wash Buffer). In the following stage the slides were incubated with primary antibody. The used antibodies were anti-estrogen receptor (clone 1D5, 1:50 solution, Dako) and anti-progesterone receptor (clone PgR 636, 1:50 solution; Dako). The incubation time was 20 minutes. Next, the slides were immersed in the buffer for another five minutes followed by 20-minute incubation with the polymer (EnVisionTM FLEX /HRP). The last buffer wash took five minutes. Then the staining procedure with EnVisionTM FLEX DAB+ Chromogen took place for 10 minutes followed by a 10-minute bath in de-ionized water. One of the last stages was the immersion in haematoxylin staining (EnVisionTMFLEX Hematoxylin) for another 10 minutes, followed by 10 minutes of washing the slides in tap water. Finally, the slides were dehydrated by immersing the slides in several isopropyl alcohols of strength growing gradually from 70% to 98% and in several xylene solutions of varying concentration. In the last step, the slide was protected with a coverslip and a commercial antifading mountant (Dako Mounting Medium).

HER-2 expression was tested with HercepTest (Dako). With HER-2 expression value at 2+, an additional staining of FISH was performed in order to determine the HER-2 gene amplification. FISH is performed in several stages. It starts with cutting the paraffin block into 4-5µm thin segments that are mounted on microscope slides. In this study the test was performed according to a standard HER-2-dedicated FISH protocol recommended by the manufacturer. The LSI

Table 1. Study group description

Parameters under evaluation		N	Number	%
Age at time of cancer diagnosis	Mean	265	63.21	–
	SD		9.73	–
	Median		62	–
FIGO and WHO histological type	Endometrioid carcinoma	265	254	95.85
	Mucosal carcinoma		0	0
	Serous carcinoma		4	1.51
	Clear-cell carcinoma		3	1.13
	Neuroendocrine neoplasm		1	0.38
	Mixed-cell adenocarcinoma		1	0.38
	Undifferentiated carcinoma		2	0.75
Grading	G1	265	144	54.34
	G2		92	34.72
	G3		29	10.94
FIGO clinical stage	IA	265	155	58.49
	IB		68	25.66
	II		24	9.06
	IIIA		15	5.66
	IIIB		0	0
	IIIC 1		1	0.38
	IIIC 2		0	0
	IVA		1	0.38
	IVB		1	0.38
BMI	< 25,0 normal weight	240	47	19.58
	(25–29.99) overweight		71	29.58
	(30–34,99) 1^0 obesity		68	28.33
	(35–39,99) 2^0 obesity		34	14.17
	(> 40) 3^0 obesity		20	8.33
Hypertension	Absent	256	96	37.5
	Present		160	62.5
Type II diabetes mellitus	Absent	256	203	78.9
	Present		53	21.1

SD — standard deviation; FIGO — International Federation of Gynecology and Obstetrics; WHO — World Health Organization

HER-2/neu and CEP17 probes from PathVysion HER-2 DNA Probe Kit (Abbott Laboratories) were used. Fluorescent in situ hybridisation is a technique where tissue is treated with proteolytic enzymes (here: proteinase K) in order to destroy proteins constituting the cell membrane. Tissue is covered with the DNA probe solution being a mixture of two molecular probes. The HER-2/neu (LSI — Locus Specific Identifier) probe, which is rendered orange with fluorochrome, binds to the HER-2 gene sequence while the second probe (CEP — Chromosome Enumeration Probe), rendered green, binds to the complementary area of chromosome 17 centromer. The samples were then denaturised and hybridized. Cell nuclei were identified by means of DAPI stain. The hybridisation effects were assessed under the fluorescence microscope. The relation between LSI (HER-2/neu) probe and CEP17 probe was quantified by the so called HER2/CEP17 ratio.

The H-Score is a semi-quantitative method for assessing the intensity of protein or receptor expression and the quantity of cells indicating individual degrees of expression. The score gives a range from 0 to 300. In case of the H-Score two cut-off values were used based on literature data. According to McCarty et al. [22], the ER expression is regarded positive when the H-Score is equal to and higher than 75. McCarty et al., criteria were also used by other authors [23]. However, according to Thinke et al. [24], the H-Score below 50 is con-

Table 2. Criteria for TNP diagnosis

Grading scale	Receptor negative expression criterion	Criteria for TNC diagnosis
H-Score	≤ 50	PR and ER ≤ 50 by H-Score and rec. HER-2 negative
H-Score	≤ 75	PR and ER ≤ 75 by H-Score and rec. HER-2 negative
Allred	≤ 4	PR and ER ≤ 4 by Allreda and rec. HER-2 negative

TNC — triple negative cancer; PR — progesterone receptor; ER — estrogen receptor

sidered negative for ER or PR expression. It is noteworthy that a threshold below 75 was described in the studies on breast cancer and endometrial cancer, as opposed to a threshold below 50 which was described solely for breast cancer [22–24]. Therefore, two cut-off values of the negative ER and PR expression were adopted for the H-Score: below 50 and below 75.

The eight-grade score by Allred et al. [25], was also used to identify ER and PR expressions. The score from 0 to 6 was used to describe the proportion of tumour cells showing positive nuclear staining — A (0 = none; 1 ≤ 1%; 2 = 2–9%; 3 = 10–33%; 4 = 34_66%; 5 > 66%) while the score from 0–4 represented the staining intensity — B (0 = none; 1 = weak; 2 = moderate; 3 = strong). The result was described as the sum of A + B = TS (Total Score). The original system used by Allred distinguished two categories: no expression when TS equalled 0–2 points, and positive expression at TS between 3–8 points. Regarding endometrial cancer, Gottwald et al., used modified TS scales for the Allred score. They assumed that for a score of 0 points — no expression was found; at 2–4 points the expression was considered weak, while at 5–8 points the expression was seen as strong [26]. Given the literature data on the percentage of endometrial cancers showing TNP, the following interpretation was adopted for the purpose of this study: the number of points equal to or below 4 — no expression of ER and PR, while the points above 4 — a positive expression.

The HER2 expression was interpreted according to standard criteria. In the absence of recommendations concerning HER2 determination in endometrial cancers, the recommendations for interpreting HER2 test results for breast carcinomas were followed [27]. In cases of doubt (HER-2 was at the level of 2+), FISH was performed with the purpose of determining the number of gene copies. Depending on the scale used to assess PR and ER expression, the criteria outlined in Table 2 were adopted for diagnosing the triple negative phenomenon.

The tissue microarray preparation was scanned with Aperio Cs scanner. The proportion of cells showing a positive response was evaluated using computer image analysis by means of the Nuclear Image Analysis virtual microscopy system. The process was made possible due to the application of algorithms (Image Score Version 11.2.0.780) evaluating the intensity of immunohistochemical reactions and their number. The evaluation of HER2 expressions was done manually.

Table 3. Percentage of TNC by type of scale

	Number	%
TNC at H-SCORE < 75		
Absent	225	84.91
Present	40	15.09
TNC at H-SCORE < 50		
Absent	236	89.06
Present	29	10.94
TNC at Allred Score		
Absent	238	89.81
Present	27	10.19

TNC — triple negative cancer

The normality of distributions of all variables was checked by Kolmogorov-Smirnov test. The statistical relationships between discontinuous variables were examined using Pearson's $\chi 2$ test. The survival analysis was employed to assess the overall survival. The results were presented with Kaplan-Meier curves. In order to decide which factors increased the survival odds, the Cox regression model was used. The results were described with HR (Hazard Ratio) with 95% confidence interval and probability. The differences were considered statistically significant when their probability was lower than 0.05.

RESULTS

Depending on the score system used, TNP was found in 10.19–15.09% of cases. The results are listed in Table 3. Depending on the criteria adopted for the diagnosis, the proportion of deaths varied from 25.71% to 29.17% of patients with TNC. Although there were discrepancies in the death rates of patients with and without TNP, no statistically significant differences were found. The proportions of deaths by the criteria adopted for the TNP diagnosis are outlined in Table 4. In the next stage of the study, the analysis of survival was performed using Kaplan-Meier curves in relation to the TNP incidence, depending on the applied diagnosis criterion. The results are givenin figures 1–3. Further analysis by the Cox regression method failed to provide the answer whether TNP was or was not a risk factor leading to the death of patients, regardless of

Table 4. Comparison of mortality rate by incidence of TNP and score type

Endometrial carcinoma (whole population under study)					
	Death –		Death +		p
TNP by H-Score < 75					
Absent	160	78.43%	44	21.57%	0.58545
Present	26	74.29%	9	25.71%	
TNP by H-Score < 50					
absent	169	78.60%	46	21.40%	0.38474
present	17	70.83%	7	29.17%	
TNP by Allred Score					
absent	169	78.24%	47	21.76%	0.63482
present	17	73.91%	6	26.09%	

TNP — triple negative phenomenon

the adopted diagnostic scoring system. The results are given in Table 5. The presence of TNP, irrespective of the criteria applied in endometrial cancer, was neither a factor increasing the risk of death nor it affected the patients' survival.

DISCUSSION

The studies describing TNP in endometrial cancer are scarce. Three research teams adopted the following criteria for determining the ER and PR status: the expression at the level 3+ was assumed to be the strong or weak expression of 50% or more of all cells, while the expression at the level 2+ was assumed to be the strong or weak expression in the range of ≥ 50% and ≤ 10% of all cells. The results classified as 1+ or 0 were considered negative, whereas 1+ meant strong or weak expression of less than10% of all cells.

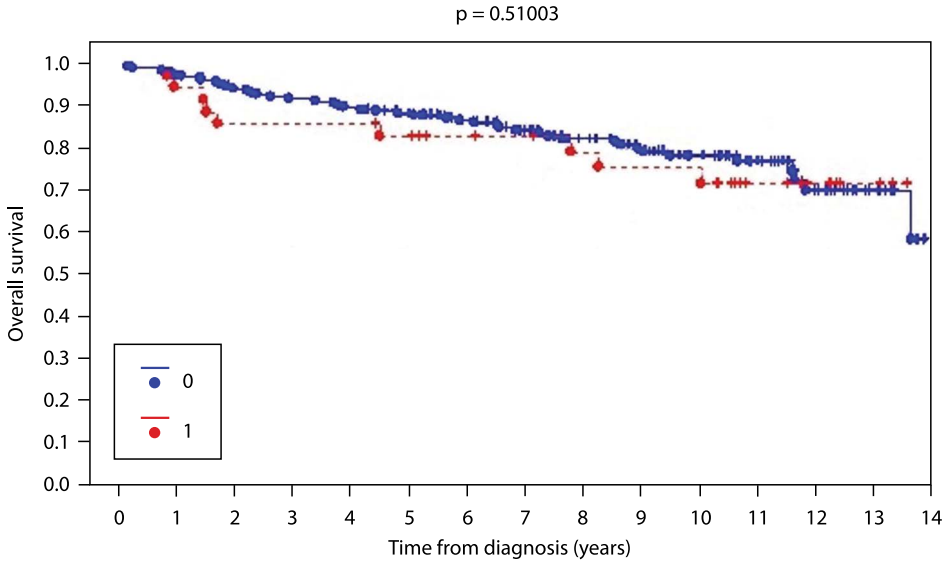

Figure 1. Kaplan-Meier survival curve in relation to TNP presence according to criteria of H-Score < 75; 0 — absent; 1 — present

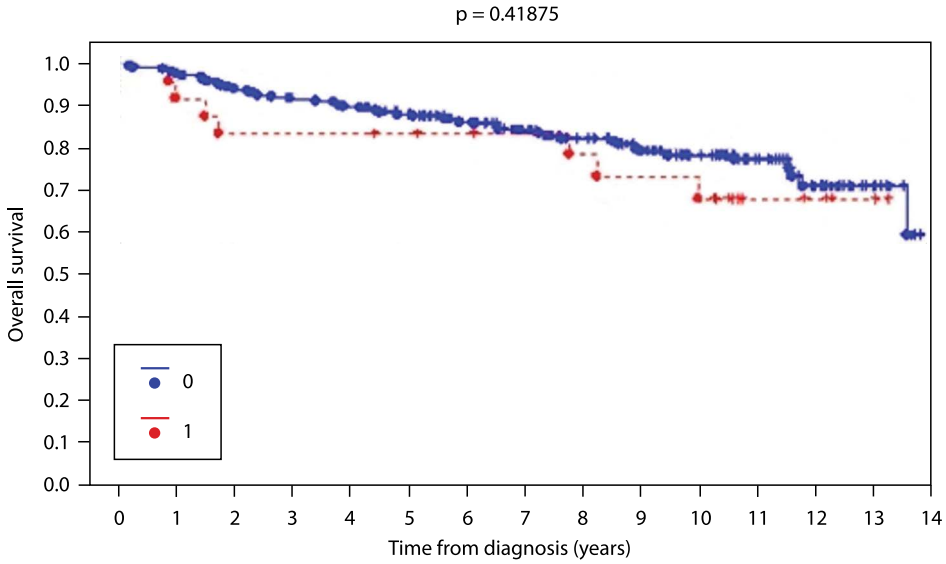

Figure 2. Kaplan-Meier survival curve in relation to TNP presence according to criteria of H-Score < 50; 0 -- absent; 1 -- present

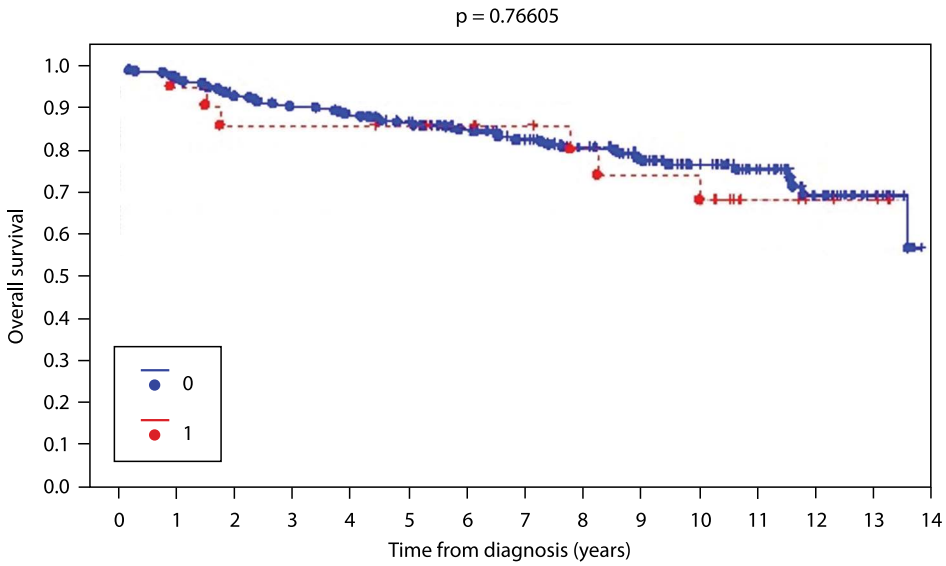

Figure 3. Kaplan-Meier survival curve in relation to TNP presence according to criterion of Alfred score; 0 -- absent; 1 -- present

Table 5. TNP – death risk factor – Cox regression analysis

Risk factors	HZ	95%	CI	p
Incidence of TNP by H-Score < 75	1.10	0.54	2.27	0.787
Incidence of TNP by H-Score < 50	1.27	0.57	2.82	0.561
Incidence of TNP by Allred Score	1.09	0.47	2.57	0.836

TNP — triple negative phenomenon; CI — confidence interval

The absence of receptor expression was defined as 0 [14, 16, 18]. It should be noted that the results of the presented score differ greatly when converted to the H-Score. Basing on the assumption that the result of below 10% of cells with positive weak or strong expression converted to the H-Score was between 10 and 30 and that all other cells did not show any expression, Voss et al. maintained that a H-Score below 150 translated to negative ER or PR expression [15].

In all the existing publications on endometrial cancer with TNP, the criteria for determining the HER2 receptor status were based on the standard adopted for breast cancer [27].

In this paper different criteria were adopted for the identification of a negative status of steroid receptors. Two scoring systems were used: H-Score and Allred score.

The Allred score is generally employed to determine the status of steroid receptors in order to diagnose the triple negative breast cancer [28]. It seemed, therefore, appropriate to examine the criteria for diagnosing TNP in breast cancer in reference to endometrial cancer.

The H-Score was the most accurate scoring system for evaluating PR and ER expressions in digital image analysis which allowed for precise determination of the percentage of cells showing particular degrees of receptor expression. In this study a decision was made to adopt two thresholds when classifying expressions of steroid receptors by means of the H-Score. The cut-off threshold of < 75 stemmed from including all cases considered negative [22]. The cut-off threshold of < 50 resulted from the assumption that the group was composed exclusively of the cases with negative status of steroid receptors [24]. It is worth noting that the above-mentioned criteria were applied to endometrial and breast cancer in the former case, while in the latter case — only to breast cancer [22–24].

When calculated according to the criteria quoted in the literature, the percentage of cancers with TNP ranged from 12% to 26%. The discrepancies in results may have been caused by specific characteristics of the examined populations. Each study was conducted on a different continent. In a study covering the European population, the percentage of endometrial cancers with TNP was 12% [18]. In one publication, the percentage of endometrial cancers showing TNP was not determined [15]. In the present study the percentage of triple negative cancers ranged from 10.19% to 15.09%. When adopting the criteria of diagnosis according to the Allrad score, the proportion of TNP was 10.19%. In the case of the H-Score < 50 it reached 10.94%, while according to the H-Score < 75 it was even higher at 15.09%. If the criteria used most often in the literature were adopted, in the population under study the percentage of endometrial cancers with TNP would range between 5.24 and 8.61% [14, 16, 18]. When adopting the TNP diagnosis criteria proposed by Voss et al., the percentage of triple negative endometrial cancers would amount to 37.09%. In view of vast

discrepancies among the above outcomes, it is necessary to adopt unified criteria for the diagnosis of TNP, as in the case of triple negative breast cancer.

Breast cancer with TNP is burdened with poor prognosis [29]. Similar observations were made for triple negative ovarian cancer [30]. This type of cancer occurs more frequently in young patients and is characterized by an aggressive course. The present study found that the incidence of TNP was correlated with older age of patients at the time of diagnosis. Data concerning the age of patients diagnosed with endometrial cancer with TNP are contradictory.

The results of one study turned out to be consistent with the results of the present study, while other authors did not show such a correlation [16, 18]. However, those authors revealed the relationship between poorer survivals of patients with TNP endometrial cancers. This relationship was not confirmed in this study, irrespective of the criteria used to diagnose TNP. It should be noted that shorter survival was correlated with the presence of TNP, regardless of the organ affected by the disease. It is worth noting, however, that the number of publications on survival in tumours of organs other than breast cancer is limited.

In this study, the system of digital image analysis was used to analyse the degree of expression of individual receptors, with the exception of the HER-2 receptor expression which was assessed manually according to the applicable criteria [27]. Significant subjectivity was observed in the assessment of receptor expression in the same histopathological preparation performed by several pathologists. In order to eliminate the above problem, programmes to count cells showing a positive response to the immunohistochemical staining reaction were developed. Endometrial cancer research has already used digital image analysis before [31, 32]. For years numerous research teams have studied the variables relating to the morphology of cell nuclei in endometrial cancer [32, 33]. Paulik et al. [34], used digital image analysis to study the degree of PR and ER expressions and the cellular nucleus parameters in breast cancer.

CONCLUSIONS

The following conclusions were reached when verifying the research hypotheses put forward for the purpose of this study:

The percentage of TNP diagnosed in endometrial cancer depends on the population tested and the criteria adopted.

The survival of patients with endometrial cancer did not depend on the presence of TNP.

Conflict of interests

None.

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Remission of HPV infection after LEEP-conization — a retrospective study

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ABSTRACT

Objectives: Human papillomavirus (HPV) infection is one of the most common sexually transmitted diseases. Long-term exposure to the HPV is a known cause of squamous intraepithelial lesions that consequently lead to cervical cancer development. The loop electrosurgical excision procedure (LEEP) conization is an established early cervical cancer treatment method. We aim to assess the remission of HPV infection after LEEP in non-vaccinated patients with pre-cancerous cervical lesions and establish the efficacy of cervical cancer prophylaxis.

Material and methods: We analyzed 31 LEEP conizations performed due to low and high-grade squamous intraepithelial lesions in 2019–2020. We obtained molecular test samples and detected DNA of 37 different HPV genotypes. After a six-month follow-up, each patient underwent subsequent high-risk HPV testing and genotyping.

Results: We observed that 54.8% of qualified patients were infected with HPV 16. We discovered complete viral remission in 64.5% of cases. After surgery, margins were negative in 71% of the patient's samples. During the follow-up, six patients got infected with new strains of HPV.

Conclusions: We found that a correctly performed LEEP conization may contribute to the remission of persistent HPV infection; a more extended follow-up period might be recommended due to a high rate of post-surgery HPV infections.

Key words: cervical cancer; loop electrosurgical excision procedure; LEEP; HPV

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INTRODUCTION

Cervical cancer remains the fourth most frequent cancer in women worldwide [1] despite the fact that this disease is theoretically wholly preventable. Persistent infection with high-risk human papillomavirus is the direct cause of the majority of cervical intraepithelial neoplasia (CIN) and invasive cervical cancers. Vaccination against HPV prevents infections with specific HPV types and, consequently, the development of cervical cancer caused by the virus's specific strains [2–4]. We have been observing a decrease in cervical cancer incidence for several decades thanks to these preventive measures and screening.

Doctors and scientists have been searching years for diagnostic tools which show the highest sensitivity, specificity, and patients' acceptance and allow to detect the disease at its earliest stage possible. These tools might be particularly useful in countries where cervical cancer incidence is still much higher than the world's average. An example of such a method is the optoelectronic method using a Truscreen. The procedure was convenient and had great potential for future use, especially in countries with limited access to colposcopy. However, despite this method's acceptable specificity, its sensitivity was significantly worse when compared to standard colposcopy and the HPV DNA test [5].

Early treatment of squamous intraepithelial lesions (SIL), especially high-grade (HSIL) is considered the most crucial method of preventing cervical cancer. Cold knife conization, loop electrical excision procedure (LEEP), and large loop

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excision of the transformation zone (LLETZ) find themselves among other established treatment procedures

According to the Updated Consensus Guidelines for the *Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors*, both ablation and excision effectively treat CIN. Randomized trials comparing different modalities show similar efficacy, ranging from 90% to 95% [6]. High-grade post-treatment disease may occur even in 18% of patients [7]; most recurrences are observed within two years post-treatment [8].

Previous studies reported that the incomplete excision of the endocervical cone margin during LEEP was a significant predictor for either persistence or recurrence of cervical intraepithelial neoplasia during follow-up [9, 10]. That is why the prolonged, careful observation of patients after surgical treatment may offer a significant chance to improve their future prognosis. However, no recommendation specifies both time and methods of subsequent follow-up. When considering other procedures, HPV testing may offer adequate sensitivity for predicting recurrence, while HPV genotyping seems helpful in increasing the post-treatment predictive value [11].

The study aimed to access the molecular remission of HPV infection in patients after LEEP — conization who refused to be vaccinated against HPV. We carried out a retrospective study to assess the ability of Pap-smear, HR-HPV testing, and their combination to identify residual or recurrent disease during the patients' follow-up.

MATERIAL AND METHODS

This retrospective study included 31 patients with squamous intraepithelial neoplasia (SIL) treated with LEEP — conization between 2019 and 2020 at Provincial Hospital in Poznań. The LEEP — conization was performed in a total of 160 women; the only inclusion criterium for the study was the lack of consent for HPV vaccination. We obtained samples from all the patients for a Pap-smear and molecular test. The latter detected DNA of 37 different HPV genotypes. After six months of follow-up, each woman underwent subsequent high-risk human papillomavirus testing and genotyping. All patients gave informed consent to participate in the study. The research was approved by the Bioethics Committee of the Medical Chamber of Wielkopolska.

Pap-smear for molecular assessment was collected with the endocervical Cyto-Brush and preserved in PreservCyt® (Hologic Corp.) and SurePath® (BD Diagnostics-TriPath) reserved for the biological samples. The probe was handed over to the independent, standardized laboratory. HPV detection was performed using the PCR method, followed by DNA enzyme immunoassay and genotyping with a reverse hybridization line probe assay. Sequence analysis was performed to characterize HPV — positive samples with unknown HPV genotypes. The molecular test detected DNA of 37 different HPV genotypes.

Each colposcopy was performed by a specialist in gynecologic oncology with 10-year experience in SmartOPTIC colposcope. We performed a test with 5% aqueous solution of acetic acid and Schiller's test with Lugol's iodine in all included cases. The colposcopic images were evaluated according to Reid's Colposcopic Index which assesses the color, lesion boundaries and surface, blood vessels and result of the iodine test. All colposcopic images were archived. We used classification created by The International Federation of Cervical Pathology and Colposcopy, recommended by the Polish Society of Colposcopy and Cervical Pathophysiology.

The excisions were performed via the colposcope after application of acetic acid 5% and Lugol's iodine. The sizes of the loops were selected according to the size of the lesion. When lesions reached high to the cervix, the lesion was excised deeper. We additionally sampled the lesions' margins. Finally, the curettage of the cervical canal was performed in order to obtain adequate endocervix samples. Between 12 to 16 paraffin blocks were prepared from each cervical specimen; each block was divided and examined in four to five sections. Histopathological analysis was performed in an independent laboratory by experienced pathologists. The follow-up schedule for all women included cytology and high-risk HPV genotyping at six months.

Calculations were performed using the statistical package of Statistica (ver. 13.1), all graphs were created with Microsoft Excel. Statistical hypotheses were verified at the level of significance of $p < 0.05$. Fisher's exact test was used to analyze the relationship between persistent HPV infection after LEEP - conization and a positive margin. This specific test was chosen due to the expected small number of positive samples. A logistic regression model was used to assess the relationship between age, HPV infection status, parity, and final histological diagnosis.

RESULTS

LEEP-conization procedure

The mean age of women admitted for planned excision was 33 years. The vast majority of patients had less than three children, and more than a half lived in a town or a city with less than 100,000 inhabitants. Table 1 presents descriptive characteristics of the study group. According to the Pap-smear results, one-third of hospitalized women were diagnosed with HSIL, one-third with LSIL, and 20% with atypical squamous cells (unable to exclude HSIL). We compiled all Pap-smear results in Figure 1. In total, 29 of 31 patients (93.5%) were positive for the HPV test before the surgery. In a group of 29 women with positive HPV test results, 17 cases (58.6%) tested positive for genotype 16.

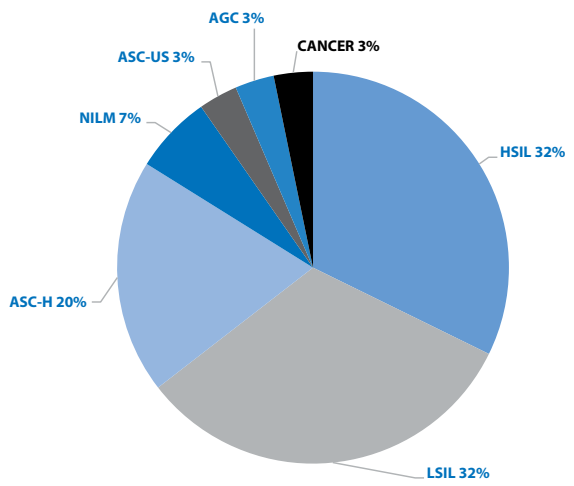
All the performed HPV test results and the occurrence of different genotypes are shown in Table 2.

According to the primary Pap-smear, HPV infection status, and colposcopy results, 25 (80.6%) of women were pre-diagnosed with HSIL while five (16.1%) with LSIL. One patient had discrepancies in the results. According to the histopathological material acquired in the study, more than half of women had HSIL lesions, and about 32% of patients had no pathological changes in tested samples. Excised margins were fully clean in 22 women (71%) in both the ectocervix and cervical canal. A negative ectocervix margin was observed in 23 patients (74.2%), while a negative cervical canal margin was found in 30 cases (96.7%).

Table 1. Descriptive characteristics of the study group

Characteristics	Category	Value
Evaluated patients, n		31
Mean age at evaluation, years		33
Area of residence, n (%)		
	> 100.000 inhabitants	13 (42)
	≤ 100.000 inhabitants	18 (58)
Thyroid disease, n (%)		
	Present	5 (16)
	Absent	26 (84)
Parity, n (%) of children		
	0	14 (45.2)
	1–2	13 (42)
	≥ 3	4 (12.9)

[1]The percentage was calculated by subtracting the remaining % values from 100%



LSIL — low-grade squamous intraepithelial lesion; HSIL — high-grade squamous intraepithelial lesion; AGC — atypical glandular cells; ASC-US — atypical squamous cells of undetermined significance; ASC-H — atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; NILM — negative for intraepithelial lesion or malignancy

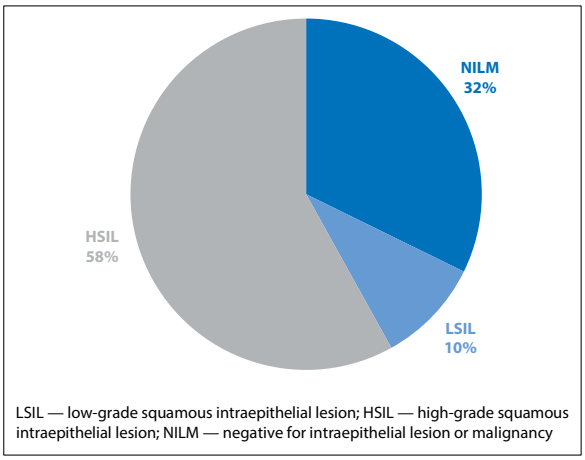
Figure 1. Pap-smear results

One patient presented a positive margin in the cervical canal despite having a negative margin in the ectocervix samples. Final histopathological results after LEEP-conization are presented in Figure 2.

Table 2. Occurrence of HPV genotypes within positive genotyping results

HPV type†	Before treatment[1], n (%)	After treatment[2], n (%)
16	17 (29.8)	1 (5.9)
58	4 (7)	1 (5.9)
73	4 (7)	1 (5.9)
31	3 (5.29)	0 (0)
33	3 (5.29)	0 (0)
45	3 (5.29)	2 (11.7)
53	3 (5.29)	2 (11.7)
66	3 (5.29)	1 (5.9)
6	2 (3.5)	0 (0)
11	2 (3.5)	0 (0)
51	2 (3.5)	1 (5.9)
54	2 (3.5)	1 (5.9)
18	1 (1.75)	0 (0)
35	1 (1.75)	0 (0)
52	1 (1.75)	0 (0)
56	1 (1.75)	1 (5.9)
59	1 (1.75)	2 (11.7)
61	1 (1.75)	0 (0)
62	1 (1.75)	0 (0)
68	1 (1.75)	1 (5.9)
82	1 (1.75)	1 (5.9)
90	0 (0)	1 (5.9)
70	0 (0)	1 (5.9)

† The patients could test positive for 1 or more HPV genotypes
[1] A total number of HPV genotypes detected among 29 cases: 57
[2] A total number of HPV genotypes detected among 11 cases: 17



LSIL — low-grade squamous intraepithelial lesion; HSIL — high-grade squamous intraepithelial lesion; NILM — negative for intraepithelial lesion or malignancy

Figure 2. Histopathological results after LEEP-conization

Table 3. Occurrence of HPV infection after LEEP-conization

Characteristics	Category	HPV status		Total	p-value
		Positive	Negative		
All cases		11 (35.5)	20 (64.5)	31 (100)	
Ectocervix margin	Positive	3 (37.5)	5 (62.5)	8 (100)	0.643
	Negative	6 (26.1)	17 (73.9)	23 (100)	
Cervical canal margin	Positive	0 (0)	1 (100)	1 (100)	1.000
	Negative	9 (30)	21 (70)	30 (100)	

Data given as a number of cases (percent)

Follow-up

In the follow-up, we performed both Pap-smear and HPV genotyping tests. Pap-smear results were normal in 30 (96.7%) women. HPV genotyping tests showed viral remission in 20 patients (64.5%), whereas in three cases (9%), the HPV infection was classified as persistent. One-third of negative Pap-smear cases were re-classified to persistent or recurrent HPV infection, based on their positive genotyping results. The persistent HPV infection was observed in three out of eight women with positive margins; however, the majority of these patients showed viral remission. Six patients (19.4%) had recurrent or persistent infection despite having a negative cervical margin. The relationship between the positive margin and persistent infection did not turn out to be statistically significant ($p > 0.05$). Seven patients tested positive for new HPV strains that hadn't been detected before. The occurrence of positive margins and HPV infections after LEEP-conization is presented in Table 3.

There was one Pap-smear positive for cancer cells, although the LEEP-conization results did not confirm the presence of any pathological changes. We also described one case with the preliminary cytological diagnosis of NILM (negative for intraepithelial lesion or malignancy), that was re-classified to HSIL after the performed histopathological examination. We assessed the correlation between age, HPV infection status, parity, and the final histological diagnosis — we found no statistically significant relationships ($p > 0.05$).

DISCUSSION

This study assessed the molecular remission of HPV infection in patients after LEEP-conization who refused vaccination against HPV. Active and effective treatment of HSIL is the primary approach to control the occurrence and development of cervical cancer. Cervical conization is one of the standardized treatments for HSIL. However, previous studies reported that residual lesions and disease recurrence might occur frequently following this surgical procedure [12]. The positive margins after cervical conization are generally considered to be a risk factor for the recurrence or persistence of SIL [13]. On the other hand, the viral clearance rate at the follow-up after conization is associated with negative excision margins, as confirmed by Cricca et al. [14]. In our study margins appeared to be clean in 22 (71%) women; the relationship between the positive margin and persistent infection did not turn out to be statistically significant. These divergent results might be caused by the insufficient size of the study group.

We also investigated the ability of Pap-smear and HR-HPV testing to identify residual or recurrent disease during the patients' follow-up. Despite the importance of early detection of treatment failure, follow-up after conservative treatment of high-grade CIN has not yet been standardized and varies in terms of timing, intervals, and methods. According to the ASCCP consensus guidelines, acceptable post-treatment management options for women with CIN 2/3 include HPV DNA testing at 6 to 12 months. Follow-up with the use of Pap-smear alone or in combination with colposcopy at six months is also acceptable [21]. Several investigators analyzed the sensitivity and specificity of HPV DNA testing compared with Pap-smear to detect residual or recurrent disease after undergone treatment [22–24]. HPV testing was found to be more sensitive than follow-up cytology, with comparable specificity of both mentioned methods [24, 25]. Women who are HPV-positive after surgery were statistically at higher risk for treatment failure [25, 27].

In research performed by Bruno et al. [26], 182 of 192 (94.7%) patients tested positive for HPV infection before the surgery. One hundred four women (57.1%) tested positive for genotype 16, 78 (42.8%) for other genotypes [26]. Our findings show a similar ratio — 93.5% positive patients to 58.6% — cases positive for HPV 16 genotype. Women with pre-treatment HPV infections had higher incidence of post-treatment HPV presence compared to women who were HPV negative at or before treatment. Women who had been previously treated for cervical disorders may be more prone to develop subsequent cervical intraepithelial lesions or even cervical cancer than women without a history of cervical disease and treatment [16]. Although a substantial proportion of post-treatment dysplasia and cancers may result from an incomplete excision of the lesion, or the persistence of the

lesion-associated HPV type, the affected patients are also at risk of developing a second cervical precancerous condition due to the acquisition of newly acquired HPV strains. Data on type-specific HPV infections associated with a higher probability of cervical disease development after treatment are limited [17–20]. Most studies did not distinguish between recurrent or residual cervical disease, and most of them did not differentiate the newly acquired HPV-related lesions.

A finding beyond this study's scope confirmed that 9 of 31 women (29%) were still infected with HPV after surgical treatment. Interestingly, in seven of these cases the detected genotypes were new and not present in any previous samples. According to a review prepared by Anne F. Rositch et al., [15] most HPV incidence estimates were recorded among women treated for cervical neoplasia using LEEP. Presented data showed that the HPV incidence ranged from 0% to 18% at 2 to 6 months post-treatment and 0% to 24% at 6 to 35 months post-treatment [15]. Our results indicate that the rate of HPV infection after LEEP-conization (29%) is above the mentioned average.

This study's limitations include its retrospective design and the fact that the entire research was conducted in a single medical facility. This fact may reduce the generalizability of our results to a broader geographic area. Further research with a prolonged follow-up period is highly recommended.

CONCLUSIONS

A negative margin from the target lesion and a positive margin from the cervical canal may indicate that the changes may be located deeper, outside of the transformation zone. Therefore, it is important to remember that a simple Pap-smear test may not show the disease's full advancement and should not be treated as the only source of clinical decisions. A more extended period of follow-up might be recommended due to a high rate of post-surgery HPV infections.

Conflict of interest

All authors declare no conflict of interest.

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Impact of clinicopathological variables on laparoscopic hysterectomy complications, a tertiary center experience

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ABSTRACT

Objectives: To analyze intraoperative and postoperative complications according to Clavian-Dindo Classification (CDC) and evaluate the influence of clinicopathological features on the feasibility and safety of total laparoscopic hysterectomy (TLH) in patients that underwent surgery in a tertiary center.

Material and methods: We retrospectively reviewed the database of 469 patients that underwent surgery for patients who underwent extra facial TLH from 2013 to 2020.

Results: A total of 86 (18.3%) peri-postoperative complications were observed. The incidence of intraoperative complications was 2% (n = 10). The overall conversion rate to open surgery was 1.9% (n = 9). A total of 76 postoperative complications were observed in 61 patients (14.3%). The incidence of minor [Grade I (n = 16, 3.4%) and II (n = 42, 8.9%)] and major complications [Grade III (n = 15, 3.2%), IV (n = 2, 0.4%) and V (n = 1, 0.2%)] were 12.3% and 3.8%, respectively.

A higher BMI and performing surgery at the first step of learning are found to be associated with intraoperative and postoperative complications ($p < 0.05$). Postoperative complications related to having a history of the cesarean section, additional comorbidities, and uterine weight ≥ 300 g ($p < 0.05$).

Conclusions: The implementation of TLH by experienced surgeons appears to have remarkable advantages over open surgery. However, the risk factor for complications should be taken into account by surgeons in the learning curve in selecting the appropriate patient for surgery.

Key words: total laparoscopic hysterectomy; complication; learning curve; Clavian-Dindo classification

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INTRODUCTION

Hysterectomy is one of the most common surgeries in gynecological procedures [1]. The vaginal hysterectomy (VH) is the preferred approach for benign cases whenever feasible. Besides, total laparoscopic hysterectomy (TLH) is a preferable alternative to open total abdominal hysterectomy (TAH) for patients in whom vaginal surgery was not suitable [2].

Traditionally, TLH has been associated with good outcomes in terms of effectively reducing symptoms, improving the quality of life and sexual functions [3]. Besides, TLH provides a bloodless dissection of anatomical spaces, better surgical precision and a view of magnified anatomy [4]. Some studies suggest that TLH appears to be associated with significantly greater rate of complications than other routes of surgical approachs [5, 6]. Many randomized controlled trials found that laparoscopic route has a tendency to cause more bladder or ureteral injuries [5, 7, 8]. Furthermore, all these complications of TLH depend mainly on the surgeon's experience [9]. Besides this learning curve, the complications specific to laparoscopic procedures such as pneumoperitoneum, and electrosurgical instruments related complications have to be considered.

The present study aimed to examine data from consecutive TLHs. The objective was to analyze intraoperative and

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postoperative complications according to Clavian-Dindo Classification (CDC) and evaluate the influence of clinicopathological features on the feasibility and safety of TLH in 469 patients that underwent surgery in a tertiary center [10].

MATERIAL AND METHODS

The present study was approved by the institutional ethics committee of Hacettepe University, in Ankara, Turkey. Informed consent was obtained from the patients before the procedures. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Study design

We retrospectively reviewed the database of 469 patients who underwent extrafacial laparoscopic hysterectomy from 2013 to 2020 at Hacettepe University Hospital in Ankara, Turkey. Exclusion criteria were invasive cervical cancer, endometrial and ovarian cancer requiring surgery more than extrafacial hysterectomy. All cases were performed by three obstetrics and gynecology residents training through gynecologic oncology as a subspecialist, assisted by obstetric and gynecology subspecialists. These three surgeons are well experienced in open surgical techniques but less experienced in laparoscopic surgery. The second surgeon, standard for all cases, was a fifth-year trainee of the obstetrics and gynecology residency program. With respect to hysterectomy, the second surgeon performed abdominal hysterectomy as a primary surgeon on approximately 30 cases and at least 10 laparoscopic surgical experiences as a surgical assistant. The manipulator was handled by a third-year trainee of the obstetrics and gynecology residency program in all cases.

Age, parity, body mass index (BMI), history of previous surgeries and cesarian sections, comorbidities, surgical findings, duration of surgery, hospital stay, intraoperative and postoperative complications, conversion to laparotomy and amount of blood losses were obtained from hospital records and patients files. Uterine weight, cervical length, and final histopathologies were obtained from pathology results. The 469 cases were divided into two periods to assess the surgeons' learning curve (90 cases in the first period, each surgeons' first 30 cases), and the remaining cases in the second period. The number of cases needed to achieve criteria on-level performance varies in many studies [11]. This criteria defined to be 30 cases in the present study.

The patients were assessed for intraoperative and postoperative complications (morbidity within 30 days). Postoperative complications were registered by a standardized system recorded (CDC) initially developed by Clavien in 1992 [12], and modified by Dindo in 2004 [10]. In this classification the severity of the complication was graded into five groups according to the type of therapy required for treatment. Grade I complications refer to minor risk events not requiring therapy. Grade II complications refer to potentially life-threatening situations that required intervention or hospitalization. Blood transfusions and total parenteral nutrition is also included. Grade III complications refer to the need for surgical, endoscopic, or radiological intervention consist of two groups; IIIA (not under general anesthesia) and IIIB (under general anesthesia). Grade IV complications refer to life-threatening complication (including central nervous system complications) requiring Intermediate Care/Intensive Care Unit management consists of two groups; IVA (single organ dysfunction) and IVB (multiorgan dysfunction). Grade V refer the death of a patient. In this study, complication was divided into minor (CDC Grade I and II) major (CDC Grade III, IV and V) categories.

Present study protocol has been approved by Institutional Review Board and Hacettepe University Ethics Committee (approval number: GO 20/501). Informed consent was not obtained from the patients due to the retrospective design of the study.

Procedures

All patients underwent pelvic examination and ultrasonography in the preoperative period. Preoperative chest X-ray, electrocardiography, and blood tests were performed. Anesthesia consultation was requested from all patients in the preoperative period. Depending on their medical history and comorbid diseases, the patients were consulted with the relevant medical departments in the preoperative period. Prophylactic antibiotics were administered 30 minutes before operation.

Operative technique

All procedures were performed under general anesthesia and in the dorsal lithotomy position. Clermont-Ferrand (Karl Storz Gmbh & Co., Tuttlingen, Germany) and RUMI uterine manipulator with a Koh cup colpotomizer (Koh Colpotomizer System; Cooper Surgical, Trimbull, CT) were preferred as a uterine manipulator, and the manipulator type was randomly selected in cases regardless of any circumstances. After installing the manipulator transvaginally, the whole abdominal cavity was insufflated with CO_2 with the help of a Veress needle from the abdominal umbilical point. After pneumoperitoneum creation, a 10 mm trochar from umblicus, and other three 5 mm trochars (a suprapubic and 2 laterals) were inserted. The procedure was performed with electrothermal bipolar vessel sealing device (LigaSure™), dissectors, graspers, monopolar and bipolar energy modalities. The first step is dissecting the round ligaments, and opening broad ligaments. Anterior and posterior leafs of

broad ligaments were dissected. Both ureters were checked in this step. The bladder is dissected and seperated from the uterus. The infundibulopelvic ligaments or utero-ovarian on both sides were coagulated and transected up to operation plan. If the ovaries are decided to be preserved, the fallopian were always removed. After coagulation and transection of uterin arteries and cardinal ligaments, circular colpotomy was performed with monopolar hook cautery and/or LigaSure. Specimens were retrieved vaginally, if it was possible. In inappropriate cases, manual morcellation was accomplished vaginally. The vaginal occlusion was provided to restore pneumoperitoneum. The vaginal vault was closed, laparoscopically or vaginally, with continuous vicryl no 1–0 sutures. The procedure ended with inspection and haemostasis.

All recommendations for postoperative period was similar among patients without any complications. The nasogastric tube was removed at the and of the surgery, urinary catheter was kept for six hours after surgery. Mobilization started on the day of surgery, and oral fluid intake started six hours after surgery. In cases without complications, discharge of the patients occurred after the first day of the operation. The patients were seen after the sixth week of the discharge date.

Statistical analysis

Descriptive analysis of clinicopathological characteristics was performed. Statistical differences between groups were analyzed using the Chi-square test and Student's t-test. Statistical analyses were performed using Statistical Package for the Social Sciences statistical software (version 23.0, SPSS Inc, Chicago, IL, USA). A p value of < 0.05 was considered to indicate statistical significance.

RESULTS

The mean age of the patients was 54.7 ± 8.2 years. Of these 469 patients, 57 (12.1 %) were nulliparious. The mean BMI of the patients was 28.2 ± 10.1 kg/m^2. One hundred and twenty-one patients (n = 121, 25.7%) had at least a history of cesarian section, and 62 patients had at least a history of abdominopelvic surgery including both open and laparoscopic surgery. Two hundred and fourty-three (n = 243, 53%) patients had at least one medical comorbidities such as diabetes, cardiovascular disease, pulmonary disease etc (Tab. 1).

Four hundred twenty-one (n = 421, 89.7%) of 469 patients underwent hysterectomy with adnexectomy. The mean operative time was 86 ± 47.3 minutes. The mean length of hospital stay was 1.6 ± 0.4 days. Of these patients, 9 patients (1.9%) had been converted to laparotomy. The mean blood loss was 98.8 ± 42.1 mL. The surgical indications are listed in Table 1.

Table 1. Baseline characteristics, clinical features and operative outcomes of 469 patient

Patients	
Age [years] (mean)	54.7 ± 8.2
Nulliparity: n (%)	57 (12.1)
BMI (kg/m^2) (mean)	28.2 ± 10.1
Previous cesarean section: n (%)	121 (25.7)
History of abdominopelvic surgery: n (%)	62 (13.2)
Comorbidities: n (%)	243 (51.8)
Operative outcomes	
Adnexectomy (USO/BSO): n (%)	421 (89.7)
Operation time (minute) (mean)	86 ± 47.3
Length of stay at hospital [days]	1.6 ± 0.4
Conversion to open surgery: n (%)	9 (% 1.9)
Reoperation	7 (% 1.5)
Blood loss [mL] (mean)	98.8 ± 42.1
Intraoperative complications: Total n (%)	10 (2)
Major vascular injury: n (%)	2 (0.4)
Bowel injury: n (%)	1 (0.2)
Ureteral injury: n (%)	2 (0.4)
Epigastic artery injury: n (%)	3 (0.6)
Bladder injury: n (%)	2 (0.4)
Surgical indications	
Fibroids: n (%)	169 (36)
Endometrial cancer: n (%)	49 (10.4)
Premalignant lesions of endometrium: n (%)	43 (9.1)
Abnormal uterine bleeding	40 (8.5)
Endometriosis: n (%)	39 (8.3)
Adenomyosis: n (%)	29 (6.1)
BRCA mutation	28 (5.9)
Premalignant lesions of cervix: n (%)	25 (5.3)
Others: n (%)	47 (10)

BMI — body mass index; USO — unilateral salpingo-oophorectomy; BSO — bilateral salpingo-oophorectomy

The incidence of intraoperative complications was 2% (n = 10), and these complications included major vascular injury (n = 2, 0.4%), bowel injury (n = 1, 0.2%), ureteral injury (n = 2, 0.4%), epigastric artery injury (n = 3, 0.6%), and bladder injury (n = 2, 0.4%) (Tab. 1). The intraoperative complications were more common in patients with high BMI, endometriosis, and previous history of cesarian section. A major vascular (50%) and the two epigastric artery injuries (66.6%) were observed in patients with ≥ 30 kg/m^2. A ureter injury (50%) was seen in a patient with endometriosis, and a bladder injury (50%) was seen in patients with a history of cesarian section. The other intraoperative ureteral injury occurred in a patient with ipsilaterally myoma uteri of the injury side. Conversion to laparotomy was required in nine

(1.9%) patients, four of those were due to intraoperative complications including major vascular injury in two cases, a bowel injury, and a ureteral injury. Two conversions were due to large size uterus that hindered the movement of the uterus, and the remaining two were due to adhesions.

A total of 76 postoperative complications were observed in 64 patients (13.6 %). Among 469 patients, 54 (11.5 %) had one complication and 10 patients (2.3%) had experienced more than one postoperative complication. All complications were categorized according to five grades of CDC as summarized in Table 2. The incidence of minor [Grade I (n = 16, 3.4%) and II (n = 42, 8.9%)] and major complications [Grade III (n = 15, 3.2%), IV (n = 2, 0.4%) and V (n = 1, 0.2 %)] were 12.3 % and 3.8 %, respectively. Eighteen patients had major complications, seven (1.5%) of the patients underwent reoperation including bowel perforation (n = 2), uterine artery bleeding (n = 2), ureterovaginal fistula (n = 1), rectovaginal fistula (n = 1), and vaginal vault evisceration (n = 1). Finally, the remaining major complications were managed with interventional radiology.

Table 2. Postoperative complications according to Clavien-Dindo classification

Clavien-Dindo Score	Total, n (%)	Complication
Grade I	16 (3.4)	4 pelvic hematomas 3 wound hematomas 3 wound infections 2 wound dehiscence 2 atelectasis 2 postoperative ileus
Grade II	42 (8.9)	8 urinary tract infection 6 blood transfusion 5 cuff cellulitis 2 deep venous thrombosis 2 uninary retantion 2 pelvic absess 2 bowel perforation 2 pelvic hematomas 2 ureteral injury 2 vaginal cuff dehiscence 2 pneumonia 2 pulmoner embolism 1 postoperative ileus 1 atelectasis 1 cardiac arrhythmia 1 myocardial infarction 1 mesenteric panniculitis
Grade IIIa	3 (0.6)	2 pelvic absess 1 pelvic hematoma
Grade IIIb	12 (2.5)	3 ureteral injury 3 ureterovaginal fistulas 2 uterine artery bleeding 2 vesicovaginal fistulas 1 rectovaginal fistulas 1 vaginal vault evisceration
Grade IV	2 (0.4)	2 bowel perforation
Grade V	1 (0.2)	1 liver failure

Among postoperative major TLH complications, a rectovaginal fistula (100%) and a ureteral injury (33.3%) occurred after the dissection of deep infiltrating endometriosis. Two vesicovaginal fistulas (100%) and a ureterovaginal fistula (33.3%) were observed in patients who had a history of cesarian section. Two uterine artery bleedings (100%) were registered in patients undergoing hysterectomy for uterine fibroids (uterine weight ≥ 300 g). One bowel perforation was secondary to unwillingly cauterization was reported on the 4th day of surgery in a patient with a BMI over 30. Mortality due to liver failure was noticed in a patient with cirrhosis.

BMI and the period of surgery are found to be associated with total complication rates (intraoperative and postoperative) in univariate analysis ($p < 0.05$). Unlike postoperative complications, intraoperative complications are significantly associated with operative time ≥ 90 minutes ($p < 0.05$). Postoperative complications had a statistically significant association with having a history of cesarian section, additional comorbidities, and uterine weight ≥ 300 g ($p < 0.05$). Univariate analysis also showed that the age, nulliparity, adnexectomy during TLH, uterine cervical length, and uterine manipulator type were not risk factors for TLH complications in present study (Tab. 3).

The number of surgeries for each of three surgeons were reported to be 168 (35.8%), 154 (32.8%), 147 cases (31.4%), respectively. There was no significant difference among the surgeons with respect to complications rate ($p = 0.89$). However, linear regression analysis reviewed a significant negative correlation between the number of surgeries performed and the rate of complications for each surgeon ($p < 0.05$).

DISCUSSION

With the preference of laparoscopic approach has the greatest increase in hysterectomy route, determining the risk factors associated with this surgical procedure is of vital importance. Laparoscopic hysterectomy is performed for a wide variety of *reasons, such as* significantly less pain, a shorter hospital stay, and a faster return to normal daily activities. Besides all these benefits of laparoscopic hysterectomy, many studies revealed that intraoperative and postoperative complications were more common in laparoscopic surgery [6, 13].

Several studies reveal a statistically significant increase in urologic complications in laparoscopic route, particularly in ureteral injury and fistula [8]. In contrary to open surgeries, the lack of certain recognition of the cervicovaginal margin in TLH cause unnecessary dissections through to the vagina. These dissections and the use of energy modalities may results with higher rates of urinary tract complications. Some may hypothesize a longer cervix and the absence of pro-

Table 3. Univariate analysis of factors associated with surgical complications

Variables	Intraoperative complications: n (%)	p	Postoperative major complications: n (%)	p
Age		NS		NS
< 60 years	7/341 (2)		13/341 (3.8)	
≥ 60 years	3/128 (2.3)		5/128 (3.9)	
Nulliparity		NS		NS
Yes	1/40 (2.5)		2/40 (5)	
No	9/429 (2.1)		16/429 (3.7)	
BMI [kg/m^2]		< 0.05		< 0.05
< 30	4/277 (1.4)		9/277 (3.2)	
≥ 30	6/175 (3.4)		9/175 (5.1)	
Previous cesarean section		NS		< 0.05
Yes	3/121 (2.4)		8/121 (6.6)	
No	7/348 (2)		10/348 (2.8)	
Comorbidities		NS		< 0.05
Yes	5/243 (2)		12/243 (4.9)	
No	5/226 (2.2)		6/226 (2.6)	
Adnexectomy		–		NS
Yes	10/421 (2.3)		16/421 (3.8)	
No	–/48 (0)		2/48 (4.2)	
Operation time		< 0.05		NS
< 90 min	2/264 (0.7)		10/264 (3.7)	
≥ 90 min	8/188 (4.2)		8/188 (4.2)	
Uterine cervical length		NS		NS
< 4 cm	6/313 (1.9)		12/313 (3.8)	
≥ 4 cm	4/140 (2.8)		5/140 (3.5)	
Uterine weight		NS		< 0.05
< 300 g	6/354 (1.7)		9/354 (2.5)	
≥ 300 g	4/135 (2.9)		9/135 (6.7)	
Uterine manipulator		NS		NS
RUMI	2/99 (2)		5/99 (5)	
Clermont-Ferrand	8/364 (2.2)		13/364 (3.6)	
Surgery periods		< 0.05		< 0.05
First 90 cases	4/90 (4.4)		7/90 (7.7)	
Remaining cases	6/379 (1.6)		11/379 (2.9)	
p < 0.05 is statistically significant				

NS — not significant; BMI — body mass index

lapse in nulliparous may contribute an intensive dissection. However, nulliparity and the length of cervix were not associated with any TLH complications in the present study. While the ureteral injuries were commonly identified postoperatively, the bladder injuries were commonly identified intraoperatively [14], this figure was consistent with our study. Moreover, there are studies reporting the rate of bladder injury was higher than ureteral injury. Contrary to finding, we reported higher complication rates in ureteral injury than bladder [13, 15].

Another variable that may lead to lower urinary tract complications is the scar tissue typically located in the lower segment of the anterior uterine wall after cesarean sections. The adhesion, due to scar tissue, leads to challenges in the dissection of vesicocervical fascia. Hence, this resulted with bladder and ureter complications in many studies [16, 17]. Although the previous cesarean section was not directly related to intraoperative complications in the present study, there were notable effects on postoperative major complications. The presence of urinary tract complications in about

half of postoperative major complications is a main cause of this significance. Anatomical distortions and the inability to obtain an optimal surgical field of vision secondary to causes such as increased blood loss, pelvic adhesions, and endometriosis are found to be associated with increased urinary tract injury [8, 17], increased length of surgery and large uterus size was the described as the other risk factor in many studies [18, 19]. As the prior reported data, deep infiltrating endometriosis was another reason for ureter complications in the present study. Although many urinary tract complications were managed with interventional procedures or surgery, there were undetected injuries resulting in vesicovaginal (n = 2) and ureterovaginal (n = 3) fistulas in our study. The surgical interventions for fistulas performed by urologists produced excellent results in our tertiary care center.

Five of the six bowel injury cases in our study were located in the rectum. A patient with rectovaginal fistula, who had a delayed diagnosis of rectal injury, was operated due to in endometriosis with rectal involvement. Only one case occured during the initial insertion of a trocar and this intraoperative small bowel perforation was managed by conversion to open surgery. Two of the remaining rectal perforations required reoperation, and of those one had deep infiltrating endometriosis. And the early recognition of two rectal perforation caused by the monopolar energy was managed conservatively with a favorable clinical outcome.

During the course of laparoscopy in obese patients, there are difficulties such as reaching the peritoneum and maintaining pneumoperitoneum, inability to give the patient proper position during surgery due to ventilation problems, and limitation in surgical exposure. The relationship between obesity and surgical complication of TLH is controversial. Many studies revealed that the TLH complications were more frequent among patients with obesity [16, 20]. Also, there are some retrospective studies showing the similar rates of complications in TLH among obese and normal weight patients [21, 22]. This study revealed the intraoperative and postoperative major complications were more likely to seen in BMI 30 and above participants.

Having comorbidities are risk factors for surgical complications in patients who undergo TLH [22, 23]. The present study revealed that the comorbidities were risk factors only for major postoperative complications. However, a weakness of the study is the failure to address which comorbidities are predictors. Specifying types of comorbidities might improve the quality of the preoperative assessment and treatment strategies.

The definition of large uterus is described differently in many studies, but is often used for uterine weights above 300, or 500 mg [19, 24]. In the case of large uterus, laparoscopic hysterectomy still is not the prominent choice in most cases. However, with increasing experience, the impression of performing TLH on large uterus was strengthed. Serial studies described a significant association between TLH complications and large uterus [25]. On the other hand, this association was not found to be significant in some published studies [19, 26]. And a prospective study advocated the uterine weight was not a predictor for TLH complications, and a higher success rate in TLH for large uterus may be associated with the surgeon's experience with only an increased operative time [27]. We determined 300 g as a cut-off value in the present study, and the large uterus had increased risk for postoperative complications.

The process of the translation of open surgical skills into laparoscopic surgery describe as a learning curve and it is one of the most frequently cited risk factor for laparoscopic complications. The new application of the laparoscopic technique in hysterectomy has led to an increased rate of surgical complications, even in the hands of highly experienced surgeons in open surgery. This conclusion strengthens the importance of supplying appropriate conditions for surgeons having no prior experience with laparoscopic hysterectomy to facilitate them to carry out this procedure with an optimal degree of qualification and reliability. In our first 90 cases, the whole complication rate was 24.4% and the rate of complications markedly reduced to 16.8% in remaining cases. This reflected the tendency for diminishing complications with increasing experience. This finding was consistent with previous reports [9, 28].

This work has numerous limitations. The first limitation was the retrospective nature of the study. The second was the sample size of the study is insufficient to conclude valid results in the heterogeneous cases with different complications. Finally, the last potential limitation was the lack of reliability for the outcomes of TLH in three surgeons that might have a different set of laparoscopic skills.

CONCLUSIONS

In conclusion, the implementation of TLH by experienced surgeons appears to have remarkable advantages over open surgery. However, risk factors for complications should be taken into account by surgeons in the learning curve in selecting the appropriate patient for surgery. Hence, the benefit and risk ratio of laparoscopic surgery in advanced cases have to be considered.

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Conflicts of interest

Authors declare no conflict of interest.

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Why do some patients with stage 1A and 1B endometrial endometrioid carcinoma experience recurrence? A retrospective study in search of prognostic factors

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ABSTRACT

Objectives: Endometrial endometrioid carcinoma (EEC) is the most encountered subtype of endometrial cancer (EC). Our study aimed to investigate the factors affecting recurrence in patients with stage 1A and 1B EEC.

Material and methods: Our study included 284 patients diagnosed with the International Federation of Gynecology and Obstetrics stage 1A/1B EEC in our center from 2010 to 2018. The clinicopathological characteristics of the patients were obtained retrospectively from their electronic files.

Results: The median age of the patients was 60 years (range 31–89). The median follow-up time of the patients was 63.6 months (range 3.3–185.6). Twenty-two (7.74%) patients relapsed during follow-up. Among the relapsed patients, 59.1% were at stage 1A EEC, and 40.9% were at stage 1B. In our study, the one-, three-, and five-year recurrence-free survival (RFS) rates were 98.9%, 95.4%, and 92.9%, respectively. In the multivariate analysis, grade and tumor size were found to be independent parameters of RFS in all stage 1 EEC patients. Furthermore, the Ki-67 index was found to affect RFS in stage 1A EEC patients, and tumor grade affected RFS in stage 1B EEC patients. In the time-dependent receiver operating characteristic curve analysis, the statistically significant cut-off values were determined for tumor size and Ki-67 index in stage 1 EEC patients.

Conclusions: Stage 1 EEC patients in the higher risk group in terms of tumor size, Ki-67, and grade should be closely monitored for recurrence. Defining the prognostic factors for recurrence in stage 1 EEC patients may lead to changes in follow-up algorithms.

Key words: endometrial endometrioid carcinoma; early stage; recurrence-free survival; ki-67; grade; tumor size

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INTRODUCTION

While the most common gynecological malignancy in developed countries is endometrial cancer (EC), it ranks second after cervical cancer in developing countries [1]. Approximately 75–90% of patients with EC present with abnormal uterine bleeding, and the most important risk factors are obesity, type 2 diabetes mellitus (DM), high fatty diet, early menarche, nulliparity, late menopause, Lynch syndrome, age > 55 years and chronic tamoxifen use [2–6].

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In the traditional classification, EC is divided into two types: estrogen-driven type 1, which includes grades 1–2 endometrial endometrioid carcinomas (EEC), and non-estrogen-driven type 2, which consists of grade 3 EEC and non-endometrioid carcinomas [7]. EEC is the most common subtype, comprising 75%–80% of EC [8].

The stage of EC can be determined using the International Federation of Gynecology and Obstetrics (FIGO) system. In the FIGO staging system, less than half of myometrial invasion is defined as stage 1A, and invasion equal to or more than half of the myometrium is defined as stage 1B EEC [9]. However, FIGO staging alone is inadequate for treatment planning in patients with stage 1 EEC. In the National Comprehensive Cancer Network (NCCN) guidelines, besides myometrial invasion, risk factors such as pathological grade, ≥ 60 years, and lymphovascular invasion are recommended for making therapy decisions. According to risk factors, observation or brachytherapy is recommended after surgery in stage 1A disease [10]. The NCCN uterine cancer guideline recommends brachytherapy ± external beam radiation therapy or radiation therapy ± chemotherapy after surgery in stage 1B disease [10]. In stage 1A and 1B EEC disease, a few patients relapse despite current treatment options.

Our study aimed to investigate the factors affecting recurrence in patients with stage 1A and 1B EEC and identify the clinicopathological features of patients who should be followed up closely for recurrence.

MATERIAL AND METHODS

Study population and data collection

Our study included 284 patients diagnosed with stage 1A/1B EEC according to the FIGO 2009 staging system between 2010 and 2018 in the Departments of Medical and Gynecological Oncology, Bursa Uludag University. The patients who could not be staged, who had a second history of malignancy, and who were under the age of 18 were excluded.

As study variables, the demographic characteristics (age, body mass index, presence of DM and parity), histopathological features (tumor size, lower uterine segment involvement, lymphovascular space invasion, and accompanying non-tumor lesion), total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy (BSO) and TAH and BSO plus bilateral pelvic paraaortic lymph node dissection (BPPLND) as surgical types, external radiotherapy, brachytherapy and chemoradiotherapy as applied treatments as well as oncological results (follow-up time, any recurrence development and recurrence-free survival) were obtained retrospectively from the patients' electronic files. In addition to all these variables, estrogen receptor (ER), progesterone receptor (PR), Ki-67 level, tumor grade and myometrial invasion were obtained from the histopathological examination.

Treatment features

Surgical treatment of EC in our institution is a total hysterectomy and bilateral salpingo-oophorectomy. Intraoperative frozen section analysis was routinely performed in all cases. Pelvic and paraaortic lymphadenectomy is also performed for women whose frozen section analysis reveals a tumor type other than EEC, grade 3 histology, cervical invasion, myometrial invasion greater than 50% depth, and tumor size greater than 2 cm.

Brachytherapy was applied to the patients with stage 1A/grade 1–2 EEC, in the presence of high-risk factors (lymphovascular space invasion and age ≥ 60). Brachytherapy was applied to all patients to patients with stage 1A/grade 3 and stage 1B. The treatment dose was given to the vaginal 1/3 apex area, 5 mm deep from the vaginal surface with a high dose rate brachytherapy device using the Ir-192 source. The doses applied to the vaginal mucosa, rectum, and bladder were calculated according to International Commission on Radiation Units and Measurements. A total dose of 18–24 gray (Gy) was planned with a fraction dose of 6–7 Gy. External radiotherapy was applied to stage 1B/grade 3 cases. The total dose of 45 Gy (1.8 Gy per fraction) was delivered to the primary tumor site and pelvic lymph nodes.

Histological examination

Hematoxylin-Eosin and immunohistochemical staining of specimens (Ki-67, ER and PR) were re-evaluated, and histopathological features (grade, myometrial invasion) were recorded. The slides of the cases were evaluated using a light microscope (model BX51TF, Olympus, Tokyo, Japan). Histological grading was performed using the FIGO grading system. Myometrial invasion depth was evaluated in two categories of being less than half (less than 50%) or more than half (50% or more) in the slide with the deepest tumor penetration. The ER assay clone used was SP1, the PR assay clone used was 1E2, and the Ki-67 assay clone used was 30–9. Only nuclear staining was considered as positive immunostaining for ER, PR, and Ki-67, and staining was scored according to the percentage of nuclear staining. Staining of > 1% of tumor cell nuclei is considered positive for ER and PR staining. For Ki-67, at least 1000 cells were counted at x400 magnification from the hot-spot areas in each sample.

Outcomes

Recurrence-free survival (RFS) was defined as the time between the date of surgical staging and the date of histologically or radiologically confirmed recurrence. Overall

survival (OS) was determined from the time of diagnosis until death from any cause.

Ethics

Our study was conducted in accordance with the 1964 Helsinki declaration. The clinical research ethics committee of the Bursa Uludag University Faculty of Medicine approved the study (Approval number: 2020-6/33). As this study is based on retrospective analysis of encrypted data, informed consent was not needed.

Statistical analysis

The continuous variables were expressed by the mean and median values, and the categorical variables were expressed by frequency and the corresponding percentage values. Survival analysis was calculated using the Kaplan-Meier method. The factors were examined by Cox Regression Analysis. The enter model was used with the parameters having a p-value below 0.20 to determine the independent factors. The data were statistically processed using IBM SPSS version 22 software. In all statistical analyses, $p < 0.05$ was accepted as statistically significant for the results. A time-dependent receiver operating characteristic (ROC) curve analysis was performed with R software version 3.4.2 and the survival ROC package version 1.0.3. The nearest neighbor estimator with a span of $\lambda = 0.05$ was used. The cut-off point that achieves this maximum Youden-J index was accepted as the optimal cut-off point. The area under the ROC curve (AUC) value was obtained from the ROC curve analysis.

RESULTS

General findings

The clinicopathological features of and treatment options for stage 1 EEC patients are presented in Table 1. The median age of patients was 60 years (range 31–89). The median body mass index (BMI) of the patients was 33.6 (range 20.4–63.7) kg/m^2. Among the patients, 118 (41.6%) had a history of DM, 88.7% were multiparous, 54.6% underwent TAH with BSO and BPPLND, 77.8% were at stage 1A, and 22.2% were at stage 1B. The median tumor size was 3.2 cm (range 0.3–10.0). 42 (14.8%) patients had no myometrial invasion, 179 (63.0%) had less than 50% myometrial invasion, and 63 (22.2%) had 50% or more myometrial invasion.

Most of the patients were in grade 1 (48.9%). The median Ki-67 index was 20 (range 1.0–90.0). Among the patients, 61 (21.5%) had lower uterine segment involvement, 16 (5.6%) had lymphovascular space invasion, and 65 (22.9%) had adenomyosis. The number of patients with a positive ER and a positive PR was 240 and 243, respectively. After surgery, 159 (56.0%) patients were treated with radiotherapy, five patients (1.7%) with chemoradiotherapy. Among the patients, 42.3% were followed up without treatment.

Table 1. Clinicopathological features and treatment options of stage 1 EEC patients

Characteristic		N	(%)
Age (median) (range, years)		60.0 (31.0–89.0)	
BMI (median) (range, kg/m^2)		33.6 (20.4–63.7)	
Diabetes mellitus	Present Absent	118 166	41.6 58.4
Parity	≥ 1 0	252 32	88.7 11.3
Surgery	TAH with BSO	129	45.4
	TAH with BSO and BPPLND	155	54.6
Stage	1A 1B	221 63	77.8 22.2
Tumor size (Median) (Range, cm)		3.2 (0.3–10.0)	
Myometrial invasion	Absent < 1/2 ≥ 1/2	42 179 63	14.8 63.0 22.2
Grade	1 2 3	139 124 21	48.9 43.7 7.4
Ki-67 (median) (range, %)		20 (1.0–90.0)	
Lower uterine segment involvement	Absent Present	223 61	78.5 21.5
Lymphovascular space invasion	Absent Present	268 16	94.4 5.6
Adenomyosis	Absent Present	219 65	77.1 22.9
Estrogen receptor status	Positive Negative Missed Data	240 11 33	84.5 3.9 11.6
Progesterone receptor status	Positive Negative Missed Data	243 8 33	85.6 2.8 11.6
Postoperative treatment	Observation Radiotherapy Chemoradiotherapy	120 159 5	42.3 56.0 1.7

EEC — endometrial endometrioid carcinomas; BMI — body mass index; TAH — total abdominal hysterectomy; BSO — bilateral salpingo-oophorectomy; BPPLND — bilateral pelvic paraaortic lymph node dissection

Oncological outcomes

The median follow-up time of the patients was 63.6 months (range 3.3–185.6). Twenty-two (7.74%) patients relapsed during follow-up. Among the relapsed patients, 59.1% were at stage 1A EEC, and 40.9% were at stage 1B. The median time between diagnosis and tumor recurrence was 33.4 (range 3.9–100) months. Tumor recurrence occurred in the vagina in nine patients, in the lung in five patients, in the peritoneum in four patients, in the bladder in one patient, in the colon in one patient, in the

intra-abdominal lymph node in one patient, and in the bone in one patient.

In our study, the one-, three-, and five-year RFS rates were 98.9%, 95.4%, and 92.9%, respectively. The OS rates for one, three, and five years were 99.3%, 95.4%, and 93.3%, respectively.

The factors affecting recurrent free survival for all FIGO stage 1 EEC patients in the study

The factors affecting RFS in FIGO stage 1 EEC patients were evaluated after univariate analysis, and grade, myometrial invasion, tumor size, ER, PR, and Ki-67 index were included in the multivariate analysis. In the multivariate analysis, grade and tumor size had a statistically significant effect on disease recurrence (p = 0.035, p = 0.018, respectively) (Tab. 2).

The time-dependent ROC curve analysis was performed to obtain a cut-off value for tumor size, which had an effect on relapse in stage 1 EEC patients. In the time-dependent ROC curve analysis for tumor size, the AUC was found to be significant for the time intervals of 26.4–32.6 and 74.2–100 (months). The cut-off values corresponding to the maximum Youden-J index were 3 cm and 2.2 cm, respectively. This finding means that a tumor size greater than 3.0 cm predicts recurrence after 26.4 months and that a tumor size greater than 2.2 cm predicts recurrence after 74.2 months significantly. No significant AUC was found for the other time points (Tab. 3). The time-dependent ROC curves of the tumor size for the 26.4–32.6 time interval and for the 74.2–100 time interval are presented in Figure 1.

The factors affecting recurrent free survival for FIGO stage 1A EEC patients

Grade, Ki-67 index, ER, adjuvant therapy and lower uterine segment involvement were included in the multivariate Cox regression analysis in which stage 1A EEC patients were evaluated. The Ki-67 index had a statistically significant effect on RFS (p = 0.019) (Table 4). A time-dependent ROC curve analysis was performed to obtain a cut-off value for the Ki-67 index. Stage 1A patients were analyzed for the Ki-67 index, and no significant AUC value was found in the time-dependent ROC curve analysis. Also, time-dependent ROC curve analysis was performed to evaluate the Ki-67 index in all stage 1 EEC patients. For Ki-67, the AUC was found to be significant for the time interval of 64.2–74.1 and 74.1–185.6 (months). The cut-off values were 30% and 20%, respectively. This means that Ki-67 values greater than 30% predicted recurrence after 64.2 months and that Ki-67 values greater than 20% predicted recurrence after 74.1 months significantly. No significant AUC was found for the other time points (Tab. 5).

The factors affecting recurrent free survival for FIGO stage 1B EEC patients

After the univariate analysis, age, BMI, grade, tumor size, and PR status of stage 1B EEC patients were included in

Table 2. Univariate and multivariate cox regression analysis of the predictors for all patients recurrence

Factor		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	p	HR	95% CI	p
Age	Years	1.001	0.959–1.044	0.962			
BMI	kg/m^2	0.997	0.943–1.054	0.918			
Diabetes mellitus	Absent (RC) vs Present	1.037	0.455–2.363	0.931			
Parity	Nulliparous (RC) vs Multiparous	1.017	0.300–3.446	0.978			
Grade		**3.914**	**2.068–7.408**	**< 0.001**	**2.508**	**1.066–5.901**	**0.035**
Myometrial invasion	< 50% (RC) vs ≥ 50%	**1.899**	**0.796–4.534**	**0.148**	0.985	0.311–3.116	0.980
Tumor size	cm	**1.303**	**1.035–1.642**	**0.025**	**1.386**	**1.058–1.818**	**0.018**
Lymphovascular space invasion	Absent (RC) vs Present	1.732	0.639–4.698	0.281			
Lymph node dissection	Absent (RC) vs Present	1.153	0.497–2.675	0.741			
Adenomyosis	Absent (RC) vs Present	1.294	0.497–2.675	0.741			
Ki-67	%	**1.027**	**1.007–1.048**	**0.009**	1.018	0.992–1.044	0.171
Estrogen receptor status	Negative (RC) vs Positive	**3.395**	**0.974–11.834**	**0.055**	6.818	0.774–60.077	0.084
Progesterone receptor status	Negative (RC) vs Positive	**3.360**	**0.776–14.558**	**0.105**	0.282	0.015–5.303	0.398
Lower uterine segment involvement	Absent (RC) vs Present	1.392	0.565–3.428	0.472			
Adjuvant therapy	Absent (RC) vs Present	3.585	0.478–26.876	0.214			

HR — hazard ratio; CI — confidential interval; BMI — body mass index; RC — reference category; *Cox regression model is statistically significant (p = 0.001)

Table 3. Time-dependent ROC curve analysis results and accuracy summaries for tumor size

Time Interval	AUC	p-value	cut-off	Youden J	Sensitivity	Specificity	LR+	LR–
[3.3–4.3)	0.005	1.000	–	–	–	–	–	–
[4.3–6.5)	0.214	0.999	–	–	–	–	–	–
[6.5–9.2)	0.245	0.893	–	–	–	–	–	–
[9.2–13.4	0.559	0.401	–	–	–	–	–	–
[13.4–20)	0.539	1.000	–	–	–	–	–	–
[20–21.3)	0.512	0.464	–	–	–	–	–	–
[21.3–22.1)	0.558	0.322	–	–	–	–	–	–
[22.1–25.8)	0.578	0.220	–	–	–	–	–	–
[25.8–26)	0.592	0.137	–	–	–	–	–	–
[26–26.4)	0.629	0.061	–	–	–	–	–	–
[26.4–32.6)	**0.635**	**0.039**	**3**	**0.250**	**0.745**	**0.505**	**1.505**	**0.505**
[32.6–34.2)	0.562	0.226	–	–	–	–	–	–
[34.2–36.3)	0.531	0.350	–	–	–	–	–	–
[36.3–38)	0.519	0.399	–	–	–	–	–	–
[38–40)	0.543	0.271	–	–	–	–	–	–
[40–46.7)	0.539	0.272	–	–	–	–	–	–
[46.7–51)	0.565	0.272	–	–	–	–	–	–
[51–60.1)	0.544	0.245	–	–	–	–	–	–
[60.1–64.2)	0.583	0.245	–	–	–	–	–	–
[64.2–74.1)	0.593	0.068	–	–	–	–	–	–
[74.1–74.2)	0.620	0.068	–	–	–	–	–	–
[74.2–100)	**0.611**	**0.034**	**2.2**	**0.159**	**0.872**	**0.286**	**1.222**	**0.446**
[100–185.6]	0.582	0.096	–	–	–	–	–	–

AUC — area under the ROC curve; LR+ — positive likelihood ratio; LR — negative likelihood ratio

the multivariate analysis, and the grade was found to have a statistically significant effect on RFS for stage 1B patients (p = 0.031) (Tab. 6). The effect of grade on RFS is presented in Figure 2.

DISCUSSION

In this study, we found tumor size and grade as prognostic factors for recurrence with multivariate analysis in stage 1 EEC patients, while we found that Ki-67 index in stage 1A EEC patients and tumor grade in stage 1B EEC patients were prognostic factors affecting recurrence.

In many studies, EC patients were evaluated according to FIGO staging as stages 1–4 [11–14] or stages 1–2 [15–17]. Although these studies provide general information about relapse-related factors and survival in EC patients, there are a limited number of studies about stage 1 EEC disease. To our knowledge, except for the study of Han et al. [18], there is no large-scale research investigating the recurrence factors in stage 1A and 1B EEC disease.

Many studies have confirmed the prognostic value of grade in EC patients [11, 16, 18]. Han et al. [18] study showed that grade was a statistically significant factor for recurrence in all patients with stage 1 EEC. However, multivariate analysis revealed that tumor grade was an independent factor for recurrence in patients with stage 1B disease, and myometrial invasion was an independent factor in patients with stage 1A disease. Likewise, in our study, we found that tumor grade is an independent prognostic factor on recurrence in stage 1 EEC patients and stage 1B EEC patients, not for stage 1A. Therefore, our study is one of the studies showing that these features are prognostic factors.

Although there are studies in which tumor size is not one of the factors affecting survival in patients with EEC [16, 18, 19], Schink JC. et al. [20] evaluated stage 1 EEC patients and reported that tumor size was a prognostic factor for survival, as in our study. In this study, the cut-off value was 2 cm. In the time-dependent ROC curve analysis for tumor size, the risk of recurrence increased after 26.4 months in patients with a tumor size greater than 3 cm and after 74.2 months in patients with a tumor size greater than 2.2 cm.

Except for resting cells (G0), Ki-67 protein is expressed at all active cell cycle stages (G1, S, G2, M) [21]. It is used as a marker of cellular proliferation; its prognostic and predictive value was shown in several cancer types, inclu-

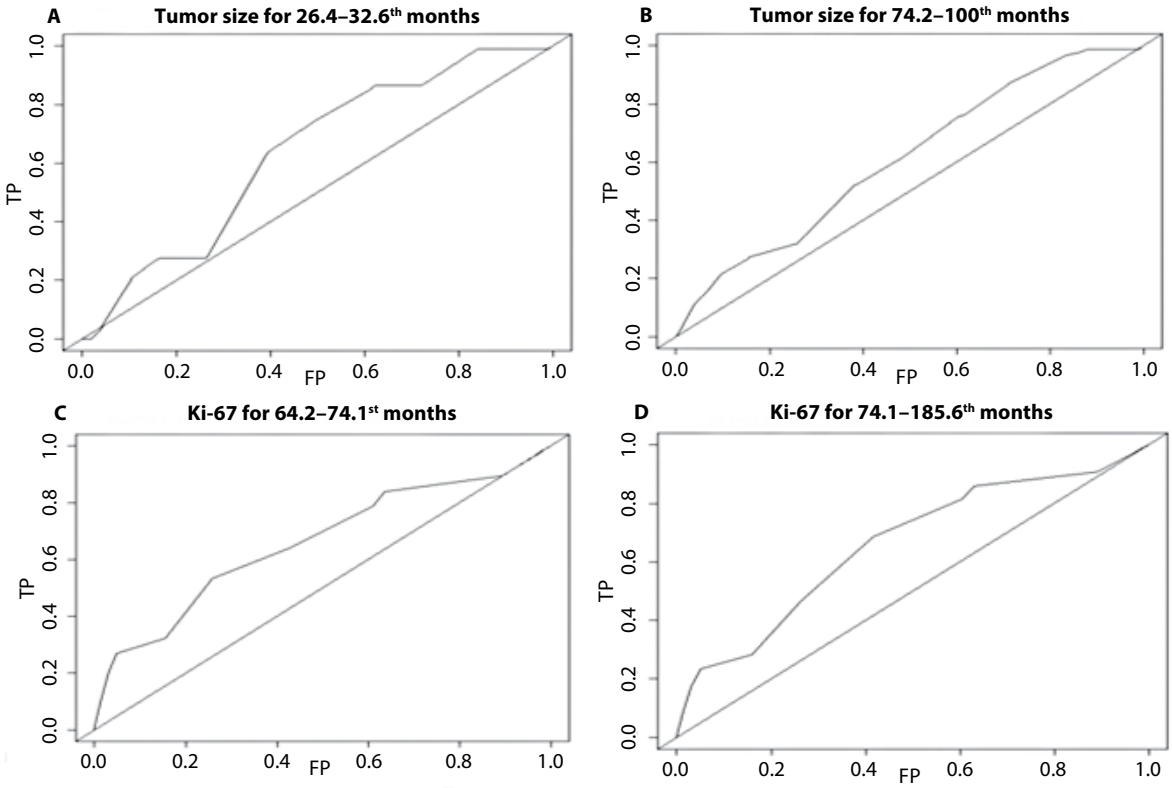

Figure 1. Time-dependent ROC curves of A) tumor size for 26.4-32.6 time interval, B) tumor size for 74.2-100 time interval, C) Ki-67 for 64.2-74.1 time interval, D) Ki-67 for 74.1-185.6 time interval

Table 4. Univariate and multivariate cox regression analysis of the predictors for stage 1A patients recurrence

Factor		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	p	HR	95% CI	p
Age	Years	1.039	0.974–1.109	0.247			
BMI	kg/m^2	0.976	0.905–1.052	0.519			
Diabetes mellitus	Absent (RC) vs Present	0.734	0.244–2.206	0.581			
Parity	Nulliparous (RC) vs Multiparous	2.030	0.263–15.676	0.497			
Grade		**2.723**	**1.127–6.580**	**0.026**	1.096	0.345–3.481	0.8777
Myometrial invasion	Absent (RC) vs Present	0.185	0.008–4.085	0.286			
Tumor size	cm	1.121	0.793–1.584	0.519			
Lymphovascular space invasion	Absent (RC) vs Present	1.706	0.377–7.729	0.488			
Lymph node dissection	Absent (RC) vs Present	1.741	0.567–5.340	0.332			
Adenomiyozis	Absent (RC) vs Present	1.959	0.640–5.994	0.239			
Ki-67	%	**1.030**	**1.001–1.060**	**0.045**	**1.036**	**1.006–1.067**	**0.019**
Estrogen receptor status	Negative (RC) vs Positive	**4.451**	**0.937–21.137**	**0.060**	5.65	0.651–49.137	0.11
Progesterone receptor status	Negative (RC) vs Positive	2.508	0.322–19.530	0.380			
Lower uterine segment involvement	Absent (RC) vs Present	**2.192**	**0.712–6.744**	**0.171**	0.683	0.134–3.474	0.64
Adjuvant therapy	Absent (RC) vs Present	**3.584**	**0.986–13.031**	**0.053**	3.255	0.651–16.262	0.151

HR — hazard ratio; CI — confidential interval; BMI — body mass index; RC — reference category; *Cox regression model is statistically significant (p = 0.001)

Table 5. Time-dependent ROC curve analysis results and accuracy summaries for Ki-67

Time interval	AUC	p-value	Cut-off	Youden J	Sensitivity	Specificity	LR+	LR–
[3.3–3.9]	0.012	1.000	–	–	–	–	–	–
[3.9–6.5]	0.495	0.511	–	–	–	–	–	–
[6.5–9.2]	0.480	0.555	–	–	–	–	–	–
[9.2–13.4]	0.592	0.341	–	–	–	–	–	–
[13.4–20]	0.480	0.541	–	–	–	–	–	–
[20–21.3]	0.469	0.582	–	–	–	–	–	–
[21.3–22.1]	0.523	0.435	–	–	–	–	–	–
[22.1–25.8]	0.465	0.601	–	–	–	–	–	–
[25.8–26]	0.452	0.668	–	–	–	–	–	–
[26–26.4]	0.507	0.474	–	–	–	–	–	–
[26.4–32.6]	0.553	0.323	–	–	–	–	–	–
[32.6–34.1]	0.520	0.425	–	–	–	–	–	–
[34.1–36.2]	0.554	0.309	–	–	–	–	–	–
[36.2–38]	0.575	0.225	–	–	–	–	–	–
[38–46.7]	0.581	0.185	–	–	–	–	–	–
[46.7–51]	0.615	0.101	–	–	–	–	–	–
[51–60.1]	0.649	0.052	–	–	–	–	–	–
[60.1–64.2]	0.641	0.057	–	–	–	–	–	–
[64.2–74.1]	**0.658**	**0.030**	**30**	**0.276**	**0.534**	**0.742**	**2.072**	**0.628**
[74.1–185.6]	**0.659**	**0.016**	**20**	**0.268**	**0.686**	**0.583**	**1.643**	**0.539**

AUC — area under the ROC curve; LR+ — positive likelihood ratio; LR– — negative likelihood ratio

Table 6. Univariate and multivariate cox regression analysis of the predictors for stage 1B patients recurrence

Factor		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	p	HR	95% CI	p
Age	years	**0.955**	**0.907–1.006**	**0.081**	0.959	0.850–1.082	0.492
BMI	kg/m^2	**1.090**	**0.983–1.208**	**0.101**	1.084	0.871–1.350	0.469
DM	Absent (RC) vs Present	1.993	0.533–7.448	0.305			
Parity	Nulliparous (RC) vs Multiparous	0.429	0.089–2.072	0.292			
Grade		**5.371**	**1.783–16.185**	**0.003**	**5.508**	**1.169–25.960**	**0.031**
Tumor size	cm	**1.344**	**0.999–1.808**	**0.051**	1.013	0.434–2.366	0.977
Lymphovascular space invasion	Absent (RC) vs Present	0.981	0.139–1.930	0.327			
Lymph node dissection	Absent (RC) vs Present	0.518	0.497–2.675	0.741			
Adenomiyozis	Absent (RC) vs Present	0.842	0.174–4.072	0.830			
Ki-67	%	1.012	0.918–1.045	0.447			
Estrogen receptor status	Negative (RC) vs Positive	1.598	0.191–13.341	0.665			
Progesterone receptor status	Negative (RC) vs Positive	**5.261**	**0.611–45.289**	**0.131**	4.099	0.357–47.125	0.258
Lower uterine segment involvement	Absent (RC) vs Present	0.498	0.103–2.416	0.387			
Adjuvant therapy	Absent (RC) vs Present	1.899	0.233–15.469	0.549			

HR — hazard ratio; CI — confidential interval; BMI — body mass index; RC — reference category; *Cox regression model is statistically significant (p = 0.001)

ding EC [22, 23]. Kitson et al. [24] investigated prognostic factors, including Ki-67 in stages 1–4 EC patients. Ki-67 was associated with worsening of cancer-specific survival in the univariate analysis. However, this significance was not detected in the multivariate analysis. Yu et al. [25], examined stages 1–4 EC patient group and found that Ki-67 was

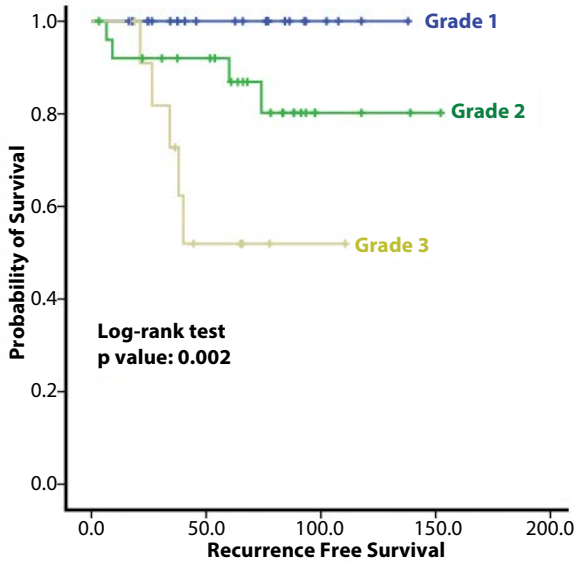

Figure 2. Kaplan-Meier curves of recurrence-free survival according to histologic grade in FIGO stage 1B endometrioid endometrial cancer. FIGO: International Federation of Gynecology and Obstetrics

associated with stage, differentiation, depth of myometrial invasion, and lymph node status. The studies investigating the importance of Ki-67 consisted mainly of all EC subtypes and stages 1–4 patient groups. To the best of our knowledge, our research is the first to show the effect of the Ki-67 index on recurrence in stage 1A disease in the multivariate analysis. In the study, no statistically significant cut-off value was determined in the time-dependent ROC analysis for Ki-67 in stage 1A EEC patients. However, in all stage 1 EEC patients, Ki-67 values greater than 30% predicted recurrence after 64.2 months, and Ki-67 values greater than 20% predicted recurrence after 74.1 months significantly.

The depth of myometrial invasion has been used for staging EEC [9]. In Han et al.'s [18] study, myometrial invasion in stage 1A EEC disease was found to be a prognostic factor in recurrence. Our study included similar patient groups, but the depth of myometrial invasion was not detected as a prognostic factor for recurrence in stage 1A EEC patients. Akar et al. [16] found that myometrial invasion was not associated with RFS and disease-specific survival in patients with stages 1–2 EEC. This finding should be compared with those of studies involving larger groups of stage 1A patients. In our study and Han et al. [18] study, age, lymphovascular involvement, lower uterine segment involvement, lymph node dissection, and adjuvant therapy were not prognostic factors recurrence in stage 1 EEC patients. In addition to Han et al., we also studied factors such as BMI, DM, parity, ER and PR status, and presence of adenomyosis. These factors were not found to be prognostic factors for recurrence.

Limitations

Our study's main limitations are its retrospective design and the limited number of relapsed patients. Moreover, there were not enough death events to analyze OS or cancer-specific survival.

CONCLUSIONS

Tumor grade and size were found to be the independent parameters for RFS in all stage 1 EEC patients. The Ki-67 index affected RFS in stage 1A EEC patients, and tumor grade affected RFS in stage 1B EEC patients. In the time-dependent ROC curve analysis, statistically significant cut-off values were determined for tumor size and the Ki-67 index in stage 1 EEC patients. Stage 1 EEC patients in a higher risk group for tumor size, Ki-67 index, and grade, should be closely monitored for recurrence. Defining the prognostic factors for recurrence in stage 1 EEC patients may lead to changes in follow-up algorithms.

Conflict of interest

The authors declare no conflict of interest.

Acknowledments

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Diagnostic accuracy of PremaQuick in detection of preterm labor in symptomatic women

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ABSTRACT

Objectives: Failure to identify women at risk of preterm labor (PTL) leads to failure to implement standard measures. This study designed to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with threatened preterm labor (TPTL).

Material and methods: One hundred and twenty-two (122) pregnant women, singleton pregnancy, < 37 weeks, admitted with TPTL included in this study, and were compared to 122 controls.
After thorough evaluation, participants were examined using sterile vaginal speculum for cervico-vaginal fluid (CVF) sampling, and PremaQuick test. The CVF sampling was followed by trans-vaginal sonographic (TVS) assessment of cervical length (CL). Participants were managed according to hospitals policy thorough their admission, and after discharge in the ante-natal clinics till delivery. After delivery, the delivery data were compared by the recorded participants` data on admission.

Results: The PremaQuick test had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value, 95.2% negative predictive value, and 96.3% accuracy in detection of PTL. The PremaQuick had significantly higher true negative rate, specificity, positive predictive value, and overall accuracy in detection of PTL compared to CL < 25 mm (p = 0.005, 0.005, 0.01, 0.002; respectively).

Conclusions: The PremaQuick is an accurate bedside test in detection of PTL in women presented with TPTL. It had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value, 95.2% negative predictive value, and 96.3% overall accuracy in detection of PTL. The PremaQuick had significantly higher true negative rate, specificity, positive predictive value, and overall accuracy in detection of PTL compared to CL < 25 mm.

Key words: diagnostic accuracy; PremaQuick; preterm labor

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INTRODUCTION

Preterm labor (PTL) is an important cause of perinatal deaths, and neonatal morbidity [1–4]. PTL occurs after excessive uterine stretch (twin or triplet pregnancies), amniotic fluid infection, or chorio-decidual hemorrhage [5, 6].

The fetal fibronectin and cervical length (CL) measured by trans-vaginal sonography (TVS) are the main diagnostic tools currently used to detect PTL [7].

The CL, and fetal fibronectin have low positive predictive value, and limited accuracy to detect PTL [8, 9].

The fetal fibronectin, and insulin growth factor binding protein-1 (IGFBP-1) are amniotic fluid markers used for prediction of PTL [10].

The fetal fibronectin test has high negative predictive value (NPV) in diagnosing PTL [11, 12]. While amniotic fluid contamination, bleeding, and unprotected intercourse are associated with false fetal fibronectin results [4].

Failure to identify women at risk of PTL leads to failure to implement standard measures with subsequent increase in perinatal deaths, and neonatal morbidity. While the false

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positive diagnosis of PTL exposes women to unnecessarily admission, tocolysis, and corticosteroids.

The IGFBP-1 released into the cervico-vaginal fluid (CVF) during the process of chorio-decidual disruption of PTL [13–15]. The IGFBP-1 is a good negative predictor of PTL [16]. The interlukein-6 (IL-6) is a marker of sub-clinical chorioamnionitis associated with PTL [17, 18].

It is crucial to have a reliable diagnostic tool rather than the currently available tests to identify women at risk of PTL [10]. PremaQuick is a bedside test containing antibodies against three amniotic fluid markers (Native, and total IGFBP-1, and IL-6).

Objectives

This study designed to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with threatened preterm labor (TPTL).

MATERIAL AND METHODS

This prospective comparative study was conducted over 15 months (June 2019 to August 2020); after ethical committees approval (approval number OB_0403_19), and registration as clinical trial (ACTRN12618001472268) [19].

One hundred and twenty-two (122) pregnant women between 20–40 years` old, singleton pregnancy, < 37 weeks` gestation, admitted with TPTL were included in this study, and compared to 122 controls, after informed consent in accordance with the Declaration of Helsinki to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with TPTL.

Women without medical disorders, with pregnancy, and intact fetal membranes, between 24–36^{+6} weeks` gestation, presented with uterine contractions (3–4 contractions/30 minutes), each contraction lasting for ≥ 30 seconds with ≤ 50% cervical effacement, and < 3 cm dilated cervix were included in TPTL group.

Pregnant women without TPTL admitted under observation for fetal wellbeing assessment because of suspected intrauterine growth retardation or for blood sugar or blood pressure monitoring due to suspected diabetes or hypertensive disorders with pregnancy, were included as controls after exclusion of intrauterine growth retardation, diabetes, and hypertensive disorders with pregnancy [15].

Women ≥ 37 weeks`, twin or triplet pregnancies, intrauterine growth retardation, medical disorders with pregnancy (diabetes and/or hypertension), dilated cervix ≥ 3 cm, rupture of membranes (ROM), fetal anomalies or intrauterine fetal death, and/or ante-partum hemorrhage were excluded from this study.

Women delivered preterm iatrogenically due to medical disorders with pregnancy (diabetes, hypertension, or intrahepatic cholestasis) or obstetrics indications [twins, triplets, or premature rupture of fetal membranes (PROM)] [20] were also excluded from this study.

The gestational age was estimated based on the first day of LMP (last menstrual period), and confirmed by ante-natal scan done before 20 weeks` [21–23].

Participants were examined abdominally to evaluate; the fundal height, uterine contractions (frequency and duration), and fetal heart, followed by laboratory investigation according to hospitals protocol.

Participants were also examined using sterile vaginal speculum (without antiseptics or lubricant) for CVF sampling, and PremaQuick test before CL assessment, and digital examination.

The sterile swab of PremaQuick kit (Biosynex, France) was placed in the posterior vagina for 15 seconds for CVF sampling, then placed in the extraction solution provided by manufacture for 10 seconds. Three drops of the extraction solution were dispensed into the wells of test device/cassette, then the test result detected within 10 minutes, and recorded.

The presence of 3C (control) lines is important for PremaQuick test validation, and score ≥ 2 means positive PremaQuick test, while score 0 or ≤ 1 means negative PremaQuick test [15].

PremaQuick test is a bedside test containing antibodies against three amniotic fluid markers: Native, and total IGFBP-1, and IL-6 [15].

The CVF sampling for PremaQuick test was followed by TVS assessment of CL by sonographer blinded to participants' clinical data using the standard guideline (to avoid potential bias) [24], and digital examination for assessment of cervical effacement, and dilatation.

Participants were managed according to hospitals policy (hospitalization, tocolysis, and corticosteroids) based on the PremaQuick test results, CL, and clinical findings.

The participants were followed in the ante-natal clinics weekly after hospital discharge till delivery. After delivery, the delivery data were compared to the recorded participants` data on admission to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with TPTL.

Statistical Analysis

Statistical analysis done using Statistical Package for Social Sciences (SPSS) version 20 (Chicago, IL, USA). The Chi-square test (x^2), and student (t) were used for analysis of qualitative, and quantitative variables, respectively.

The sensitivity, specificity, predictive values, and accuracy of PremaQuick test and CL in dectection of PTL were calculated and compared. The relative risk (RR) of PTL in women with positive PremaQuick test, and CL < 25 mm was also calculated. P-value < 0.05 was considered significant.

RESULTS

One hundred and twenty-two (122) pregnant women, singleton pregnancy, < 37 weeks` gestation, admitted with TPTL were compared to 122 controls in this study to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with TPTL.

There was no significant difference between the TPTL group, and controls regarding the mean maternal age, and gestational age at enrollment (30.7 ± 7.1 years, and 32.4 ± 4.1 weeks vs 33.1 ± 6.3, and 35.2 ± 3.7; respectively) (p = 0.9 and 0.1; respectively).

In TPTL group, the PremaQuick test had higher true positive rate in detection of PTL compared to CL < 25 mm (95.1% (116/122) vs 71.3% (87/122); respectively), but this difference was statistically insignificant (p = 0.1).

In controls, the PremaQuick test had significantly higher true negative rate in detection of PTL compared to CL < 25 mm (97.5% (119/122) vs 56.6% (69/122); respectively) (p = 0.005) (Tab. 1).

The PremaQuick test had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value (PPV), 95.2% negative predictive value (NPV), and 96.3% overall accuracy in detection of PTL. While the CL < 25 mm had 71.3% sensitivity, 56.6% specificity, 62.1% PPV, 66.3% NPV, and 63.9% overall accuracy in detection of PTL (Tab. 1).

The PremaQuick had significantly higher true negative rate, specificity, PPV, and overall accuracy in detection of PTL compared to CL < 25 mm (p = 0.005, 0.005, 0.01, 0.002; respectively) (Tab. 1).

The relative risk of PTL in women presented with TPTL was higher with positive PremaQuick test (RR 20.3 (95% CI: 9.29–44.36), p = 0.0001) compared to CL < 25 mm (RR 1.85 (95% CI: 1.37–2.49), p = 0.0001). In addition, the number of women delivered preterm after positive PremaQuick test was significantly higher than those delivered preterm after CL < 25 mm (116/119 versus 87/140; p = 0.01) (Tab. 2).

DISCUSSION

Failure to identify women at risk of PTL leads to failure to implement standard measures. The false positive diagnosis of PTL exposes women to unnecessarily admission, tocolysis, and corticosteroids. It is important to have a reliable diagnostic tool rather than the currently available tests to predict women at risk of PTL [10].

Table 1. Accuracy of the PremaQuick test, and cervical length (CL) in detection of Preterm labor

Variables	PremaQuick Number (%)	CL < 25 mm Number (%)	p-value
TPTL group (122 women) True positive (TP) False negative (FN)	 116/122 (95.1%) 6/122 (4.9%)	 87/122 (71.3%) 35/122 (28.7%)	0.1
Controls (122 women) True negative (TN) False positive (FP)	 119/122 (97.5%) 3/122 (2.5%)	 69/122 (56.6%) 53/122 (43.4%)	0.005*
Sensitivity (TP ÷ TP + FN) × 100	116 ÷ (116 + 6) × 100 = (95.1%)	87 ÷ (87 + 35) × 100 = (71.3%)	0.1
Specificity (TN ÷ TN + FP) × 100	119 ÷ (119 + 3) × 100 = (97.5%)	69 ÷ (69 + 53) × 100 = (56.6%)	0.005*
Positive predictive value (PPV) (TP ÷ TP + FP) × 100	116 ÷ (116 + 3) × 100 = (97.5%)	87 ÷ (87 + 53) × 100 = (62.1%)	0.01*
Negative predictive value (NPV) (TN ÷ TN + FN) × 100	119 ÷ (119 + 6) × 100 = (95.2%)	69 ÷ (69 + 35) × 100 = (66.3%)	0.07
Accuracy (TP + TN ÷ TP + TN + FP + FN) × 100	116+119 ÷ (116 + 119 + 3 + 6) × 100 = (96.3%)	87 + 69 ÷ (87 + 69 + 53 + 35) × 100 = (63.9%)	0.002*

* Significant difference; Chi-square test (x^2) used for statistical analysis; TPTL — threatened preterm labor

Table 2. Relative risk of PTL with positive PremaQuick test, and cervical length (CL) < 25 mm

Variables	PTL (bad outcome)	Good outcome (No PTL)	RR (95% confidence interval) p-value
PremaQuick Positive test group (119) Negative test group (225)	 116 6	 3 119	20.3 (9.29–44.36) 0.0001*
CL < 25 mm Positive group (140) Negative group (104)	 87 35	 53 69	1.85 (1.37–2.49) 0.0001*

* Significant difference; CL — cervical length; PTL — preterm labor; RR — relative risk

PremaQuick is a bedside test containing antibodies against three amniotic fluid markers (Native, and total IGFBP-1, and IL-6). Therefore, one hundred and twenty-two (122) pregnant women, < 37 weeks`, admitted with TPTL were compared to 122 controls in this study to evaluate the accuracy of PremaQuick test in detection of PTL in women presented with TPTL.

In this study, the PremaQuick test had significantly higher true negative rate in detection of PTL compared to CL < 25 mm (97.5% (119/122) vs 56.6% (69/122); respectively), (p = 0.005). It also had significantly higher specificity, PPV, and overall accuracy compared to CL < 25 mm (p = 0.005, 0.01, and 0.002; respectively) in detection of PTL.

Similarly, Abu-Faza et al. [15], found the CL < 25 mm had low specificity, and low positive predictive value in detection of PTL.

Schmitz et al. [25], also, found the CL ≤ 25 had 75% senstivity, 63% specificity, and 24% PPV in detection of PTL.

Nikolova et al. [26], concluded that the PAMG-1 (placental alpha microglobulin-1) is better predictor of imminent PTL when compared with phosphorylated-IGFBP-1 (ph IGFBP-1) alone or in combination with CL.

Melchor et al. [27], found the positive predictive value of PAMG-1 was significantly higher than the phIGFBP-1 or fetal fibronectin in detection of spontaneous PTL within seven days.

The phIGFBP-1 alone as an amniotic fluid marker has limited predictive ability to detect women at risk for PTL [28]. Therefore, the PremaQuick test designed against three amniotic markers (Native, and total IGFBP-1, and IL6) to increase its accuracy in detection of PTL.

In this study, the PremaQuick test had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value, 95.2% negative predictive value, and 96.3% overall accuracy in detection of PTL. The PremaQuick test had significantly higher specificity, positive prtedictive value, and overall accuracy in detection of PTL compared to CL < 25 mm (p = 0.005, 0.01, and 0.002; respectively).

Similarly, Asiegbu et al. [29], found the the PremaQuick test had 96.3% senstivity, 97.6% specificity, 89.7% PPV, 99.2% NPV, and 97.3% accuracy, in detection of PTL within 14 days in women with TPTL between 28–36^{+6} weeks` gestation.

Eleje et al. [30], also found the PremaQuick test had 100.0/87.5% sensitivity, 94.1/96.9% specificity, 70.5/87.5% PPV, 100.0/96.9% NPV and 95.0/95.0% accuracy in detection of PTL within 7/14 days in women with singleton pregnancy presented with TPTL < 35 weeks`, respectively. They concluded that the PremaQuick test is an accurate test in detection of PTL in women with singleton pregnancy presented with TPTL [30].

Abu-Faza et al. [15], also found the PremaQuick test had higher specificity and positive predictive value in diagnosing PTL compared to CL.

In this study, the relative risk of PTL in women presented with TPTL was higher with positive PremaQuick test (RR 20.3 (95% CI: 9.29–44.36), p = 0.0001) compared to CL < 25 mm (RR 1.85 (95% CI: 1.37–2.49), p = 0.0001). In addition, the number of women delivered preterm after positive PremaQuick test was significantly higher than those delivered preterm after CL < 25 mm (116/119 vs 87/140; p = 0.01).

Abu-Faza et al. [15], also found the odds, and relative risk (RR) of PTL within 7–14 days in symptomatic women were significantly higher for PremaQuick (12.9, and 8.4; respectively) compared to CL < 25 mm (1.4, and 1.1; respectively).

This study found the PremaQuick is an accurate bedside test in detection of PTL in symptomatic women presented with TPTL. It had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value, 95.2% negative predictive value, and 96.3% overall accuracy in detection of PTL. Its true negative rate, specificity, PPV, and overall accuracy in detection of PTL were significantly higher than CL < 25 mm.

The current study was the first registered, prospective, comparative, multicenter study conducted to evaluate the accuracy of PremaQuick test in detection of PTL in symptomatic women presented with TPTL.

Women refused to give consent and participate, and shipping of the PremaQuick kits were the limitations faced during this study.

The accuracy of PremaQuick test in detection of PTL should be compared with other amniotic fluid markers as PAMG-1 (AmniSure test) or IGFBP-1 (Actim-PROM test) in future studies.

CONCLUSIONS

The PremaQuick is an accurate bedside test in detection of PTL in women presented with TPTL. It had 95.1% sensitivity, 97.5% specificity, 97.5% positive predictive value, 95.2% negative predictive value, and 96.3% overall accuracy in detection of PTL. The PremaQuick had significantly higher true negative rate, specificity, positive predictive value, and overall accuracy in detection of PTL compared to CL < 25 mm.

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Conflict of interest

No conflict of interests related to this study.

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Maternal serum adipokines and inflammatory markers at late gestation and newborn weight in mothers with and without gestational diabetes mellitus

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ABSTRACT

Objectives: Maternal obesity increases the risk of gestational diabetes mellitus (GDM) and is positively correlated with neonatal obesity increasing the risk of adiposity in both young and adult offspring. Maternal secreted factors from adipose tissue such as adipokines and inflammatory cytokines may regulate fetal growth. This study investigated associations between maternal adipokines and inflammatory markers at late gestation, and neonatal anthropometric characteristics in mothers with and without GDM.

Material and methods: The study included 65 women with GDM and 65 pregnant women with normal glucose tolerance evaluated at the time of term elective Caesarean section. Adiponectin, leptin, resistin, adipsin, neutrophil gelatinase-associated lipocalin (NGAL), nerve growth factor (NGF), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-alpha) concentrations were measured in maternal serum by the multiplex immunoassay using Magpix technology. C-reactive protein (CRP) was measured with a particle-enhanced turbidimetric immunoassay and neonatal anthropometric variables were assessed. The association of birthweight with individual biomarkers was analyzed using multivariate logistic regression adjusted for maternal factors.

Results: Adiponectin, leptin, resistin, adipsin, NGAL and NGF were not significantly associated with higher birthweight. The maternal factors in association with higher birthweight observed in GDM were CRP, MCP-1 and TNF-alpha levels. Regression analysis showed that TNF-alpha was an independent risk factor for higher birthweight ($p = 0.046$).

Conclusions: These results suggest an involvement of maternal inflammatory markers at late gestation and fetal growth in mothers with GDM, and that TNF-alpha could play a major role.

Key words: gestational diabetes mellitus; adipokines; cytokines; maternal obesity; birthweight

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INTRODUCTION

Obesity is a major clinical problem in women of reproductive age worldwide [1]. It is currently estimated that three in four women of reproductive age in Mexico are classified as overweight or obese [2]. Maternal obesity increases the risk of infertility, stillbirth, gestational diabetes mellitus (GDM), preeclampsia, pregnancy-induced hypertension, thromboembolism and complications during delivery. In addition, maternal obesity is positively correlated with neonatal and childhood obesity in the offspring with negative consequences that include increased risk of metabolic syndrome and type 2 diabetes mellitus (T2DM) later in life [3].

The prevalence of GDM, which is a state of glucose intolerance that develops during pregnancy, is rising worldwide [4]. In Mexico, it ranges from 10.3% to 30.1%, depending on the diagnostic criteria used [5, 6]. GDM increases the risk of T2DM, and associate with higher risk of large for gestational age (LGA) products, who subsequently develop an increased

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risk of obesity and diabetes in adulthood [7]. It is established that the transport of nutrients other than glucose from the mother to the fetus as amino acids and/or lipids could contribute to excessive fetal growth [8].

As pregnancy increases adiposity, attention has been drawn to the role of maternal factors associated with adipose tissue (adipokines and inflammatory markers) in mediating fetal growth [9–12]. Adiponectin, the most commonly found adipokine, is produced exclusively by adipose tissue and is suggested to have various properties, including insulin-sensitizing, anti-atherogenic, and anti-inflammatory. Deficient concentrations in plasma of adiponectin correlate with GDM and obesity [13]. This may be due to reduced levels of adiponectin causing a reduction of glucose uptake in skeletal muscle, accompanied by an increase in production of hepatic glucose. In addition, studies have shown that maternal adiponectin is inversely associated with birthweight [14, 15].

Leptin is released into the circulation in white adipose tissue in proportion to the amount of lipid stores and it has been suggested that increased maternal leptin concentrations in the third trimester of pregnancy may be attributed to the placenta, and not maternal adipose tissue. Leptin regulates placental growth, nutrient transfer, angiogenesis, and trophoblast invasion and enhances the mobilization of maternal fat stores to the fetus. Moreover, most studies have found increased leptin concentrations in GDM [13] and maternal leptin levels have been shown to be associated to fetal growth, and an association between maternal leptin levels during pregnancy and offspring adiposity at two years of age has also been observed [16].

Tumor necrosis factor-alpha (TNF-alpha) is a multifunctional cytokine, and a central regulator of inflammation. TNF-alpha is defined as a cytokine which is the product of monocytes, macrophages, T-cells, neutrophils, fibroblasts and adipocytes. TNF-alpha induces insulin resistance in skeletal muscle and adipose tissue. Plasma TNF-alpha levels correlate with body mass index (BMI) and insulin resistance. High TNF-alpha levels from early gestation are associated with subsequent GDM [13]. There is also evidence that TNF-alpha transcript is significantly increased in placentas from women with GDM at term, compared to non-diabetic control women [17]. It appears that TNF-alpha can activate docosahexaenoic acid, an essential ω-3 polyunsaturated fatty acid that accumulates in placenta of offspring from mothers with GDM, causing increased adiposity in newborns [18].

C-reactive protein (CRP) is a nonspecific marker of inflammation in the body that increases gradually throughout pregnancy to approaching labor. Monocyte chemotactic protein-1 (MCP-1) is a chemokine secreted by monocytes, macrophages, lymphocytes, endothelial cells, decidua, myometrium and placenta. It is a chemotactic factor that attracts and activates monocytes and macrophages into sites of inflammation. MCP-1 increases during normal pregnancy and even more during labor, suggesting that MCP-1 modulates the immune system as pregnancy advances. Several studies have reported that maternal serum CRP and MCP-1 concentrations are negatively correlated to birthweight [19, 20].

Adipsin is a protease with close homology to human complement D. It is secreted from muscle, lung, peripheral nerves, placenta and adipose tissue. Adipsin is the rate-limiting enzyme in the formation of acylation stimulating protein, a factor contributing to lipid storage in adipose tissue. Adipsin levels are significantly higher in obesity and positively related to BMI. Furthermore, higher levels of adipsin have been reported in GDM [21]. A recent study found no association between cord blood adipsin and birthweight [22].

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2, is a glycoprotein secreted by neutrophils, epithelial cells and adipocytes. It is abundantly present in damaged epithelia during inflammation and cardiovascular disease. NGAL concentrations are elevated in obesity, T2DM and GDM [23].

Resistin is a pro-inflammatory adipokine expressed in adipocytes, pancreatic islets, mononuclear cells, macrophages, placenta and liver. In rodents, resistin represents a clear pathogenic factor in the severity of insulin resistance. However, in humans, this adipokine correlates with insulin resistance as a consequence of obesity. Some studies have suggested that elevated circulating resistin is a risk factor for GDM. One study found mRNA expression of gene-encoded resistin was increased in adipose tissue from GDM when compared to a non-GDM group, and the expression level was related to insulin resistance [24]. Conflicting findings have been found regarding cord serum resistin and birthweight [25, 26].

Nerve growth factor (NGF) is one of the neurotrophic factors, and associates with the survival, development and function of central nervous system basal forebrain cholinergic neurons, as well as peripheral embryonic and sympathetic sensory neurons. In addition, NGF appears to play an important role in placental and fetal growth. In small for gestational age (SGA) infants born at term, NGF levels are markedly higher than appropriate for gestational age (AGA) and LGA infants [27]. The NGF gene is also found in white adipose tissues, and it increases in the presence of inflammatory cytokines, including TNF-alpha. In addition, levels of circulating NGF are upregulated in obesity and metabolic syndrome [28].

In summary, most studies related to maternal adipokines and birthweight have yielded inconsistent findings. In addition, very few studies have investigated maternal CRP,

MCP-1, resistin, adipsin, NGAL and nerve growth factor NGF levels. The aim of this study was to determine whether there is an association between maternal plasma adiponectin, leptin, resistin, adipsin, NGAL, NGF, CRP, MCP-1 and TNF-alpha concentrations at late gestation, GDM, and the fetal growth.

MATERIAL AND METHODS

Research Design and Study Population

Data for this cross-sectional study was obtained from a larger study of women who were recruited from the Hospital of Gynecology and Obstetrics 3, Medical Center La Raza, IMSS (Mexico City), or the Hospital of Gynecology and Obstetrics 221, IMSS (Toluca, State of Mexico), for an elective caesarean section, for a study on adipokine gene expression in omental adipose tissue in GDM (data not published). The original study was approved by the Institutional Review Board of the Instituto Mexicano del Seguro Social (IMSS) in Mexico City (R-2018-785-026) and all participants gave written informed consent. The study included singleton births to mothers over 18 years of age from January 2019 to December 2019. To eliminate any confounding effects from the labor process, only those undergoing a term (37–41 weeks of gestation) elective Caesarean section were considered for the study (indications for Caesarean included breech presentation, previous Caesarean section, and/or when macrosomia was suspected by ultrasonography at 38 weeks of gestation). Women with pre-gestational diabetes or hypertension and autoimmune, immunosuppressive, kidney, heart, infectious diseases or smoking and alcohol habits as well as pregnancies complicated by fetal anomalies were excluded from the study. The present study included term births to both normal glucose tolerant (NGT) women and women with GDM. Gestational age was estimated by last menstrual period and confirmed by ultrasonographic measurements at first trimester in all subjects. All pregnant women were screened for GDM at 24–28 weeks of gestation and were classified according to the results of the screening. Women with GDM were diagnosed according to the International Association of Diabetes and Pregnancy Study Groups criteria by one or more abnormal glucose value during a 75 g-oral glucose tolerance test (OGTT), with fasting levels $\geq$ 5.1 mmol/L, 1h $\geq$10 mmol/L or 2 h $\geq$ 8.5 mmol/L. Management after GDM diagnosis was started with medical nutrition therapy (1600–1800 kcal/day with restriction of carbohydrates to 35–40%), and moderate physical activity (30 minutes of moderate-intensity aerobic exercise at least five days a week) and subsequent evaluation of glycemic control with fasting glucose and postprandial blood glucose at two hours after meals at 2–4 week intervals. Insulin therapy (0.7–1.0 units/kg of body weight daily) was prescribed for women who did not achieve glycemic control with diet (fasting glucose levels < 5.27 mmol/L, and postprandial blood glucose values < 6.66 mmol/L at 2 hours). Of the women with GDM, 75% (n = 49) were treated by diet and moderate physical activity only, and the remainder (n = 16) received the aforementioned insulin therapy for glycemic control.

Information about current and previous pregnancies was collected on a baseline questionnaire using medical records. Demographic characteristics included maternal age, history of GDM and family history of diabetes in a first-degree relative. Maternal pre-pregnancy BMI was calculated from patient-reported pre-pregnancy weight and the height measured during the first visit of gestation. BMI at the time of delivery was also calculated.

Birth size measures

Anthropometric measures (weight, length, foot length, head circumference, chest circumference, abdominal circumference, and ponderal index) were assessed at birth in the term infants. Ponderal index was calculated as 100 × [birthweight (g)/length (cm^3)]. The infants were classified as small for gestational age (SGA), appropriate for gestational age (AGA) or large for gestational age (LGA) according to weight for gestational age of Mexican children [29]. LGA and SGA were defined as > 90th and < 10th percentile, respectively. Macrosomia was considered in cases of birth weight ≥ 4000 g. Information about the infant's sex, and Apgar scores came from birth certificates. Apgar scores were noted soon after delivery at one minute and at five minutes.

Biochemical analysis

Maternal fasting blood samples for biochemical analysis were obtained by venipuncture together with the samples for routinely performed laboratory tests at the day of the scheduled Caesarean section. The samples could clot for at least 30 minutes before centrifugation at 1000 g, which was continued for 15 minutes. Serum aliquots were frozen at –70° C until assayed. Levels of glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured in fresh samples on an ARCHITECT Plus c4000 Clinical Chemistry Analyzer (Abbot Diagnostics, Abbott Park, IL, USA). Levels of low- density lipoprotein (LDL) cholesterol were estimated with use of the Friedewald formula. CRP was measured with a particle-enhanced turbidimetric immunoassay (MULTIGENT CRP Vario kit; Sentinel CH, Milan, Italy) on the ARCHITECT Plus c4000 Clinical Chemistry Analyzer (Abbot Diagnostics, Abbott Park, IL, USA). Adiponectin, leptin, resistin, adipsin, NGAL, NGF, MCP-1, TNF-alpha, and insulin were measured through the multiplex immunoassay using Magpix technology (Milliplex Map, Billerica, MA, USA). Insulin resistance was calculated using the homeostasis model as-

sessment for insulin resistance (HOMA-IR) method, where HOMA-IR = fasting insulin concentration [μU/mL] × fasting glucose concentration [mmol/L]/22.5 [30].

Statistical analysis

Kolmogorov-Smirnov test was used to assess data distribution. Results are presented as medians with interquartile range (IQR). Differences between groups were analyzed by Mann-Whitney test and Kruskal-Wallis test. To assess the correlation of the data, Spearman's correlation test was performed. Logistic regression analysis with LGA as a dependent variable was performed with adjustment for the following covariates: length of gestation, infant sex, maternal demographic factors, maternal anthropometric measures, plasma glucose levels, lipids, insulin, adipokines and inflammatory proteins. We used IBM SPSS Statistics 23.0 (IBM SPSS Inc., Chicago, IL) for statistical analysis, and $p < 0.05$ was defined as significant.

RESULTS

The demographic and clinical data of mothers are shown in Table 1. Women with GDM had a higher age, weight, BMI, and parity, previous GDM, family history of diabetes and higher glucose levels at OGTT screening and glucose concentration at late gestation than women with normal glucose tolerance. They did not significantly differ from women without GDM in maternal weight gain at the time of term elective Caesarean section, total cholesterol, triglycerides, HDL, LDL, insulin and HOMA-IR.

Results of the adipocitokines are presented in Table 2. There were no significant differences in adiponectin, leptin, resistin, NGAL, NGF, and CRP levels between subjects with GDM and controls. Women with GDM had higher adipsin, MCP-1, and TNF-alpha levels. These observed differences did not remain after adjusting for age and weight, and insulin treatment did not significantly affect adipokine levels between women taking insulin therapy and those not.

Elevated pre-pregnancy maternal BMI in GDM and NGT subjects, was found to be positively associated with adipsin ($r = 0.320$, $p = 0.002$), leptin ($r = 0.369$, $p = 0.001$), MCP-1 ($r = 0.410$, $p = 0.001$), and TNF-alpha levels ($r = 0.341$, $p = 0.001$).

Gestational age at delivery was significantly lower among GDM in comparison with healthy pregnant women (Tab. 3).

Table 1. Characteristics of women with and without GDM

	NGT (n = 65)	GDM (n = 65)	p
Maternal age [years]	26 (22–31)	32 (28–35)	0.001
Pregravid weight [kg]	60.0 (54.8–67.0)	78.1 (63.5–96.5)	0.001
Weight at delivery [kg]	70.3 (65.7–77.3)	85.2 (72.5–104.5)	0.001
Pre-pregnancy BMI [kg/m^2]	25.1 (23.0–27.1)	32.5 (26.3–38.3)	0.001
BMI at delivery [kg/m^2]	29.6 (27.0–31.5)	34.8 (30.8–41.4)	0.001
Maternal weight gain [kg]	10.2 (7.1–12.5)	8.3 (4.2–12.3)	0.202
Parity n [%] Nulliparous One or greater	 24 (36.9) 41 (63.1)	 9 (13.8) 56 (86.2)	0.008
Family history of diabetes n [%]	7 (10.8)	23 (35.4)	0.004
Past history of GDM n [%]	0 (0.0)	12 (18.5)	0.001
Blood glucose at OGTT [mmol/L]			
Fasting	4.0 (3.8–4.2)	5.4 (4.6–5.8)	0.001
1 h	7.5 (6.5–8.8)	12.8 (11.0–13.0)	0.001
2 h	6.4 (5.5–7.4)	9.8 (8.7–10.5)	0.005
Fasting glucose at delivery [mmol/L]	4.2 (3.8–4.8)	4.7 (4.1–5.3)	0.05
Triglycerides at delivery [mmol/L]	2.9 (2.5–3.6)	3.4 (2.2–3.9)	0.280
HDL at delivery [mmol/L]	2.5 (2.2–3.0)	2.4 (1.9–3.0)	0.319
LDL at delivery [mmol/L]	1.8 (1.3–2.5)	1.4 (0.84-2.1)	0.081
Total cholesterol at delivery [mmol/L]	60.1 (52.0–67.0)	55.8 (49.3–66.0)	0.188
Fasting insulin at delivery [mmol/L]	56.0 (37.6–82.7)	60.4 (43.4–81.7)	0.902
HOMA-IR at delivery	1.4 (0.9–2.3)	1.7 (1.3–2.5)	0.149

Data are presented as medians (interquartile range) as well as counts and percentages; NGT — normal glucose tolerant; GDM — gestational diabetes mellitus; BMI — body mass index; OGTT — oral glucose tolerance test; HDL — high-density lipoprotein cholesterol; LDL — low- density lipoprotein cholesterol; HOMA-IR — homeostasis model assessment-insulin resistance

Table 2. Maternal adipokines and inflammatory markers for normal pregnancy and GDM at delivery

	NGT (n = 65)	GDM (n = 65)	p
Adiponectin [pg/mL]	213.5 (77.6–766.4)	220.9 (64.6–605.1)	0.490
Resistin [pg/mL]	54.6 (38.6–68.8)	43.9 (32.4–62.9)	0.097
Adipsin [pg/mL]	2.5 (1.9–3.1)	3.1 (2.4–3.9)	0.011
NGAL [pg/mL]	164.9 (112.0–234.7)	159.7 (109.7–247.2)	0.520
NGF [pg/mL]	2.1 (1.6–2.6)	2.1 (1.6–2.9)	0.487
Leptin [pg/mL]	7.4 (4.7–11.3)	8.4 (4.9–17.0)	0.538
CRP [nmol/L]	6.9 (3.7–10.8)	9.5 (5.0–22.9)	0.096
MCP-1 [pg/mL]	93.3 (68–137)	126.5 (102.4–165.2)	0.005
TNF-alpha [pg/mL]	2.9 (2.3–3.5)	3.9 (2.8–4.7)	0.006

Data are presented as medians (interquartile range); NGT — normal glucose tolerant; GDM — gestational diabetes mellitus; NGAL — neutrophil gelatinase-associated lipocalin; NGF — nerve growth factor; CRP — C-reactive protein; MCP-1 — monocyte chemotactic protein-1; TNF — alpha, tumor necrosis factor-alpha

Table 3. Neonatal characteristics grouped by maternal glucose tolerance status during pregnancy

	NGT (n = 65)	GDM (n = 65)	p
Gestational age [weeks]	39 (38–40)	38 (38–39)	0.013
Sex, female	30 (46.2)	41 (63.0)	0.109
Birth weight [g]	3100 (2875–3300)	3400 (3000–3900)	0.001
Birth length [cm]	49 (48–50)	50 (49–52)	0.037
Ponderal index [g/cm^3]	2.5 (2.4–2.76)	2.7 (2.5–2.95)	0.05
Nenonatal foot length [cm]	8 (7–8)	8 (7–8)	0.560
Neonatal head circumference [cm]	34 (33.25–35)	35 (34–36)	0.084
Neonatal chest circumference [cm]	33 (32–34)	32 (31–34)	0.510
Neonatal abdominal circumference [cm]	30 (30–32)	31 (29–33)	0.412
Birth weight for gestational age n [%] Appropriate for gestational age Large for gestational age	 63 (97) 2 (3)	 44 (68) 21 (32)	0.001
Macrosomia n [%]	1 (0.65)	10 (6.5)	0.001
Apgar 1´ Score	8 (8–8)	8 (8–8)	0.639
Apgar 2´ Score	9 (9–9)	9 (9–9)	0.519

Data are presented as medians (interquartile range) as well as counts and percentages; NGT — normal glucose tolerant; GDM — gestational diabetes mellitus

However, offspring of mothers with GDM had higher weight, height, and ponderal index than offspring of mothers without GDM. The rate of LGA was significantly higher in the GDM group than in patients with a non-GDM pregnancy. The occurrence of macrosomia was markedly higher in GDM group; there were ten macrosomic newborns in GDM and one in the NGT group. The rate of girls, foot length, head circumference, chest circumference, abdominal circumference, and Apgar score did not significantly differ between both groups.

Pre-pregnancy BMI, biochemical maternal parameters and adiponectin, leptin, resistin, adipsin, NGAL and NGF were not significantly associated with higher birthweight. The maternal factors in association with higher birthweight observed were CRP, MCP-1, and TNF-alpha levels. Among women with GDM, the median maternal plasma concentrations of CRP and MCP-1 showed no significant differences between mothers of AGA and LGA neonates (Fig. 1). However, the median maternal plasma CRP and MCP-1 concentration were higher in mothers of LGA neonates with GDM than in mothers with NGT and AGA newborns. Furthermore, among women with GDM, the median maternal plasma TNF-alpha concentration was higher in women with an LGA neonate than in those with an AGA newborn, and TNF-alpha concentration was higher in women with GDM and LGA neonate than in those with NGT and AGA newborn.

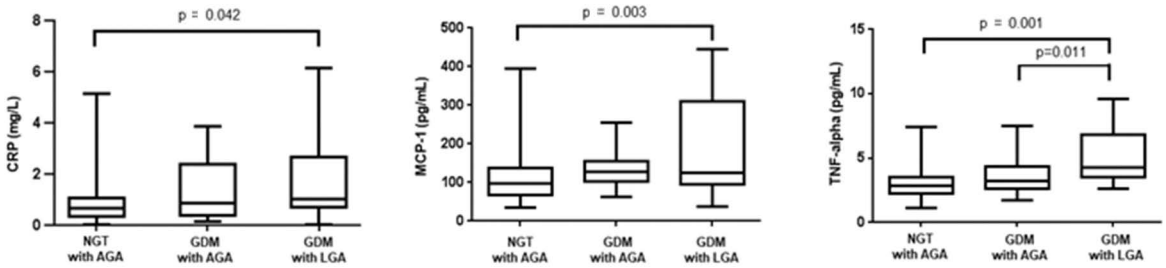

Figure 1. Box and whisker plots of serum CRP, MCP-1 and TNF-alpha levels between the study groups and/or cases of an LGA product. Among women with GDM, there were no statistically significant differences in concentrations of CRP and MCP-1 in maternal plasma between patients with AGA and LGA products. However, the median maternal plasma CRP and MCP-1 concentration were higher in pregnant women with GDM and LGA neonates than in those with NGT and AGA newborns. Furthermore, among women with GDM, the maternal plasma concentrations of TNF-alpha were higher in cases of GDM and LGA neonate than in NGT and AGA newborn; NGT — normal glucose tolerant; GDM —gestational diabetes mellitus; AGA — appropriate for gestational age; LGA — large for gestational age; CRP — C-reactive protein; MCP-1— monocyte chemotactic protein-1; TNF-alpha — tumor necrosis factor-alpha

On logistic regression analysis, the significant predictor of having a LGA infant, after adjustment for covariates, was TNF-alpha level (odds ratio: 1.55, 95% confidence interval: 1.04–2.32, p = 0.046).

DISCUSSION

In our study, we evaluated the association between specific maternal adipokines and inflammatory markers at late gestation and fetal growth in mothers with GDM. Notably, offspring of mothers with GDM had higher weight, height and ponderal index than offspring of mothers without GDM, and the rate of LGA was significantly higher in the GDM group than in patients with a non-GDM pregnancy. Gestational diabetes mellitus is a common predisposing factor for having infants who are born large for gestational age and subsequently develop an increased risk of obesity and T2DM in adulthood [7]. Maternal blood glucose level is associated with birthweight. However, even with strict glycemic control, women with GDM have higher frequency of macrosomic infants [31]. It has been suggested that other nutrients, such as triglycerides and amino acids, contribute to excessive fetal growth [8]. In this study, we did not find any relation of either maternal fasting glucose or triglyceride concentration at term with newborn weight. Similarly, none of the metabolic measures, such as glucose values at OGTT, total cholesterol, HDL, LDL, insulin and HOMA-IR, were associated with birth weight. Moreover, although mean values of OGTT indices at screening were significantly elevated in GDM over healthy controls, indicating increased insulin resistance in GDM group, there was no difference between studied groups regarding lipid profile, insulin and HOMA-IR at term. This could be a consequence of metabolic control performed in GDM, which was evaluated at 2–4-week intervals until delivery. Notably, maternal weight gain during pregnancy did not differ between the study groups.

Recently, maternal adipokines and inflammatory cytokines have been identified as independent risk determinants of fetal overgrowth [9–12]. In the present study, fetal growth was associated with higher CRP, MCP-1, and TNF-alpha levels in mothers with GDM. The association between newborn weight and TNF-alpha was independent of established risk factors of GDM such as age, prepregnancy BMI, parity, and family history of T2DM. Similarly, recent findings from Kumarathasan et al., demonstrated a proinflammatory status in LGA healthy mothers, as higher inflammatory index compared to the AGA group [19].

Some studies have reported, contrary to our finding, that maternal serum CRP and MCP-1 concentrations are negatively correlated to birthweight. Thus, CRP at 28 weeks' gestation in healthy pregnant women, as well as maternal MCP-1 at third trimester (32–34 weeks), were associated with SGA births [19, 20]. The discrepancy between our finding and others might be related to gestational age at sampling. Further, our population differs from others in relation to GDM, which could affect the maternal inflammatory state. GDM associates with changes in inflammatory profiles, both maternal, fetal and placental, mirrored by an increase in circulating inflammatory molecules [32].

Obesity is associated with pathologic sequelae, including chronic inflammation and adipocyte dysregulation. Maternal obesity, defined by an elevated pre-pregnancy BMI, is a risk factor for fetal macrosomia. In our study, maternal BMI was not associated with birthweight. However, maternal BMI was in correlation with the serum adipsin, leptin, MCP-1, and TNF-alpha levels, suggesting that one of the main sources of cytokine production is the adipose tissue, although it has also been documented that the feto-placental unit could also be an important source of TNF-alpha and leptin [13, 17].

In the current study, we did not find an association between maternal adipokines adiponectin, leptin, adipsin

NGAL, NGF and resistin levels and fetal growth. Previous data have shown that some of these adipokines are associated with fetal growth. However, most studies have examined maternal adipokines in midpregnancy, or are focused on cord blood. In our study, maternal adipokines were measured at the time of Caesarean section. It has been demonstrated that blood levels of adipokines change throughout pregnancy in relation to the increased fat accretion in the first two trimesters of pregnancy and to the changes in clearance in latter stages of pregnancy. In addition, the gestational diabetes participants in our study had higher pre-pregnancy BMI, parity, and birthweight than pregnant controls, which are factors associated with higher circulating blood volume. The increase in plasma volume would result in lower circulating adipokine levels. Other discrepancies may also be due to the different genetic backgrounds or environmental condition of the populations studied, study design, sample size of the population, the diagnosis criteria of GDM patients, the status of glycemic control, and the assay methods used to measure adipokines.

The cross-sectional design together with the limited sample size, must be considered as a limitation of our study. We did not assess the associations between the adipokines at various points of time in gestation and birth outcomes. We also relied on self-reported pre-pregnancy weight, which is susceptible to reporting bias. Besides, the difference in age and BMI between women with GDM and women with a non-GDM pregnancy must be considered, although adjustments for these variables were made. In addition, there was a lack of HbA1C, and cord plasma measurements and of other, more specific, measures of maternal adiposity, that is, intra-abdominal maternal visceral adipose tissue (VAT). BMI conveys no information about the quantity, quality, location, or metabolic function of fat depots. Measurement of VAT by ultrasound in early pregnancy strongly predicts GDM and is associated with birthweight [33, 34]. Excess VAT leads to increased release of fatty acids and secretion of pro-inflammatory substances, with consequent changes in fetal growth.

Strengths of this study include the assessment of several maternal adipokines, while much of the prior literature is focused on cord blood or neonatal blood and the control of numerous pregnancy factors, including mode of delivery, gestational age, maternal fasting status at delivery and maternal smoking.

Overall, the major present findings reveal that circulating maternal CRP, MCP-1, and TNF-alpha are higher in term GDM and LGA neonates than in those with term NGT and AGA newborns. Although it is not possible to determine exactly if the source of these inflammatory markers is placenta or adipose tissue, this provides a further evidence for a possible association of these inflammatory markers with metabolic processes in the feto-placental unit involved in fetal growth. This inflammatory profile can alter developmental programming and have long-lasting influence on offspring. In support of this, Perrin et al. [35], in a cohort of infants born prior to 28-weeks' gestation, reported that obesity at age two years is predicted by perinatal systemic inflammation.

CONCLUSIONS

These results suggest an involvement of maternal inflammatory markers at late gestation and fetal growth in mothers with GDM, and that TNF-alpha could play a major role. Further mechanistic studies are necessary to investigate the precise roles of these inflammatory markers in birthweight.

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Authors' contributions

RS, JV, and MHV planned and designed the study. JV, MIPC, LEMG, AAM, and YG performed laboratory biomarker analysis. RS, JV, and MFDV drafted the manuscript and RS performed the data analysis. All authors provided significant intellectual contribution in interpreting data and critical review of the manuscript. All authors approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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Impact of advanced maternal age on maternal and neonatal outcomes in preterm birth

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ABSTRACT

Objectives: The aim of this study was to investigate the influence of advanced maternal age on the maternal and neonatal outcomes of preterm pregnancies.

Material and methods: The characteristics of patients admitted to the Department of Obstetrics and Gynecology, The First Affiliated Hospital of Fujian Medical University between January 2015 and March, 2019 were retrospectively reviewed. The maternal and neonatal outcomes were compared between advanced maternal age group (≥ 35 years) and younger age group (18–34 years). Statistical analysis was performed by applying the SPSS software.

Results: The study population consisted of 986 pregnancies with preterm delivery and 1094 liveborn preterm infants. Multivariate analyses demonstrated that mothers of advanced age were more likely to suffer iatrogenic preterm birth, placenta previa, preeclampsia, gestational diabetes mellitus and postpartum hemorrhage, but less likely to suffer multiple gestation. In terms of neonatal outcomes, advanced maternal age was associated with a decreased rate of low birthweight in an adjusted model without multiple gestation. However, with multiple gestation included in the adjusted model, advanced maternal age was only associated with an increased rate of hyperbilirubinemia.

Conclusions: Advanced maternal age was a risk factor for adverse pregnancy outcomes including iatrogenic preterm birth, placenta previa, preeclampsia, gestational diabetes mellitus, postpartum hemorrhage, and a protective factor for multiple gestation. Regarding neonatal outcomes, advanced maternal age was related to a decreased rate of low birthweight or an increased rate of hyperbilirubinemia depending on the adjustment for multiple gestation.

Key words: advanced maternal age; multiple gestation; preterm birth; pregnancy outcome; preeclampsia

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INTRODUCTION

Conception at an advanced maternal age (35 years or older) has been an increasing trend worldwide over the past decades [1]. In China, the advanced pregnancies increased by over 10% in the decade from 2004 to 2014, accounting for about 31% of total pregnancies in 2016 [2]. Numerous women delay childbearing to achieve their educational and career-related goals. Additionally, the better access to contraception and developments of assisted reproductive technology (ART) also contribute to an increasing incidence of delayed childbearing [3]. Advanced maternal age is related to a range of unfavorable pregnancy outcomes [4]. Several studies demonstrated that women with advanced maternal age in their nulliparous singleton pregnancies were more likely to undergo gestational diabetes mellitus (GDM), gestational hypertension, preeclampsia, cesarean section, small for gestational age infants, and more admission requirement for the neonatal intensive care unit [5, 6]. In pregnancies by ART or spontaneous conception, advanced maternal age also increased the risk of gestational hypertensive disorders, placenta previa, cesarean delivery, preterm birth, low birthweight, and small for gestational age [3]. Those findings, however, were mainly observed in infants born at term. For premature infants who suffer higher risks for adverse outcomes, the data are scarce and inconsistent according to few studies. The studies by Eventov-Friedman et al. [7] and DiLabio et al. [8] demonstrated that maternal age did not contribute to poor short- and long-term neurodevelopmental outcomes of preterm infants. Whereas Kanungo et al. [9] found an association of advanced maternal age with increased survival without major morbidity and a decreased risk of mortality for preterm newborns.

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Objectives

In view of the increasing proportion of delayed childbearing, the present study aimed to investigate the impact of advanced maternal age on the short-term outcomes of mothers with preterm delivery and of their premature infants.

MATERIAL AND METHODS

Study population

This retrospective study enrolled women who underwent preterm delivery (a gestational age of < 37 weeks) at the Department of Obstetrics and Gynecology, The First Affiliated Hospital of Fujian Medical University (Fuzhou, China) between January 2015 and March 2019. Maternal and neonatal variables were reviewed from the medical records. The exclusion criteria included maternal age younger than 18 years, induced labor, intrauterine fetal death or stillbirth and missing information of interest. According to the maternal age at delivery, the participants were categorized into the advanced maternal age group (maternal age ≥ 35 years) and the reference group (maternal age 18–34 years). The maternal and neonatal characteristics were compared between the two groups. The study was accordant with the Declaration of Helsinki and was approved by our institutional review board. Informed consent was obtained from the participants for the use of their records for the evaluation of medical practices.

Variables and definitions

The maternal characteristics included age, height, body mass index (BMI), education, gravidity, parity, appropriate pregnancy interval (1.5–5 years), prenatal examination, history of premature birth and abortion, method of conception (conception spontaneously or by ART), pre-existing disease (chronic disease prior to the pregnancy) and pregnancy outcomes. Pregnancy outcomes included multiple gestation, gestational age, cause of preterm birth (for spontaneous or iatrogenic reasons), cesarean delivery, placenta previa, placental abruption, intrauterine growth retardation (IUGR, fetal weight below 10th percentile by ultrasound examination), abnormal amniotic fluid volume (amniotic fluid index ≤ 5 cm or > 20 cm), intrahepatic cholestasis of pregnancy (ICP, new onset pruritus and total bile acid level of ≥ 10 µmol/L), preeclampsia (pregnancy-induced hypertension accompanied by daily proteinuria of > 300 mg), GDM (positive result of 75 g oral glucose tolerance test during 24–28 weeks of gestation) and postpartum hemorrhage (PPH, blood loss of more than 500 ml or 1000 mL within the first 24 h after vaginal delivery or caesarean section, respectively).

Neonatal outcomes included sex, birthweight, Apgar scores at 1 and 5 min, neonatal intensive care unit (NICU) admission, neonatal morbidity and mortality. Neonatal morbidity included fetal abnormalities, neonatal asphyxia, anemia, hypoglycemia, sepsis, pneumonia, hyperbilirubinemia, hypoxic ischemic encephalopathy (HIE), myocardial damage, and necrotizing enterocolitis. Neonatal asphyxia was defined as oxygen deprivation of organs or brain before, during or just after birth. Anemia was defined as hematocrit < 39%. Neonatal hypoglycemia was defined as plasma glucose concentration of below 45 mg/dL. Neonatal sepsis was defined by positive blood culture results for either bacteria or fungus. Neonatal hyperbilirubinemia was diagnosed based on the serum bilirubin level of more than 10 mg/dL (171 µmol/L). Hypoxic ischemic encephalopathy was diagnosed based on impaired placental gas exchange complicated by encephalopathy. Myocardial damage was diagnosed based on serum cardiac troponin T of > 0.1 µg/L. Necrotizing enterocolitis was defined according to Bell's criteria at stage ≥ 2 [10]. Neonatal mortality was defined as infant death before hospital discharge.

Statistical analysis

The statistical analysis was performed using SPSS software version 19.0 (IBM Corp, Armonk, NY, USA). The continuous data were presented as mean ± standard deviation (SD) and categorical data presented as number (percentage). The χ^2 test was employed for the comparison of categorical variables. The normality assessment followed by the Student's t test (data with normal distribution) or the Mann-Whitney U test (data with skewed distribution) was applied for the comparison of continuous variables. Univariate and multivariate binomial logistic regression analyses were employed to analyze the independent value of advanced maternal age for maternal outcomes which was adjusted for height, BMI, education level, parity, method of conception, pre-existing disease, and for neonatal outcomes which was additionally adjusted for GDM, preeclampsia or multiple gestation. A p-value less than 0.05 was considered statistically significant.

RESULTS

Maternal and neonatal characteristics of patients

A total of 1014 preterm deliveries occurred in our hospital between January 2015 and March 2019. According to the exclusion criteria, 986 patients were included in the study with 230 (23.33%) women at the age of ≥ 35 years and 756 (76.67%) women at the age of 18–34 years. The maternal and neonatal characteristics according to maternal age groups are summarized in Table 1. Mothers in the advanced age group had significantly higher frequency of gravidity and parity, as well as higher proportion of the histories of abortion and pre-existing disease compared to those in the reference group ($p < 0.05$). Whereas mothers of advanced

Table 1. Maternal and neonatal characteristics according to maternal age group*

	Maternal age group		Statistic value	p-value
	18–34 years	≥ 35 years		
Maternal, number	n = 756 (76.67)	n = 230 (23.33)		
Age [years]	28.51 ± 3.47	37.63 ± 2.42	Z = –23.03	**< 0.001**
Height [cm]	160.42 ± 4.59	159.69 ± 4.17	Z = –2.57	**0.010**
BMI [kg/m^2]	26.51 ± 3.34	27.41 ± 8.03	Z = –1.86	0.063
Education			χ^2=1.25	0.264
High school or less	389 (51.46)	128 (55.65)		
College or university	367 (48.54)	102 (44.35)		
Gravidity			Z = –10.36	**< 0.001**
1	275 (36.38)	8 (3.48)		
2	218 (28.84)	67 (29.13)		
≥ 3	263 (34.78)	155 (67.39)		
Parity			Z = –9.92	**< 0.001**
0	402 (53.17)	35 (15.22)		
1	306 (40.48)	165 (71.74)		
≥ 2	48 (6.35)	30 (13.04)		
Appropriate pregnancy interval				
Nulliparous	402 (53.17)	35 (15.22)		
Yes	222 (29.37)	50 (21.74)	χ^2 = 69.12	**< 0.001**
No	132 (17.46)	145 (63.04)		
Regular prenatal examination	650 (85.98)	184 (80.00)	χ^2 = 4.83	**0.028**
History of premature birth	25 (3.31)	14 (6.09)	χ^2 = 3.59	0.058
History of abortion	316 (41.80)	162 (70.43)	χ^2 = 57.90	**< 0.001**
Use of ART	46 (6.08)	19 (8.26)	χ^2 = 1.36	0.244
Pre-existing disease	208 (27.51)	82 (35.65)	χ^2 = 5.63	**0.018**
Neonatal, number	n = 848 (77.51)	n = 246 (22.49)		
Sex, male	479 (56.49)	135 (54.88)	χ^2 = 0.20	0.655
Birthweight [g]	2281.47 ± 607.62	2312.76 ± 712.14	Z = –1.20	0.232
Apgar score at 1 min	8.89 ± 2.12	8.72 ± 1.99	Z = –1.46	0.143
Apgar score at 5 min	9.57 ± 1.27	9.52 ± 1.30	Z = –0.58	0.561
NICU stay [days]	12.84 ± 15.14	12.92 ± 17.40	Z = –1.00	0.320

*Data are presented as number (percentage) or mean ± standard deviation; ART — assisted reproductive technology; BMI — body mass index; NICU — neonatal intensive care unit

age were less likely to have higher height as well as to undergo appropriate pregnancy interval and regular prenatal examination than those of younger age ($p < 0.05$). There were a total of 1094 live births, with 246 preterm infants born to mothers of advanced age and 848 preterm infants born to mothers aged 18–34 years. There was no significant difference regarding neonatal sex, birthweight, Apgar scores at 1 and 5 min, and NICU stay between the 2 groups.

Impact of advanced maternal age on maternal outcomes

The impact of advanced maternal age on pregnancy outcomes is shown in Table 2 and 3. Compared to women with 18–34 years of age, women aged ≥ 35 years had significantly decreased proportion of multiple gestation and spontaneous preterm birth but increased proportion of iatrogenic preterm birth and cesarean delivery ($p < 0.05$). Moreover, pregnancy complications including placenta previa, preeclampsia, GDM, and PPH were more frequently seen in women with advanced age than in women with 18–34 years of age ($p < 0.001$) (Tab. 2). Based on the results of unadjusted logistic regression analysis (Tab. 3), the incidences of pregnancy outcomes including iatrogenic preterm birth, cesarean delivery, placenta previa, preeclampsia, GDM and PPH were more likely in mothers with advanced age, whereas multiple gestation was less likely to

Table 2. Pregnancy outcomes according to maternal age group*

	Total (n = 986)	Maternal age group		c^2	p-value
		18–34 years (n = 756)	≥ 35 years (n = 230)		
Multiple gestation	111 (11.26)	94 (12.43)	17 (7.39)	4.49	**0.042**
Gestational age				0.44	0.514
28 to < 32 weeks	137 (13.89)	102 (13.49)	35 (15.22)		
32 to < 37 weeks	849 (86.11)	654 (86.51)	195 (84.78)		
Cause of preterm birth				25.31	**< 0.001**
Spontaneous reason	499 (50.61)	416 (55.03)	83 (36.09)		
Iatrogenic reason	487 (49.39)	340 (44.97)	147 (63.91)		
Cesarean delivery	715 (72.52)	524 (69.31)	191 (83.04)	16.68	**< 0.001**
Placenta previa	164 (16.63)	104 (13.76)	60 (26.09)	19.34	**< 0.001**
Placental abruption	51 (5.17)	39 (5.16)	12 (5.22)	< 0.01	1.000
Intrauterine growth retardation	155 (15.72)	117 (15.48)	38 (16.52)	0.66	0.458
Abnormal amniotic fluid volume	42 (4.26)	33 (4.37)	9 (3.91)	0.09	0.854
Intrahepatic cholestasis of pregnancy	48 (4.87)	38 (5.03)	10 (4.35)	0.18	0.731
Preeclampsia	178 (18.05)	118 (15.61)	60 (26.09)	13.09	**< 0.001**
Gestational diabetes mellitus	169 (17.14)	107 (14.15)	62 (26.96)	20.35	**< 0.001**
Postpartum hemorrhage	88 (8.92)	52 (6.88)	36 (15.65)	16.70	**< 0.001**

*Data are presented as number (percentage)

Table 3. Impact of advanced maternal age on pregnancy outcomes by the univariate and multivariate analyses

	≥ 35 years			
	Crude OR (95% CI)	p-value	Adjusted OR[a] (95% CI)	p-value
Multiple gestation	0.56 (0.33–0.96)	0.036	0.33 (0.16–0.64)	0.001
Gestational age (very preterm)	0.87 (0.57–1.32)	0.508	0.88 (0.56–1.38)	0.578
Iatrogenic preterm birth	2.17 (1.60–2.94)	< 0.001	1.65 (1.18–2.31)	0.004
Cesarean delivery	2.17 (1.49–3.16)	< 0.001	1.50 (0.99–2.27)	0.055
Placenta previa	2.21 (1.54–3.17)	< 0.001	1.64 (1.11–2.43)	0.013
Placental abruption	1.01 (0.52–1.97)	0.972	1.11 (0.54–2.27)	0.776
Intrauterine growth retardation	1.08 (0.73–1.61)	0.703	1.32 (0.85–2.04)	0.219
Abnormal amniotic fluid volume	0.89 (0.42–1.89)	0.766	0.81 (0.37–1.79)	0.602
Intrahepatic cholestasis of pregnancy	0.86 (0.42–1.75)	0.676	0.75 (0.35–1.62)	0.465
Preeclampsia	1.91 (1.34–2.72)	< 0.001	1.98 (1.32–2.96)	0.001
Gestational diabetes mellitus	2.24 (1.57–3.20)	< 0.001	2.44 (1.65–3.61)	< 0.001
Postpartum hemorrhage	2.51 (1.60–3.96)	< 0.001	1.89 (1.16–3.08)	0.011

[a]Adjusted for height, body mass index, education level, parity, method of conception and pre-existing disease; CI — confidence interval; OR — odds ratio

be seen in the older mothers. After adjusting for the potential confounders (Tab. 3), the differences regarding multiple gestation [adjusted odds ratio (OR) 0.33, 95% confidence interval (CI) 0.16–0.64], iatrogenic preterm birth (adjusted OR 1.65, 95% CI 1.18–2.31), placenta previa (adjusted OR 1.64, 95% CI 1.11–2.43), preeclampsia (adjusted OR 1.98, 95% CI 1.32–2.96), GDM (adjusted OR 2.44, 95% CI 1.65–3.61) and PPH (adjusted OR 1.89, 95% CI 1.16–3.08) remained statically significant, with the cesarean delivery near a significant level (adjusted OR 1.50, 95% CI 0.99–2.27).

Impact of advanced maternal age on neonatal outcomes

The impact of advanced maternal age on neonatal outcomes is shown in Table 4 and 5. The rates of hyperbilirubinemia, NICU admission and neonatal mortality were more

Table 4. Neonatal outcomes according to maternal age groups

	Total (n = 1094)	18–34 years (n = 848)	≥ 35 years (n = 246)	c^2	p-value
Low birthweight < 2500 g	620 (56.67)	494 (58.25)	126 (51.22)	3.84	0.050
Apgar score < 7/1 min	104 (9.51)	76 (8.96)	28 (11.38)	1.30	0.255
Apgar score < 7/5 min	30 (2.74)	23 (2.71)	7 (2.95)	0.02	0.895
Fetal abnormalities	61 (5.58)	46 (5.54)	15 (6.10)	0.16	0.685
Asphyxia	186 (17.00)	135 (15.92)	51 (20.73)	3.13	0.077
Anemia	218 (19.93)	163 (19.22)	55 (20.36)	1.18	0.278
Hypoglycemia	313 (28.61)	245 (28.89)	68 (27.64)	0.15	0.703
Sepsis	167 (15.27)	126 (14.86)	41 (16.67)	0.48	0.488
Pneumonia	117 (10.69)	91 (10.73)	26 (10.57)	0.01	0.942
Hyperbilirubinemia	817 (74.68)	616 (72.64)	201 (81.71)	8.29	**0.004**
Hypoxic ischemic encephalopathy	12 (1.10)	9 (1.06)	3 (1.22)	0.04	0.834
Myocardial damage	403 (36.84)	318 (37.50)	85 (34.55)	0.71	0.399
Necrotizing enterocolitis	26 (2.38)	20 (2.36)	6 (2.44)	0.01	0.942
NICU admission	906 (82.82)	691 (81.49)	215 (87.40)	4.68	**0.030**
Neonatal mortality	26 (2.38)	16 (1.89)	10 (4.07)	3.90	**0.048**

*Data are presented as number (percentage); NICU — neonatal intensive care unit

Table 5. Impact of advanced maternal age on neonatal outcomes by the univariate and multivariate analyses

	≥ 35 years					
	Crude OR (95% CI)	p-value	Adjusted OR[a] (95% CI)	p-value	Adjusted OR[b] (95% CI)	p-value
Low birthweight < 2500 g	0.75 (0.57–1.00)	0.050	0.68 (0.49–0.93)	0.017	0.77 (0.55–1.07)	0.113
Apgar score < 7/1 min	1.31 (0.83–2.06)	0.256	1.14 (0.69–1.90)	0.611	1.10 (0.66–1.83)	0.723
Apgar score < 7/5 min	1.05 (0.45–2.48)	0.910	0.80 (0.31–2.07)	0.644	0.76 (0.30–1.98)	0.580
Fetal abnormalities	1.13 (0.62–2.07)	0.686	1.05 (0.55–2.02)	0.878	1.05 (0.55–2.04)	0.875
Asphyxia	1.38 (0.97–1.98)	0.078	1.24 (0.84–1.84)	0.282	1.20 (0.81–1.78)	0.374
Anemia	1.21 (0.86–1.71)	0.279	1.12 (0.77–1.64)	0.549	1.20 (0.82–1.77)	0.343
Hypoglycemia	0.94 (0.69–1.29)	0.703	0.81 (0.57–1.14)	0.230	0.83 (0.59–1.18)	0.305
Sepsis	1.15 (0.78–1.68)	0.488	1.14 (0.75–1.74)	0.529	1.17 (0.77–1.79)	0.455
Pneumonia	0.98 (0.62–1.56)	0.942	0.85 (0.51–1.40)	0.518	0.87 (0.52–1.45)	0.593
Hyperbilirubinemia	1.68 (1.18–2.40)	0.004	1.45 (0.99–2.12)	0.060	1.48 (1.01–2.18)	0.047
Hypoxic ischemic encephalopathy	1.15 (0.31–4.28)	0.834	1.27 (0.30–5.43)	0.744	1.13 (0.26–5.00)	0.868
Myocardial damage	0.88 (0.65–1.19)	0.399	0.86 (0.62–1.18)	0.343	0.88 (0.64–1.22)	0.437
Necrotizing enterocolitis	1.04 (0.41–2.61)	0.942	1.26 (0.45–3.47)	0.662	1.38 (0.50–3.84)	0.538
NICU admission	1.58 (1.04–2.39)	0.032	1.28 (0.81–2.00)	0.289	1.35 (0.86–2.12)	0.197
Neonatal mortality	2.20 (0.99–4.92)	0.054	2.23 (0.91–5.47)	0.078	2.30 (0.93–5.71)	0.072

[a]Adjusted for height, body mass index, education level, parity, method of conception, pre-existing disease, gestational diabetes mellitus and preeclampsia; [b]Adjusted for the above factors and multiple gestation; CI — confidence interval; NICU — neonatal intensive care unit; OR — odds ratio

common in the advanced maternal age group compared with the reference group ($p < 0.05$). The rates of other neonatal outcomes were similar between the two groups (Tab. 4). According to the results of unadjusted logistic regression analysis (Tab. 5), advanced maternal age was significantly associated with higher incidences of hyperbilirubinemia and NICU admission. After adjusting for the potential confounders including height, BMI, education level, parity, method of conception, pre-existing disease, GDM and preeclampsia, however, the association of advanced maternal age with a decreased rate of low birthweight (adjusted OR 0.68, 95% CI 0.49–0.93) was significant. The association of advanced maternal age with increased rates of hyperbilirubinemia (adjusted OR 1.45, 95% CI 0.99–2.12) and neonatal mortal-

ity (adjusted OR 2.23, 95% CI 0.91–5.47) was marginally significant. With multiple gestation included in the adjusted model, the association of advanced maternal age with an increased rate of hyperbilirubinemia (adjusted OR 1.48, 95% CI 1.01–2.18) was statistically significant, whereas the difference of low birthweight (adjusted OR 0.77, 95% CI 0.55–1.07) between maternal age groups was not statistically significant. There was no significant difference between the two groups regarding the rates of other neonatal outcomes in the two adjusted models.

DISCUSSION

Delayed childbearing is increasingly common over the past decades, which is regarded as a risk factor for adverse maternal and perinatal outcomes [11]. The present study found that, with the potential confounders controlled, mothers giving birth at an advanced age were more likely to undergo iatrogenic preterm birth, placenta previa, preeclampsia, GDM, PPH but less likely to undergo multiple gestation. Regarding the neonatal outcomes, there was a significant association of advanced maternal age with a decreased rate of low birthweight after controlling for the confounders without multiple gestation. However, with the multiple gestation adjusted, advanced maternal age was only associated with an increased risk of hyperbilirubinemia.

Tseng et al. [12] found an association of advanced maternal age with preeclampsia by univariable analysis. The present study, after adjustment for main confounding factors, observed the independent role of maternal age for preeclampsia. Women of advanced maternal age were approximately 2.0 times more likely to suffer preeclampsia than those under 35 years of age (Tab. 3). The proper explanation could be that aging promotes vascular pathologies including endothelial dysfunction and pathological remodeling of the microcirculation, which characterize preeclampsia [13]. Aging-associated vascular dysfunctions and vasculopathies are also hallmarks of diabetes [14]. According to our results, GDM was almost twice (27% and 14%, respectively) as common in women aged ≥ 35 years compared with women aged < 35 years. Moreover, the positive association of maternal age with GDM in preterm pregnancies remained significant even after adjustment for main potential confounders, which was consistent with the previous study by Lai et al. [15].

Placenta previa is an unfrequently seen complication which occurs to 0.4–3.2% of pregnancies [3, 16, 17]. Tseng et al. [12] found no effect of advanced maternal age on placenta previa by univariate analysis in a cohort of very low birthweight preterm infants. The present study, however, found that placenta previa was approximately 2 times more prevalent for mothers aged ≥ 35 years than that for mother aged below 35 years (Tab. 2). With adjustment for main confounders, advanced maternal age remained an independent contributing factor for placenta previa with an adjusted odds ratio of 1.64, which was in accordance with one previous report [16]. That can be partially explained by atherosclerosis-induced vascular endothelial dysfunction of the uterus, leading to insufficient perfusion of the placenta. As a result, the placenta tends to implant in the lower uterine segment, thereby increasing the risk of placenta previa [18].

According to Schummers et al. [19], the risk of iatrogenic preterm delivery increased independently with maternal age, especially for those ≥ 35 years. Consistently, the present study demonstrated that advanced maternal age was a risk factor for iatrogenic preterm delivery, independent of height, BMI, education level, parity, method of conception and pre-existing disease. Among iatrogenic preterm births, preeclampsia was reported to be the most common indication [20]. Placental implantation abnormalities, especially for placenta previa, are also the major contributors to medically indicated preterm delivery [21]. The development of those pregnancy complications in advanced-maternal-age women, is mainly attributed to placental insufficiency, as demonstrated by lower placental weight-to-birth weight ratios, decreased uteroplacental spiral vasculature volume and abnormal placental-derived hormones and biomarkers [22].

Women of advanced age are more likely to undergo cesarean section [23] and PPH [24]. The present study found that advanced maternal age was an independent risk factor for PPH and a marginally independent risk factor for cesarean section. Decreased number of oxytocin receptors and maternal complications such as preeclampsia and GDM with age are the proposed causes of cesarean section and PPH [16, 25]. Magnesium sulfate, a routine medicine for patients with preeclampsia, also has the side effect of compromising postpartum uterine contractility [26]. As a result of prolonged labor, cesarean delivery is often performed, leading to uterine muscle fatigue or damaged contraction at the uterine incision site, and eventually making a patient susceptible to uterine atony. Moreover, preeclampsia may lead to thrombocytopenia, platelet dysfunction, and disseminated intravascular coagulation [26]. Under these conditions, mothers of advanced maternal age are relatively vulnerable to PPH.

In this study, the impact of advanced maternal age on short-term neonatal outcomes was also evaluated. With adjustment for height, BMI, education level, parity, method of conception, pre-existing disease, GDM and preeclampsia, mothers of advanced age were less likely to deliver infants with low birth weight. However, with multiple gestation included in the above adjusted model, only the association of advanced maternal age with hyperbilirubinemia

was found. Previous studies have reported that advanced maternal age was not associated with adverse neonatal outcomes in the cohort of preterm infants, with limited controlling of the potential confounding [7, 12]. This study, after adjusting for more possible factors including multiple gestation, further demonstrated the not significant impact of advanced maternal age on neonatal outcomes except for hyperbilirubinemia. Research regarding the impact of maternal age on neonatal hyperbilirubinemia are limited. Our results were consistent with the study by Boskabadi et al. [27], which demonstrated that maternal age above 35 years during pregnancy is a maternal risk factor for neonatal hyperbilirubinemia.

There remains controversial regarding the effect of advanced maternal age on the birthweight of infants, depending on the controlling of the confounders. According to the unadjusted analysis by DiLabio et al. [8], the birthweight in pregnancies of mothers aged ≥ 35 years was not significantly different compared with mothers aged 20–34 years in a cohort of preterm infants with < 29-week gestation. Adane et al. [28] reported a significant association of maternal age ≥ 35 years with low birthweight, after controlling for the potential confounders. However, the status of multiple gestation was not included in the adjusted model. In the present study, multiple gestation was identified as a considerable factor in the association between advanced maternal age and low birth weight. Similarly, Simchen et al. [29] reported that the association of advanced maternal age with low birth weight was primarily attributed to multiple gestation. Among cohorts of singleton pregnancies, multiple analysis found no significant difference regarding the rate of low birthweight infants between advanced and younger ages [2, 6, 16]. Mothers with advanced maternal age tend to have higher educational and socioeconomic statuses, which may be related to less common maternal obesity and fetal macrosomia. That may help to explain the not increased birth weight in the older mothers [30].

There are several limitations in our study. Firstly, random bias may exist due to the retrospective nature and the relatively small sample size in a single center population. Secondly, though our study controlled common maternal characteristics, some degree of undetermined confounding is possible owing to the missing capture of other sociodemographic factors (i.e., socioeconomic status) that affect perinatal outcome. Thirdly, the study only investigated the effect of advanced maternal age on the neonatal short-term complications. Long-term follow-up regarding the neurodevelopmental status is required to comprehensively evaluate the impact of advanced maternal age on the outcomes of preterm birth infants. Despite of those limitations, the findings of our study are strengthened by the selection of population with preterm delivery and the ability to control for multiple gestation apart from common risk factors in the analysis of neonatal outcomes in this preterm population.

CONCLUSIONS

In a preterm infant population, advanced maternal age was a risk factor for adverse pregnancy outcomes including iatrogenic preterm birth, placenta previa, preeclampsia, GDM, PPH, and a protective factor for multiple gestation. In terms of neonatal outcomes, advanced maternal age was associated with a decreased rate of low birthweight or an increased rate of hyperbilirubinemia according to the independence of multiple gestation. Multiple gestation should be considered in the analysis of the risk factors for neonatal outcomes in the cohort of preterm infants. Timely diagnosis of those complications to take maternal monitoring and better management is warranted to improve pregnancy outcomes for women conceived at an advanced age.

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LL: conception, study design, manuscript draft and corresponding author; JHL and XFD: data interpretation and critical revision of the manuscript; JBW: data acquisition; LHC and JFH: data process and statistical analysis.

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Conflict of interest

All authors declare no conflict of interest.

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Expectations of pregnant women for antenatal care services and factors affecting anxiety severity during the COVID-19 pandemic

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ABSTRACT

Objectives: We aimed to evaluate the difficulties pregnant women encountered while receiving health care, their demands for antenatal care, and their mental state during the COVID-19 pandemic.

Material and methods: A total of 447 pregnant women were included in this cross-sectional study. The data were collected through a face-to-face questionnaire, which assessed participants' demographic, individual, and obstetric characteristics, their opinions regarding the COVID-19 pandemic, expectations from their antenatal care services, and their Beck Anxiety Inventory (BAI) scores.

Results: During the COVID-19 pandemic, it was determined that 17.2% of the pregnant women participating in our study could not go to antenatal follow-ups and almost half (45.9%) demanded that their follow-ups be reduced due to the risk of coronavirus transmission. The BAI scores were found to be significantly higher in participants with low-income levels, chronic diseases, those in the third trimester, those with high-risk pregnancy either previous or current, and those who got pregnant unintentionally. Young age, unintentional conception, advanced pregnancy week, previous high-risk pregnancy, and failure to receive regular antenatal care were independent variables that predicted moderate-severe anxiety in logistic regression analysis.

Conclusions: In order to minimize the adverse effects of the COVID-19 pandemic on the mental health of pregnant women, it is important to develop support programs that contribute to the well-being of the mother and fetus by recognizing the pregnant women at risk in the antenatal period.

Key words: antenatal care; anxiety; Beck Anxiety Inventory; coronavirus; COVID-19; pandemic

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INTRODUCTION

The 2019 Coronavirus Disease (COVID-19), which first emerged in the city of Wuhan, Hubei Province in China in December 2019, was declared a "Public Health Emergency of International Concern" on January 31, 2020, and as a pandemic on March 11, 2020 by the World Health Organization (WHO) [1].

Based on the limited data currently available related to COVID-19, there is no increased susceptibility or risk of severe morbidity and mortality among pregnant women compared to the non-pregnant women in the general population. However, viral diseases during pregnancy can cause adverse maternal and fetal outcomes. The association of COVID-19 with complications such as premature birth, fetal growth restriction, preeclampsia, gestational diabetes, hypertension, severe maternal disease, increased admission to the intensive care unit, and maternal death has been reported in the late pregnancy period (> 24 weeks) [2, 3]. Indeed, in a study by Zaigham and Andersson, it was reported that COVID-19 during pregnancy may be associated with severe maternal morbidity and the possibility of maternal-fetal transmission cannot be completely ruled out [4].

Based on the studies examining previous outbreaks, people are more likely to experience psychological problems

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during public health emergencies, such as COVID-19. Pregnant women, who are one of the most susceptible, fragile, and vulnerable groups of the society, are expected to be affected by uncertainties regarding clinical impacts of this disease, decrease in support from families and friends due to isolation and quarantine, financial problems, and travel restrictions. It is noteworthy that especially outbreaks, pregnant women experience anxiety due to disruptions in their routine antenatal follow-ups, changes in health services (online meeting instead of face-to-face meeting, use of telemedicine, etc.), and difficulties in reaching the hospitals and physicians from which they receive care [5].

Objectives

in this study, we aimed to evaluate the difficulties pregnant women faced while receiving healthcare services during the COVID-19 pandemic, as well as changes in their lifestyle, their anxiety and to determine their expectations from prenatal care services.

MATERIAL AND METHODS

This study was carried out in the pregnancy follow-up outpatient clinics at the University of Health Sciences, Zeynep Kamil Women and Children Diseases Training and Research Hospital in Istanbul, Turkey between June-July 2020. During the pandemic, our hospital continued to serve women with low and high-risk pregnancies without any interruption by rearranging the working conditions.

Population and sample

The population of this cross-sectional study consisted of 447 pregnant women who applied to the pregnancy outpatient clinic of the hospital throughout the study period. All participants were between 19 and 45 years of age, were literate, had no communication problems, and gave consent to fill out the questionnaire upon being informed about the significance and objective of the study. Participants that had any clinical manifestations or symptoms of COVID-19, who were suspected of/diagnosed with COVID-19, and those previously diagnosed with any psychiatric disorder were excluded from the study.

The sample size was calculated through the analysis, which was conducted using the OpenEpi software (version 3). Based on the anxiety rate, the outcome of which was unpredictable and predicted as 50%, a total of 377 participants were found to be sufficient with 5% alpha error and 99% power to represent the number of pregnant women (500) who applied to our hospital for the first time within one month.

Data collection tools

The data were collected through a questionnaire, which assessed participants' demographic, individual, and the obstetric characteristics, their opinions regarding the COVID-19 pandemic, expectations from their antenatal care services, and the Beck Anxiety Inventory (BAI) scores.

Pregnancy introduction form has been prepared in line with the literature. In this form, there were 6 sections that questioned the socio-demographic characteristics and medical history, obstetric history, changes in the lives of participants during the pandemic, their opinions about the COVID-19 pandemic, their concerns about COVID-19 infection, and the service they need during the pandemic [6–8]. Before the study, a preliminary version of the questionnaire was applied to a different set of pregnant women and the items of the questionnaire were revised accordingly. These patients that participated in the preliminary questionnaire were excluded from the study.

The participants' anxiety level and severity were assessed through the BAI, which was developed by Beck et al. [9] and adapted to Turkish by Ulusoy et al. [10]. It has been determined that the scale had an adequate reliability and validity [11, 12]. BAI assesses the frequency of anxiety symptoms that are experienced by the individual. According to BAI, the scores are categorized as follows: 0–7 points indicate minimal anxiety, 8–15 points indicate mild anxiety, 16–25 points demonstrate moderate anxiety, and 26–63 points point to severe anxiety [10].

Ethical statement

Ethical approval for our study was obtained from the Ethics Committee of Health Sciences University, Zeynep Kamil Women and Children Diseases Training and Research Hospital (decision no 108, dated 03.06.2020). Before starting the survey, participants were informed that the survey was for research purposes and that their identities would be kept confidential within the scope of the confidentiality principle, and their written consents were obtained in this regard.

Statistical methods

The Statistical Package for the Social Sciences (SPSS Inc., version 17; Chicago, IL, USA) was used for statistical analyses. Data were expressed as numeric (%) or mean ± standard deviation (SD) and median (min–max) values where appropriate. Kolmogorov–Smirnov tests were performed for distribution of continuous data. Statistical analyses were performed by using Student t-test for normally distributed data and Mann–Whitney U test for non-normally distributed data. For categorical values, p-values were calculated using the chi-square test (with Fisher exact test for groups with less than five subjects expected in a cell). The relationship between two sets of data was analyzed by Spearman's rank correlation test. Multivariate analysis was used for logistic regression analysis (Backward LR). P value of less than 0.05 was considered to show a statistically significant result.

RESULTS

The mean age of the 447 pregnant women who participated in the study is 29.4 ± 5.8 years. The socio-demographic data of the participants are presented in detail in Table 1.

Evaluation of obstetric characteristics showed that the mean gravida was 2.39 ± 1.40 and mean week of gestation was 23.67 ± 9.18. It was found that 59.7% of the participants were multiparous, 20.6% experienced problems in their previous pregnancy, and 34% had a risk in their current pregnancy (6.9% had thyroid disease, 6% had diabetes mellitus, 4.7% had chronic HT, 4.3% had chronic respiratory disease and asthma) and 5.4% were smokers. It was determined that 82.1% of the participants attended antenatal follow-ups regularly during the pandemic, and 51.3% of those who did not participate in follow-ups attributed this to fear of being infected with the disease (Tab. 1).

Evaluation of the changes experienced by participants during the pandemic showed that 15.2% experienced shorter sleep duration and 57.5% experienced a decrease in their daily activities. It was determined that almost half of the participants (45.9%) asked the health institutions to reduce the frequency of pregnancy follow-ups throughout the pandemic due to the concern of being infected with the disease, and the pregnant women who wanted to reduce the frequency of follow-ups were mostly in their first trimester (38%) (Tab. 1).

In our study, it was determined that participants (73.8%) were most frequently worried about the risk of transmission of COVID-19 from another patient during or after delivery at the hospital. The second most common concern (72.5%) was that their babies would be harmed if they became infected with COVID-19 during pregnancy, followed by the worry that their spouse or relative could not be present during the delivery (68.7%). In addition, nearly half (45.9%) of the participants did not want to attend the follow-up because they were worried about being infected with the COVID-19 in the hospital setting, participants whose spouses were working were concerned that their spouses might infect them with COVID-19 (49.9%), 75% were unsure about breastfeeding during the pandemic or did not know if breastfeeding was safe. Moreover, 28.6% of the participants said they might opt for cesarean section instead of vaginal delivery, 33.8% would increase interventions to hasten the delivery, 59.7% indicated that sufficient measurements were being taken in the hospital, and 79% of them thought that they may get infected with COVID-19 from healthcare staff in the hospital and that they were indecisive related to this issue (Tab. 2).

Among the participants, 95.3% requested designating isolated and clean areas for pregnant women to receive health care service in hospitals, 91.7% requested to be informed about the pregnancy follow-up process and screening tests during the pandemic, 90.6% requested to be examined by the appointment system to avoid contact while attending the pregnancy follow-ups. Also, 82.8% requested free examination and delivery services in private hospitals to reduce the volume during the pandemic, 87.9% requested to be informed about protective and preventive ways against COVID-19 infection, and 77.9% of them requested including psychological support in the health care services during the pandemic (Tab. 3).

When all the participants were evaluated, the mean BAI score was 13.25 ± 11.27. It was determined that BAI scores of those who had low-income levels, who had chronic diseases, who were in the third trimester, those at risk in the previous and current pregnancy, and those who become pregnant unintendedly were significantly higher ($p < 0.05$) (Tab. 4).

Among the participants, 39.6% had minimal anxiety, 24.2% had mild anxiety, 19.9% had moderate anxiety, and 16.3% had severe anxiety. Significantly higher level of moderate-severe anxiety was found in those who conceived unintentionally, those who had a problem in their previous and current pregnancy, those who were in the third trimester, did not receive regular antenatal follow-up ($p < 0.05$) (Tab. 5).

Logistic regression analysis (backward LR) was used to determine the target pregnancy group that independently affected the anxiety level and had a high risk of moderate to severe anxiety. While performing the multivariate analysis, variables that were significant in univariate analyzes and variables that were expected to be related according to the literature and may be confusing were included in the model. According to multivariate analysis, moderate-to-severe anxiety was less frequent in oldest participants [odds ratio (OR) 0.96; 95% CI 0.92–0.99; $p = 0.033$]. However, it was more frequent in those who conceived unintentionally (OR 2.02; 95% CI 1.17–3.50; $p = 0.012$), those in the later weeks of pregnancy (OR 1.09; 95% CI 1.02–1.18; $p = 0.016$), those with high-risk pregnancies (OR 2.09; 95% CI 1.17–3.49; $p = 0.012$), and those who did not have regular antenatal follow-ups (OR 2.51; 95% CI 1.41–4.48; $p = 0.002$).

DISCUSSION

The COVID-19 pandemic [1] has had devastating effects all over the world. In most countries, health systems have faced collapse, and all elective surgeries and outpatient services, except emergency cases, have been partially or completely stopped. Each hospital implemented its own emergency action plan [13].

In a study conducted by Lebel et al. [14] with 1987 pregnant women, most of whom had high education and income levels, 89% of the participants stated that there were changes in pandemic-related antenatal care, and 90% of them stated that the person who was supposed to provide them with social support was not allowed at birth. In the same study, 35% of the pregnant women reported that they changed

Table 1. Participants' socio-demographic, obstetric characteristics and distribution of changes experienced during the COVID-19 pandemic (n = 447)

Participants		mean ± SD	median (min–max)
Age [years]		29.4 ± 5.8	29 (19–44)
Duration of marriage [years]		6.4 ± 5.4	5 (1–30)
Gravida		2.4 ± 1.40	2 (1–9)
Parity		1.0 ± 1.0	1 (0–6)
Week of gestation [weeks]		23.7 ± 9.2	29 (26–39)
Mean age of their children [years]		5.9 ± 3.9	5 (1–28)
		n	%
Educational status	Elementary school	94	21.0
	Secondary school	108	24.2
	High school	125	28.0
	University	120	26.8
Working status during pandemic	Formal	17	3.8
	Flexible work	38	8.5
	Work from home	44	9.6
	Not working	349	78.1
Smoking habit	Smoker	24	5.4
	Non-smoker	387	86.6
	Quit during pregnancy	36	8.1
Social security status		364	81.4
Income of family	Low	188	42.1
	Medium	238	53.2
	High	21	4.7
Family type	Core	376	84.1
	Extended	71	15.9
Chronic diseases		101	22.6
Unintended pregnancy status		89	19.9
Pregnancy trimester	1st trimester	145	32.4
	2nd trimester	146	32.7
	3rd trimester	156	34.9
Multiparity		267	59.7
Had problems during previous pregnancy		92	20.6
Risk in current pregnancy		152	34.0
Adequate knowledge of pregnancy, delivery and puerperium		322	72.0
Postpartum care training during pregnancy		150	33.6
Regular antenatal follow-ups		367	82.1
Reasons for not following (n = 80)	For fear of getting infected	41	51.3
	Hospital could not serve due to pandemic	17	21.3
	Couldn't find an appointment	22	27.5
Demand to reduce the number of pregnancy examinations during the pandemic		205	45.9
Demand to reduce the number of examinations according to pregnancy trimester (n = 205)	1st trimester	78	38.0
	2nd trimester	72	35.2
	3rd trimester	55	26.8
Sleep time in pregnancy during pandemic	4–6 hours	68	15.2
	7–8 hours	203	45.4
	9–10 hours	146	32.7
	≥ 11 hours	30	6.7
Daily activity change during the pandemic	Decreased	257	57.5
	Not changed	169	37.8
	Increased	21	4.7

Table 2. Participants' concerns about COVID-19 infection (n = 447)

	Yes		Undecided		No	
	n	%	n	%	n	%
I do not want to go to follow-ups because I might get COVID-19 infection from hospitals.	205	45.9	145	32.4	97	21.7
I think that enough precautions are being taken at the hospital where I go for antenatal care.	267	59.7	137	30.6	43	9.6
I think the disinfectants I use during pregnancy will harm my baby.	100	22.4	175	39.1	172	38.5
I think that COVID-19 infection can be transmitted from healthcare professionals.	161	36.0	192	43.0	94	21.0
My husband is working and I'm afraid he might bring home COVID-19 infection.	223	49.9	95	21.3	129	28.9
I think my baby will suffer if I get COVID-19 infection during pregnancy.	324	72.5	93	20.8	30	6.7
I think that even if there is no infection, there will be a risk of transmission from another patient at the hospital or after birth.	330	73.8	95	21.3	22	4.9
I think there will be increase number of interventions in this period to accelerate birth.	151	33.8	167	37.4	129	28.9
I'm afraid of not getting physical/emotional support during delivery.	198	44.3	139	31.1	110	24.6
It worries me that my husband or a relative will not be there during the delivery.	307	68.7	60	13.4	80	17.9
Experiencing pain while wearing a mask constantly worries me.	289	64.7	77	17.2	81	18.1
I think I will be referred to a planned cesarean section instead of a vaginal delivery.	128	28.6	119	26.6	200	44.7
If I am COVID-19 positive, I think I can breastfeed my baby.	136	30.4	152	34.0	159	35.6
I am worried about going to the healthcare facility for my baby's vaccinations and follow-ups.	223	49.9	99	22.1	125	28.0

Table 3. The subjects that participants want to be included in the service processes during the COVID-19 pandemic (n = 447)

	Yes		Undecided		No	
	n	%	n	%	n	%
Designation of isolated and sterile areas in hospitals for pregnant women to receive service	426	95.3	18	4.0	3	0.7
Providing examination by appointment system due to the least need for contact while going to pregnancy controls	405	90.6	36	8.1	6	1.3
Continuing pregnancy education through online classes	304	68.0	110	24.6	33	7.4
Providing consultations and care services for pregnant women via telemedicine and online system	335	74.9	89	19.9	23	5.1
Performing pregnancy follow-ups in primary care family health centers to reduce the density in hospitals during the pandemic	326	72.9	92	20.6	29	6.5
During the pandemic, pregnancy follow-ups were carried out as home visits to reduce the density in hospitals	225	50.3	122	27.3	100	22.3
Providing free examination and delivery services in private hospitals to reduce the patient volume during pandemic periods	370	82.8	53	11.9	24	5.4
Informing about COVID-19 infection control and prevention methods	393	87.9	29	6.5	25	5.6
Informing about pregnancy follow-up process and screening tests during pandemic periods	410	91.7	18	4.0	19	4.3
Informing about pregnancy follow-up process and screening tests during pandemic periods	348	77.9	68	15.2	31	6.9

birth plans such as location, social support and childcare due to the pandemic, 74% had problems in accessing other health services during pregnancy, and 9% could not access psychological counseling services. In our study, 82.1% of the participants went to antenatal follow-ups regularly during the pandemic. However, 51.3% of the participants who did not go to antenatal follow-up did not do so due to fear of being infected. The fact that most of the pregnant women participating in the study received antenatal care may be related to the uninterrupted service of our hospital during the pandemic. In our study, most of the participants stated that they preferred practices that were revised in accordance with the preventive measures of the pandemic and required less contact, instead of the existing practices in antenatal care processes during the COVID-19 pandemic period. In addition, 45.9% of the participants asked health institutions to reduce the frequency of their pregnancy follow-ups due to the concern of COVID-19 transmission and 38% of those who wanted to reduce the frequency of follow-up were in their first trimester.

In another study conducted by Akgör et al. [15] with 297 pregnant women, more than half of the participants

Table 4. Factors affecting participants' Beck anxiety scale score (n = 447)

		Beck anxiety scale score			p value
		Mean	(SD)	Median	
Education status	Elementary school	13.14	11.40	10.00	0.697
	Secondary school	12.76	11.23	9.50	
	High school	14.17	11.58	12.00	
	University	12.82	10.97	9.00	
Working status	Not working	13.28	11.18	10.00	0.786
	Working	13.14	11.64	10.00	
Working status during pandemic	Formal	11.47	12.26	9.00	0.065
	Flexible work	13.50	12.93	8.50	
	Work from home	11.65	9.77	9.50	
	Others	24.00	9.35	23.00	
Education status of spouse	Elementary school	11.89	9.78	9.00	0.218
	Secondary school	12.24	11.80	9.00	
	High school	14.63	11.78	11.00	
	University	13.23	10.79	11.00	
Income level	Low	14.95	12.02	12.00	**0.038***
	Medium	12.14	10.63	9.00	
	High	10.57	9.77	11.00	
Chronic diseases	Present	15.58	12.12	13.00	**0.028***
	Absent	12.57	10.94	10.00	
Pregnancy trimester	1st trimester	8.70	8.07	7.00	**< 0.001***
	2nd trimester	9.23	8.51	7.00	
	3rd trimester	21.24	11.77	21.00	
Parity	Nullipar	12.75	11.79	9.00	0.195
	Multipar	13.58	10.92	11.00	
Had problems during previous pregnancy	Yes	15.99	12.40	14.00	**0.025***
	No	12.58	10.94	9.00	
Risk in current pregnancy	Present	15.76	12.39	14.00	**< 0.001***
	Absent	11.96	10.44	9.00	
Intended pregnancy	Intended	12.15	11.00	9.00	**0.003***
	Unintended	17.65	11.32	17.00	
Postpartum care training during pregnancy	Present	12.11	10.95	9.00	0.126
	Absent	13.82	11.41	11.00	

*Statistically significant at $p < 0.05$

were concerned about delaying their appointments and not reaching their specialists, despite having been provided with uninterrupted health care during the COVID-19 pandemic. The authors reported that this situation could be related to the probability of health system collapse and disinformation on social media.

Xian et al. [16] reported that regular physical activity during pregnancy had a protective effect on anxiety and depression. Indeed, in the study of Kahyaoğlu et al. [17] it was reported that the risk of anxiety and depression increased in pregnant women who did not engage in regular physical activity. In our study, it was determined that the daily activities of 57.5% of the participants decreased due to the social isolation and quarantine practices applied in the pandemic.

In our study, 72.5% of participants worried that their baby may be harmed when infected with COVID-19. Moreover, 68.7% of pregnant women were concerned that their spouse or a relative will not be with them at birth. As a matter of fact, approximately one third participants stated that they were worried about interventions that would accelerate labor, while 28.6% of them stated that they could be referred to a planned cesarean in the management of their

Table 5. Factors affecting participants' Beck anxiety level (n = 447)

		Minimal-mild anxiety n = 285		Moderate-severe anxiety n = 162		p value
		mean ± SD	median (min–max)	mean ± SD	median (min–max)	
Age		32.2 ± 5.5	32 (20–44)	30.7 ± 4.7	31 (21–40)	0.303
Duration of marriage		9.5 ± 5.4	9 (1–30)	8.4 ± 4.9	7.00 (1–20)	0.188
Mental state		7.4 ± 2.2	8 (2–10)	5.6 ± 2.0	5 (1–10)	**< 0.001***
Gravida		3.2 ± 1.2	3 (1–9)	3.1 ± 1.3	3 (2–8)	0.816
Parity		1.7 ± 0.9	1 (0–6)	1.6 ± 0.8	1 (1–4)	0.929
Pregnancy week		20.7 ± 8.7	20 (10–38)	28.0 ± 8.9	31 (11–39)	**< 0.001***
Child age		6.1 ± 4.1	5 (1–28)	5.6 ± 3.7	4.00 (1–19)	0.317
		n	(%)	n	(%)	
	Elementary school	62	21.8	32	19.8	0.961
	Secondary school	69	24.2	39	24.1	
	High school	79	27.7	46	28.4	
	University	75	26.3	45	27.8	
Family income	Low	108	37.9	80	49.4	0.058
	Medium	162	56.8	76	46.9	
	High	15	5.3	6	3.7	
Working status	Yes	66	23.2	32	19.8	0.403
	No	219	76.8	130	80.2	
Working status during the pandemic	Formal	13	4.6	4	2.5	0.695
	Flexible work	25	8.8	13	8.0	
	Work from home	28	9.8	15	9.3	
	Not working	219	76.8	130	80.2	
Family type	Core	243	85.3	133	82.1	0.379
	Extended	42	14.7	29	17.9	
Chronic diseases	Yes	11	3.9	10	6.2	0.267
	No	274	96.1	152	93.8	
Smoking habit	Smoker	17	6.0	7	4.3	0.613
	Non-smoker	247	86.7	140	86.4	
	Quit during pregnancy	21	7.4	15	9.3	
Pregnancy trimester	1st trimester	120	42.1	25	15.4	**< 0.001***
	2nd trimester	110	38.6	36	22.2	
	3rd trimester	55	19.3	101	62.3	
Parity	Nullipar	118	41.4	62	38.3	0.516
	Multipar	167	58.6	100	61.7	
Intended pregnancy	Intended	243	85.3	115	71.0	**< 0.001***
	Unintended	42	14.7	47	29.0	
Had problems during previous pregnancy	Yes	46	16.3	45	27.8	**0.004***
	No	237	83.7	117	72.2	
Risk in current pregnancy	Yes	83	29.1	69	42.6	**0.004***
	No	202	70.9	93	57.4	
Regular antenatal follow-up	Yes	244	85.6	123	75.9	**0.010***
	No	41	14.4	39	24.1	
Professional training during pregnancy	Yes	48	16.8	21	13.0	0.275
	No	237	83.2	141	87.0	

*Statistically significant at $p < 0.05$

deliveries. In the study by Xian et al. [16], it was reported that 12.8% of pregnant women wanted to perform a planned cesarean section instead of waiting for a hospital birth.

Fetal health is one of the main concerns of the expectant mother during pregnancy. In the study by Ahorsu et al. [18], it was shown that pregnant women felt fear and anxiety about fetal and neonatal health during the pandemic. It has been reported that these pregnant women want to terminate their pregnancy early or deliver via cesarean section due to the stress and anxiety caused by the risk of transmission. Uncertainty regarding the duration of the COVID-19 pandemic is another factor that increases the anxiety level of pregnant women [19].

There are data showing that prenatal anxiety and depression affect maternal and infant health both physically and psychologically in the short and long term. Among these, an increased risk of abortion, preterm delivery, low birth weight, low Apgar score at birth, and long-term cognitive and behavioral problems in the mothers themselves and their children have been reported [20–23].

Studies have reported higher levels of anxiety and depression in pregnant women compared to non-pregnant women [24, 25]. In studies conducted before the COVID-19 pandemic, 5–13% anxiety, 4–15% depression and 0.9–3.8% combination of anxiety and depression were reported in pregnant women [26–28]. During the pandemic, the prevalence of anxiety in pregnant women has been reported to vary between 63% and 68% [14, 29, 30]. In the study by Kahyaoğlu and Küçükkaya [17], this rate was found to be 64.5% and 56.3% for anxiety and depression, respectively. As a result of the study, the authors reported that low education level, lack of regular physical activity, having to make face-to-face hospital visits and not having enough information about the effects of COVID-19 on pregnancy were the most important factors associated with the development of anxiety and depression in pregnant women.

In our study, we found moderate and severe anxiety levels as 19.9% and 16.3%, respectively. Multivariate analysis revealed that moderate-severe anxiety was associated with unintended pregnancy, previous or current high-risk pregnancy, not having regular prenatal follow-up, and being in the third trimester. We also found that our patients had similar anxiety levels compared to other studies [14, 29, 30]. However, we think that the lower levels of moderate and severe anxiety in our participants are related to integrated health services, effective information and family supports during pregnancy.

This study is one of the leading researches that identified the levels of concern, demand, and anxiety of pregnant women regarding the COVID-19 pandemic in Turkey. Most of the previous COVID-19-related survey studies were performed with participants who use social media and the internet and those who had a low-risk pregnancy, higher income, and educational status. Furthermore, online surveys have less likelihood of being responded to. The strengths of our study were that we conducted our surveys face-to-face and thus we were able to reach individuals with low/medium education and income rates, which are difficult to access online. Moreover, we were able to reach individuals with high-risk pregnancies. Hence, we believe that our study will help establish a scientific basis for development of health policies that will optimize maternal and infant health by determining the health needs of various pregnancy groups.

However, our study has some limitations. The cross-sectional nature of survey research is one of the primary limitations of this study. Another limitation is that the study was performed solely with literate pregnant women who applied to our hospital during a limited period. Thus, obtained data may not be generalizable to the entire population of Turkey. Another limitation is that the information on COVID-19 has not been fully verified yet, and scientific data and information are updated continuously.

CONCLUSIONS

The detection of anxiety, albeit minimal, in the majority of pregnant women participating in the study once again demonstrated the importance of exposure to stress factors during the pandemic. In order to minimize the adverse effects of COVID-19 pandemic on the mental health of pregnant women, it is important to develop support programs that will contribute to the well-being of the mother and fetus by recognizing those at risk in the antenatal period.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Abnormal liver function tests in pregnant patients with COVID-19 — a retrospective cohort study in a tertiary center

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ABSTRACT

Objectives: The current study aimed to describe the incidence of abnormal liver function tests (LFTs) in pregnant COVID-19 patients, explore the association between LFTs with current medication, and provide a reference for medical therapy of pregnant patients with COVID-19.

Material and methods: This retrospective single tertiary center cohort study included 122 pregnant patients with confirmed COVID-19 admitted and treated from April 1, 2020, to May 31, 2020. We defined abnormal LFTs as the elevation of the following liver enzymes in serum per our hospital's laboratory reference range standards: AST > 35 U/L, ALT > 35 U/L, and TBIL > 1.2 mg/dL. We evaluated patients for demographic and clinical features, laboratory parameters, medications, and hospital length of stay (LOS).

Results: Patients in this cohort had clinical presentations of fever (84.4%), dry cough (78.6%), and shortness of breathing (6.5%). In total, 17 (13.9%) patients had abnormal LFTs during hospitalization. Critically ill patients were three-fold higher in the abnormal LFTs group (11.8%) than in the normal LFTs group (3.8%, p = 0.16). The proportion of patients who used hydroxychloroquine and lopinavir/ritonavir were significantly higher in patients with abnormal LFTs (88.2% and 35.3%, respectively) than those with normal LFTs (62.9% and 15.2%, p = 0.04 and p = 0.04, respectively). The hospital length of stay (LOS) was significantly longer in the abnormal LFTs group (8.2 ± 5.8 days) than in the normal LFT group (6.0 ± 2.8 days, p = 0.02).

Conclusions: SARS-CoV-2 may induce liver injury and the LFT abnormality was generally mild in pregnant patients with COVID-19. Abnormal LFTs are associated with prolonged hospital LOS. Drug use was the most crucial risk factor for liver injury during hospitalization. The use of lopinavir/ritonavir and hydroxychloroquine were significantly higher, and the course of treatment of these drugs was significantly longer in pregnant women with abnormal LFTs than the patients with normal LFTs. Therefore, pregnant women with COVID-19 who received antiviral treatment should be closely monitored for evaluating LFTs.

Key words: COVID-19; liver function test; pregnancy; SARS-CoV-2

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INTRODUCTION

The coronoviridae family of viruses has risen as a global threat to public health and can cause both respiratory and multisystemic diseases in numerous human species and humans [1, 2]. Of these, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) originated in Wuhan City in Hubei Province, central China, was responsible for the Coronavirus Disease 2019 (COVID-19) in December 2019, and rapidly spread across the globe [3]. As of August 2020, more than 20 million patients globally had been infected with Covid-19, and more than 700 000 deaths are associated with this virus [4]. Researchers reported that approximately 15% of patients infected with COVID-19 progress severe health complications, about 5–10% require intensive care unit due to the severe pneumonia type symptoms, and with 3–5% of high mortality risk [5]. As this disease continues to spread

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sustainably and indiscriminately across the world, it is expected to see pregnant patients with COVID-19 canvassed across all trimesters of gestation [6]. Therefore, further epidemiological and clinical features should be clarified to enhance our perception of the virus's correct extent, develop diagnostic and treatment abilities and diminish its overall morbidity and mortality.

COVID-19 patients typically present with fever, weakness, dry cough, and shortness of breathing [7]. SARS-CoV-2 has also been associated with different degrees of liver injury [8]. Previous studies reported that 14–76% of COVID-19 patients experience abnormal liver function tests (LFTs), primarily increased levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [9, 10]. The extent and underlying mechanisms for liver injury in COVID-19 patients are not fully understood, but the pathogenesis seems multifactorial. The primary liver injury mechanism in COVID-19 patients is considered to be the binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2) receptor, which is known as the host cell entry receptor and highly expressed cholangiocytes, and then damages these bile duct cells [11]. Cholangiocytes are dynamic players in many aspects of liver physiology, including regeneration and innate and adaptive immune response mechanisms, and the disruption of these cells' functions induces a systemic inflammatory response leading to hepatobiliary damage [12]. Moreover, an autopsy analysis of liver biopsy specimens from a COVID-19 patient showed moderate microvesicular steatosis and mild inflammation in the portal and lobular area, suggesting that the liver injury might be caused by SARS-CoV-2 infection or drug-induced liver injury (DILI) [13]. Studies also indicated that this virus could cause acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF), driving to hepatic ischemia and hypoxia reperfusion injury in severe COVID-19 patients [14, 15].

Till present, a few data exist in the literature that has elaborately investigate the prevalence and severity of abnormal LFTs, their association with baseline LFTs before COVID-19 hospitalization, and clinical characteristics of liver failure among pregnant patients with COVID-19. Hence, the current study aimed to describe the incidence of abnormal LFTs in pregnant COVID-19 patients, explore the association between LFTs with current medication, and provide a reference for medical therapy of pregnant patients with COVID-19.

MATERIAL AND METHODS

This retrospective single tertiary center cohort study included 122 pregnant patients with confirmed COVID-19 admitted and treated from April 1, 2020, to May 31, 2020, at Kanuni Sultan Süleyman Training and Research Hospital isolation ward. All patients had an exposure history and clinical presentation of COVID-19, including respiratory symptoms or fever. We diagnosed patients with COVID-19 based on the World Health Organization (WHO) interim guidance [16]. The ethics committee of the hospital approved the study (approval date: 10.06.2020, approval number: 2020.06.67).

We detected SARS-CoV-2 nucleic acid in all patients by real-time nasopharyngeal swab polymerase chain reaction (PCR). Pregnant COVID-19 patients underwent clinical assessment of vital signs and symptoms, laboratory analysis, and radiologic chest evaluation at admission. We performed a chest X-ray and/or computed tomography (CT) for pneumonia diagnosis. As expected, concerns relating to the possible teratogenic impacts to the fetus from radiation exposure are inevitable. The accepted cumulative dose of ionizing radiation in the course of pregnancy is 5 rad, and no single diagnostic examination exceeds this upper limit. The exposure amount to the fetus from a two-view chest X-ray of the pregnant woman is 0.00007 rad, and chest CT (10 slices with a slice thickness of 10 mm) exposes the fetus to < 0.1 rad [17]. Thus, Wang et al. suggested that, if indicated, in a pregnant patient with suspected COVID-19, chest X-ray and chest CT can be conducted safely [18]. Before undergoing chest X-ray and CT examinations, pregnant patients with COVID-19 signed an informed consent form and had their lower abdomen and pelvis covered with a lead blanket. We classified pregnant patients into mild or severe cases based on the results from symptoms, clinical findings, and chest radiography [10]. We identified patients with mild symptoms (i.e., fever, dry cough, expectoration) with or without mild changes on chest imaging as mild cases. We defined mild changes in chest radiography by multiple small patchy shadows and interstitial changes, primarily in the outer region of the lung and below the pleura. We described patients with severe pneumonia by any of the following findings' presence: increased respiration rate (RR, ≥ 30/minute), hypoxia (resting oxygen saturation ≤ 93%), the partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) ≤ 300 mmHg in blood gas analysis, or respiratory or other organ failure occurrence that requires intensive care unit (ICU) admission, or shock.

Since there was no standard guidance on drug choice, therapeutic management was accommodated according to the clinical findings and guidelines [16]. All pregnant patients received supportive treatments, including intravenous fluid supplementation and maintenance of electrolyte and acid-base homeostasis, and a prophylactic dose of lower molecular weight heparin for preventing thromboembolic complications (LMWH) [19]. We closely monitored vital signs and finger oxygen saturation and gave oxygen treatment to hypoxemic patients. Since no antiviral therapy or antibiotic regimen was accepted for COVID-19 treatment, we treated patients with lopinavir/ritonavir, hydroxychloroquine, or

azathioprine [20]. The decision of antiviral treatment regimen and/or antibiotic regimen was based on the infectious disease specialist's discretion.

We excluded patients with gestational hypertensive disorders, HELLP syndrome, intrahepatic cholestasis of pregnancy, pre-existing liver disease, other infections, and co-existing morbidities, including renal disease, collagen vascular disease, chronic hypertension, known malignancy, and ischemic heart disease.

Age, gestational week, gravida, parity, and BMI were obtained by examining patients' medical records. The gestational week was examined by sonographic measurement and confirmed according to the last menstrual period and a first-trimester ultrasound exam [21].

The CBC values of the patients were measured with Mindray BC 6800, an automatic blood counting device using laser and impedance measurement techniques. Hemoglobin (Hb), white blood cell count (WBC), neutrophil count, lymphocyte count, platelet (PLT) count, D-Dimer, ferritin, C-reactive protein (CRP), AST, ALT, total bilirubin (TBIL), direct bilirubin, indirect bilirubin, amylase, and lipase values were all derived from patient' medical files.

As COVID-19 is a recently identified infectious disease, there is no consensus or guidance on liver injury definition and classification. Therefore, abnormal LFTs were defined as the elevation of the following liver enzymes in serum per our hospital's laboratory reference range standards: AST > 35 U/L, ALT > 35 U/L, and TBIL > 1.2 mg/dL. We obtained admission values of LFTs and peak values of aminotransferases during hospitalization.

We discharged pregnant patients treated when the symptoms and clinical findings improved significantly, with no fever for at least three days, and obvious absorption of inflammation in pulmonary imaging. Patients who did not meet the discharge criteria continued hospitalization for treatment and close follow-up. We also recorded the hospital length of stay (LOS). We followed up on the patient outcomes until August 31, 2020. The delivery mode was determined by standard obstetric indications [22].

Statistical analysis

Continuous variables were presented as means ± standard deviations if normally distributed and medians [interquartile ranges (IQRs)] if not normally distributed, while categorical variables were given as percentages. The chi-squared (χ^2) test was used to compare categorical variables between the groups, while the Kolmogorov–Smirnov test was employed to assess whether the variables were normally distributed. A Student's t-test or Mann–Whitney U test was used to compare the continuous variables between the groups according to whether they were normally distributed or not. Spearman's rho correlation coefficient was calculated to describe the degree of correlation between the parameters. In order to determine the independent predictors of liver dysfunction, variables found to be associated at a $p<0.1$ level according to univariate analysis, were included in the multivariate logistic regression analysis by using the Backward LR method with the results reported as the odds ratios (OR) and 95% confidence intervals (CI). The threshold of statistical significance was established at $p<0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences version 24.0 software program (IBM Corp., Armonk, NY, USA).

RESULTS

During the study period, a total of 134 pregnant women with COVID-19 were admitted to our hospital. After applying the exclusion criteria and withholding patients with missing medical records, 122 patients were included in our study.

Patients in this cohort had clinical presentations of fever (84.4%), dry cough (78.6%), and shortness of breathing (6.5%). There were two (1.6%) pregnant patients with abnormal LFTs on admission. One of them progressed to severe pneumonia during hospitalization and required ICU admission. Of patients with normal baseline LFTs, 15 patients developed elevated LFTs during hospitalization. One of them suffered from severe pneumonia and was admitted to ICU. In total, 17 (13.9%) patients had abnormal LFTs during hospitalization. Critically ill patient rates were three-fold higher in the abnormal LFTs group (11.8%) than in the normal LFTs group (3.8%). This difference was not statistically significant due to the low sample size ($p = 0.16$). All patients were discharged from the hospital by June 18th. No patient died in our study cohort.

The demographic variables, clinical characteristics, and the perinatal outcomes of the participants were summarized in Table 1. The mean age was significantly higher in the abnormal LFTs group (33.2 ± 6.1 years) than in patients with normal LFTs (28.1 ± 6.6 years). There were no significant differences between the two groups in terms of gravidity, parity, maternal weight, height, previous history of abortion, and the gestational week at admission.

The proportion of patients who used hydroxychloroquine and lopinavir/ritonavir were significantly higher in patients with abnormal LFTs (88.2% and 35.3%, respectively) than those with normal LFTs (62.9% and 15.2%, $p = 0.04$ and $p = 0.04$, respectively). Also, patients with elevated LFTs received significantly longer duration of treatment with hydroxychloroquine (4.7 ± 2.1 days) and lopinavir/ritonavir (2.7 ± 4.1 days) than those with normal LFTs (3.0 ± 2.5 days and 0.7 ± 2.1 days, $p = 0.01$ and $p < 0.01$, respectively). According to this, the hospital LOS was significantly longer in the abnormal LFTs group (8.2 ± 5.8 days) than in the normal LFTS group (6.0 ± 2.8 days, $p = 0.02$).

Table 1. Demographic and clinical parameters of the study cohort

Variables	All population (n = 122)	Abnormal LFTs (–) (n = 105)	Abnormal LFTs (+) (n = 17)	p-value
Age, years	28.8 ± 6.8	28.1 ± 6.6	33.2 ± 6.1	**< 0.01**
Height, cm	161.8 ± 5.9	161.7 ± 5.9	162.3 ± 6.1	0.71
Weight, kg	76.3 ± 12.8	75.6 ± 12.8	80.5 ± 12.6	0.18
Gravidity, n	2.7 ± 1.5	2.5 ± 1.4	3.3 ± 1.7	**0.05**
Parity, n	1.3 ± 1.2	1.2 ± 1.1	2.1 ± 1.6	**< 0.01**
Previous abortion, n (%)	30 (24.6)	27 (25.7)	3 (17.6)	0.47
Gestational week at admission	29.4 ± 9.3	29.2 ± 9.4	30.6 ± 8.6	0.57
Birth weight, g	3115 ± 634	3173 ± 607	2741 ± 713	**0.05**
Hydroxychloroquine usage, n (%)	81 (66.4)	66 (62.9)	15 (88.2)	**0.04**
Hydroxychloroquine usage time, days	3.3 ± 2.5	3.0 ± 2.5	4.7 ± 2.1	**0.01**
Lopinavir/Ritonavir usage, n (%)	22 (18)	16 (15.2)	6 (35.3)	**0.04**
Lopinavir/Ritonavir usage time, days	0.98 ± 2.5	0.7 ± 2.1	2.7 ± 4.1	**< 0.01**
Azithromycine usage, n (%)	24 (19.7)	19 (18.1)	5 (29.4)	0.28
Azithromycine time, days	0.84 ± 1.8	0.77 ± 1.8	1.2 ± 2.2	0.33
Severe disease, n (%)	6 (4.9)	4 (3.8)	2 (11.8)	0.16
Hospital length of stay, days	6.3 ± 3.4	6.0 ± 2.8	8.2 ± 5.8	**0.02**

We presented the laboratory parameters of the study population in Table 2. The peak AST value on admission was 40 U/L, during hospitalization was 207 U/L. The peak ALT value on admission was 37 U/L, during hospitalization was 200 U/L. AST and ALT elevations were generally (82.3%) mild, defined as < 5 times the upper reference limit. There were no significant differences between the groups in terms of neutrophil count, lymphocyte count, CRP, and D-Dimer value. Serum ferritin level was significantly higher in patients with elevated LFTs (243.6 ± 642 ng/mL) than in the normal LFTs group (48.9 ± 50 ng/mL, $p < 0.01$). Platelet count was demonstrated to be significantly higher in patients with abnormal LFTs (305 ± 169 (/mm^3 ×10^3) than those in patients with normal LFTs (231 ± 71/mm^3 × 10^3, $p < 0.01$).

We showed factors that were found to be independently associated with liver dysfunction in univariate analysis and multivariate logistic regression analysis in Table 3. On multivariate analysis, maternal age, serum ferritin levels, and serum platelet counts were associated with abnormal LFTs.

DISCUSSION

The current study demonstrates the results of LFTs and clinical outcomes in hospitalized pregnant patients with confirmed SARS-CoV-2 infection in a tertiary referral hospital. In our study cohort, 13.9% of pregnant patients with COVID-19 had elevated LFTs during hospitalization. Also, the pooled incidence of elevated aminotransferases determined during hospitalization appeared to be higher than that of at admission (1.6%), suggesting the disease progression and the toxicity of drugs used during hospitalization may both contribute to liver injury.

Rabaan et al. stated that SARS-CoV-2 shares similarities in terms of pathogenicity and structure with other coronaviruses, including SARS-CoV and MERS-CoV [23]. A study during the SARS outbreak in 2004 reported that abnormal LFTs were common (70%) in patients with SARS and might be associated with virus replication in the liver [24]. Also, liver specimens of SARS autopsies have demonstrated hepatocyte mitoses, fatty degeneration, central lobular necrosis, and lymphocytic infiltration, suggesting that SARS-CoV-2 can damage the liver tissue [25].

The liver plays an essential role in host defense against microorganisms and is frequently involved in most systemic infections as it receives a dual blood supply from the systemic and portal circulation. Various studies stated abnormal aminotransferase levels in COVID-19 patients and abnormal LFTs are common in hospitalized COVID-19 patients [10, 12, 26, 27]. The prevalence of AST elevations ranged between 4–53%, and that of ALT elevations ranged between 4–33% among COVID-19 patients in Chinese cohorts [26]. Sultan et al. reported that the pooled prevalence estimates of elevated LFTs were 15.0% [28]. Fan et al. found significantly higher (37.2%) abnormal LFTs than previously reported rates, and elevated liver cell injury markers (AST, ALT) are more common. They suggested that liver damage in COVID-19 patients might be directly induced by the liver cells' viral infection [29]. Hundt et al. concluded that since the

Table 2. Laboratory parameters of the study population

Variables	All population (n = 122)	Abnormal LFTs (–) (n = 105)	Abnormal LFTs (+) (n = 17)	p-value
AST, (mean) (median, [IQR])	22.1 ± 12.5 20 [16–25]	20.9 ± 8.3 19 [15–24]	34.9 ± 22.5 29 [22–40]	**< 0.01**
AST (peak), (mean) (median, [IQR])	34.4 ± 76 20 [15–25]	19.5 ± 5.6 18 [15–24]	145.6 ± 195 46 [42–207]	**< 0.01**
ALT, (mean) (median, [IQR])	17 ± 15 14 [9–19]	15.6 ± 8.4 13 [9–16]	35.8 ± 30.5 26 [19–37]	**< 0.01**
ALT (peak), (mean) (median, [IQR])	29.5 ± 73.5 15 [10–20]	15.7 ± 5.4 14 [10–17]	147.4 ± 180.9 43 [41–200]	**< 0.01**
Total Bilurubin, (mean) (median, [IQR])	0.4 ± 0.3 0.28 [0.2–0.39]	0.3 ± 0.2 0.27 [0.2–0.36]	0.7 ± 0.5 0.69 [0.28–0.84]	**< 0.01**
Indirect Bilurubin, (mean) (median, [IQR])	0.15 ± 0.09 0.13 [0.09–0.19]	0.1 ± 0.08 0.13 [0.09–0.18]	0.8 ± 2.4 0.18 [0.12–0.31]	**< 0.01**
Direct Bilurubin, (mean) (median, [IQR])	0.4 ± 2.1 0.14 [0.09–0.20]	0.4 ± 2.2 0.13 [0.09–0.17]	0.5 ± 0.5 0.20 [0.15–0.64]	0.96
Amylase, (mean) (median, [IQR])	65 ± 26 59 [49–79]	65 ± 24 58 [50–77]	79 ± 39 79 [38–95]	0.33
Lipase, (mean) (median, [IQR])	27 ± 19 23 [18–29]	24 ± 10 22 [18–28]	50 ± 44 30 [26–64]	**< 0.01**
Neutrophil, median, (mean) (median, [IQR])	5.7 ± 2.4 5.3 [4.1–7.1]	5.6 ± 2.3 5.1 [4.1–7.0]	6.6 ± 2.9 6.3 [4.1–8.7]	0.98
Lymphocyte, (mean) (median, [IQR])	1.4 ± 0.5 1.3 [1.0–1.8]	1.3 ± 0.6 1.3 [1.0–1.7]	1.7 ± 0.6 1.7 [1.0–2.1]	0.17
Platelet, median, (mean) (median, [IQR])	237 ± 100 221 [181–278]	231 ± 71 219 [181–277]	305 ± 169 253 [172–401]	**< 0.01**
CRP, median, (mean) (median, [IQR])	26 ± 28 16 [4.3–28]	31.8 ± 40 16 [4.3–39]	25.7 ± 30 16 [4.5–40]	0.55
D-Dimer, median, (mean) (median, [IQR])	2.3 ± 2.2 1.7 [1.1–2.5]	2.2 ± 2.1 1.6 [1.1–2.5]	2.1 ± 1.7 2.0 [1.3–3.8]	0.80
Ferritin, median, (mean) (median, [IQR])	83.8 ± 302 31.2 [16.8–62.2]	48.9 ± 50 30 [16.5–52.8]	243.6 ± 642 75.8 [55.8–160]	**< 0.01**

Table 3. Univariable and multivariable logistic regression analysis for determining the predictors of the liver dysfunction

	Univariate		Multivariate*	
Variables	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**
Maternal age	1.116 (1.031–1.207)	< 0.01	1.156 (1.044–1.281)	**< 0.01**
Ferritin	1.010 (1.002–1.018)	< 0.01	1.011 (1.002–1.020)	**0.01**
Platelet	1.007 (1.001–1.013)	0.02	1.008 (1.001–1.015)	**0.02**
†Parity	1.703 (1.145–2.532)	0.01		
†Hydroxychloroquine	0.226 (0.049–1.040)	0.06		
†Lopinavir/Ritonavir	3.034 (0.982–9.375)	0.05		

* Multivariate logistic regression analysis by using Backward LR method; † Not included in multivariate analysis according to Backward LR method

ACE2 receptor is dominantly expressed in cholangiocytes than in hepatocytes, the primary mechanism of liver injury is not due to the cytopathic effect of SARS-CoV-2 [27]. In our study, 13.9% of pregnant patients during hospitalization had abnormal LFTs. We also found that AST and ALT elevations were generally mild (1–2x the upper limit of normal). Likewise, Bertolini et al. reported that mild elevations of ALT and AST were often detected observed in COVID-19 patients on admission, and this elevation did not drive to notable liver dysfunction [26].

The liver is the major organ for drug metabolic processes and detoxification, and maintenance of function is crucial

to participate in all feasible COVID-19 treatment modalities. Since an effective antiviral agent for COVID-19 has not been developed yet, supportive and symptomatic therapies are essential. Also, antiviral drugs previously utilized to treat other coronavirus infections have been considered as the first choice to treat COVID-19 patients [30]. All these drugs are used for the treatment or management of COVID-19 patients, including antiviral drugs (lopinavir/ritonavir, hydroxychloroquine), antibiotics, antipyretics (acetaminophen), corticosteroids, and herbal medicines are potentially hepatotoxic [26]. Fan et al. reported that the proportion of patients who were treated with lopinavir/ritonavir was significantly higher in patients with liver injury than in patients with sustained normal LFTs [29]. Wu et al. stated that compared with patients suffering mild clinical signs and symptoms, severe COVID-19 patients require longer antiviral treatment duration and multiple drug combinations. The number of drugs used ≥ 3 might be associated with liver injury [31]. In our study, the use of lopinavir/ritonavir and hydroxychloroquine were significantly higher, and treatment durations of these medications were significantly longer in the abnormal LFTs group than in the normal LFTs group. We also found that abnormal LFTs during hospitalization are associated with prolonged hospital LOS. This finding might be due to the toxicity of drugs used during hospitalization and the clinical course of the disease.

Regardless of the mechanisms implicated in the liver injury of COVID-19 patients, immune-mediated pathway activation considers to be crucial [12]. Virus particles spread through the respiratory mucosa, and then infect other cells, which induces a cytokine storm in the body and generates a series of immune responses [32]. COVID-19 patients present significant inflammatory marker activation, including neutrophils, CRP, and cytokines that might contribute to pulmonary and extrapulmonary injuries [12]. Cytokine storm syndrome (CSS) is an uncontrolled or excessive proinflammatory cytokine release to external stimuli, which is correlated with disease severity [33]. Fan et al. found that patients with abnormal LFTs had higher inflammatory markers, including procalcitonin and CRP, and higher fever rates, which might be associated with the immune response following SARS-CoV-2 infection [29]. However, Zhao et al. indicated that abnormal LFTs in mild COVID-19 patients may not be associated with inflammatory status [34]. It was also considered that specific inflammation induced by SARS-CoV-2 is more prone to induce abnormal LFTs than general inflammation caused by other microorganisms. We did not find any significant differences between the groups regarding neutrophil count, lymphocyte count, and CRP. However, serum ferritin levels were significantly higher in pregnant women with abnormal LFTs than in the group with normal LFTs. Kernan et al. demonstrated that ferritin is a pivotal marker of and pathogenic player in the inflammatory process through its signaling as a component of the innate immune response and lymphocyte function modulation [35]. However, the accurate mechanism by which ferritin contributes to the course of this disease remains elusive.

The prevalence of pre-existing liver disease in patients with SARS-CoV-2 infection varies from 1–11% [26]. Previous studies stated that COVID-19 patients with pre-existing liver diseases might be more prone to suffer abnormal LFTs [10, 12, 29, 31]. Also, the highest proportion of deaths in COVID-19 patients were detected in elderly patients with underlying liver diseases [36]. Our study cohort consisted of reproductive-aged pregnant patients. We also excluded patients with pre-existing liver diseases from this study. We consider that these criteria could explain our lower rates of severe COVID-19 patients than the previous research.

Patients with abnormal LFTs during hospitalization had a significantly higher risk of worsening to severe COVID-19 than patients with normal LFTs. Progressing to severe pneumonia describes the clinical situation with a high mortality rate that requires ICU admission or mechanical ventilation [10]. In this study, while 3.8% of patients with normal LFTs exacerbate severe COVID-19, 11.8% of pregnant women with elevated aminotransferases progressed to severe pneumonia. However, this difference was not statistically significant. We consider that the absence of significant difference was due to the low sample size.

There are some limitations to this study. This study has been designed retrospectively and has the potential to contain limitations of such studies [37]. Because of the dependence on information reported in the patient record, we could not identify risk factors for all pregnant patients with COVID-19. We also evaluated abnormal LFTs rather than liver injury, because the description of liver damage was unclear in pregnant patients with COVID-19. The main strength of this study is that a few data exist in the literature that has comprehensively examine the prevalence and severity of abnormal LFTs.

CONCLUSIONS

We demonstrated that SARS-CoV-2 may induce liver injury and the LFT abnormality was generally mild in pregnant patients with COVID-19. Abnormal LFTs are associated with prolonged hospital LOS. Drug use was the most crucial risk factor for liver injury during hospitalization. The use of lopinavir/ritonavir and hydroxychloroquine were significantly higher, and the course of treatment of these drugs was significantly longer in pregnant women with abnormal LFTs than the patients with normal LFTs. Therefore, pregnant women with COVID-19 who received antiviral treatment should be closely monitored for evaluating LFTs.

Conflict of interest

The authors declared no conflict of interest.

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Serum S100B protein concentrations in SGA/FGR newborns

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ABSTRACT

Objectives: Fetal growth restriction (FGR) is associated with chronic fetal hypoxia, poor perinatal outcome and increased perinatal mortality. There are no reliable methods to detect cell damage in the central nervous system (CNS) in these patients. The findings of increased an acidic calcium-binding protein (S100B) concentration in biological fluids of infants after brain injury have supported the use of S100B as a biochemical marker of CNS damage.
The purpose of the study was to assess blood S100B concentrations in small for gestational age (SGA) and appropriate for gestational age (AGA) newborns and to evaluate the usefulness of S100B for early detection of hypoxia.

Material and methods: The investigation was carried out between November 2011 and April 2014. Serum S100B protein level was assessed in cord blood collected from newborns after birth. Medical records of mothers of neonates studied were reviewed for pregnancy induced hypertension (PIH), preeclampsia, maternal smoking during pregnancy and abnormalities in umbilical artery (UA) Doppler ultrasound examination.

Results: The study was carried out in 88 SGA neonates and 80 AGA neonates. The median value of S100B protein concentration in the SGA study group was significantly higher than in AGA controls ($p < 0.001$). Cord blood serum S100B concentration in SGA neonates with prenatal normal UA Doppler ultrasound findings ($n = 32$) did not differ from that SGA neonates with abnormal prenatal UA Doppler findings ($n = 25$) ($p = 0.74$), but was significantly higher than in AGA newborns ($p < 0.001$).

Conclusions: Elevated S100B protein levels in cord blood collected from SGA newborns may be helpful in detecting infants at higher risk of postnatal neurologic disturbances at an early stage.

Key words: S100B protein; biomarker; SGA; FGR, newborn; fetal hypoxia; CNS; neurological damage

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INTRODUCTION

Small for gestational age (SGA) neonates have been defined as those with birthweight below a threshold, the 10th centile. Infants who are SGA may suffer intrauterine fetal growth restriction (FGR). FGR is defined as a persistent suppression of fetal growth potential that occurs in response to a decrease in oxygen and nutrients supply from the mother to the fetus. Clinical evidence suggests that feto-placental insufficiency and fetal pre-exposure to decreased oxygen is associated with chronic fetal hypoxia and increased perinatal mortality [1]. FGR is associated with poor perinatal outcome, perinatal brain injury and is the strongest risk factor for an unexplained intrauterine death [2, 3]. About 15% of infants with FGR develop some degree of neurological damage [4, 5]. Most SGA/FGR fetuses remain unnoticed until birth, even when routine third-trimester ultrasound is performed [6]. However, not all fetuses measuring less than the 10th percentile for their gestational age are at risk for adverse outcomes and might be constitutionally small. Available diagnostic tools and routine procedures

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used when hypoxia is suspected (blood pH, lactate levels, cerebral ultrasound) may not be able to detect cell damage and subclinical lesions in the nervous system. Recently, novel potential biomarkers of brain injury and hypoxia have been identified [7–9]. One of them, S100B is an acidic calcium--binding protein expressed and released by astrocytes and mainly concentrated in the central nervous system. Secretion of S100B is an early process of the glial response to metabolic injury (oxygen, serum and glucose deprivation) [9–12]. Previous evidence that S100B concentrations are increased in the presence of brain injury suggests that higher S100B levels in pregnancies with FGR reflect fetal chronic hypoxia [7, 13, 14]. Since increased concentration of S100B can easily be found in biological fluids (cerebrospinal fluid, peripheral and cord blood, urine and amniotic fluid), the protein can be used as a biomarker of brain damage in growth restricted newborns [13–18].

The aim of this study was to assess blood S100B concentrations in small for gestational age (SGA) and appropriate for gestational age (AGA) newborns and to evaluate the usefulness of S100B for early detection of hypoxia. We hypothesized that serum levels of the S100B marker might differ in SGA and AGA infants.

MATERIAL AND METHODS

Patients

The investigation was performed in a tertiary referral center for neonatal intensive care. All neonates who were born SGA at the Department of Neonatology, Medical University of Łódź, between November 2011 and April 2014 were enrolled in the study.

Birth weight and head circumference in newborns were measured and percentile were assessed. SGA was defined as birth weight ≤ 10th centile. The control group included AGA infants (defined as having birth weight between 10th and 90th centile) delivered consecutively at term. Infants who died at birth, those who had congenital malformations, and children of mothers who refused to participate were excluded. The gestational age of newborns was determined according to the date of the last menstrual period and the first trimester ultrasound exam. Apgar score was determined at five minutes after birth.

SGA newborns were divided according to anthropometric indices into those with symmetrical and those with asymmetrical fetal growth restriction. SGA newborns have been categorized as "asymmetrical" when the birth weight was disproportionally restricted as compared to head circumference and "symmetrical" when both: weight and head circumference were proportionately small. Asymmetrical growth restriction was defined as any birth weight > 1 SD less than the corresponding head circumference.

Medical records of mothers of neonates studied were reviewed for gestational hypertension (GH), preeclampsia, maternal smoking during pregnancy and abnormalities in umbilical artery (UA) Doppler ultrasound examination. An abnormal pulsatility index PI for UA was defined as above the 95th centile for gestational age for uncomplicated pregnancies.

The study was approved by the local ethics committee (approval No. RNN/175/05/KE). Written informed consent was obtained from all mothers of enrolled neonates.

Blood samples

Cord blood was obtained from newborns directly after birth. Serum was obtained by centrifugation at 3000 × *g* for 5 min and stored at –70° C (for up to 6 months) until the biochemical assay.

S100B Assay

S100B concentrations were quantified by an enzyme-linked immunosorbent assay (ELISA; EIAab Science Co., Wuhan, China), according to the manufacturer's instructions. Samples were analyzed in duplicate and compared with S100B standard. The lower limit of detection of the ELISA for S100B is 1 pg/mL.

Statistical analysis

Descriptive statistics were used to describe characteristics of the study group. Continuous data were presented as means with standard deviations (SD) or median with minimum and maximum values. Categorical variables were described as frequencies and percentages. The Shapiro-Wilk test was evaluated to determine if the data were normally distributed. Group comparisons for normally distributed data were performed using the *t* test. When the normality assumptions were not satisfied, Mann-Whitney U test for continuous variables and the Fisher's exact test for binary variables were used. All analyses were performed with the use of IBM SPSS Statistics for Windows V. 25.0.0 (IBM Corp. Armonk, New York, USA). We considered a two-tailed p value less than 0.05 to be significant.

RESULTS

The study was carried out in 88 SGA neonates and 80 AGA neonates. Birth weight was lower in the SGA group than in the AGA group ($p < 0.001$). There were no significant differences in maternal age, gender characteristics, mode of delivery and cord blood lactate concentration between the SGA and AGA groups. There was statistically significant difference in the Apgar score and gestational age (lower in SGA group) (Tab.1). All SGA infants were above or equal 32 weeks of gestation.

None of the newborns had chromosomal abnormalities or TORCH infection. In the SGA group, maternal smoking during pregnancy, gestational hypertension (GH) and preeclampsia were observed significantly more often than in the AGA group (Tab. 1). Absent end-diastolic flow in UA was found in 13 cases of SGA group with abnormal prenatal Doppler.

Cord blood serum concentration of S100B protein at birth was significantly higher in the SGA infants than in the AGA group ($p < 0.001$) (Tab. 2).

UA Doppler examination was performed in 57 out of 88 (65%) mothers of newborns from the study group. Cord blood serum S100B concentration in SGA neonates with prenatal normal UA Doppler ultrasound findings (n = 32, median 69.3 [0.3–463.5]) did not differ from that SGA neonates with abnormal prenatal UA Doppler findings (n = 25, median 44.4 [6.8–1110.2]) ($p = 0.74$) but was significantly higher than in AGA newborns (n = 80) ($p < 0.001$) (Tab. 2).

S100B concentration was significantly higher in both SGA with symmetrical (n = 29) and asymmetrical (n = 59) fetal growth restriction than in non-growth restricted AGA neonates ($p < 0.001$) (Tab. 2).

Ultrasound examination of central nervous system was performed in all SGA infants within first 48 hours of life. Four out of 88 SGA infants had abnormal RI in the anterior cerebral artery (< 0.6) in the first head ultrasound examination.

We found no statistically significant differences in S100B concentration in terms of the anterior cerebral artery Doppler scanning between SGA newborns with abnormal RI (< 0.6) and normal RI (> 0.6).

DISCUSSION

The study showed that serum S100B concentrations in the SGA infants were higher than in the AGA group. Our findings confirm the previous results of Gazzolo et al. [13], who studied S100B levels in cord blood from FGR new-

Table 1. Neonate and maternal demographic and obstetric characteristics of SGA and AGA group

Characteristic	SGA group	AGA group	p-value
Male neonates, n (%)	88 (52)	80 (56)	0.64
Mean birth weight, g (SD)	2132 (543)	3491 (366)	0.001
Mean gestation, weeks (SD)	37.1 (3.0)	39.1 (1.0)	< 0.001
Median Apgar score at 5 min (min–max)	10 (5–10)	10 (8–10)	< 0.001
Cord blood lactate, mg/dL (SD)	45.61 (16.1)	45.6 (14.7)	0.99
Mean maternal age, years (SD)	31.1 (5.0)	30.0 (4.6)	0.15
Mode of delivery, n (%)			0.13
Spontaneous VD	20 (23)	33 (41)	
Operative VD	1 (1)	0	
Cesarean section	67 (76)	47 (59)	
Smoking during pregnancy, n (%)	20 (23)	4 (5)	0.002
GH, n (%)	22 (25)	3 (4)	< 0.001
Preeclampsia, n (%)	5 (6)	0	0.06
Antenatal Doppler changes:			
PI > 95th centile in UA, n (%)	25 (28)	2 (3)	< 0.001
Absent end-diastolic flow in UA, n (%)	13 (15)		

The bold p-values highlight whether the differences observed were statistically significant. SD — standard deviation; SGA — small for gestational age; AGA — appropriate for gestational age; GH — gestational hypertension; UA — umbilical artery; VD — vaginal delivery

Table 2. Cord blood S100B concentrations in AGA, all SGA, those SGA that had normal UA PI and SGA newborns with symmetrical growth

	Group	n	Median	Min–max	p-value
S100B (pg/mL)	AGA †	80	25.7	0.1–395.8	
	SGA	88	64.3	0.1–2025.0	< 0.001
	SGA with normal UA PI	32	69.3	0.3–463.5	< 0.001
	"Symmetrical" SGA	29	58.5	0.3–95.9	< 0.001

The bold p-values highlight whether the differences observed were statistically significant. AGA group serves as a reference (†) when compared separately with the SGA group and the SGA with normal UA PI. AGA — appropriate for gestational age; SGA — small for gestational age; UA — umbilical artery; PI — pulsatility index

born delivered by elective caesarean section and published the first paper about S100B protein and FGR correlation in 2002. In this study higher S100B levels in umbilical blood of FGR newborn were demonstrated.

Since S100B protein, during an active brain injury, is released from a damaged tissue into circulation, its concentration increases at an early stage of hypoxia in both cerebrospinal fluid and cord blood. The best sources for biomarkers are fluids obtained the least invasively and shortly after birth [8]. As S100B protein is highly concentrated in central nervous system, and has a half-life about one hour, is released by kidney tissue and increases in biological fluids at an early stage. Cord blood and urine seems to be a perfect source for further studies of the potential use of S100B measurements in FGR pregnancies and newborns. Gazzolo et al. [19], reported that higher concentrations of S100B were detected in cord blood of FGR fetuses who developed intraventricular hemorrhage after birth. In addition, the authors observed that the highest maternal S100B concentrations were found in group of fetuses with prenatal brain-sparing effect in prenatal ultrasound examination. In another study, Florio et al., found higher S100B concentrations in urine samples taken shortly after birth in FGR newborns compared to matched AGA controls. They noticed highest S100B concentrations in the neonates with abnormal neurologic follow-up but also significantly high in FGR infants with uneventful neurologic follow-up at one week of age [15]. However, no differences in S100B serum concentration of FGR and AGA infants were found in a small study containing 20 infants in each group [16]. In another study, Gazzolo et al. [13], found a correlation between circulating S100B protein and the fetal middle cerebral artery pulsatility index (MCA PI) that might suggest cerebral cell damage in growth restricted fetuses. This study showed no difference in S100B levels between SGA newborns with no abnormalities in prenatal Doppler examination (normal UA PI) and neonates with normal growth (AGA).

In our study, we attempted to investigate whether S100B concentration depends on prenatal hemodynamic disturbances. When SGA newborns were grouped according to normal and abnormal prenatal UA Doppler examination (UA PI > 95th centile), we did not find any differences in serum S100B concentration between either group. In contrast to Gazzolo et al. [13], we observed significantly higher S100B concentrations in SGA newborns with normal Doppler examination compared to AGA newborns. However, the prenatal ultrasound examinations were not performed in all cases and thus these results should be interpreted with caution.

In this study we also divided SGA group according to the traditional biometric birth measurements to those with symmetrical and asymmetrical growth restriction. Factors that cause symmetric growth restriction tend to develop early during fetal life. Since all SGA newborns studied were older than 32 weeks of gestation, we could speculate that beside growth restricted newborns, "symmetrical SGA" group included constitutionally and physiologically small infants. Both "symmetrical" and "asymmetrical" neonates had higher cord blood S100B concentrations compared to AGA neonates but these non-growth restricted infants could not be recognized on a group level.

The importance of biometric as well as functional parameters for identification of FGR was reported by Baschat [20], then the consensus-based definition of FGR was established in the Delphi criteria [21]. In our study we found that assessment of S100B concentration may be helpful in identifying FGR while Doppler findings are normal. Further studies however are needed to confirm our results.

CONCLUSIONS

Increased S100B protein concentration in blood collected from SGA newborns indicates secretion of this marker in response to hypoxia occurring in intrauterine growth restriction. Examination of cord blood S100B concentration may be helpful in identifying SGA newborns at a higher risk of postnatal neurological sequelae at an early stage while prenatal Doppler examination is normal, standard clinical and laboratory parameters are silent, and an early-stage neurologic follow-up is uneventful.

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Conflict of interests

The authors declare that they have no competing interests.

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Molecular classification of endometrial carcinoma, is it the new era of precision medicine?

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ABSTRACT

For many years endometrial cancer has been subdivided into oestrogen — dependent (type I) and oestrogen — independent (type II), according to classical Bokhman classification. Histopathological evaluation including type and grade of tumour, along with clinical factors have been considered as very important prognostic factors that impact treatment decision. However, histologically similar tumours may have different outcomes. Recent molecular findings and new histopathological parameters have given new concept on risk stratification. The Cancer Genome Atlas Research Network (TCGA) of tumours have brought new insights into endometrial cancer management. Four molecular subgroups have been described: POLE ultramutated (POLE mut), p53 mutant (p53abn), mismatch repair deficient (MMRd) and non-specific molecular profile (NSMP). This new subdivision has been recently introduced in the European risk stratification system.

Key words: molecular classif endometrial cancer; POLE mutations; p53 mutations; MMRd; NSMP

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INTRODUCTION

Endometrial cancer (EC) is the second most common gynaecologic malignancy that affects thousands of women globally. In 2018, 417 367 new cases were diagnosed worldwide and 97 370 patients died [1]. Although nearly 80% of EC patients are diagnosed in stage I–II according to 2018 FIGO classification [2], some of the apparently early-stage EC, have fatal outcomes. There are some several international guidelines concerning adjuvant treatment in early EC, however the recommendations are ambiguous. Guidelines of the European Society of Medical Oncology (ESMO) [3] or National Comprehensive Cancer Network (NCCN) [4] present a wide spectrum of options from patients' observations to adjuvant chemoradiotherapy.

Until now, the 4th edition of the World Health Organization (WHO) classification of tumours of female reproductive organs was based on histological morphology completed by immunohistochemical prognostic markers [5]. The treatment indications were based on these findings, but in some cases, histopathological interobserver variations have been demonstrated. This fact explains the reason why scientists started to search for new prognostic factors to precise optimal indications to adjuvant therapy in EC patients. Recently, the Cancer Genome Atlas Research Network (TCGA) brought important knowledge regarding molecular profile of EC [6]. 373 cases of EC were analysed using next generations sequencing (NGS) test, and these cases were stratified to four different subgroups. These four subgroups include: ultra-mutated EC which presents pathogenic variants in the exonuclease domain of DNA polymerase-epsilon (POLE), hypermutated EC characterised by microsatellite instability (MSI), a low copy number with a low mutational burden and a high copy number with TP53 mutations. Validation of prognostic factors by molecular stratifications have restructured EC classifications. This project included a comprehensive analysis of endometrioid, serous and mixed histology. Nowadays, based on transcriptomic, genomic and proteomic characterization EC is categorised as follows: POLEmut EC (pathogenic polymerase – epsilon variants), MMRd EC (mismatch repair protein deficiency), NSMP EC

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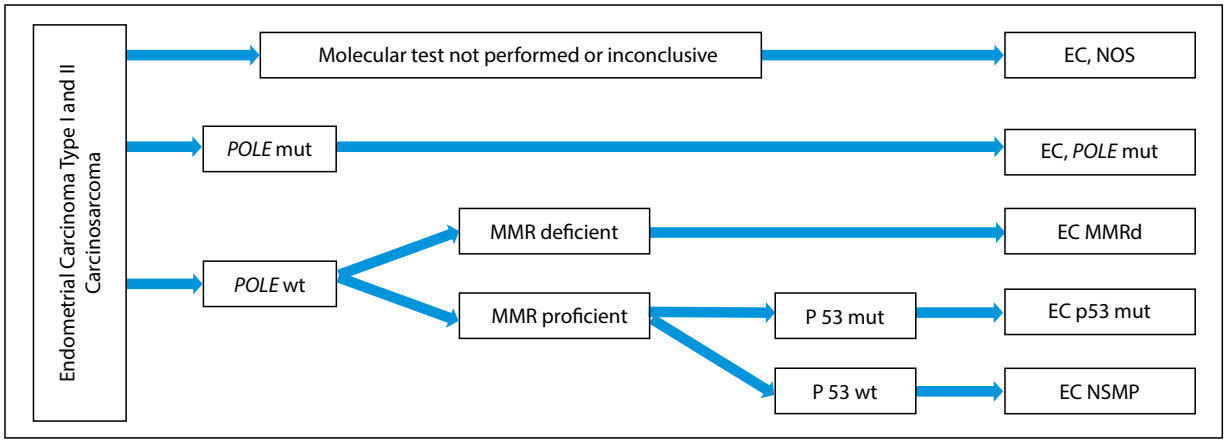

Figure 1. Proposition of diagnostic algorithm of endometrial cancer including molecular testing

(nonspecific molecular profile) and p53mut EC (mutation in p53). In case of inconclusive or not performed molecular test the term NOS (not otherwise specified) should be used. New algorithm of pathological/molecular examination of EC was presented on Figure 1.

CLINICAL SIGNIFICANCE OF NEW MOLECULAR CLASSIFICATION OF ENDOMETRIAL CANCERS

EC, *POLE* mut

POLE mutated variants comprise about 7% of all EC [6]. *POLE* gene encodes polymerase epsilon (ε), which can correct DNA synthesis errors and helps protect against genome instability. Loss of function in the DNA polymerase ε is important in tumorigenesis of EC. Tumours with *POLE* exonuclease domain mutations (EDMs) have shown to increase spontaneous mutation rates and are referred as "*POLE* ultra-mutated".

POLE mutations are assessed using PCR amplification and Sanger sequencing, while other subgroups of EC are evaluated by immunohistochemical staining [7]. Five hot spots were recognized: P286R, V411L, S297F, A456P and S459F. This variant of tumour despite the presence of poor pathologic features (high grade and deep myometrium invasion) has good prognosis with improved progression free survival (PFS) [8, 9]. PORTEC 1 and 2 trials show that in intermediate and high intermediate groups of EC *POLE* mut and *POLE* wild type (wt) 10-years cancer specific survival were 97.7% and 89.7% (p=0.11), respectively [10]. *POLE*-mutant tumours have a risk of recurrence approximately one third of that in other types of EC. In PORTEC trials any of POLEmut grade 3 patient recurred in comparison with 30.9% of grade 3 tumours in the rest of subgroups. These findings support opinion that *POLE* mut EC has intrinsic factors beneficial for survival independent of adjuvant treatment [11]. In this type of EC high mutational load have been demonstrated and immunogenic reactions due to huge lymphocyte T infiltration of tumour was observed [12]. Additionally, loss of increased radiation sensitivity in *POLE* mut embryonic stem cells was estimated [13]. Improved overall survival was also observed in *POLE* mut high risk EC [14]. Apart from mutations in *POLE* gene multiple alterations in molecular profile could be found in EC (POLEmut and p53 mut, POLEmut and MMRd, etc.) — known as double classifier. Those additional mutations do not influence survival; patients have good prognosis and should be managed as *POLE* mut [11].

EC, MMRd

This type of molecular profile is frequent in EC (approximately 25–30%). It is defined by loss of nuclear expression of one or more MMR proteins (MLH1, MSH2, MSH6 and PMS2) within tumour cell [6, 15]. MMRd EC has an intermediate prognosis. Similar to *POLE mut* also had abundance of tumour infiltrating lymphocytes (TILs). Exploratory analysis of NRG/GOG 210 study showed that adjuvant treatment improves PFS in MMRd EC in contrast to *POLE* mut [16]. Long term analysis of PORTEC 2 trial revealed similar effectiveness of vaginal brachyterapy in comparison with external beam radiotherapy (EBRT) in reducing pelvic lymph node recurrence risk in MMRd group in the absence of other unfavourable prognostic factors (substantial LVSI, p53 mut, L1CAM) [18]. Patients with high risk (HR) MMRd EC had no benefit from addition CT to EBRT [14]. Assessment of lympho-vascular space invasion (LVSI) in histopathologic protocol is crucial as it is one of the most important prognostic factors in EC. It should be stated that focal LVSI (single focus of LVSI around the tumour) have no impact on prognosis in opposite to substantial LVSI (extensive LVSI). Substantial LVSI is observed in up to 8.9% of MMRd EC [17]. In low-risk endometrial cancer patients, MMRd increases of ovarian metastasis and synchronous gonadal involvement [19].

EC, NSMP

Non-specific molecular profile (NSMP; also called low copy number) EC should be diagnosed in patients with p53 wild type expression, MMR proficient and absence of pathogenic mutations in POLE gene. This subtype has worse prognosis than the *POLE* mut and MMRd types. According to newly developed Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), L1 cell-adhesion molecule (L1CAM) is significant indicator of high-risk disease in EC. In NSMP subgroup univariate analysis showed higher risk of fatal outcome in L1CAM positive patients compared to L1CAM negative counterpart. Disease specific survival in L1CAM positive group had HR 6.94 (95% CI 2.56–18.74; $p < 0.001$) [18]. After hysterectomy, patients with p53 wt/NSMP, L1CAM-positive tumours were at similar risk for fatal outcome when compared to patients with p53 mut disease. These patients should be subjected to adjuvant therapy even if ESMO criteria indicate low risk group with no adjuvant treatment necessary. Adjuvant EBRT with or without chemotherapy depend on patients' status, should be used [20]. In cases of p53 wt/NSMP L1CAM negative, addition of chemotherapy did not improve survival [21].

EC, p53 mut

This high copy number type of EC presents a very aggressive course and the worst outcome. Comparing 5 years PFS between subgroups: p53 mut, *POLE* mut, MMRd and NSMP, the results were as follows: 50%, 98%, 74% and 76%, respectively [21]. Budak et al. [22] showed that high p53 expression correlates with advanced stage of endometrial cancer. Adjuvant chemoradiotherapy (CRT) improved overall survival only in this subtype of EC. HR patients with p53 mut included to PORTEC 3 trial achieved better 5 years PFS in CRT group in comparison with radiotherapy group: 61% vs 37%; $p < 0.001$ [14]. Additionally, amplification of *ERBB2* gene is found quite often [21]. The prevalence of homologous recombination deficiency (HRD) had been determined in 46% of EC p53 mut [23].

NEW POTENTIAL TARGETED THERAPY IN EC

Both MSI and *POLE* mut subtypes express essential immunogenicity because of a high mutational burden [24]. Addition of PDL1 or anti PD-1 agents (atelizumab, nivolumab or pembrolizumab) can be effective in recurrent or metastatic MSI or *POLE* mutations EC [25–27].

In EC cases, showing the amplification of *ERBB2* gene and overexpression of HER2, trastuzumab therapy may be used. Trastuzumab is a monoclonal antibody directed against the HER2 receptor. The ongoing study may confirm any benefit of adjusting trastuzumab therapy in recurrent EC. The trial including patients with stages III/IV or recurrent HER2 EC presents improved PFS from 8 to 13 months ($p = 0.003$) [27, 28]. Subgroup of EC p53 mut serous type express germline *BRCA1/2* mutations, and therefore poly (ADP ribose) polymerase inhibitors may show any efficacy in treatment modality. Currently, several clinical trials investigating the efficacy of PARP inhibitors in recurrent or metastatic EC have been ongoing. Based on previous information from "ProMisE" (Proactive Molecular Risk Classifier for Endometrial Cancer), ESGO (European Society of Gynaecological Cancer), ESTRO (European Society for Radiotherapy and Oncology) and ESP (European Society of Pathology) prepared recommendations on risk stratification presented in Table 1.

The PORTEC 4a study aims to determine efficiency of molecular integrated risk profiles in endometrial cancer. This trial intends to compare molecular profile-based adjuvant therapy versus adjuvant treatment based on standard pathological characteristics.

SUMMARY

The new insights into molecular classification give novel information to better understand the biology of endometrial cancer. New stratifications based on molecular and clinical factors will allow to treat EC patients more precisely. New recommendations will hopefully help to avoid over an undertreatment of endometrial cancer patients. About 50% of patients in the *POLE* subgroup (excellent prognosis) were classified as ESMO high-risk, and about 25% of patients in the high copy number subgroup would be classified as ESMO low/intermediate risk. These data raise the question of proper adjuvant therapy. The EC molecular classification will permit to introduce a new therapeutic modality in these cases. Results of prospective PORTEC 4a trial are still awaited.

Table 1. Risk stratification according to histopathological criteria and molecular classification [30] (by courtesy of International Journal of Gynaecological Cancer)

Risk group	Molecular classification unknown	Molecular classification known*
Low	• Stage IA endometrioid + low grade& + LVSI negative or focal	• Stage I–II **POLEmut** endometrial carcinoma, no residual disease • Stage IA MMRd/NSMP endometrioid carcinoma + + low-grade& + LVSI negative or focal
Intermediate	• Stage IB endometrioid + low-grade& + LVSI negative or focal • Stage IA endometrioid + high-grade& + LVSI negative or focal • Stage IA non-endometrioid (serous, clear cell, undifferentiared carcinoma, cacinosarcoma, mixed) without myometrial invasion	• Stage IB **MMRd/NSMP** endometrioid carcinoma + + low-grade& + LVSI negative or focal • Stage IA **MMRd/NSMP** endometrioid + high-grade& + LVSI negative or focal • Stage IA **p53abn** and/or non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) without myometrial invasion
High-intermediate	• Stage I endometrioid + substantial LVSI regardless of grade and depth of invasion • Stage IB endometrioid high-grade& regardless of LVSI status • Stage II	• Stage I **MMRd/NSMP** endometrioid carcinoma + substantial LVSI regardless of grade and depth of invasion • Stage IB **MMRd/NSMP** endometrioid carcinoma high-grade& regardless of LVSI status • Stage II **MMRd/NSMP** endometrioid carcinoma
High	• Stage III–IVA with no residual disease • Stage I–IVA non-endometrioid (serous, clear cell, undifferentiared carcinoma, cacinosarcoma, mixed) without myometrial invasion, and with no residual disease	• Stage III–IVA **MMRd/NSMP** endometrioid carcinoma with no residual disease • Stage I–IVA **p53abn** endometrial carcinoma with myometrial invasion, with no residual disease • Stage I–IVA **NSMP/MMRd** serous, undifferentiated carcinoma, carcinosarcoma with myometrial invasion, with no residual disease
Advanced metastatic	• Stage III–IVA with residual disease • Stage IVB	• Stage III–IVA with residual disease of any molecular type • Stage IVB of any molecular type

According to the binary FIGO grading, grade 1 and grade 2 ECs are considered as low-grade and grade 3 ECs are considered as high-grade

Conflict of interest

All authors declare no conflict of interest.

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Safety and efficiency of COVID-19 vaccination during pregnancy and breastfeeding

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ABSTRACT

Despite the development of effective and safe vaccines, the contributions of pregnant women in clinical trials of vaccines have been excluded. Similarly, vaccine trials did not include breastfeeding women. This article is an overview of studies on immunization during pregnancy and breast-feeding. The manuscript is intended to collect the current data on the effectiveness and safety of COVID-19 vaccines in order to facilitate medical decision.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) spread throughout the world and on March 11th, 2020, The World Health Organization (WHO) declared it a pandemic [1]. Efforts of scientists from around the world have focused on the search for an effective drug or the creation of a vaccine that protects against the development of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Research on the structure and replication cycle of the virus allowed for the development of effective and safe vaccines [2]. It is known, however, that the participation of pregnant women in clinical trials of therapeutics and vaccines have been eliminated [3]. Hence, when the first COVID-19 vaccines were approved for use, the eligibility of pregnant women for vaccination was not considered. In Poland, decisions were made based on the American College of Obstetricians and Gynecologists (ACOG) recommendations that women should be able to make their own decision about COVID-19 vaccination [4]. The same applies to women who are breastfeeding as the vaccine trial did not include this population [5]. Although, in this case different scientific societies have been more inclined to recommend vaccination of these women [6].

This article is an overview of several studies on immunization during pregnancy and breast-feeding. The manuscript is intended to collect the current data on the effectiveness and safety of COVID-19 vaccines in order to facilitate medical decision.

Humoral and mucosal immunity

There are two ways that infant immune systems can be supported by the mothers' antibodies - the transplacental IgG transfer of antibodies and secretory antibodies included in milk. It is known that IgG antibodies produced after vaccination during pregnancy cross the placenta and provide children passive innate immunity until the third month after birth [7]. A transport of maternal IgG to the fetus results in about 90% of the maternal serum level of IgG antibodies in the full-term newborn in the moment of delivery [8]. These antibodies play a duel role on mucosal membranes and in the circulation. In turn, breast milk antibodies do not enter neonatal circulation. Secretory IgA (SIgA) represents the major immunoglobulin in breast milk, followed by secretory IgM and IgG. Maternal milk antibodies coat infant mucosal surfaces and have a protective role, especially against enteric infections. Differing from many species of animal, IgG from breast milk in humans are not transported across the intestinal epithelium into the neonatal circulation [9].

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VACCINATION OF PREGNANT WOMEN

COVID-19 vaccine research in pregnancy

In Poland, at the beginning of COVID-19 vaccination, the vaccines were offered to healthcare professionals first [10]. Thus, in a study by Zdanowski et al. [11] sixteen women being medical doctors, who were vaccinated with two doses of BNT162b2 mRNA COVID-19 vaccine, were included. The first dose of vaccine was taken between the 29th and 36th week of pregnancy and the second dose between the 32nd and 40th week of pregnancy. Study showed high level of anti-S IgG antibody in cord serum at birth in all included patients (mothers and infants). During publication of results, the study was still in progress and then included 150 female patients who had been vaccinated against SARS-CoV-2 during pregnancy [11].

A study on a larger group of pregnant women was conducted by Gray et al. [12]. Eighty-four pregnant women, 16 nonpregnant and 31 lactating women were qualified into a prospective cohort study. Laboratory parameters were quantified basically, at the second vaccine dose, at two to six weeks after the second dose, and during delivery. Also, concentrations of immunoglobulin were juxtaposed with immunoglobulin level in pregnant women 1–3 months from natural SARS-CoV-2 infection. The study revealed that vaccine-induced antibody concentrations were comparable in all groups of women. What is more, all immunoglobulin levels after vaccination were significantly higher than levels after COVID-19 during pregnancy [12].

Prabhu et al. [13] enrolled 122 pregnant women into study, whom by the time of delivery, 55 were vaccinated one dose, and 67 were vaccinated two doses. At birth level of immunoglobulin was established — 87 women produced an IgG response, 19 — IgM and IgG response, and 16 women had no determinable antibody response (the latter received only one dose of vaccination, up to four weeks before delivery).

Optimizing neonatal immunity

Based on the above information, immunization against COVID-19 resulted in the presence of antibodies anti-SARS-CoV-2 in the blood of mothers and newborns. However, it has not been established what the concentration of antibodies in the newborn has a protective effect, and in which period during pregnancy is the best term to vaccinate pregnant women as newborns gain the best possible post-vaccination protection.

In a study performed by Gray et al. [12], the titers of antibodies in the serum of the mothers, induced by the vaccine, did not differ depending on the trimester in which the vaccination was performed. When it comes to placental transport of immunoglobulin, specific IgG were detectable in all examined newborns. The cord with the lowest IgG level belonged to a mother who was vaccinated first dose 17 days before delivery. This result may suggest that two doses of the vaccine can be necessary to gain optimal humoral immune transfer to the newborn. Interestingly, in this study, a significant improvement of transferred specific IgG subclasses into the cord with time from boost was found. It suggests time from vaccine might be a key factor determining the rate of transfer of IgG subclasses after maternal vaccination [12]. It is known the amount of maternal IgG transferred across the placenta to the cord depends on the time of vaccination [14].

Zdanowski et al. [11] described the correlation between the week of pregnancy and the concentration of antibodies in the serum of cord blood. There was a significant positive correlation between the number of weeks from the first dose of the vaccine and the level of anti-S antibodies in the cord blood serum. In addition, there was also a significant positive correlation between the gestational week of the first dose and the gestational week of the second dose and the respective cord-to-maternal ratio. It is also interesting that the patient who received the second dose just seven days before delivery, had high levels of anti-S antibodies in the cord blood [11].

According with study of Prabhu et al. [13] as the number of weeks from vaccination elapsed, the number of women who had an antibody response and who conferred passive immunity to their neonates increased. The earliest detection of antibodies in mother blood was noted five days after the first dose of vaccine as well as the earliest detection of antibodies in cord blood was noted 16 days after this first dose. Women vaccinated with only one dose had detectable IgG in 44% of cord blood sample, whereas women vaccinated both doses had detectable IgG in 99%. Maternal IgG levels were increasing starting two weeks after the first vaccine dose and were linearly associated with cord blood IgG levels. What is more, the placental transfer ratio corresponded with the number of weeks since the day of maternal second vaccination [13].

Vaccination at the end of the second or during the third trimester may be most effective, as is the case with Tdap vaccines [15]. However, it remains further confirmation as vaccines like Tdap aim to boost preexisting antibodies, while COVID-19 vaccination is administered *de novo*.

Postvaccination reactions/side effects in mothers and offspring

Based on information tracked by the Centers for Disease Control and Prevention (CDC), no significant differences in side effects in pregnant vs nonpregnant women at the age of 16 to 54 years was found. Verification revealed that after the second dose incidence of fever occurred up to 32% [16]. Hence, it may raise concerns for pregnant women and their offspring as literature supported a 1.5- and nearly 3-fold in-

creased risk of neural tube defects, congenital heart defects, and oral clefts with fever exposure in the first trimester [17].

Shimabukuro et al. [18] based on data available from CDC and Food and Drug Administration (FDA) reported the following local and systemic reactions as the most frequent: injection-site pain, fatigue, headache, and myalgia after each dose of vaccines in pregnant women. However, side effects were observed more frequently after dose number two for both vaccines. Only less than one percent of women during first day after the first dose and eight percent after the second revealed temperature at or above 38°C. According to V-safe Pregnancy Registry among 827 women who had a completed pregnancy, the pregnancy ended in a live birth in 712 (86.1%), in a spontaneous abortion in 104 (12.6%), in stillbirth in 1 (0.1%), and in other results (ectopic pregnancy/induced abortion) in 10 (1.2%). 92.3% of spontaneous abortions occurred before 13 weeks of gestation, and 98.3% of live birth were among women vaccinated first dose in the third trimester. Preterm birth occurred in 9.4%, small size for gestational age (SGA) was noted in 3.2% and major congenital anomalies in 2.2%, among the latter no woman was vaccinated in the 1st trimester or periconceptional period. The calculated proportions of mentioned incidents turned out to be like these available from the literature.

In turn, after analysis data from Vaccine Adverse Event Reporting System (VAERS) authors stated 221 reports involving COVID-19 vaccination among pregnant persons; 70.1% involved nonpregnancy-specific adverse events, and 29.9% involved events such as spontaneous abortion (46 cases), stillbirth, premature rupture of membranes, and vaginal bleeding, with 3 reports for each [18].

Zdanowski et al. [11] state no mothers had severe pregnancy or neonatal complications.

Further monitoring is necessary to assess maternal and neonatal safety associated with maternal COVID-19 vaccination.

VACCINATION OF BREASTFEEDING WOMEN

Antibodies in breast milk

Gray et al. [12] showed that in the milk of 31 vaccinated women an increase in the titer of IgA, IgG and IgM antibodies was observed, the highest in the case of IgA and IgG. Milk samples were taken after the first and second doses of the vaccine, and between two and six weeks after the mother received the second dose. The greatest increase in the titer of IgA and IgM antibodies was noted after the first dose of the vaccine. In turn, an increase in IgG antibodies was found after the second dose with a parallel increase in their concentration in the mother's serum [12]. A prospective cohort study of breastfeeding women (either exclusive or partial) who chose to be vaccinated was performed in Israel by Perl et al. [19]. Breast milk samples were collected before vaccination and then once weekly after two weeks, two from the first dose till six weeks after this dose. 84 women completed the study, delivering 504 breast milk samples. IgA antibodies were present in 62% of the samples two weeks after the first dose, and one week after the second dose in the majority (86%) of the tested samples. The highest concentration of IgG antibodies was recorded at week five and six (97% of samples). No serious adverse event was reported in mother or infant during the study [19]. Kelly et al. [20] also conducted a study on the presence of specific antibodies in breast milk. Although the study was conducted on a group of only five women, it was confirmed anti spike IgG and IgA levels were significantly elevated relative to the pre-vaccine baseline at all time points [20]. Another longitudinal cohort study included 61 lactating women. In all samples of maternal serum and breastmilk specific IgG were found with a significant positive correlation between the SARS-CoV-2 IgG levels in the serum and breastmilk samples. The same antibody was detected in the oral mucosa of 3 of 5 (60%) breastfed infants, while neither of the dried blood spot samples from 21 infants were positive for these antibodies [21].

In mentioned above study performed by Gray et al. [12] authors examined also the levels of antibodies in breastmilk of lactating mothers finding high induction of IgG, IgA, and IgM after the first and second dose. While levels of IgA and IgM did not rise with boosting, a boost in breastmilk IgG levels was observed (corelating with the boost observed in maternal serum) [12].

Side effects in breastfeeding woman and infants

Regarding side effects of vaccination, Bertrand et al. [22] found that > 85% of 180 participating women reported any symptoms for both the Pfizer-BioNTech and Moderna vaccines following either dose. After the second dose, women who received the Moderna vaccine reported more frequently systemic side effects (chills, muscle or body aches, vomiting, fever) as well as localized symptoms (pain, redness, swelling, or itching at the injection site). Despite the low percentage of reported milk production disturbances, in all cases milk production have reached previous level within 72 hours. Among 180 breast-fed infants whose mother received the SARS-CoV-2 mRNA vaccine, no serious side effects were observed. Single women reported that their children were irritable, had difficulty falling asleep, or conversely, they experienced drowsiness [22].

Summarizing the above reports, it seems that response after COVID-19 vaccination is like previous studies performed in lactating women that have shown high levels of breast milk IgA and IgG production for up to six months after vaccination for influenza and pertussis [23, 24]. According to the fact that SARS-CoV-2 IgG was not detected in the infants

serum, it seems that vaccination during pregnancy may provide better protection to the infants because of transplacental passage of antibodies. As CDC reported that vaccine effectiveness ranges from 91% against the alpha variant to 66% against the delta variant in frontline workers [25], it remains unclear whether vaccines will be effective against the new virus variant. That is because the omicron variant has more than 30 mutations in the spike protein, the part that has been the primary target for current vaccines [26].

CONCLUSION

Pregnant women are particularly vulnerable to infectious diseases because of alterations in their respiratory and cardiovascular system as well as immune changes that occur during pregnancy.

What is more, pregnant women are more likely to require mechanical ventilation than non-pregnant women [27] and SARS-CoV-2 infection during pregnancy generates higher risk for preterm birth [28]. These facts and the potential contamination of newborn suggest pregnant women should be able to vaccinate against COVID-19. It is worth paying attention to the fact that none of currently produced vaccines contain a live, weakened (attenuated) virus. Many expert committees emphasize that these preparations are safe for breastfeeding women as well as state possibility of vaccination should be offered to all pregnant women, after being adequately informed of the benefits and risks [6]. They should have the same right to decide about vaccination as other adults. Vaccines still are the best defense against many infectious diseases, including COVID-19.

Conflict of interest

Author declare no conflict of interest.

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Urogynecology Section of the Polish Society of Gynecologists and Obstetricians Guidelines on the management of recurrent pelvic organ prolapse

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ABSTRACT

Objectives: The aim of the publication was to present the Guideline of the Urogynecology Section of the Polish Society of Gynecologists and Obstetricians (PSGO) for the management of recurrent pelvic organ prolapse, based on the available literature, expert knowledge and opinion, as well as everyday practice.

Material and methods: In 2005, 2006 and 2010, the panel of PSGO experts published guidelines for the diagnosis and treatment of patients with lower urinary tract symptoms (LUTS). This publication presents an update of those recommendations and concerns recurrent POP treatment.

Main conclusion: The analysis of data revealed that sacrocolpopexy with the use of commercial sets or polypropylene hernia mesh is the method of choice for the surgical repair of recurrent vaginal vault prolapse. However, a significantly higher risk of surgical and postoperative complications after sacrocolpopexy, as compared to vaginal surgeries, should be considered when making treatment decisions. In other types of recurrent POP, the choice of surgery method should be tailored to the individual needs of each patient and may depend on the medical center.

Key words: pelvic organ prolapse; recurrence; reoperation

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INTRODUCTION

1. Types of pelvic organ prolapse (POP) recurrence:
   — POP recurrence within the previously operated site,
   — POP or progression of a pre-existing prolapse within the non-operated compartment (*e.g.*, surgical correction of anterior vaginal wall prolapse and postoperative symptomatic posterior vaginal wall prolapse),
   — POP recurrence within the previously operated compartment, but in a different anatomic location (*e.g.*, surgical repair of the central defect of the anterior vaginal wall and postoperative presentation of the

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lateral defect of the anterior vaginal wall — the so-called 'masked defect').

2. Objective assessment of the surgical success:
   — anatomic assessment (typically using the POP-Q scale),
   — reoperation rate for same site or new site recurrent prolapse,
   — reoperation rate due to complications (mesh exposure, pain, different types of postoperative voiding dysfunctions — urine retention, urinary incontinence, overactive bladder).
3. Subjective (patient-reported) assessment of the surgical success:
   — subjective assessment of the postoperative success by the patient (e.g. using the Patient Global Impression of Improvement (PGI-I) scale),
   — validated quality of life (QoL) questionnaires after POP surgery [1].
4. Reoperation rate:
   — reoperation rate after traditional (native tissue) repair —16/1000,
   — reoperation rate after implant surgery — 7/1000, (RR = 0.44; CI 0.24–0.81),
   — POP recurrence assessed objectively (POPQ > 2): traditional (native tissue) — 41%, synthetic implants — 10.1–18.7% (RR = 0.34; CI 0.25–0.46) [1-–4]

Objectives

The aim of the Urogynecology Section of the Polish Society of Gynecologists and Obstetricians (PSGO) was to develop this Guideline for the management of recurrent pelvic organ prolapse, based on the available literature, expert knowledge and opinion as well as everyday practice.

Material and methods

In 2005, 2006 and 2010, the panel of PSGO experts developed guidelines for the diagnosis and treatment of patients with lower urinary tract symptoms (LUTS). This publication presents an update of those recommendations and concerns recurrent POP treatment.

RECOMMENDATIONS

Recommendations on the management of POP recurrence in the anterior compartment

Recurrence after traditional (native) surgery in the anterior compartment: consider using prosthetic materials [90–95% of total anatomic success; at two years of follow-up 53% of the patients presented with POP-Q ≤2 and 42% with POP- Q ≤1 as compared to only 55% anatomic success using native tissue reoperation] [5, 6]. Recent findings of a retrospective study conducted in Australia among 196 patients, also demonstrated better anatomic outcome (point Ba = 0 cm of the POP-Q) after the repair of the recurrent anterior vaginal wall defect using prosthetic materials as compared to native tissue reoperation — 25% recurrence in the TVM group vs > 40% in the classic reoperation group [2]. Also, the patients from the implant group reported a significantly higher subjective improvement in the quality of life (88% vs 66%, $p < 0.01$). The risk for yet another reoperation was significantly lower in the implant group (7.4% vs 23.9%, $p < 0.01$), but the high rate of mesh exposure (15%) and the related need for reoperation (9%), raise serious concern regarding the use of the prosthetic material method, despite its greater effectiveness [5–12].

Conclusions

The use of prosthetic materials in reoperations due to recurrent prolapse of the anterior vaginal wall results in better anatomic and functional outcome, however mesh exposure and postoperative pain syndrome, most often associated with excessive retraction of the synthetic material, constitute a significant issue. Reoperations with the use of prosthetic material should be conducted by a team with extensive experience performing urogynecological surgeries. We still wait for the results of studies using lighter weight new-generation implants.

Recommendations on the management of POP recurrence in the posterior compartment

According to the 2017 ICI guidelines, **prosthetic materials (biologic and synthetic) may be used in the posterior compartment** in the rectovaginal space for primary surgery and for recurrent POP in that compartment [1]. As far as reoperation due to recurrent prolapse in the previously operated posterior compartment is concerned, the literature offers only one study comparing the effectiveness of the traditional versus synthetic implant repairs. Those authors found that the use of synthetic prosthetic material resulted in significantly better anatomic outcome as compared to native tissue repair (anatomic success: 92.5% vs 59.1%; $p = 0.01$, subjective feeling of prolapse only in: 7.5% vs 24.1%; $p = 0.02$, need for yet another reoperation: 7.5% vs 19.5%; $p = 0.08$). An analysis of the composite outcomes also confirmed the superiority of prosthetic material repairs (56.6% vs 23.0%; $p < 0.01$), however implant-related complications mesh exposures continue to be a problem — mesh removal surgery was necessary in 15.1% of the patients after synthetic implant repair [2, 3, 13, 14].

Conclusions

The use of synthetic prosthetic materials increases the chances for permanent recovery in patients reoperated due to recurrent prolapse in the posterior compartment. High

rate of mesh exposures which require surgical management remains an unresolved issue. Therefore, reoperation without using synthetic material may be another option in selected cases.

Recommendations on the management of POP recurrence in the central compartment

A. Reoperations due to recurrent POP after primary repair in the central compartment in women with **preserved uterus** present a serious challenge to the decision-making and surgical processes. We must consider two different group of patients:
   — recurrences after traditional vaginal surgeries — modified Manchester repair (Fothergill operation), sacrospinous ligament (SSLF), uterosacral ligament suspension (USLS), medial closure of the vaginal walls.
   — recurrences after vaginal surgeries using synthetic prosthetic materials (commercial sets for sacrospinous ligament suspension from the anterior or the posterior approach)

B. Reoperations due to recurrent **vaginal vault prolapse** after hysterectomy (abdominal or vaginal).

The literature offers limited and inconclusive data on the techniques of reoperation for recurrent prolapse in the central compartment. However, after critical analysis of the available data, it seems safe to conclude that in patients with preserved uterus/cervix and after failed native tissue repair (Manchester-Fothergill, SSLF, USLS, median closure of the vaginal walls), a transvaginal repair surgery using synthetic materials may be considered in case of a two-compartment defect (central and anterior or central and posterior). The use of second generation meshes with sacrospinous ligament fixation in the treatment of the central compartment disorders may be associated with a better anatomic effect as compared to the first-generation implants.

In case of defects in three compartments, abdominal surgery (classic, laparoscopic, robotic) is often recommended - hysterosacropexy, cervico-sacropexy. Such management is also often recommended in patients with recurrent prolapse who underwent primary transvaginal surgery with synthetic materials.

In patients with vaginal vault prolapse after hysterectomy, regardless of whether the prolapse is primary or after vaginal repair surgery (native or with prosthetic materials), classical or laparoscopic sacrocolpopexy are often recommended. The risk for POP recurrence in the central compartment is higher in patients operated from the transvaginal as compared to the transabdominal approach (RR 1.89; 95% CI 1.33 to 2.70) — in absolute numbers: 41% vs 23% [20]. A meta-analysis demonstrated sacrocolpopexy to be more effective in terms of anatomic success as compared to transvaginal reoperations but is associated with the risk for gastrointestinal (2.7%) and implant-related (4.2%) complications, as well as thromboembolic events (0.6%) [16–19]. Significantly higher invasiveness of sacrocolpopexy, and the related risk for surgical and postoperative complications, should be considered when making therapeutic decisions. Importantly, objective data on reoperation techniques for recurrent prolapse in the central compartment remain limited and inconclusive.

Conclusions

The analysis demonstrated that sacrocolpopexy, with the use of commercial sets or polypropylene hernia mesh, should be recommended as the procedure of choice for recurrent vaginal vault prolapse. However, while making surgical decisions, one should consider a significantly higher risk for peri- and post-operative complications after sacrocolpopexy as compared to the vaginal approach. Therefore, the choice of surgery should be tailored to the individual needs of every patient and may vary between medical centers.

GUIDELINE SUMMARY

In accordance with the 2017 ICI recommendations and the "Consensus of the 2nd IUGA Grafts Roundtable", the use of synthetic prosthetic materials is justified in all cases of recurrent prolapse, regardless of the POP compartment. At the same time, it is also allowed to perform these surgeries without using synthetic materials. The choice of surgery should be strongly personalized. Among others, special attention should be paid to the risk for complications.

Conflict of interest

All authors declare no conflict of interest.

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Twin-to-Twin Transfusion Syndrome in monochorionic, monoamniotic twin pregnancy with common umbilical cord insertion

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Key words: Twin-to-Twin Transfusion Syndrome; TTTS; monochorionic monoamniotic twin pregnancy; common umbilical cord insertion; multiple pregnancy

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Twin-to-Twin Transfusion Syndrome, TTTS, monochorionic monoamniotic twin pregnancy, common umbilical cord insertion, multiple pregnancy. Initial clinical features of Twin-to-Twin Transfusion Syndrome (TTTS) result from discordance in the intravascular volume between twins. Monochorionic (MC) twins share a single placenta, and, in fact, all have some component of TTTS due to the presence of intertwin placental vascular anastomoses. However, most MC multiple pregnancies represent a balanced blood flow through the placental anastomoses, avoiding significant TTTS-specific consequences [1, 2].

A 24-year-old patient at 28 weeks of MC, monoamniotic twin pregnancy was hospitalized due to decreased fetal movements of one twin. The patient received antenatal corticosteroid therapy at 26 weeks of pregnancy (betamethasone 2 × 12 mg intravenously with an interval of 24 h between doses). Fetal cardiac rhythm was regular, 150 beats per minute (bpm) in the presenting twin (twin A) and 170 bpm in the non-presenting twin (twin B). Both fetuses were in a transverse lie. After 15 minutes of cardiotocography, a deceleration (up to 60 bpm) was noted in twin B. Fenoterol was administered according to the intrauterine resuscitation guidelines. Fetal well-being was assessed by ultrasound Doppler examination of the Umbilical Artery (UA), Umbilical Vein (UV) and Ductus Venosus (DV).

Twin A had an estimated fetal weight (EFW) of 1250 g, corresponding to 28 weeks and 4 days. Blood flow in the UA, UV, and DV was normal with no signs of brain sparing effect.

Twin B had an EFW of 1030 g, corresponding to 26 weeks and 1 day. Doppler examination revealed reversed end-diastolic flow in the UA, a pulsatile flow in the UV, and reverse a-wave flow in the DV with signs of brain sparing effect.

There was an excessive accumulation of amniotic fluid. Moreover, normal fetal activity and breathing in Twin A and abnormal fetal activity and breathing in twin B were found.

The patient qualified for an emergency cesarean section. Two girls, 1190 g and 1060 g were born. Typical signs of TTTS were confirmed by neonatologists. During the cesarean section, the MC monoamniotic pregnancy was confirmed. Examination of the placenta revealed a single, common, central umbilical cord insertion (single trunk). The umbilical cord of the donor was half the size of the recipient's one (Fig. 1).

The currently applied management options of TTTS include expectant management, amnioreduction (AR), serial amnioreduction (SAR), fetoscopic laser photocoagulation of placental anastomoses techniques (non-selective technique, selective laser photocoagulation, sequential selective laser photocoagulation, Solomon technique) and selective feticide [1–3].

During the literature review, we did not find any previous reports of TTTS in MC, monoamniotic twin pregnancy with common umbilical cord insertion. Such a case should be considered during diagnostics, as its treatment options are limited compared to TTTS with normal umbilical cord insertion. In the presented case, fetoscopic laser photocoagulation of placental anastomoses techniques could not be applied. First of all, TTTS was diagnosed at 28 weeks of pregnancy, whereas today, these techniques could be applied between 16 and 26 weeks. Furthermore, due to the common umbilical cord insertion, such a procedure was technically impossible to perform. AR and SAR are palliative management options of TTTS. In the presented case, an immediate intervention was required due to the deteriorating health of the fetuses. Single AR would not sufficiently improve that. Because of threatened asphyxia of one twin, an emergency cesarean section was the only available treatment option.

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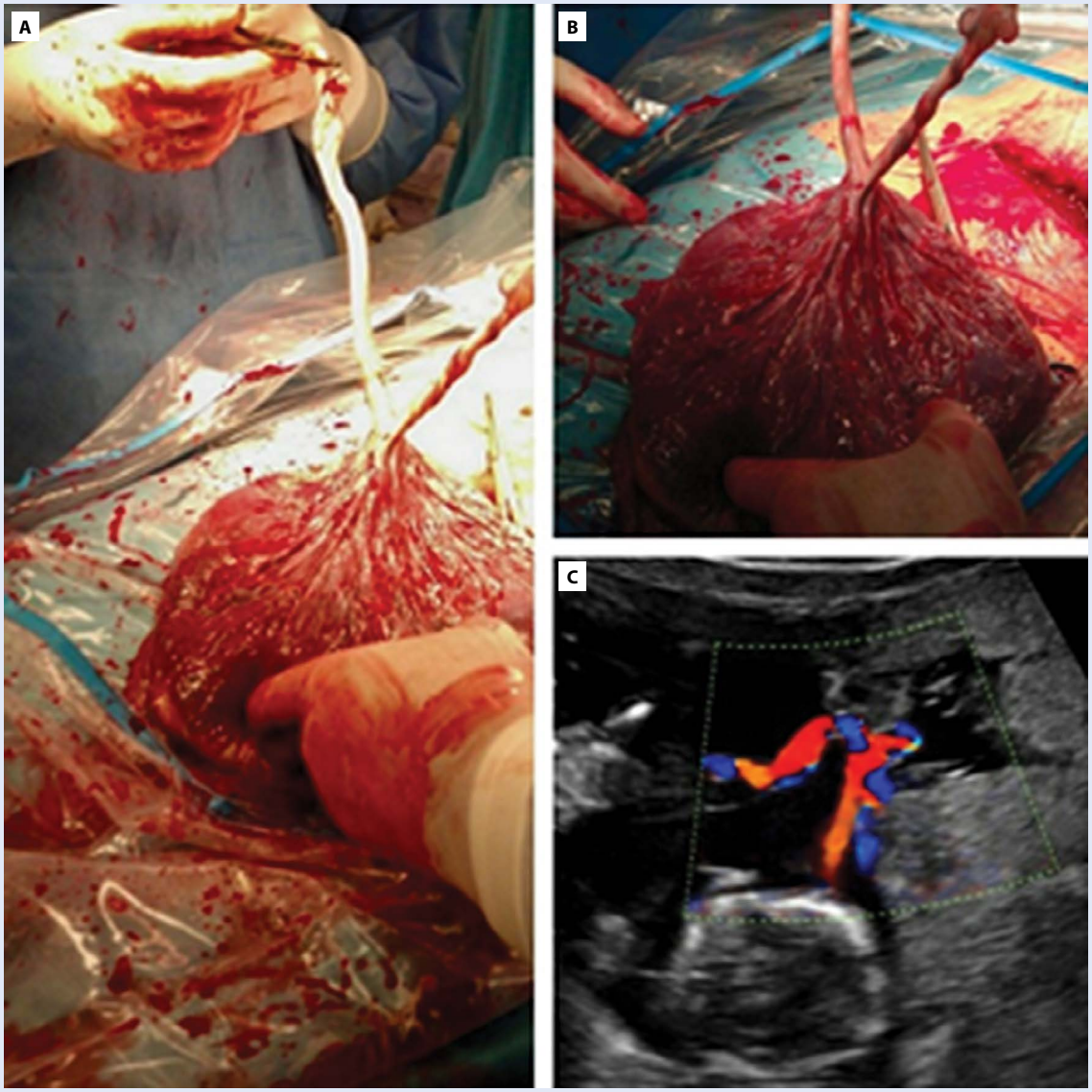

Figure 1. A–B. Common, central umbilical cord insertion (single trunk); **C.** Close proximity of umbilical cord insertions in twins at 12 weeks of pregnancy

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Conflicts of interest

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