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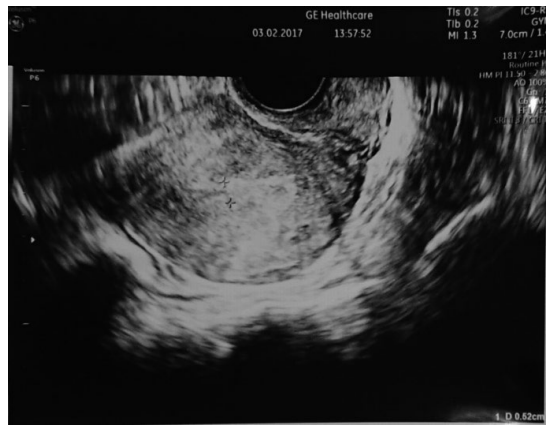

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Minimally invasive surgery in gynecology. Reconciling the past with a view to the future

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In recent years, in the surgical techniques of gynecology, the improvement of care has taken place after the active introduction of innovative research methods (magnetic resonance imaging, spiral computed tomography). More specifically, the term "minimally invasive" in surgery means, first, minimal trauma accessing a pathological process requiring elimination or correction; second, minimal intervention within the intraperitoneal environment; and finally, maintaining or restoring the correct anatomical and topographical relationships of the pelvic structures [1].

Hysterectomy is the world's second-most common gynecological surgical procedure. There are three main surgical approaches: total abdominal hysterectomy (TAH), vaginal hysterectomy (VH), and total laparoscopic hysterectomy (TLH). With technological progress, these procedures are more frequently performed using a minimally invasive technique, laparoscopy, and thanks to the use of diathermy bipolar vascular closure systems, the oldest of the surgical techniques, transvaginal removal of the uterus, is experiencing a renaissance. Since 2016, numerous activities have been initiated with the participation of the Polish Society of Gynecology and Obstetrics to increase the role of minimally invasive techniques in gynecology and gynecological oncology. Training programs were created (such as the LAP-GYN certified training path), which after a few years brought success in popularizing minimally invasive methods in gynecology. Referring to the data of the National Health Fund on the number and methods of uterine excision, initial progress was obtained in the number of uteri removed transvaginally and laparoscopically. In 2016, 31,118 uterus removals were performed, of which 27,099 (90%) were transabdominal, only 2019 (6.5%) laparoscopically, and 1,113 (3.5%) transvaginally. The National Health Fund data show a significant increase in the number of minimally invasive procedures performed two years after the dissemination of these methods began. In 2018, approximately 32,000 hysterectomies were performed, of which 3,783 (12%) were transvaginal [2].

According to the most recent ACOG recommendations, minimally invasive methods should always be used first. Vaginal hysterectomy is the method of choice whenever possible. Laparoscopic hysterectomy is the preferred alternative to open abdominal hysterectomy for patients in whom vaginal hysterectomy is not indicated or not possible.

In the case of each patient, the individual clinical situation should be considered, and it should be determined which hysterectomy route will most safely facilitate the removal of the uterus and optimize the patient's treatment outcomes [3]. The choice of hysterectomy route for non-oncological reasons may depend on the size and shape of the vagina and uterus. The other point is surgical access to the uterus (*e.g.*, pelvic adhesions); the extent of ectopic disease; the need for parallel procedures; and the surgeon's training and experience are important. It is required hospital technology, facilities, and support; determining whether the operation is urgent or scheduled; and determining the patient's preferences for the procedure [3, 4]. Undoubtedly, with the growing experience in vaginal hysterectomy, a volume greater than 300 cm^3 is no longer a contraindication to transvaginal hysterectomy. In the case of significant uterine hypertrophy, it is necessary to use one or more techniques to reduce the size of the uterus [5]: hemisection, trachelectomy, wedging, coring, and myomectomy.

The use of minimally invasive laparoscopic procedures in gynecological surgery is becoming more popular due to faster convalescence, shorter hospital stays, and a lower risk of peri- and postoperative complications. Despite the significantly longer method implementation path compared to TVH, the ability to complete the operation laparoscopi-

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cally increases with experience. There are some clinics that have argued that this plateau occurs in the 20th, 25th, or 75th patients [6]. Despite the increasing number of procedures, including laparoscopic hysterectomy (LH), there is still a risk of complications associated with the technique used. One of the most intriguing is ureteral injury, with a worldwide incidence of less than 1% for laparoscopic hysterectomy and still higher than for the vaginal technique (< 0.9%) [7].

We have made huge milestones through the increased availability of surgical devices and instruments, a significant increase in the awareness of gynecologists, and the possibility of participating in training in minimally invasive surgical techniques in gynecology. Thanks to the use of laparoscopic procedures in gynecology and gynecological oncology, many patients do not have to deal with the problems of trans-abdominal operations. The use of minimally invasive methods is associated with plenty of benefits for the patient: shorter procedure times, less postoperative pain, and often less suture material left in the patient's body. It also has smaller scars or, in the case of TVH, no scars. For the healthcare system, this means lower hospitalization costs and lower system costs. And unquestionable patient satisfaction is the pinnacle of minimally invasive surgery treatment [8].

Recently, for three days, from 3.11–5.11.2022, we had the pleasure of participating in the 1st International Congress of Operative Gynecology, which took place in Katowice [9]. A virtual operating room was created where operations were shown live, and practical experience was shared. A group of outstanding foreign and domestic experts showed how to operate cheaply, effectively, and, above all, safely.

In conclusion of the considerations on minimally invasive surgical techniques in gynecology, we would like to encourage gynecologists to actively participate in numerous programs, courses, and educational paths. Although laparoscopy remains the most popular minimally invasive surgical technique, technological progress allows the use of other methods as well. More and more boldly, the world looks towards surgical robots or surgical procedures using minimally invasive techniques through natural body orifices.

Conflict of interest

All authors declare no conflict of interest.

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Epidural anaesthesia and myomectomy-associated blood loss — a prospective randomised controlled study

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ABSTRACT

Objectives: The management of anaesthesia for patients with large myomas is particularly important due to disruption of hemodynamic as a result of massive haemorrhage, the prolonged duration of surgery and requirement for additional interventions. This study evaluated the effect of anaesthetic technique on blood loss in patients undergoing myomectomy due to large fibroid uterus.

Material and methods: A total of 156 patients that underwent myomectomy were randomized into two equal groups according to the type of anaesthesia: Epidural anaesthesia group and General anaesthesia group. The volume of blood loss and blood products transfusion was reviewed for each patient.

Results: The intraoperative blood loss and need for blood transfusion were significantly higher in general anaesthesia group ($p < 0.001$). The mean hematocrit change was 2.5 ± 1.5 vs 3.7 ± 2.9 % ($p = 0.001$) for both groups.

Conclusions: In the myomectomy planning of women with a large fibroid uterus, the team of gynecologists and anaesthesiologists should take care to choose the most optimal technique for anaesthesia.

Key words: epidural anaesthesia; general anaesthesia; myomectomy; blood loss

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INTRODUCTION

Uterine leiomyomas are the most common benign tumor of the reproductive system in women of childbearing age [1]. A hysterectomy is the optimum treatment for symptomatic fibroids. However, for patients who seek future pregnancy or preservation of the uterus, myomectomy is a popular option [2]. Abdominal fibroid enucleation is often preferred over laparoscopic myomectomy in the cases of large and multiple leiomyomas [3]. Intraoperative bleeding requiring blood transfusion is the most common complication of an abdominal myomectomy [4] and, when uncontrolled, may require a hysterectomy [5].

The amount of blood transfusion in open myomectomy depends on the size of the uterus and the number and site of fibroids [3]. The overall rate of a blood transfusion during abdominal myomectomy is 13.5 to 58.2% [6], with an unpredictable rate of hysterectomy about 2% after uncontrolled bleeding [2, 6]. Therefore, during myomectomy, effective measures to reduce blood loss are desirable [2].

A number of medical and surgical techniques have been attempted to significantly reduce bleeding during myomectomy. This includes peri-operative vaginal misoprostol and dinoprostone, intraoperative vasopressin, intrauterine bupivacaine and epinephrine, intravenous tranexamic acid and ascorbic acid, gelatin-thrombin matrix, loop ligation of the pseudo-capsule of the leiomyoma, and by using a foley catheter as a cervical tourniquet [7]. However, each technique has its limitations; therefore, blood loss control remains the main goal for both anaesthesiologists and gynaecologists [8].

To our knowledge, no published study had systematically assessed the effectiveness of epidural anaesthesia and myomectomy. Hypotensive technique during anaesthesia allowed for a significant reduction in blood loss without compromising the perfusion of vital organs [9], thereby

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reducing transfusion requirements and reducing the risk of allogeneic transfusion [10].

This study was conducted to determine the amount of blood loss during surgery and to find the optimum perioperative anaesthetic method in patients undergoing myomectomy for treatment of large fibroids. The primary outcome measure was estimated blood loss during myomectomy. The secondary outcome measures were the amount of blood transfusions during surgery, intraoperative recourse to hysterectomy and post-operative change in haematocrit.

MATERIAL AND METHODS

Study design

This prospective randomized clinical study was conducted between June 2014 and May 2019 at Sabah Maternity Hospital — a tertiary health facility in Kuwait. The Sabah Maternity Hospital has 320 beds, with an annual rate of about 4000 gynaecologic admissions.

Ethical consideration

The study protocol was approved by the ethical review committee of the Sabah Maternity Hospital, Kuwait before commencement of the study and it was post-registered at ClinicalTrials.gov (NCT04629573). Written and informed consent was obtained from all patients after thorough explanations about study purpose, design, and procedures to each patient.

Eligibility criteria

Patients over 18 years of age, American Society of Anesthesiologist (ASA) I and II, undergoing myomectomy to treat large uterine leiomyomas (with a diameter of at least 10 cm as diagnosed by transvaginal ultrasonography) were included in this study. Exclusion criteria: Patients with a history of previous uterine surgery (myomectomy, caesarean delivery), coagulopathy or other bleeding diathesis and severe anaemia (Hb under 7.0 g/dL) were excluded from the study.

Study groups

A total of 156 patients were randomized into two equal groups according to the type of anaesthesia, 78 patients in each group: Epidural anesthesia group (Group I) and General anesthesia group (Group II).

Randomization and blinding

The management protocol for each group was prepared by the researchers before the study began and sealed one protocol per envelope with a computer-generated number assigned prior to study initiation. Randomization was performed by selecting sequentially numbered envelopes and opening them before induction of anaesthesia by the anaesthetist and managing the participant according to the attached protocol. The study will not be double-blind, as healthcare professionals and patients cannot afford to ignore a strategy to which the woman is allocated.

Interventions

Anaesthesia procedures:

Upon arrival to the operative theatre, an 18- gauge IV catheter was inserted and 500 mL of NaCl (0.9%) was administered, 1 mg of intravenous midazolam was given as premedication. Patients were monitored by ECG, pulse oximetry and non-invasive blood pressure regularly every five minutes.

Lumbar epidural. Epidural procedures were performed by attending anaesthesiologists who performed at least 50 epidural procedures during their studies The Patient was placed in a sitting position, the skin in the lumbar region was cleaned and sterilization procedures were followed throughout the procedure. The skin and subcutaneous tissue were infiltrated with 2 mL of 1% lidocaine at the site of epidural placement (L2-3 or L3-4 interspace). The lumbar epidural space was localised, using a midline approach with an 18-gauge Tuohy epidural needle by loss of resistance technique with 2 mL of saline, 20-gauge epidural catheter was inserted in each patient. An initial bolus dose of 20 mL bupivacaine 0.5% plus 100 mcg Fentanyl was given through the epidural catheter, followed by continuous infusion of bupivacaine 0.5% plus 1mcg/mL Fentanyl (3–5 mL/h).

General anaesthesia. Anaesthesia was started with IV 1 µg/kg fentanyl, 2 mg/kg propofol and 0.15 mg/kg cisatracurium to facilitate endotracheal intubation. After tracheal intubation, anaesthesia was maintained with sevoflurane in O2 and air (FiO2 of 0.5), and intravenous infusion of 1µg/kg/min cisatracurium and 0.5 µg/kg/h fentanyl. Volume-Controlled ventilation was performed in all patients. Cisatracurium and fentanyl infusion was discontinued at the end of surgery, neuromuscular blockade was reversed, and the patient was extubated and send to post-anaesthesia care unit.

Myomectomy. Abdominal myomectomy was performed using standard conventional techniques. One of the surgeons oversaw all operations. The myomectomy was performed by excision of uterine fibroids and closing the uterine defect in several layers. The use of electrical devices, type of suture, and adhesion barriers were at the surgeon's discretion.

Data collection

All participants had standard preoperative evaluations including pelvic ultrasound for the size, number and site of leiomyomas. T2-weighted MRI imaging of leiomyoma was a more precise method for measuring leiomyoma volume [11]. Age, parity, body mass index, surgical history, number of previous caesarean sections, indication of myomectomy,

intraoperative anaesthetic management, ASA classification, pre and postoperative Hgb and Hct values, number of fibroids, fibroid dimension and location, suture type, number of layers of closure, concomitant procedures, duration of operation, intraoperative complications.

Outcome measures. The primary outcome was estimated intraoperative blood loss. Intraoperative blood loss was estimated by measuring the volume of blood in suction bottle; other losses were calculated using the gravimetric methods by scanning the pre-weighted abdominal towels and repeating the measurement with a weight difference of 1 gram equal to 1 mL of blood. Secondary outcomes include need for blood transfusions, need for hysterectomy and total blood loss calculations.

Blood loss calculations. The patient's estimated blood volume (EBV) using the Nadler equation [12]:

$$EBV\ (L) = 0.3561 \times height\ (m)\ 3 + 0.03308 \times weight\ (kg) + 0.1833\ (for\ female)$$

The amount of intra and post-operative blood loss (TBL) was determined by a comprehensive method that is widely used to measure blood loss perioperatively [13]:

$$TBL\ (L) = EBV\ (L) \times (preoperative\ Hct - postoperative\ Hct) / / average\ Hct$$

Average Hct is the average of haematocrit before and after surgery. In our study, Postoperative Hct was defined as Hct on the day three after myomectomy. To reduce the risk of haemodilution at the expense of blood loss, our patients didn't receive more than 2000 mL of IV fluid intra or post-operative.

Blood transfusions. Blood transfusion was initiated when the maximum calculated allowable blood loss (transfusion trigger) was exceeded. Ttransfusion of blood and blood products was also initiated with signs of cardiovascular instability or inadequate perfusion or oxygenation. In addition, fresh-frozen plasma (unit), tranexamic acid, human fibrinogen concentrates, crystalloid (mL), and colloid (mL) transfusions were also reviewed.

Sample size calculation. The sample size was calculated by Epi Info software Version 7.2 for Windows. Based on previous study [14], if the standard deviation of blood loss is 1721 mL, this study should consist of 73 patients in each group (sample size), to achieve a power of 95% and 5% of significance level (two sided) and to detect a true difference in means between the test and the reference group of 964 mL. Ten patients were added to the sample to compensate for dropouts.

Statistical analysis

SPSS for Windows 23.0 statistical program (IBM Corp., Armonk, NY, US) was used for data analysis. Continuous variables were represented as median (minimum-maximum) and categorical variables were expressed as frequencies and percentages. Pairwise comparisons were performed using the Mann-Whitney U test. Chi-square test was used to compare the categorical variables. $P < 0.05$ value was considered significant.

RESULTS

A total of 202 women were selected for the eligibility criteria within the study. Then, 156 women of the total selected were participated in our study, 78 in each group as show in consort flow diagram.

The mean age of the participants was 39.15 ± 4.14 (range, 22–45) years; 66 (42.3%) were nulliparous, and 94 (60.2%) had no children. Common symptoms were excessive and or prolonged menstruation (n = 61; 39.1%), abdominal pain (n = 25; 16%), inability to conceive (n = 33; 21.15%), mechanical complaints (n = 31; 19.87%) and 6 (3.8%) had other symptoms (Tab. 1).

Both treatment groups were similar with no statistically significant difference in demographic data and preoperative Hb level (Tab. 1). However, the treatment groups differed in characteristics of the fibroids (size, type, volume and numbers), due to lack of stratification of patients according to the size of the myomas. The mean diameter of the largest fibroid was significantly higher in women allocated to group I (Epidural anesthesia) than women allocated to group II (general anesthesia) 14.5 ± 2.3 vs 11.7 ± 1.6 cm; p = 0.035; 95% CI 0.1–2.0). Other characteristics of the fibroids at baseline such as type (subserous, interstitial and submucous), volume of the uterus and the leiomyoma volume planned for excision did not reveal a significant difference (Tab. 2).

Table 3 showed that group II participants recorded higher values in mean intraoperative blood loss [984 ± 345 vs 745 ± 289 mL, (p = <0.001)] and blood products transfusion including PRBCs, FFP and Platelets [48 (61. 53%) vs 29 (37.17%), (p = 0.008)] than group I. Blood transfusions were performed on one patient (1.2%) in group I, and three patients (3.8 %) in group II postoperative. In group II (under general anaesthesia), four patients and in group I, two patients (under epidural anaesthesia but subsequently converted to general anaesthesia) were subjected to hysterectomy.

The mean preoperative haematocrit was 31.8 ± 3.6 vs 31.9 ± 4.2% (p = 0.87), the mean post-operative haematocrit was 27.4 ± 2.1 vs 24.01 ± 1.3% (p = < 0.001), and the mean haematocrit change was 4.4 ± 1.5 vs 7.8 ± 1.9% (p = 0.03) for groups I and II, respectively (Fig. 1). Of the 40 patients who received intraoperative blood transfusion in both groups (group I 15 and group II 25), the change in haematocrit between group I and group II were in the range of 2–10.5% and this was statistically significant.

Table 1. Demographic and clinical data of the two studied groups

Variables	Epidural Anesthesia (n = 78)	General Anesthesia (n = 78)	p value
Age (years)	38.2 ± 4.9	39.4 ± 3.8	0.165
Parity	1 (0–3)	2 (0–5)	0.128
BMI (kg/m^2)	25.2 (19.6–36.7)	25.8 (20.2–38.7)	0.439
Hemoglobin (mg/dL)	9.8 ± 1.6	9.5 ± 1.3	0.722
Indication for surgery • Heavy menstrual bleeding • Abdominal pain • Sub fertility • Mechanical complaints • Others	 29 15 16 13 5	 32 10 17 18 1	0.593
Comorbidity • Hypertension • Diabetes mellitus • Hypercholesterolemia • Hypothyroidism • Cardiovascular accidents	 12 10 15 19 2	 10 7 20 21 1	0.872
Previous abdominal surgery	18 (23)	12 (15.3)	0.285

Qualitative data were described using numbers and percent while normally quantitative data were expressed in Mean ± SD and abnormally distributed data were expressed in median (Min-Max.); * — Significant p value < 0.05; BMI — Body mass index

Table 2. Procedure characteristics of the two studied groups

Variables	Epidural Anesthesia (n = 78)	General Anesthesia (n = 78)	p value
Number of Fibroids • More than 5 • Less than 5	 19 59	 16 62	0.243
Size of Fibroid • Aggregate weight (g) • Largest diameter (cm) • Fibroid volume (cm^3)	 364 (80–877) 16.8 (11.5–20.2) 112 (78–145)	 322 (92–795) 16.1 (10.2–19.4) 96 (71–128)	0.812
Fibroid location • Subserous • Interstitial • Submucous	 15 (19.23%) 34 (43.58%) 29 (37.17%)	 18 (23%) 27 (34.62%) 33 (42.3%)	0.061
Layers of closure • 2 layers • 3 layers	 22 (28.2%) 56 (77.8%)	 27 (34.62%) 51 (65.38%)	0.853
Associate surgery • Ovarian cystectomy • Oophorectomy • Adhesolysis • Endometriosis (excision or ablation)	 6 2 4 5	 8 1 7 7	0.653
Suture type • Vicryl • PDS	 70 8	 70 8	0.402
Uterine cavity opened	18 (23%)	12 (115.3%)	0.054

Qualitative data were described using numbers and percent while normally quantitative data were expressed in Mean ± SD and abnormally distributed data were expressed in median (Min-Max.). * — Significant p value < 0.05

Table 3. Perioperative outcomes of the two studied groups

Variables	Epidural Anesthesia (n = 78)	General Anesthesia (n = 78)	p value
Operative time (min)	101 ±0.693	106 ±1.525	0.167
Estimate blood loss (mL)	745 ± 289	984 ± 345	< 0.001*
Blood products transfusion • RBCs • Fresh Frozen plasma • Platelets	 15 (19.23%) 11 (14.1%) 3 (3.84%)	 25 (32%) 19 (24.35%) 4 (5.12%)	0.008
Need for hysterectomy	2 (2.56%)	4 (5.12%)	0.523
Postoperative Complications • ileus • DVT or PE • Infection or Fever • wound infection	 8 1 5 7	 9 1 7 6	0.204
Hospital stay (Less than 2 days)	46	40	0.004*

Qualitative data were described using numbers and percent while normally quantitative data were expressed in Mean ± SD and abnormally distributed data were expressed in median (Min-Max.); * — Significant p value < 0.05. RBCs — Red Blood Cell; DVT — Deep Venous Thrombosis; PE — Pulmonary Embolism

DISCUSSION

Myomectomy can increase the morbidities and the rate of mortality [15], especially when hysterectomy is performed [16]. Intraoperative complications, such as DIC, fluid overload, ARDS, and hemodynamic disturbances that may necessitate blood transfusion may threaten the patient health [17]. Therefore, anaesthetic management in these patients must be well-planned.

General anaesthesia has been reported to be less effective in reducing blood loss in myomectomy compared with epidural anaesthesia. Our study showed that the mean of intraoperative blood loss, blood transfusion rate and number of transfused blood units were higher (statistically significant) among group II (patients who underwent general anaesthesia) compared to group I (those who underwent epidural anaesthesia). However, change in haematocrit level after myomectomy and blood transfusion rate were more pronounced in the group under general anaesthesia. In this study, the mean blood loss in group under epidural anaesthesia was greater than in previous reports [18–20]. Systematic reviews have shown that these studies had lower amount of blood loss due to decreased uterine size to < than 24 weeks [20], 8.7 ± 4.6 weeks [19] and 15.7 ± 2.6 weeks [18] compared with 18.50 ± 4.72 weeks in this study. Our study showed increase in blood loss during myomectomy because blood loss is proportionate to the size of the uterus. The number of fibroids was associated with increase in blood loss, which explains the lower bleeding amount in the Turkish study with the average number of leiomyomas of 5.5 ± 1 [18] compared with 8.25 ± 3.56 in this study. The calculation of blood loss during abdominal myomectomy remains paramount. The studies in Turkey [18]

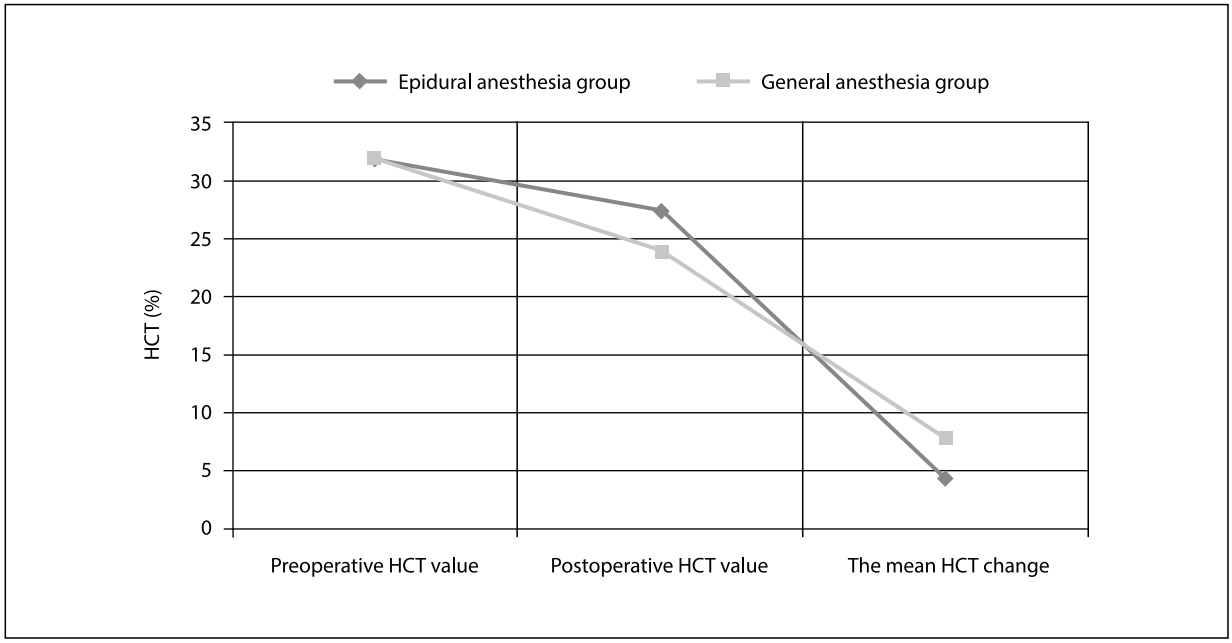

Figure 1. Preoperative, postoperative hematocrit (HCT) level and the mean HCT change in the two groups

and Iran [19] , measured blood loss from the blood in the suction bottle but our study evaluated other losses plus the suction bottle using weight measurement method, this justify the increase in blood loss in this study.

In our study, the intra-operative blood transfusion rate was 19.8%, similar to reports with previous studies 15.3% [18] and 24% [20]; some studies reported no blood transfusion requirement [19]**,** our study has myomas with larger size, uterine sizes more than 24 weeks in some patients and presence of multiple fibroids in many patients.

Maximum blood loss was 2580 mL in patients who underwent general anaesthesia and 1650 mL in patients who underwent epidural anaesthesia. Three or fewer units of RBC transfusion were required only in 11 (14.1%) patients who underwent epidural anaesthesia, while one patient only required more than three units of transfusion. While 13 (16.66%) patients undergoing general anaesthesia required three or fewer RBC transfusion units and 6 (7.7%) required more than three units. Accordingly, transfusion of RBCs and human fibrinogen concentrate were significantly higher ($p < 0.001$) in group II (patients under general anaesthesia) compared to group I (patients under epidural anaesthesia).

On the time, there is no consensus when to start blood transfusion during myomectomy procedure, in our study transfusion was started when the calculated maximum tolerated blood loss or signs of cardiovascular instability from massive bleeding was reached. However, another study used a loss of 2000 ml as an indication to start blood transfusion [19].

The previous studies suggest that epidural anaesthesia may be a better and safer option than general anaesthesia for surgeries with massive bleeding that need to initiate blood transfusion, as inhaled anaesthetics can cause uterine relaxation and thereby increase blood loss and increase the need for blood transfusion [21]. An earlier study reported that regional anaesthesia reduced intraoperative blood loss due to sympathetic blockade [22]. Lilker et al. [23] concluded that most patients undergoing surgeries with massive bleeding could tolerate prolonged surgery and excessive blood loss under epidural anaesthesia.

Only two patients in this study who underwent epidural anaesthesia were switched to general anaesthesia because of the hemodynamic instability caused by severe bleeding. In our study hysterectomy was the main cause of the change to general anaesthesia where hysterectomy was performed in six patients (3.8%), four under general anaesthesia and two starting to have epidural anesthesia but switching to general anaesthesia before hysterectomy.

Several studies [24, 25] have shown that angiogenesis is a prerequisite for tumor growth. VEGF-C is an important factor in the development of angiogenesis, increases mitogenic and vascular permeability activity, and is specific to endothelial cells. Zhang et al. noted that there is a correlation between anaesthesia and serum VEGF-C level. They also suggest in their study that the recurrence rate of myomas and progression of postoperative leiomyomas can be reduced in patients undergoing spinal and epidural anesthesia [26].

The strengths of this study include the prospective nature, randomized design, the detailed registry of surgi-

cal parameters, the accuracy of estimated blood loss and the use of a regression model to account for potential confounding factors related to operative blood loss. A discussion of how epidural anaesthesia affects excessive blood loss may be more clinically useful and further research is needed.

CONCLUSIONS

In women with large fibroid uterus planning for surgical removal, gynecology-anaesthesia team should be careful to choose the best anaesthetic technique, taking into account the location and the size of myoma, expected volume of blood loss, and surgical complications, for patient safety.

Ethical approval

An informed written consent was obtained from the patient for the publication.

Conflict of Interest

The authors declare no conflicts of interest.

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Vulvodynia in prepubertal girls: diagnosis

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ABSTRACT

Objectives: To identify specific features of vulvodynia in prepubertal girls, highlight potential triggers and concomitant diseases, outline diagnostic criteria is neglected problem in adolescent gynecology.

Material and methods: A retrospective study, based on medical records of an outpatient clinic, a cohort of 54 vulvodynia cases was evaluated, aged 3–10 years, seen between January 2016 and July 2018.

Results: The study cohort presented with pain (61%), sometimes aggravated at night, pruritus (44%) and a range of other varied and unusual vulvar complaints (26%). Concomitant diseases and/or psychological problems were present in 61% of cases. Overactive pelvic muscles accompanying symptoms like urological or gastrological problems were noted in half of children. Several potential triggers were identified in a third of the cases that were emotionally stressful to the children. From the commencement of symptoms, 93% of the girls have consulted more than one doctor with 43% seeing more than three doctors, without receiving a diagnosis of vulvodynia.

Conclusions: A diagnosis of vulvodynia needs to be considered in the absence of vulva pathology with wide range of vulvar pain, pruritus and discomfort. All persistent or recurrent vulvar discomfort must be taken into consideration as a vulvodynia symptom, also various non-specific, worrisome complaints. Comorbid urological and gastrological symptoms associated with overactive pelvic muscles should not be overlooked. Chronic pain can be triggered by the psychological distress in some prepubertal girls. Proper diagnosis may prevent long-term negative sequelae, what emphasizes the need for professional education of healthcare providers in adolescent vulvar pain and discomfort.

Key words: prepubertal; vulvar pain; vulvodynia

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INTRODUCTION

Vulvodynia is suspected in adult women when vulvar pain or discomfort persists for more than three months and occurs in the absence of any evident pathology [1]. The same applies in the case of prepubertal girls. Vulvar skin disorders, as an identifiable cause of complaints need to be excluded [1–4]. Any vaginal discharge must be noted. Labial adhesions, when seen in prepubertal girls, are common and asymptomatic [5]. Foreign bodies in the vagina are not felt by the patient, unless they protrude to the vestibule, and these are rarely the cause of vulvar pain, vaginal discharge is present in such cases.

The prevalence of chronic vulvar pain in children is unknown, but in the general population vulvodynia is estimated to occur in 8.3–16% of adult women [6, 7]. In keeping with recent nomenclature [1], vulvodynia in prepubertal girls, can be described as spontaneous or mixed, being exacerbated by vulvar touching (often for hygienic purposes) or pressure, when wearing tight clothes or playing, such as cycling. Unlike in adults, provoked vulvodynia is not as much of an issue in young girls except due to touch [8].

For affected children vulvar pain is mostly generalized. Because young girls are not familiar with their anatomy even localized pain is difficult for them to describe in any detail. Caregivers note that children usually complain of various symptoms but not pain *per se* [4, 8], and this can be misleading in terms of a diagnosis. Onset of pain is usually primary in nature, with potential triggers occasionally identified [3, 8]. However, a constant form of vulvar pain is not typical in prepubertal girls. Children are often too occupied by daily activities to be conscious of persistent pain, hence it presents more as an intermittent or reactive pain.

Diagnosis, medical assessment and treatment of vulvodynia in young girls is comparable to adults [2, 7, 9, 10],

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though some differences need to be highlighted. Persistent vulvar pain or discomfort prevalence in children is unknown, though not rare, when moderate, recurrent rather than continuous complaints are considered. Given that healthcare practitioners are generally not familiar with vulvar pain in women, when pathology does not exist, it becomes an even a more neglected health issue in prepubertal girls.

MATERIAL AND METHODS

The study is based on a retrospective review of medical records from January 2016 to July 2018. A total of 54 cases were identified in the 3–10 years age group. All cases were seen in outpatient center specializing in vulvar diseases, including vulvodynia, but also with experience in child and adolescent gynecology. The study was approved by the appropriate IRB.

Cases were included if they satisfied the diagnostic criteria for vulvodynia, as outlined in the 2015 consensus statement by the International Society for the Study of Vulvovaginal Disease, International Society for the Study of Women's Sexual Health, and International Pelvic Pain Society [1].

The groups characteristics, including list of complains, duration, trigger factors, concomitant diseases and psychological problems, were all extracted from the medical records. Assessment, differential diagnosis and treatment was based on the center's clinical protocol and experience.

Diagnosis of vulvodynia was reached following the previously outlined assessment procedure [10]. Differential diagnosis of vulvar pain was made based on persistent or recurrent clinical symptoms, occurring over a period of more than three months, with no identifiable vulvovaginal pathology of vulvar and perianal skin, mucosa and discharge, upon pinworms exclusion in children.

The specific steps that were followed to reach a diagnosis, including:

1. All reported symptoms were noted in the patient history, as provided by parents and child: previous pathology, past treatments, reports of symptom triggers, past injuries (especially of a musculoskeletal nature), postural issues (postural alignment of hips, extremities and spine), prior surgery, prolonged use of medication, concomitant diseases, hygiene habits (use of herbs, hipbaths, vulvar skin applications, soaps, and other remedies). Also noted were any behavioral and psychological issues.
2. Vulvar and anal examination: specific focus on vulvar and perianal skin, mucosa and discharge (if present) for diagnosis other than vulvodynia. Parents were assured that in prepubertal girls, normal vestibular mucosa is more often red rather than pink, due to lack of estrogens [5]. Vaginal discharge was always noted in the presence of any foreign bodies (toilet paper or toy), these exams were possible when the child was asked to take deep breaths in and out, thus dilating the vaginal opening. Looking for causes of skin irritation, redness, small fissures, blisters like herpes simplex lesions, varicella and other ulcers (aphthous, Behcet or Crohn disease related), where present, these were identified. Signs of genital trauma, abuse [5] or any other injuries were noted. In any chronic vulvar pain cases the anus was also visually examined so as not to overlook possible anal fissure (which commonly appears at the 12 or 6 o'clock position — visualize a pelvic clock as reference). During all physical exams special attention and care was exercised to look for pinworms.

   Cotton swab vulvar pressure as a Q-tip test and pelvic floor exam were not deemed necessary in young children and seem more applicable to exams of postmenarchal girls.
3. Lab tests: a diagnostic swab was taken from the vestibulum when discharge or skin erythema was noted, or reported by the mother, to exclude the presence of specific bacteria. Vulvovaginal yeast infections are rare in prepubertal girls [5]. Pinworms should also be excluded by a tap test. It is recommended that a small piece of transparent adhesive tape be used to take samples from skin around the anus, three days in a row. The tape as a specimen slide was sent to the laboratory to exclude presence of pinworms eggs. From a patient with urinary symptoms, a urine sample needs to be taken for analysis and culture.

Vulvodynia diagnosis is taken on the base of exclusion of any other visible or laboratory detectable pathology as the cause of symptoms.

Statistical analyses were performed using Statistica v. 9.1, provided by Statsoft Polska.

RESULTS

The age range of this study cohort of the 54 cases was 3–10 years of age, with a mean age of 5.8 years, constituting a prepubertal group of vulvodynia cases.

Vulvar pain and discomfort were perceived and experienced in different ways, with only two descriptors per patient being included in the summary data presented in Table 1.

The duration of symptoms was more than three months (up to maximum two years), often reported as remissions and recurrences. In many cases the exact time when the symptoms begun was not clear.

The diagnosis of vulvodynia in this cohort of prepubertal girls was made at the time of the first gynecological consultation at the vulvar specialist clinic. Prior to the diagnosis, 50 out of the 54 cases (93%) consulted other doctors who included general practitioners, pediatricians, urologists, dermatologists and gynecologists. Since the onset of vul-

Table 1. Vulvar complains in vulvodynia prepubertal girls (one or two leading ailments)

Symptom	Number of patients (% of cohort)	Pain characteristics/discription
Pain	34 (61%) patients	• Burning, twitching, soreness, pulling/ drawing, tingling, shooting, stabbing, twinge • Often worse at nighttime
Pruritus	24 (44%) patients	• Touching and scratching • Rubbing
Uncommon vulvar complaints	14 (26%) patients	• "Something irritating" • "Needles inside" • "Hair tickling inside" • "Air bubbles inside my bottom" • "Poking and prodding" • "Something moving in my bottom" • "Wet all the time" especially after voiding, needing lots of toilet paper to keep wiping • "My underwear is always wedging between buttock cheeks"

vodynia symptoms 23 girls (43%) had been seen by three or more healthcare practitioners. Due to recurrent bladder symptoms four of the children (7%) were hospitalized. None of the 54 cases had received a diagnosis of vulvodynia prior to attending the clinic.

Concomitant diseases and psychological or emotional problems were noted in 33 cases (61%), presenting with one or more associated comorbidities. Symptoms and comorbidities were divided into 4 groupings:

1. Symptoms associated with overactive pelvic muscles in 27 cases (50%) which included:
   a) Urological symptoms in 18 cases (33%): with dysuria, frequency and urge, sometimes associated with incontinence (consistent with Overactive Bladder). This was often highlighted with accounts such as "she is so occupied by her play, that she keeps her bladder full and finally is dripping on the way to the toilet", or urinary tract infection symptoms (mostly without bacteriuria), difficulty initiating voiding and nocturnal enuresis.
   b) Gastrological symptoms in 17 cases (31%): with abdominal pain, irritable bowel syndrome, constipations, diarrhea, bowel incontinence (especially when not passing stool for a long time) and anal fissure.
2. Musculo-skeletal issues in five cases (9%): presenting with postural defects and abdominal scar.
3. Other general variables in five cases (9%): with allergies, recurrent respiratory tract infections, and headache.
4. Psychological and emotional factors in 11 girls (20%): including previous participation in psychotherapy, distress, anxiety, vulnerability, tics and childhood masturbation.

Some potential stressful triggers identified by parents in 18 cases (33%) included: arrival of a newborn sibling, mother returning to work, a new babysitter, commencing kindergarten or school, and definite family problems.

DISCUSSION

Vulvodynia in prepubertal girls is a neglected healthcare problem. Comprehensive assessment of these condition is difficult, and diagnosis is often based on reported symptoms and professional experience. Among the wide spectrum of vulvar symptoms, persistent pain characterizes almost two thirds of all vulvodynia cases. Rating and character of pain was different, although sometimes aggravated at night with crying and waking up, what was the most distressing for the child and family. Almost half of vulvodynia girls seen complained of vulvar itch, and in these cases, it was essential that the presence of pinworms be excluded. A diagnosis of vulvodynia needs to be considered in the absence of vulvar pathology, although is often considered as vulvovaginal infection. The most symptomatic yeast vulvovaginal infections are seldom in children.

All persistent or recurrent vulvar discomfort must be taken into consideration as a vulvodynia symptom, also when children attempt to describe various non-specific, worrisome complaints (which was the case in every fourth of the affected girls), these included continuous wetness after voiding or bubbling-like sensations [11]. Vulvar pressure avoidance, pain on touching for hygienic purposes in children, this was considered as the equivalent of adult provoked vulvodynia symptoms [3, 4, 8, 11]. The management of this disorder requires a highly skilled approach [4, 8, 11, 12].

The 2015 consensus terminology and classification statement noted comorbid pain syndromes as potential factors associated with vulvodynia [1]. Sixty one percent of prepubertal vulvodynia cases in this study presented with at least one comorbidity (physical or emotional).

Vulvodynia is associated with pelvic diaphragm dysfunction and should be considered as the part of regional anourogenital pain syndrome [13]. Concomitant complains of pelvic floor were seen in half of the prepubertal study population. Dysuria was noted in a third of the vulvodynia girls, and anal and bowel problems also presented in a third of study cohort. Urinary symptoms are the most reported associated symptoms [11, 13, 14]. On the basis of literature, dysuria, urinary frequency, incontinence (due to urge) were present in 80.6% of premenarchal and in 62.5% of postmenarchal adolescents with vulvodynia [11]. The incidences of urological symptoms were less often observed in this study consisting of 3–10 years old children.

Pelvic floor dysfunction can be the consequence of other fascial and musculoskeletal defects in distant parts of the body in the case of vulvodynia. Abdominal scar tissue in early childhood is considered as a risk factor for chronic functional pain for reminder of life [15].

The cotton swab (Q-tip) test, first proposed by Friedrich in 1987, for the assessment of Vulvar Vestibulitis Syndrome [16] forms an important part of the diagnostic criteria for vulvodynia in adults. It consists of gently touching the vestibular hymen in approximately five points to identify areas of pain in vulvodynia patients. However, guided by the "First Do No Harm" principle [4], it is the view of the authors that it is not necessary to carry out a cotton swab vulvar touching test in the assessment of prepubertal girls. Other authors describe the procedure as useful in adolescences [3, 4, 8, 11, 14], but it may not be as appropriate for children. Children (and parents) are afraid of contact being made with the vulva, and it is difficult to draw information from little girls about sensitive areas that are painful. Based on the same principle, rectal pelvic floor muscles exams are not routinely performed. Diagnosis of vulvodynia is based on a clinical review and does not require a diagnostic biopsy [10].

Psychological predisposition and emotional states such as anxiety, distress and depression are all considered as contributor to functional chronic pain and vulvodynia [3, 4, 8, 12, 17]. This was shown to be the case in one fifth of the study cohort. The role of potential stressors needs to be assessed at an individual level. Early vulvodynia symptoms may suggest sexual abuse in the etiology of vulvar pain. Based on detailed history taking, external gynecological examination, abuse and violence must be excluded. In the literature no link has been established between physical or sexual abuse in the etiology of vulvodynia, though neglect, childhood chronic stress has been identified as predisposing factors [11, 18].

Some trigger factors such as life events that are noted in vulvodynia patients, may also be pertinent in children and adolescents [3, 8]: sexual and nonsexual physical contact, vaginal or vulvar infection, vulvar irritants in the form of herbal soap, wearing inappropriate cloths and use of antibiotics. Study authors consider several potential triggers that were evident in a third of the vulvodynia cases, that were clearly emotionally stressful events to the lives of children. Psychological distress plays crucial role in worsening of discomfort in chronic pain patients [12]. This includes numerous unnecessary consultations and medical procedures, in every age, prior to a diagnosis being established [9, 11, 12]. In the study cohort, from commencement of symptoms, almost all children have consulted at least one specialist and almost half seeing more than three doctors, without their vulvodynia being diagnosed. Chronic vulvar pain patients also seek medical consultations on account of concomitant diseases and/or psychological problems, often seen in prepubertal vulvodynia girls, highlighting the need for a holistic approach.

Vulvodynia diagnosis should be communicated to patients and their parents, explanation the disease and education is crucial [4, 9, 11]. Early diagnosis means commencement to treatment, proper educational, psychological stress reduction, physiotherapeutic intervention may prevent long-term negative physical and psychological sequelae [4], what emphasizes the need for professional education of healthcare providers in adolescent vulvar pain and discomfort.

Education on vulvar hygiene is essential for both children and parents, making them aware of possible allergens and irritants, and hence the recommendation for the use of natural emollients (such as coconut oil) is useful [4, 5, 11]. Vulvar pressure by some inappropriate cloths should be avoided, according to children's preferences.

Topical lidocaine treatment can be recommended in emergency situations, not as a long-term management [9].

According to literature, general pharmacological treatment in adolescent with severe vulvar pain, if administered with caution, may facilitate progress. In the case of adolescents, short-term therapy with tricyclic antidepressants (TCAs) can be used with persistent vulvar symptoms [3, 8, 11, 14], though more recent reviews do not recommend TCAs in vulvodynia [4, 9]. Gabapentin has also been suggested, should be used with caution [3, 4]. Serotonin reuptake inhibitors (SSRIs), and rather serotonin and norepinephrine reuptake inhibitors (SNRI), are a preferred option for adolescents [3]. Duloxetine (SNRI) and pregabalin (anticonvulsant) may be advocated in adult cases, to decrease central and peripheral sensitization. With duloxetine there are no safety concerns in adolescents and children older than seven years for depressive disorders is accepted [19]. Pregabalin may be recommended in children, shown to be as safe and well tolerated in cases of epilepsy [20]. Any surgical vestibular approach (including partial hymenectomy) must be avoided in children.

Psychologic distress plays crucial role in worsening of discomfort in chronic pain patients. This includes numerous unnecessary consultations and medical procedures, in every age, prior to a diagnosis being established [9, 11, 12]. In the study cohort, from commencement of symptoms, 93% of children have consulted at least one specialist and 43% seeing more than three doctors, without their vulvodynia being diagnosed. Chronic vulvar pain patients also seek medical consultations on account of concomitant diseases and/or psychological problems (seen in 61% of prepubertal vulvodynia girls), highlighting the need for a holistic approach. Early diagnosis and proper intervention may prevent long-term negative physical and psychological sequelae [4].

Vulvar recurrence and chronic complaints in children are rare in the area of research and scientific publication. Thisstudy addresses many aspects of the problem, comprehensive diagnostic management is outlined, although study population is too small to elucidate various statistical relationships.

CONCLUSIONS

A diagnosis of vulvodynia needs to be considered in the absence of vulva pathology with wide range of vulvar discomfort. Pain characterizes almost two thirds and while half of all vulvodynia cases complained of vulvar itch. Pinworms and vulvovaginal infection should be excluded, although yeast infection is seldom in children. All persistent or recurrent vulvar discomfort must be taken into consideration as a vulvodynia symptom, also when children attempt to describe various non-specific, worrisome complaints. Comorbid urological and gastrological symptoms (in half of vulvodynia patients) associated with overactive pelvic muscles should not be overlooked. Chronic pain can be triggered by the psychological distress in some prepubertal girls [11, 18]. Proper diagnosis may prevent long-term negative sequelae [4], what emphasizes the need for professional education of healthcare providers in adolescent vulvar pain and discomfort.

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Conflict of interest

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A survey of knowledge, attitudes and awareness of the HPV and HPV vaccine among obstetricians and gynecologists across Poland

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ABSTRACT

Objectives: The objective of this study was to assess general knowledge regarding human papillomavirus (HPV) and the attitude to primary prevention in form of HPV vaccination (HPVv) among Polish obstetricians and gynecologists (OBGYNs). In addition, we wanted to study the willingness of physicians to promote the HPVv among patients, based on their general attitude to vaccinations as well as HPV-related knowledge. The gynecologists were also asked to assess their patients' awareness of HPV infection.

Material and methods: A questionnaire consisting of 25 questions was used to collect the data and with support of the Polish Society of Gynecologists and Obstetricians (PTGiP) and the Polish Society of Colposcopy and Cervical Pathology (PTKiPSM) sent via their mailing lists to all members and beyond. The total amount of 213 fully filled questionnaires were gathered and analyzed using descriptive statistics.

Results: Most of the surveyed OBGYNs showed a good knowledge of HPV and HPVv. They were able to correctly identify the high-risk oncogenic HPV types (hrHPV) and admitted to using HPV genotyping in their daily practice and actively promoting HPVv, being in majority supporters of mandatory vaccinations in general. Almost 90% confirmed the importance of informing patients about sexually transmitted diseases (STDs). On the other hand, there was a group of OBGYNs with clearly insufficient knowledge about the HPV and its prevention.

Conclusions: General knowledge of Polish physicians about HPV is good, independent of gender and age. The acceptance of all vaccines is high, but the low availability of the HPV vaccines seems to be the biggest problem stopping patients from getting them.

Key words: HPV; human papillomavirus virus; HPV vaccine; human papillomavirus vaccine

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INTRODUCTION

Human papillomavirus (HPV) is the most common viral infection of the reproductive tract, which causes different conditions, including precancerous lesions with potential progress to cancer. Due to transmission by skin-to-skin contact, the prevalence is high but most of the infections do not cause any symptoms and resolve spontaneously. In women, persistent infection with high-risk HPV (hrHPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) may lead to high-grade squamous intraepithelial lesions (HSIL) and, if untreated, to cervical cancer. The most popular oncogenic HPV types are 16 and 18 — they are responsible for 71% of cases of cervical cancer. Almost 90% of all squamous-cell carcinomas of the cervix are positive for hrHPV DNA (16, 18, 33, 45, 58) [1, 2]. According to European Cancer Information System provided by European Commission, for women in the European Union aged 15–44 cervical cancer is the second most common type of cancer after breast cancer [3]. Based on mentioned data, implementation of primary prevention is necessary. Primary prevention in form of HPVv is already available and aims to prevent the spread of HPV in many countries. Its acceptance varies from country to country, largely dependent on the state of knowledge about diseases caused by HPV as well as cultural, social, and religious factors. As of year 2021, 40 out of 53 World Health Organization's Europe Region countries (WHO/ER)[1] have

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founded HPVv national immunization programs, Poland not being one of them yet.

A primary prevention of HPV related disease is available from year 2006, when the quadrivalent vaccine was first licensed, followed by the bivalent vaccine in 2007 and the nonavalent vaccine in 2014. Fully or partially funded HPVv was provided for girls in 25/53 (47%) WHO/ER countries and for both boys and girls in 15/53 (28%) countries [4]. All three available vaccines prevent infections with HPV-type 16 and 18, the quadrivalent covers also type 6 and 11, the nonavalent extends additional protection against type 31, 33, 45, 52, 58. The vaccines can be administered in males and females from the age of nine years to protect against conditions caused by specified types of HPV: cancer of the cervix or anus, precancerous lesions in the genital area (cervix, vulva, vagina or anus) as well as genital warts.

Objectives

The aim of this study was to assess the general attitude of Polish OBGYNs towards vaccination programs, their knowledge regarding HPV, cervical cancer prevention and HPVv, and its implementation into everyday medical practice. The doctors were also asked to assess their patients´ knowledge about HPV. In Poland a free HPVv is founded by some of the local governments and even then, only for girls and within a very strict age limit. Most of the patients must pay out-of-pocket to receive the immunization in the private sector. It was expected that the results of the study could contribute to health policy in terms of health manpower development for vaccine introduction to the public in the future. According to the Ministry of Health, the National Oncologic Strategy (NOS) claims starting of girls' public vaccination in Poland at the turn of the year 2021/2022.

MATERIAL AND METHODS

An anonymous cross-sectional online survey was conducted between January and May 2021. The study was designed to obtain information about the knowledge of, attitudes toward HPV infection and vaccinations and its possible correlations among Polish OBGYNs. The Polish Society of Gynecologists and Obstetricians (PTGiP) and Polish Society of Colposcopy and Cervical Pathology (PTKiPSM) supported the survey by disseminating the link for the inquiry via their mailing list to all members and beyond. A goal of the study was to include as many participants as possible. At the initial stage, no formal calculation of sample size was carried out. One reminder was sent out after two months.

The questionnaire survey (Appendix A,) was designed by the authors and consisted of 25 questions exploring following categories: demographics (age, gender, type and period of medical practice, average number of patients admitted weekly), general opinion on preventive vaccinations, basic HPV knowledge, attitude towards the HPVv and other vaccines. The questionnaire was preceded by preliminary information which consisted of an explanation of the purpose of the study and details on how to contact the authors. The participants were informed that the survey was part of a scientific study. Participation in the inquiry was anonymous and voluntary. The form did not allow participants to continue, unless they had answered all the questions. Part of the questions allowed multiple answers, whereas in some questions there was only one answer possible. A few questions had the option to add a free text answer if desired. Questions were designed according to similar literature already published.

The analysis was conducted mainly with the use of descriptive statistics. The results are presented in the form of frequency tables and cross tables. Attitude assessment was categorized on a VAS 10-point scale. Level of attitude was grouped into three classes: 1–6, 7–8 and 9–10.

RESULTS

Sociodemographic background characteristics of the participants

The total amount of 213 fully filled questionnaires were gathered. According to the current data provided by the Supreme Medical Council (*Naczelna Izba Lekarska*), there were 6.698 active OBGYN-specialists registered in Poland but due to the opportunistic nature of participant recruitment, a response rate could not be calculated. Most of the completed surveys (n = 121; 56.8%) were done by women. The group of OBGYNs 30–40 years of age was the main one (100, 46.9%), while physicians < 30 years of age accounted for only 2.8% (n = 6). The majority indicated town with over 500,000 inhabitants as their place of residence (74.6%; n = 159). Inhabitants of villages accounted for 4.2% (n = 9). Almost all professionals (n = 192; 90.1%) mentioned private practice as their place of employment. For most of them, it was the only place of employment - these doctors constituted 23.9% of the study group (n = 51). Others combined employment in a private practice with employment in a hospital or out-patient department. From the 213 OBGYNs who completed the survey, the clinical experience ranged from less than 5 years (n = 23; 10.8%) to more than 20 years

1 Albania, Andorra, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Republic of Moldova, Romania, Russian Federation, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkey, Turkmenistan, Ukraine, United Kingdom of Great Britain and Northern Ireland, Uzbekistan

Table 1. Baseline characteristics of surveyed Polish OBGYNs (n = 213)

Variable	n (%)
Age	
< 30 years	6 (2.8%)
30–40 years	100 (46.9%)
41–50 years	28 (13.1%)
51–60 years	41 (19.2%)
> 60 years	38 (18.0%)
Gender	
Female	121 (56.8%)
Male	92 (43.2%)
Place of practice (multiple options can be selected)	
City > 100,000 residents	159 (74.6%)
City < 100,000 residents	62 (29.1%)
Village	9 (4.2%)
Type of practice (multiple options can be selected)	
Private practice	192 (90.1%)
National healthcare practice (office)	136 (63.8%)
National healthcare practice (hospital)	81 (38.0%)
The period of practicing in gynecological and obstetric care	
< 5 years	23 (10.8%)
5–10 years	63 (29.6%)
11–20 years	42 (19.7%)
> 20 years	85 (39.9%)
Number of patients per week	
< 30	40 (18.8%)
30–60	108 (50.7%)
61–90	32 (15.0%)
> 90	33 (15.5%)

(n = 85; 39.9%). 50.7% (n = 108) of respondents reported seeing 30–60 patients per week, 18.8% (n = 40) saw < 30 patients per week and 15.5% (n = 33) saw > 90 patients per week. An overview of demographic data is given in Table 1.

The state of doctors' and patients' knowledge about HPV and vaccination

Majority of respondents (n = 162, 76.1%) assessed the patients' knowledge about HPV and HPVv as insufficient. 77.9% (n = 166) of health care professionals correctly identified type 16 and 18 as highly oncogenic. 51.2% (n = 109) properly knew all three types (bi-, quadri- and nonavalent) of HPV vaccine. Of all participants, 89.2% (n = 190) admitted to perform HPV genotyping.

Doctors' attitudes towards vaccination

More than 90% of OBGYNs carried out the conversation about STDs with patients and 96.2% (n = 205) presented the possibility of HPVv. In total, 84.5% (n = 180) were supporters of mandatory vaccinations in general, while 99.5% (n = 212) considered the HPVv as important. However, only 79.3% (n = 169) rated their confidence in the HPVv at 9 or more on the VAS scale. In terms of safety and effectiveness, 83.6% (n = 178) and 80.8% (n = 172) respondents marked a value of 9–10 on the VAS scale, respectively. The lower and upper age limit for the HPV vaccination considered by the respondents varied (Tab. 2). Most surveyed physicians saw the lower age limit for HPVv between 9–12 years of age (n = 132, 62%), followed by the age 13–16 (n = 49, 23%). The vaccine was being offered to older patients as well: 46.9% (n = 100) of surveyed OBGYNs did not see any age limit stopping them from presenting their patients with the possibility of HPVv or the limit was above 50 years. On the other hand, 105 of the respondents (n = 105) advised only women younger than to 50 to get the vaccine. The survey showed that 90.1% (n = 192) of participants offered the HPVv to patients that have already started sexual intercourse. However, only 77.5% (n = 165) presented such possibility to patients that have undergone cervical ablation or surgery. Of all 213 OBGYNs, 152 (71.4%) used a 3-dose HPVv schedule.

Doctors' opinions towards HPVv

As many as 151 respondents (70.9%) considered the availability of HPV vaccine in Poland as very limited. 77% (n = 164) believed, that HPV vaccinations should be mandatory and reimbursed for both girls and boys. Almost all surveyed physicians (n = 207, 97.2%) did not agree with the statement that HPVv favors early sexual initiation.

DISCUSSION

The knowledge about high-risk oncogenic HPV types is obligatory to every OBGYN: from all 14 hrHPV types, 5 alone (16, 18, 33, 45, 58) account for approximately 90% of the squamous cell carcinomas of the cervix which are positive for HPV DNA [1, 2]. The survey's findings revealed that the awareness on HPV subtypes of Polish professionals were at a high level but still with a need for further education. Very high number of respondents recognized the hrHPV types 16 and 18 correctly (98.1% and 98.6% respectively). Still, it is alarming that 33.3% saw type 6, 11, 42, 43 as high-oncogenic. Low-risk HPV strains (lrHPV), such as HPV 6 and 11, as well as 42 and 43, cause over 90% of genital warts, which rarely develop into cancer [5]. Compared to similar studies, the OBGYNs in the present study showed slightly higher knowledge about hrHPV: 84.1% of family physicians and 45% of pediatricians in the USA did [6, 7]. A study conducted among Polish doctors during residency in pediatrics, gynecology and obstetrics, and dermatology and venereology showed that their knowledge about HPV was low, independent of sex, age, and specialization [8].

The first HPVv was licensed in year 2006 and since then there are three highly efficacious vaccines available in the market: bivalent, quadrivalent and nonavalent. The nonavalent vaccine turned out to be the most popular among Polish gynecologists, with 91.1% respondents recognizing it, followed by quadrivalent (73.2%) and bivalent (65.3%). The nonavalent and quadrivalent vaccines offer similar protection

Table 2. Results of the HPV survey conducted among Polish OBGYNs (n = 213)

Question	n (%)
What is your overall attitude to mandatory vaccinations?	
I am a supporter of mandatory vaccinations	180 (84.5%)
I am a supporter of vaccinations, but I believe that it should not be mandatory	33 (15.5%)
How do you rate the mandatory vaccinations in terms of SAFETY on a scale from 1 to 10?	
9–10	178 (83.6%)
7–8	34 (16.0%)
< 7	1 (0.4%)
How do you rate the mandatory vaccinations in terms of EFFECTIVENESS on a scale from 1 to 10?	
9–10	172 (80.8%)
7–8	40 (18.8%)
< 7	1 (0.4%)
Which of the following HPV genotypes are considered highly oncogenic? (Multiple options can be selected)	
16	209 (98.1%)
18	210 (98.6%)
6	11 (5.2%)
11	15 (7.0%)
42	17 (8.0%)
43	28 (13.1%)
Do you order genotyping for HPV types 16 and 18?	
Yes	190 (89.2%)
No	23 (10.8%)
What types of HPV vaccines do you know? (open question)	
Bivalent	139 (65.3%)
Quadrivalent	156 (73.2%)
Nonvalent	194 (91.1%)
I don't know any	6 (2.8%)
Do you consider HPV vaccination as important?	
Yes	212 (99.5%)
No	1 (0.5%)
Do you carry out the conversation with patients about sexually transmitted diseases?	
Yes	200 (93.9%)
No	13 (6.1%)
Do you carry out the conversation with patients about the possibility of HPV vaccination?	
Yes	205 (96.2%)
No	8 (3.8%)
What is the lower age limit when you present a patient with the possibility of HPV vaccination? (open question)	
< 9	20 (9.3%)
9–12	132 (62.0%)
13–16	49 (23.0%)
> 16	11 (5.2%)
I do not offer my patients this vaccination	1 (0.5%)

Table 2 (cd.). Results of the HPV survey conducted among Polish OBGYNs (n = 213)

Question	n (%)
What is the upper age limit when you present a patient with the possibility of HPV vaccination? (open question)	
< 20	4 (1.9%)
20–30	29 (13.6%)
31–40	33 (15.4%)
41–50	39 (18.3%)
> 50 and/or without age limit	100 (46.9%)
Other:	
Until menopause	4 (1.9%)
Until first sexual intercourse	3 (1.4%)
I do not offer my patients this vaccination	1 (0.5%)
Do you propose HPV vaccination to patients who already had first sexual intercourse?	
Yes	192 (90.1%)
No	21 (9.9%)
Do you propose HPV vaccination to patients who have undergone cervical ablation or surgery?	
Yes	165 (77.5%)
No	48 (22.5%)
How do you assess your patients' knowledge of HPV and HPV vaccination?	
Satisfactory	7 (3.3%)
Acceptable	38 (17.8%)
Insufficient	162 (76.1%)
I have no opinion	6 (2.8%)
What primary dosing schedule of the HPV vaccination do you use?	
1-dose	6 (2.8%)
2-dose	55 (25.8%)
3-dose	152 (71.4%)
How do you assess the availability of the HPV vaccine in Poland?	
All vaccine variants are available (2-, 4-, and 9-valent)	26 (12.2%)
Only 2-valent and 4-valent vaccine variants are available	21 (9.9%)
The HPV vaccine is very difficult to obtain	151 (70.9%)
I have no opinion	15 (7.0%)
In your opinion, should HPV vaccination be mandatory and reimbursed?	
Yes, for girls and boys	164 (77.0%)
Yes, but only for girls	37 (17.4%)
No	10 (4.7%)
I have no opinion	2 (0.9%)
Do you think that HPV vaccination favors early sexual initiation?	
Yes	6 (2.8%)
No	207 (97.2%)
How do you rate your confidence in the HPV vaccination on a scale of 1 to 10?	
9–10	169 (79.3%)
8–7	32 (15.0%)
< 7	12 (5.6%)

against a combined outcome of cervical, vaginal, and vulval precancer lesions or cancer [9]. Alarmingly, almost 3% admitted to not knowing any of the above. Among doctors who actively use the vaccines, most of them (71.4%) preferred the 3-doses regime, followed by 25.8% who administered two doses. According to Centers for Disease Control and Prevention and based on the available immunogenicity evidence, a 2-dose schedule (0, 6–12 months) had efficacy equivalent to a 3-dose schedule (0, 1–2, 6 months) if the HPVv series was initiated before the 15th birthday [10]. It is interesting that

there were physicians who admitted in the survey to giving only one dose. Long-term observational studies are needed to determine the effectiveness of reduced-dose schedules against HPV-related cancer endpoints, and whether adopting these schedules improves vaccine coverage rates [9].

To successfully implement new recommendations and methods into everyday practice it is important to not only possess the necessary knowledge but also to use it in everyday practice. Health care workers are the real faces of any immunization systems and their knowledge and skills are crucial to the success of immunization programs [2]. According to a 2020 survey of gynecologists' behaviors and attitudes, 91% of respondents believed co-testing (HPV and liquid based cytology) was valuable for their patients' health and more than 8 in 10 were likely to use co-testing for screening [11]. Among Polish gynecologists, 89.2% recommended HPV-testing but still almost 11% did not, even though HPV-based screening is recommended by The Polish Society of Gynecologists and Obstetricians (PTGiP) and Polish Society of Colposcopy and Cervical Pathology (PTKiPSM) [12]. Research on knowledge, attitudes, and beliefs among Canadian physicians reported that 75% obstetricians--gynecologists (n = 395), 60% family physicians (n = 408), and 48% pediatricians (n = 461) were aware that persistent HPV is a necessary cause of cervical cancer [13, 14].

Healthcare providers play the key role in influencing parents' decision making to allow their children to receive HPVv [15]. There is a continued need to increase parental knowledge about the HPVv to close the gap on vaccine nonadherence [16]. According to previous studies, patients' knowledge about HPV remained low and was influenced by gender, education, income, race, and other sociodemographic characteristics [17, 18]. Over 76.1% of surveyed OBGYNs thought that their patients did not know enough about problems connected with HPV infection. Only 21.1% of patients were estimated to have a satisfactory knowledge about HPV. This topic certainly needs more attention and further studies should follow in order to assess the real dimensions of patients' knowledge and attitude toward this STD. On the other hand, 93.9% of surveyed gynecologists claimed to talk with their patients about STDs, with only 6.1% refraining from it. At this point it has to be reminded that inadequacies in physician knowledge may serve as a barrier to the appropriate diagnosis and treatment of STDs [19].

According to WHO there are six pillars of a strong immunization program, among them the most important two: reaching every person and staff training [20]. Therefore, the acceptance for any vaccine is related to various factors for decision making in vaccination of the population, the acceptance by healthcare providers being one of them [21]. Among Polish gynecologists most of the respondents were in favor of mandatory vaccinations provided by the National Health Care Provider and implemented in national vaccination program (84.5%). The minority found them necessary but not mandatory (15.5%). Those results show a very positive attitude of OBGYNs towards immunization programs and correspond with other studies where a large majority were in favor of the mandatory vaccination law (91%) [22]. The safety of mandatory vaccinations was rated very high, with 83.6% of gynecologists giving the highest marks (9–10/10), 16% finding them safe (7–8/10) and only 0.4% grading them lower (< 7/10). The respondents assessed the effectiveness of mandatory vaccines as high, almost 81% of them gave maximal scores.

Acceptability of HPVv has been studied worldwide. Previous studies demonstrated that HPVvs acceptability was generally positive among healthcare providers. Physicians were expected to have good acceptance and positive attitude toward HPVv. They were also expected to have good knowledge on HPV infection and its relation to cervical cancer. Moreover, healthcare providers were considered the primary and most trusted source of health and vaccine information for the public. According to available data, rumors about vaccine safety had been one of the principal obstacles for the acceptance of HPV vaccination by the public [23]. Therefore, it is positive that the HPVv is very important for Polish gynecologists: 99.5% found this vaccination relevant and almost 94% claimed to talk with their patients about the possibility of immunization. They also showed high trust in the effectiveness of the HPVv: almost 80% respondents agreed with its potential.

Available HPVv can be used in males and females from the age of nine years but most of international vaccination programs start with children at the age of 12 [24]. The questionnaire's results showed that in Poland most of the gynecologists advise to perform the vaccination by girls at the age from 9 to 12 (62%), followed by female teenagers at the age from 13 to 16 (23%). Surprisingly, there are physicians who advise to get the vaccine under 9 years (9.3%), which is not allowed by the European Medicines Agency (EMA) or over 16 years (5,2%). An annual, web-based survey of American healthcare professionals including physicians and nurse practitioners from year 2012 showed that only 14.5% of providers recommended the vaccine to all age-eligible females and 20,2% recommended it to females aged 11–26 years, more frequently to girls older than 11––12 years and another study brought similar results, where fewer physicians strongly recommended HPV vaccination for 11- to 12-year-old female patients than for older female patients [25, 26]. A big part of the respondents did not find any age limit at which the vaccine is not advisable — almost 47% gynecologists would advise to vaccine women over 50 years old and/or without any age limit. It is indeed

possible that some individuals over the age of 50 may also benefit from vaccination, but the benefit has not been well researched yet. According to a study, which has been published in Lancet in 2016, in women older than 25 years, the HPV 16/18 vaccine continues to protect against infections, cytological abnormalities, and lesions associated with HPV 16/18 and cervical intraepithelial lesions CIN1+ irrespective of HPV type, and infection with non-vaccine types HPV 31 and HPV 45 over seven years of follow-up. Part of enrolled participants had a history of HPV infection or disease [27]. It was interesting to find out that over 90% of surveyed doctors would advise a patient who already hat sexual initiation to get the vaccine but only 77.5% offered to vaccine a patient after ablative or surgical procedure at the cervix. From August 2019 there is an ongoing randomized, double blinded, placebo controlled Dutch trial with primary objective of the efficacy of nonavalent HPV vaccination in women with HSIL who underwent a loop electrosurgical excision procedure (LEEP) in preventing recurrent HSIL after at 24 months follow-up. The recruitment is planned to end in August 2022, with results to follow [28]. The current knowledge indicates that patients having vaccination after LEEP experience a slightly lower risk of recurrence than women who had not, although not statistically significantly different.

It is reassuring that over 97% respondents did not associate HPVv with earlier age at the first intercourse and that 77% surveyed gynecologists supported mandatory and refundable vaccination for both girls and boys. On the other hand, a little over 17% found immunization of only women necessary and almost 5% did not think these vaccines should me mandatory and implemented in the national vaccine program. In comparison, in a recent study almost 36% of British healthcare professionals admitted to not being adequately informed about HPV-related topics and having interest in more frequent training [29]. The above results show the importance of continuous education among physicians in order to close the knowledge gap.

Still, the biggest problem in Poland seemed to be the lacking availability of the vaccine with over 70% of the respondents finding the availability of any HPVv low. The reasons are related to the rapid growth in this vaccination worldwide and the increasing number of national vaccination programs including boys. The production of the vaccines in question takes two years, moreover the responsible pharmaceutical companies due to low production capacities are not able to meet all demand and thus HPVv are supplied first to markets that already have HPV national vaccination programs. According to the Polish Ministry of Health, the return of full availability of the HPVv is expected at the turn of the year 2021/2022. A national vaccination program is to follow.

CONCLUSIONS

The findings of this study suggested that the knowledge of Polish health care providers was sufficient; however, their awareness should be improved in terms of basic knowledge of HPV, cervical screening, and the efficacy of the vaccine. Overall, the OBGYN's attitude was positive to HPVv and the acceptance of HPVv was high. Most of them had good attitude about the severity of HPV-related diseases and the benefit of HPVv. The barrier that might influence vaccination achievement was the lack of accessibility of the vaccine. As a result, health policymakers should consider appropriate training programs for healthcare providers to gain more knowledge and improve their attitude. Those programs could potentially increase the acceptance for HPVv among gynecologists in the future and thereby also increase patient awareness of the risks of HPV infection and the benefits of available primary prevention.

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Conflict of interest

No conflict of interest was declared by the authors.

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APPENDIX A

The questionnaire for physicians concerning vaccination against HPV

Dear Sir or Madam,

Under the scientific supervision of professor Robert Jach, we conduct a questionnaire study at the Clinical Department of Gynecological Endocrinology and Gynecology of the University Hospital in Krakow, which is aimed at understanding the approach of physicians to vaccination against HPV.

The conclusions drawn from the data collected thanks to Your kindness will allow us to analyze the knowledge, acceptance and implementation of primary prevention of cervical cancer in daily gynecological practice.

The questionnaire consists of 25 questions and takes approximately 5 minutes to complete.

Please send any questions and additional comments to the e-mail address: rabaran@su.krakow.pl

*Required

Demographic data

1. Gender*
   - ❑ female
   - ❑ male
2. Age*
   - ❑ < 30 years
   - ❑ 30–40 years
   - ❑ 41–50 years
   - ❑ 51–60 years
   - ❑ > 60 years
3. Place of practice (multiple options can be selected)*
   - ❑ city > 100,000 residents
   - ❑ city < 100,000 residents
   - ❑ village
4. Type of practice (multiple options can be selected)*
   - ❑ private practice
   - ❑ national healthcare practice (office)
   - ❑ national healthcare practice (hospital)
5. The period of practicing in gynecological and obstetric care*
   - ❑ < 5 years
   - ❑ 5–10 years
   - ❑ 11–20 years
   - ❑ > 20 years
6. How many patients do you admit on average per week? (please provide number)*

   .....

Opinion on preventive vaccinations

7. What is your overall attitude to mandatory vaccinations?*
   - ❑ I am a supporter of mandatory vaccinations
   - ❑ I am a supporter of vaccinations, but I believe that it should not be mandatory
   - ❑ I am opposed to vaccinations
   - ❑ I have no opinion
8. How do you rate the mandatory vaccinations in terms of SAFETY on a scale from 1 to 10?*

1	2	3	4	5	6	7	8	9	10
❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

9. How do you rate the mandatory vaccinations in terms of EFFECTIVENESS on a scale from 1 to 10?*

1	2	3	4	5	6	7	8	9	10
❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Knowledge about HPV

10. Which of the following HPV genotypes are considered highly oncogenic? (multiple options can be selected)*
    - ❑ 6
    - ❑ 16
    - ❑ 42
    - ❑ 11
    - ❑ 18
    - ❑ 43
11. Do you order genotyping for HPV types 16 and 18?*
    - ❑ Yes
    - ❑ No
12. What types of HPV vaccines do you know?*

    .....

Practice

13. Do you consider HPV vaccination as important?*
    - ❑ Yes
    - ❑ No
14. Do you carry out the conversation with patients about sexually transmitted diseases?*
    - ❑ Yes
    - ❑ No
15. Do you carry out the conversation with patients about the possibility of HPV vaccination?*
    - ❑ Yes
    - ❑ No
16. What is the lower age limit when you present a patient with the possibility of HPV vaccination?*

    .....

17. What is the upper age limit when you present a patient with the possibility of HPV vaccination?*
    .....
18. Do you propose HPV vaccination to patients who have already started sexual intercourse?*
    - ❑ Yes
    - ❑ No
19. Do you propose HPV vaccination to patients who have undergone cervical ablation or surgery?*
    - ❑ Yes
    - ❑ No
20. How do you assess your patients' knowledge of HPV and HPV vaccination?*
    - ❑ Satisfactory
    - ❑ Acceptable
    - ❑ Insufficient
    - ❑ I have no opinion
21. What primary dosing schedule of the HPV vaccination do you use?*
    - ❑ 1-dose
    - ❑ 2-dose
    - ❑ 3-dose
22. How do you assess the availability of the HPV vaccine in your country?*
    - ❑ All vaccine variants are available (2-, 4-, and 9-valent)
    - ❑ Only 2-valent and 4-valent vaccine variants are available
    - ❑ The HPV vaccine is very difficult to obtain
    - ❑ I have no opinion
23. In your opinion, should HPV vaccination be mandatory and reimbursed?*
    - ❑ Yes, for both girls and boys
    - ❑ Yes, but only for girls
    - ❑ No
    - ❑ I have no opinion
24. Do you think that HPV vaccination favours early sexual initiation?*
    - ❑ Yes
    - ❑ No
25. How do you rate your confidence in the HPV vaccination on a scale of 1 to 10?*

1	2	3	4	5	6	7	8	9	10
❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Thank you very much for your participation in the survey.

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Relationship of cystatin C, Hs-CRP, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio with isolated oligohydramnios

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ABSTRACT

Objectives: We evaluated inflammatory parameters in pregnant women with isolated oligohydramnios.

Material and methods: This prospective cross-sectional study enrolled 54 pregnant with isolated oligohydramnios (IO) and 54 matched by gestational week, healthy pregnant with normal amniotic fluid. Maternal plasma levels of cystatin C, hs-CRP, neutrophil-lymphocyte ratios (NLR), platelet-lymphocyte ratios (PLR), and pregnancy outcomes were compared between two groups.

Results: Cystatin C, hs-CRP, and PLR were significantly higher in the IO group than that in the control group ($p < 0.05$). In the IO group, the rate of primary cesarean section, fetal distress, neonates with meconium-stained, and need for neonatal intensive care unit was higher, and Apgar scores were significantly lower than those in the control group ($p < 0.05$). There was no significant difference between the groups for meconium-stained neonate rates and the intensive care unit's need in the late-term (410/7–416/7 weeks). Cystatin C, hs-CRP, and PLR were significantly higher in the IO group than the control group ($p < 0.05$). Cystatin C was positively correlated with the need for neonatal intensive care and negatively correlated with Apgar scores. The PLR was positively correlated with the rate of meconium-stained neonates ($p < 0.05$). Cystatin C and hs-CRP had significant value in predicting IO ($p < 0.05$).

Conclusions: Maternal serum levels of Cystatin C and hs-CRP may support the diagnosis and prediction of perinatal outcomes as possible biochemical markers in IO cases. In particular, a high level of cystatin C may indicate the need for neonatal intensive care and low Apgar scores. In addition, late-term IO may show similar results in meconium and neonatal intensive care needs compared to without oligohydramnios.

Key words: cystatin C; Hs-CRP; neutrophil-lymphocyte ratio; oligohydramnios; platelet-lymphocyte ratio

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INTRODUCTION

Oligohydramnios is a common clinical complication in pregnancy. Its incidence ranges from 1/60 to 1/750. Hill et al. [1] reported an average of 0.9%. There are many factors that play a role in its etiology. Some of these factors are fetal genitourinary system anomalies, placental insufficiency, maternal hypertensive, renal diseases, rupture of amniotic membranes, and history of maternal drug use such as angiotensin-converting enzyme inhibitor and nonsteroidal anti-inflammatory drugs [2]. Isolated oligohydramnios (IO) also called idiopathic oligohydramnios accounts for about half of the cases of oligohydramnios [2]. It is thought that this situation is caused by decreased placental perfusion and hypoxia, which leads to decreased of fetal urine production [3].

The diagnose of oligohydramnios is made, when the amniotic fluid index is < 5 cm or single pocket measurement is < 2 cm measured by two-dimensional ultrasound [4]. The incidence of oligohydramnios increases in the term periods of pregnancy. Its incidence rises to 15–20% at 42 weeks of gestation [5]. Amniotic fluid surrounds the fetus after the first few weeks of pregnancy. It has important functions in the growth and development of the fetus [6]. Fetal, maternal, and obstetric adverse effects may occur in oligohydramnios. Adverse outcomes such as admission to the neonatal intensive care unit (NICU), cesarean delivery

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for fetal distress, and meconium aspiration syndrome have been shown to increase even in low-risk pregnancies with oligohydramnios [7].

Studies have shown a close relationship between oligohydramnios and intrauterine growth retardation, small gestational age, prolonged labor, low Apgar score, and an increase in neonatal intensive care [8, 9]. Therefore, evaluation of amniotic fluid has been used as an integral component of obstetric screening and evaluation of fetal well-being for many years. However, it is not possible to measure the amniotic fluid in terms of volume and shape because it is constantly dynamic due to maternal and fetal factors [10]. Amniotic fluid measurement methods applied in the clinic are subjective and determining the amniotic fluid volume is directly related to the doctor's experience [11].

Cystatin C is an extracellular cysteine protease inhibitor present in many tissues and fluids in the body. Cysteine is involved in many processes such as inflammation and tumor metastasis by regulating protease activity [12]. CRP is a very sensitive acute phase reactant. CRP blood levels can be measured precisely (up to values below 0.2 mg/L) with new methods, i.e., hs-CRP. Recent studies have shown that increased CRP levels have a predictive value in diseases involving chronic inflammation such as cardiovascular diseases [13]. Studies of the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) have shown a significant association with cancer and many diseases with inflammation; these parameters are beginning to be considered as possible inflammation markers [14, 15].

The pathophysiology of oligohydramnios is not fully known, but it is thought to develop secondary to chronic stress [16]. Oligohydramnios develops without fetal renal agenesis or obstructive uropathy and is thought to be due to fetal response to chronic stress [16, 17]. It is caused by a decrease in fetal perfusion and urinary output secondary to the fact that the blood flow due to chronic stress is directed to vital organs such as the brain, heart, adrenal gland, and kidneys [18]. Fetal hypoxia [19] and acidosis [9] develop due to impaired fetal perfusion. Therefore, an increase in fetal and neonatal morbidity and mortality is observed in oligohydramnios [17]. There are studies arguing that poor perinatal outcomes which have been shown to be associated with oligohydramnios do not increase in isolated oligohydramnios. Other studies argue the opposite [20]. These poor perinatal outcomes are thought to be due to the relationship between oligohydramnios and placental insufficiency [21]. As a result, fetal perfusion disorder and secondary fetal hypoxia and acidosis may develop from placental insufficiency [22]. A cellular inflammatory response occurs from fetal hypoxia [23]. Both placental and fetal inflammation develop if due to acidosis [24]. Inflammation that occurs in the fetus and placenta may cause an inflammatory response in the mother [25].

Objectives

In this study, we wanted to evaluate the relationship of oligohydramnios with levels of cystatin C, hs-CRP NLR, and PLR. They are associated with inflammatory diseases in recent studies [26, 27]. We suspect that they play a role in the pathophysiology of oligohydramnios. Thus, we wanted to evaluate whether these parameters could be used as clinical markers due to their association with oligohydramnios, whether they could support the diagnosis of oligohydramnios as inflammatory parameters, and whether these parameters have value in terms of obstetric outcomes.

MATERIAL AND METHODS

Ethical approval for this study was obtained from the Ethics Committee of Amasya University (protocol #46). Those included in the study were selected from pregnant women over 32 weeks of gestation who applied to the Obstetrics and Gynecology outpatient clinics of Amasya Sabuncuoğlu Şerefeddin Training and Research Hospital between 8 November 2019 and 15 November 2020. The diagnosis of oligohydramnios was made in two ways: amniotic fluid index (AFI) less than 5 cm or less than 2 cm in the deepest single pocket (SDP) [10]. Patients diagnosed with oligohydramnios with both methods were included in the study. Patients diagnosed with oligohydramnios according to one method and not diagnosed with oligohydramnios according to the other method were not included in the study. In terms of dynamic changes in amniotic fluid, amniotic fluid was measured at two different times during the day (2–4 hours after the first measurement). A Mindray DC-7 ultrasound device was used to measure the amniotic fluid. The amniotic fluid evaluation was performed by a single clinician (A.T.T.) to minimize differences between measurements.

Pregnant women with chronic disease (such as diabetes mellitus, Addison's disease, adrenocortical insufficiency) or obstetric disease (such as gestational diabetes, hypertensive diseases of pregnancy, intrauterine growth retardation, premature rupture of membranes) that may trigger inflammation (such as collagen tissue disease, maternal infectious diseases) were excluded. Pregnant women with a history of drug use (such as a nonsteroidal anti-inflammatory or angiotensin-converting enzyme inhibitor) and with fetal congenital anomalies were not included in the study.

The study group consisted of 54 pregnant women with IO who meet the study criteria and 54 healthy pregnant women with normal amniotic fluid at the same gestational week as the matched control group. Of the pregnant women who were diagnosed with IO, 16 were 41 weeks, 25 were 40 weeks, eight were 39 weeks, two were 38 weeks, one was 36 weeks, and two were 34 weeks. All pregnant women were included in the study after being informed about the study and obtaining their consent. In terms of demo-

graphic characteristics of both groups, age, parity, height, weight, educational status, previous cesarean section, and previous non-cesarean abdominal surgery were questioned.

The obstetric outcomes of both groups (delivery type, primary cesarean rate, cesarean section indications, low birth weight, neonates with meconium, neonatal intensive care need, as well as 1st and 5th minute Apgar scores) were compared. The relationship of cystatin C, hs-CRP, NLR, and PLR with obstetric results was investigated. Here, 41-week pregnant women in the groups were evaluated separately in terms of neonatal intensive care need and rates of babies with meconium.

Blood samples were collected into one hemogram tube and two biochemistry tubes to evaluate cystatin C, hs-CRP, neutrophils, lymphocytes, thrombocytes, as well as neutrophil-lymphocyte and thrombocyte-lymphocyte ratios. Neutrophil, lymphocyte and thrombocyte levels were measured with laser optics (X N-1000, Siemens, Japan), Hs-CRP with an immunoturbidimetric test (Cobas C 702, Roche, Japan), and cystatin C level with an immunonephelometric test (Dade Behring, Germany).

Power analysis

The sample size was determined with the G * Power 3.1 program via a statistician who made a statistical analysis of the study similar to Figueroa et al. [28] using a disease prevalence of 0.7%. We analyzed the number of women diagnosed with new pregnancy in one year at the health institution where the study was conducted (n = 1600–1700) as well as effect width (d = 0.71) and a two-tailed hypothesis method 36. The confidence interval was determined as 95%, and the margin of error was 5%. As a result of the calculation, we determined that there should be 53 women in the control group and 53 in the study group or 106 women in total.

Statistical analysis

The data were analyzed using the SPSS 21.0 program. Whether the continuous variables were suitable for normal distribution according to the groups was evaluated with the Kolmogorov-Smirnov test. A Mann-Whitney U test was used to compare variables that did not conform to a normal distribution. One-way analysis of variance (ANOVA) was used to compare normally distributed data according to three or more groups, and the Kruskal-Wallis test was used to compare non-normally distributed data. The Chi-square test or Fisher's exact probability test was used to determine whether the frequency distributions of categorical variables were homogeneously distributed among groups. A Chi-square test was used to test the relationships between categorical variables, and the Z test (Bonferroni method) was used in the post hoc analysis of variables that were statistically different between groups. The $p < 0.05$ was considered statistically significant. Spearman's rho correlation coefficient was used to examine the relationship between non-normally distributed quantitative variables. ROC curve analysis was used to find threshold values. The sensitivity, specificity, positive predictive, and negative predictive values of these limits were calculated in the presence of significant limit values. Cases with Type 1 error level below 5% were considered statistically significant upon evaluation of the area under the curve.

RESULTS

The groups were similar in terms of demographic characteristics. Comparison of the groups according to demographic parameters is shown in Table 1. Cystatin C, hs-CRP, and PLR levels were significantly higher in pregnant women with oligohydramnios than the control group ($p < 0.05$). No statistically significant difference was observed between the groups in terms of NLR. The distribution of Cystatin C, hs-CRP, PLR, and NLR values among groups is shown in Table 2.

Table 1. Demographic characteristics of the groups

		IO group (n = 54) (%)	Control group (n = 54) (%)	p
Age [year]		28.19 ± 5.36	27.87 ± 6.07	0.776
BMI [kg/m^2]		29.85 ± 3.91	30.56 ± 3.76	0.336
Parity	Nulliparous	21 (38.9%)	18 (33.3%)	0.689
	Multiparous	33 (61.1%)	36 (66.7%)	
Education	Primary school	10 (18.5%)	12 (22.2%)	0.767
	Middle School	9 (16.7%)	6 (11.1%)	
	High school	22 (40.7%)	25 (46.3%)	
	University	13 (24.1%)	11 (20.4%)	
PCS		7 (13.0%)	9 (16.7%)	0.588
PAS		8 (14.8%)	11 (20.4%)	0.614

p — values were calculated with the Independent test (age, BMI) and Chi-square test; IO — isolated oligohydramnios; PAS — previous abdominal surgery (except cesarean); PCS — previous cesarean surgery

Table 2. Comparison of laboratory results of the groups

	IO group (n = 54)	Control group (n = 54)	p
Cystatin C [mg/L]	0.69 ± 0.06	0.65 ± 0.06	**0.006**
Hs-CRP [mg/L]	2.75 ± 0.62	2.47 ± 0.77	**0.041**
Neutrophils [×10^9/L]	4.52 ± 0.81	4.24 ± 0.80	0.078
Lymphocytes [×10^9/L]	2.29 ± 0.55	2.43 ± 0.64	0.235
Platelets [×10^9/L]	305.50 ± 59.66	288.37 ± 68.10	0.167
NLR	2.08 ± 0.61	1.89 ± 0.68	0.134
PLR	140.82 ± 43.70	124.56 ± 37.02	**0.039**

p — values were calculated with the Independent test; IO — isolated oligohydramnios; NLR — neutrophil to lymphocyte ratio; PLR — platelet to lymphocyte ratio

Table 3. Sensitivity, specificity, positive predictive, and negative predictive values for cystatin C, Hs-CRP, NLR, and PLR

	AUC (95 CI)	p	Cut-off	Sensitivity	Specificity
Cystatin C [mg/L]	0.636 (0.531–0.74)	**0.015**	≥ 0.675	0.574	0.574
Hs-CRP [mg/L]	0.656 (0.549–0.762)	**0.005**	≥ 2.615	0.704	0.667
NLR	0.589 (0.482–0.697)	0.109	—	—	—
PLR	0.607 (0.501–0.714)	0.054	—	—	—

p — values were calculated with the ROC analyses; AUC — area under the curve; NLR — neutrophil to lymphocyte ratio; PLR — platelet lymphocyte ratio

Cystatin C, hs-CRP, NLR, and PLR were evaluated with Receiver Operator Characteristics Curve (ROC). The area under the curve in ROC was statistically significant for cystatin C and hs-CRP ($p < 0.05$). However, there was no statistical significance of the area under the curve in the ROC for NLR and PLR ($p > 0.05$) (Tab. 3).

When both groups were compared in terms of obstetric results, primary cesarean section, fetal distress, neonates with meconium, and neonatal intensive care need were higher in the oligohydramnios group, but these were not statistically significant ($p > 0.05$) (Tab. 4). The 1st and 5th minute Apgar scores were significantly lower in the IO group compared to the control group ($p < 0.05$) (Tab. 4). There was no difference between the groups in terms of birth weight.

The relationship of cystatin C, hs-CRP, NLR, and PLR parameters with obstetric results were also evaluated. PLR was positively correlated with rates of meconium neonates ($r = 0.274$, $p = 0.004$). Cystatin C was positively correlated with neonatal intensive care need and negatively correlated with 1st and 5th minute Apgar scores ($r = 0.237$, $p = 0.013$). Correlation analyses according to the groups are shown in Table 5.

Since meconium is more expected in the late-term period, meconium-stained infants' rates and the NICU needs were also compared between the groups in the late-term. Evaluation of 41-week pregnant women in terms of meconium rates and neonatal intensive care needs showed no significant difference between the groups (Tab. 6).

Gestational week distribution according to NICU needs: seven infants at 41 weeks and 8 at 40 weeks were in the IO group (n: 15). Three infants at 41 weeks, 3 at 40 weeks, and one at 39 weeks were in the control group (n: 7).

Gestational week distribution of meconium-stained infants: six infants at 41 weeks, four at 40 weeks, one at 39 weeks, and one infant at 38 weeks were in the IO group (n: 12). Three infants were at 41 weeks and three at 40 weeks in the control group (n: 6).

DISCUSSION

We evaluated maternal serum levels of the inflammation parameters cystatin C, hs-CRP, NLR, and PLR to determine whether the fetal and placental inflammation that develops in isolated oligohydramnios causes an inflammatory response in the mother. Cystatin C, hs-CRP, and PLR levels were significantly higher in the oligohydramnios group than the control group. Significantly higher values of the aforementioned parameters suggest that there is an inflammatory process in the pathophysiology of oligohydramnios and that a maternal inflammatory response may occur against this inflammation. There was a significant area under the curve (AUC) in the ROC of cystatin C and hs-CRP. This suggests that these two parameters may be suitable for use as diagnostic decision-makers in oligohydramnios ($p < 0.05$) (Tab. 3). In addition, when the obstetric results of both groups were compared, the 1st and 5th minute Apgar scores were significantly lower in the IO group versus the control group ($p < 0.05$). Cystatin C elevation was positively correlated with neonatal intensive care need and negatively correlated with 1st and 5th minute Apgar scores. Although PLR did not have

Table 4. Comparison of obstetric outcomes of groups

		IO group (n = 54) (%)	Control group (n = 54) (%)	p
Birth weight [g]		3141.11 ± 478.20	3288.43 ± 484.2	0.115
Type of delivery	Vaginal	34 (72.2%)	39 (72.2%)	0.304
	Cesarean	20 (37.0%)	15 (27.8%)	
Birth week [week]	34-34^6 week	2 (3.7%)	2 (3.7%)	1.00
	36-36^6 week	1 (1.9%)	1 (1.9%)	
	38-38^6 week	2 (3.7%)	2 (3.7%)	
	39-39^6 week	8 (14.8%)	8 (14.8%)	
	40-40^6 week	25 (46.3%)	25 (46.3%)	
	41-41^6 week	16 (29.6%)	16 (29.6%)	
Primary cesarean		13 (42.1%)	6 (11.1%)	0.072
Cesarean indication	Fetal distress	10 (50.0%)	3 (20.0%)	0.092
	Previous cesarean	7 (35.0%)	9 (60.0%)	
	CPD	1 (5.0%)	3 (20.0%)	
	Prolonged labor	2 (10.0%)	0 (0.0%)	
Low birth weight		4 (7.4%)	3 (5.6%)	0.696
Meconium		12 (22.2%)	6 (11.1%)	0.121
Need of NICU		15 (27.8%)	7 (13.0%)	0.056
1st minute AS		7.89 ± 1.14	8.43 ± 0.83	**0.006**
5st minute AS		9.09 ± 0.78	9.54 ± 0.60	**0.001**

AS — apgar score; CPD — cephalopelvic dystocia; IO — isolated oligohydramnios; NICU — Neonatal Intensive Care Unit; p — values were calculated with the independent test (APGAR scores, birth weight) and Chi-square test

Table 5. Relationship of cystatin C, hs-CRP, NLR and PLR with obstetric outcomes

		Cystatin C [mg/L]	Hs-CRP [mg/L]	NLR	PLR
Low birth weight	R	0.034	–0.042	0.038	0.054
	P	0.723	0.670	0.693	0.578
Primary cesarean	R	0.101	0.156	0.006	0.061
	P	0.298	0.106	0.953	0.533
Fetal distress	R	0.177	0.129	–0.026	0.033
	P	0.067	0.184	0.788	0.732
Meconium-stained	R	0.128	–0.095	0.112	0.274
	P	0.186	0.326	0.247	**0.004**
1st minute Apgar Score	R	0.237	–0.054	0.105	0.097
	P	**0.013**	0.580	0.279	0.319
5th minute Apgar Score	R	–0.288	–0.015	–0.106	–0.121
	P	**0.003**	0.877	0.274	0.213
Neonatal intensive care need	R	0.237	–0.054	0.105	0.097
	P	**0.013**	0.580	0.279	0.319

NLR — neutrophil to lymphocyte ratio; P — values were calculated with the Spearman's rho correlation test; PLR — platelet lymphocyte ratio; R — Spearman's rrho correlation coefficient

decision-maker validity, it had a positive correlation with the meconium rate.

While ultrasonography is sufficient to evaluate the normal volumes of amniotic fluid, it is poor at identifying abnormal volumes [18]. In addition, the experience of the doctor, the fetal position, the possibility of temporary changes in amniotic fluid, and the existence of different ultrasound criteria that diagnose anomalous amniotic fluid

Table 6. Comparison of both groups at the 41 $^{0/6}$–41$^{6/7st}$ gestational week in terms of neonatal intensive care need and meconium-stained rates

Comparison of 41-week gestation of both groups in terms of neonatal intensive care needs					
	IO group NICU (–) n (9)	**IO group NICU (+) n (7)**	**Control group NICU (–) n (13)**	**Control group NICU (+) n (3)**	**p**
Cystatin C [mg/L]	0.69 ± 0.07	0.73 ± 0.06	0.66 ± 0.09	0.67 ± 0.01	0.344
Hs-CRP [mg/L]	2.57 ± 0.56	2.42 ± 0.81	2.63 ± 0.90	2.67 ± 0.10	0.955
NLR	1.72 ± 0.43	1.76 ± 0.35	2.09 ± 0.79	2.23 ± 0.05	0.354
PLR	138.04 ± 43.59	139.41 ± 48.71	139.36 ± 46.13	140.82 ± 13.39	0.985
Comparison of 41-week gestation of both groups in rates of meconium					
	IO group Meconium (–) n (10)	**IO group Meconium (+) n (6)**	**Control group Meconium (–) n (13)**	**Control group Meconium (+) n (3)**	**p**
Cystatin C [mg/L]	0.68 ± 0.07	0.74 ± 0.07	0.66 ± 0.09	0.67 ± 0.01	0.321
Hs-CRP [mg/L]	2.64 ± 0.63	2.28 ± 0.70	2.63 ± 0.90	2.67 ± 0.10	0.891
NLR	1.64 ± 0.40	1.89 ± 0.31	2.09 ± 0.79	2.23 ± 0.05	0.264
PLR	116.03 ± 33.08	176.32 ± 34.55	139.36 ± 46.13	140.82 ± 13.39	0.052

IO — solated oligohydramnios; NICU — neonatal intensive care unit; NLR — neutrophil-to-lymphocyte ratio; p — values were calculated with the Kruskal-Wallis test and one-way analysis of variance test; PLR — platelet lymphocyte ratio

volume led to a decrease in the reliability of ultrasound [29]. For this reason, we used ultrasound evaluation from the same physician to provide both AFI and SDP results and made measurements twice a day.

Accuracy rate can reach up to 87% when measured with the ultrasound index made by measuring three dimensions [30]. It has been suggested that invasive methods such as indicator dilution techniques give the most accurate measurement, but they are not practical for clinical use [19]. Therefore, existing methods continue to be used, and we used both (AFI, SDP).

The main clinical importance of oligohydramnios is due to the increase in fetal and neonatal morbidity and mortality [17]. These poor outcomes are thought to be due to placental insufficiency developing in oligohydramnios [21]. In addition, decreased uteroplacental flow may cause an increase in the release of chemokines and activation of neutrophils in the later stages of pregnancy. This is more common in healthy pregnancies and can overstimulate an inflammatory response [31]. Platelets, like neutrophils, increase the secretion of cytokines at the onset of inflammation, and increased cytokines contribute to increased inflammation by enhancing new neutrophil and platelet synthesis [32]. In light of these studies, we think that it is clinically important to investigate the relationship between the inflammatory process in the mother with IO, inflammatory parameters, and obstetric outcomes. In the IO group, we found high levels of cystatin C, hs-CRP, and PLR among these inflammation markers support that an inflammatory process plays a role in the pathogenesis of oligohydramnios. Although the current literature is constantly developing, there is still limited information in terms of the pathophysiology and management of IO.

While some studies suggest increased risks in IO, other studies report the opposite [2]. In our study we tried to exclude negative obstetric outcomes due to secondary factors (such as diabetes, hypertension *etc.*). In addition, there is no method recommended to accurately predict perinatal morbidity and mortality in IO, and there is no consensus for cut-off values [33]. Here, when the obstetric results of the groups were compared, the 1st and 5th minute Apgar scores were significantly lower in the IO group than the control group. When the correlation of these inflammatory markers with obstetric outcomes was evaluated, the PLR level was positively correlated with the number of neonates with meconium, and the level of cystatin C was positively correlated with the need for neonatal intensive care. Cystatin C levels were negatively correlated with 1st and 5th minute Apgar scores. These results may support the idea that pregnant women with oligohydramnios with high cystatin C need to be more careful in terms of fetal well-being. This could contribute positively to obstetric outcomes by allowing the necessary preparations to be made in terms of intensive care conditions and Apgar scores. Oligohydramnios, meconium association, and the need for neonatal intensive care were associated more frequently in pregnant women at 41 weeks of gestation and were accepted as late-term in our study [34]. Thus, 41-week pregnant women were re-evaluated to reduce bias in terms of perinatal outcomes. We think that pregnant women in our study had IO cases even if they were late-term (41$^{0/7}$–41$^{6/7}$ week); thus, they had similar intensive care needs and meconium rates as the control group. There was no difference in birth weight between the groups like the results of studies suggesting that isolated oligohydramnios is not associated with impaired fetal growth or increased risk of adverse perinatal outcomes [20].

The strength of our study is that this is the first study evaluating the relationship between inflammatory parameters and oligohydramnios. Cases were matched with controls, and mean birth weight was evaluated in IO cases that were not complicated by intrauterine growth retardation; secondary factors were excluded in the comparison of obstetric results. The evaluation was also done for late-term weeks. Markers such as PLR and hs-CRP were used, which can be easily examined in the clinic. We suspect that it may be possible to use these to predict poor perinatal outcomes.

One limitation of our study was that while we did have sufficient statistical power, we could not perform a large series. In addition, a maternal comparison of inflammatory parameters could be done as well as a fetal comparison; however, we did not evaluate fetal levels in our study. This was similar to another study investigating the relationship of cystatin C in cases with intrauterine growth retardation [35].

CONCLUSIONS

In summary, cystatin C, hs-CRP, and PLR, increase in inflammation and were found to be higher in IO especially when cystatin C and hs-CRP were statistically and significantly higher in ROC analysis. This suggests that they could be used as markers in both the diagnosis of oligohydramnios and in predicting perinatal outcomes in suspected cases. In addition, a high cystatin C level may indicate a poor prognosis in terms of fetal well-being. It may be clinically helpful to determine prognostic parameters that would support the diagnosis of IO and enable to take precautions in terms of the risks.

Conflict of interest

All authors declare no conflict of interest.

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Effectiveness of paracervical block in endometrial sampling procedures for pain control: a randomized controlled clinical trial

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ABSTRACT

Objectives: We aimed to evaluate the effect of paracervical block (PCB) on endometrial sampling procedures, to assess the effect on pain of waiting between PCB and intervention, and to compare the effectiveness of PCB with oral non-steroidal anti-inflammatory drugs (NSAID) for decreasing the pain levels associated with endometrial biopsy.

Material and methods: A total of 123 participants were divided into four groups as Group 1: Waiting 1 minute after PCB, Group 2: Waiting 3 minute after PCB, Group 3: Control group, and Group 4: Waiting 60 minute after taking oral NSAIDs. The success of analgesic measures used for endometrial biopsy during and 30 minutes after the procedure was compared with the Numeric Pain Rating Scale (NPRS) system.

Results: The Numeric Pain Rating Scale (NPRS) 0 score was 2.60 (± 2.42) in Group 1; 1.60 (± 1.73) in Group 2; 5.30 (± 2.10) in Groups 3; 5.63 (± 1.99) in Groups 4. NPRS 30 score was 0.80 (± 0.88) in Group 1; 0.43 (± 0.81) in Group 2; 1.90 (± 1.32) in Groups 3; 2.70 (± 1.41) in Groups 4. The pain was significantly less in the paracervical block groups compared to control and oral NSAIDs groups. However, there was no significant difference in NPRS 0 ($p = 0.196$) and NPRS 30 ($p = 0.191$) scores between Group 1 and Group 2. There was no significant difference in NPRS 0 and NPRS 30 scores between control group and oral NSAID group.

Conclusions: Paracervical block (PCB) is an effective method and superior to oral NSAIDs. Waiting 1 minute or 3 minutes after PCB were equally effective.

Key words: endometrial sampling; numeric pain rating scale; oral NSAIDs; pain control; paracervical block

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INTRODUCTION

Currently, office-based endometrial procedures are preferred over diagnostic dilation and curettage. Short operation time, low uterine perforation risk, no need for an operating room, and low cost are the benefits of sampling procedures.

Office sampling procedures do not cause severe pain, but several approaches are used by clinicians to reduce the discomfort due to the operation. Oral nonsteroidal anti-inflammatory drugs (NSAID) taken 30 minutes before the procedure, intrauterine instillation of local anaesthetics, topical 10% lidocaine spray, and paracervical block are the accepted methods [1–3].

Frankenhauser (or uterovaginal) plexus contains fibers derived from the inferior hypogastric plexus (T10-L1) and sacral nerve roots (S1–S4). Paracervical block targets this plexus before it enters the uterus at the level of the internal cervical os. The paracervical block is a single-shot nerve block that involves a one-time injection of local anaesthetic adjacent to the uterovaginal nerve plexus. The block provides analgesia during the cervical pass of the sampling device or manipulation of the cervix. A study showed that paracervical block decreased the pain during intrauterine device placement in 64 nulliparous women [4].

Objectives

The paracervical block seems to work within a few minutes after injection, but the optimal waiting time between injection and the procedure is not known. This prospec-

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tive, randomized controlled, non-blinded study aimed to determine the effect of a waiting time of 1 minute and 3 minutes after paracervical block in endometrial sampling procedures using the Pipelle cannula on pain during and after endometrial sampling and compare with NSAIDs taken before the procedure.

MATERIAL AND METHODS

We enrolled 123 participants who underwent endometrial biopsy due to abnormal uterine bleeding in the current study in the Ege University Hospital from September 29, 2020, through November 3, 2020. Inclusion criteria were absence of major psychiatric symptoms, Turkish language comprehension, and age 35 years or older. Pregnancy, pelvic infections, current heavy menstrual bleeding, and NSAID allergy were the exclusion criteria (Fig. 1). Two participants with findings of pelvic infection and a pregnant participant were excluded from the study. Participants were divided into 4 groups using a computer-generated randomization list. These groups were Group 1: Waiting 1 minute after paracervical block, Group 2: Waiting 3 minute after paracervical block, Group 3: Control group, and Group 4: Waiting 60 minute after taking oral NSAIDs. The Numeric Pain Rating Scale (NPRS) system was used to assess pain for each case during and 30 minutes after the procedure. According to this system, "0" indicated no pain, and "10" points represented the most severe pain.

Before the procedure, the cervix and vaginal vault were prepared with povidone-iodine. A vaginal speculum was used for optimal exposure and manipulation of the cervix. All procedures were performed without grasping the cervix with a tenaculum. Two-point (at 4 and 8 o'clock position only) technique was used for the paracervical block for Group 1: Waiting 1 minute after paracervical block and Group 2: Waiting 3 minute after paracervical block. For these groups, a total of 10 ml of 2% prilocaine (VEM İlac, Tekirdag, Turkey) was injected at 4 o'clock and 8 o'clock positions approximately 10 mm into the cervical stroma at the cervicovaginal junction with a 22-gauge hypodermic needle. The capped needle model was used for Group 3: Control group. After cervical and vaginal preparation with povidone-iodine, a capped needle touched the cervicovaginal junction at the 4 and 8 o'clock position. Participants in Group 4 took 550 mg of naproxen sodium (Abdi Ibrahim, Turkey) 60 minutes before the procedure.

For all procedures, a low-pressure sampling device (Pipelle Cannula, Medbar Medical Equipment Inc.) was inserted into the cavity, and endometrial samples were obtained using a corkscrew rotation combined with a repeating cephalic-caudal motion. The procedure was repeated

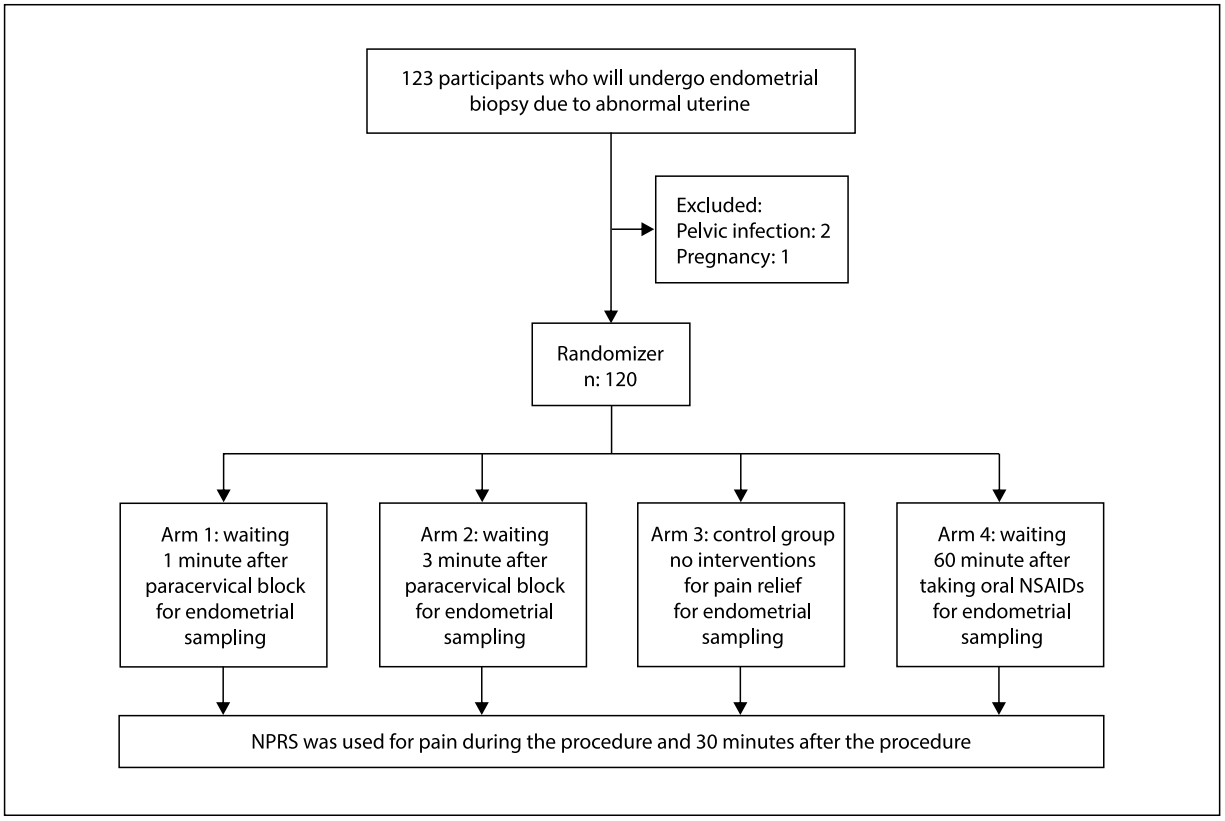

Figure 1. Participant's Flow Chart; NSAID — non-steroidal anti-inflammatory drugs; NPRS — Numeric Pain Rating Scale

twice in each case to ensure standardization. All endometrial biopsies were performed by the same operator.

The NPRS system was explained to all groups after the procedure, and the participants were requested to grade the pain during the procedure. Thirty minutes after the procedure, the participants were asked to rate current pain according to the NPRS system.

According to previous trials, we calculated those 123 participants would be required for 80% power with a type I error (α) rate of 5% to detect this difference with 3% drop-out rate [4, 5]. Categorical variables were analysed with frequency tables, and descriptive statistics were calculated for continuous variables. The Shapiro-Wilk normality test was used to analyse whether continuous data were normally distributed. As the data were not normally distributed, the Kruskal-Wallis test was used for comparing more than two independent groups. When the difference between the groups was statistically significant, Bonferonni correction was made in post hoc comparisons after the Kruskal-Wallis test. Spearman's correlation coefficient was used to analyze the relation to between continuous variables. The significance level was taken as 0.05 in all hypothesis tests. All statistical analyses were performed using the IBM SPSS Version 25.0 statistical package program.

The study was approved by Ege University Institutional Ethics Committee with 20-6.1T/67 reference number on 25 June 2020. Written informed consent was obtained from all participants. All procedures performed in the current study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Trial registry name is "Effectiveness of Paracervical Block in Endometrial Sampling Procedures for Pain Control" and Clinical Trial Registration Number is NCT04572828. Initial participant was enrolled on September 29, 2020 [6].

RESULTS

Mean age was 48.23 (± 11.74) years in Group 1; 46.23 (± 5.33) years in Group 2; 51.78 (± 8.21) years in Groups 3; 46.53 (± 7.10) years in Groups 4. There was no significant difference in demographic data between groups (Tab. 1, Tab. 2). Mean endometrial thickness was 8.66 (± 2.86) mm in Group 1; 8.71 (± 2.93) mm in Group 2; 8.74 (± 2.61) mm in Groups 3; 9.66 (± 3.02) mm in Groups 4. Sixty-six of the participants were in the premenopausal period, and the remaining 54 were in the postmenopausal period.

NPRS 0 score was 2.60 (± 2.42) in Group 1; 1.60 (± 1.73) in Group 2; 5.30 (± 2.10) in Groups 3; 5.63 (± 1.99) in Groups 4. NPRS 30 score was 0.80 (± 0.88) in Group 1; 0.43 (± 0.81) in Group 2; 1.90 (± 1.32) in Groups 3; 2.70 (± 1.41) in Groups 4 (Tab. 3).

NPRS 0 score was significantly less in Group 1 compared to control ($p < 0.001$) and oral NSAIDs groups ($p < 0.001$). NPRS 30 score was significantly less in Group 1 compared to the control group ($p < 0.001$) and the oral NSAIDs group ($p < 0.001$). As expected, NPRS 0 score was significantly less in the waiting 3 minute after paracervical block group compared to Group 3 ($p < 0.001$) and Group 4 ($p < 0.001$). Further, NPRS 30 score was significantly less in the waiting 3 minute after paracervical block group compared to Group 3 ($p < 0.001$) and Group 4 ($p < 0.001$). However, there was no significant difference in NPRS 0 ($p = 0.196$) and NPRS 30 ($p = 0.191$) scores between Group 1: Waiting 1 minute after paracervical block and Group 2: Waiting 3 minute after paracervical block (Fig. 2). Furthermore, there was no significant difference in NPRS 0 ($p = 0.643$) and NPRS 30 ($p = 0.064$) scores between Groups 3: Control group and Groups 4: Waiting 60 minute after taking oral NSAIDs (Fig. 3).

The current study showed that menopausal status, day of menstruation, and endometrial thickness had no effect on pain during the endometrial sampling procedure. There were no patient-reported adverse effects and major complications during the trial.

DISCUSSION

Low-pressure endometrial sampling devices such as the Pipelle cannula are the most popular biopsy devices in modern gynaecological practice [7]. The diagnostic value of low-pressure sampling devices depends on the types of indications, BMI, age, and menopausal status [8]. Although endometrial sampling with a Pipelle is relatively painless, a mild-moderate pain is reported by women in the absence of measures to minimize discomfort during the operation [1]. These techniques are sedoanalgesia, oral NSAID intake before the procedure, and administration of a paracervical block and topical local anaesthetic spray application to the cervix [9]. Sedoanalgesia ensures sufficient conditions for cervical dilatation and uterine intervention. According to a prospective randomized double-blind study, intravenously administered lidocaine reduced the pain scores compared to the control group during colposcopic cervical biopsy and endocervical curettage [10]. However, PCB offers an alternative for cervical dilatation and uterine intervention in high-risk patients for sedoanalgesia or if no anaesthesiologist is available. Cervical dilatation or cervical pass with a sampling device is one of the significant causes of pain associated with the procedure [11]. Therefore, one should aim to minimize the pain experienced during this part of the procedure with a paracervical block.

Paracervical block (PCB) is an effective and easy-to-perform method for gynaecologists, especially before intrauterine interventions, although many nerve blocks are performed by anaesthesiologists. However, the efficiency of

Table 1. Demographic data — 1

Trial arms			Statistic	Standard error
Gravida	Waiting 1 minute after paracervical block	Mean	2.93	0.389
		Standard deviation	2.132	
		Minimum	0	
		Maximum	9	
	Waiting 3 minutes after paracervical block	Mean	2.47	0.190
		Standard deviation	1.042	
		Minimum	0	
		Maximum	4	
	Control group	Mean	3.13	0.481
		Standard deviation	2.636	
		Minimum	0	
		Maximum	15	
	Waiting 60 minutes after taking oral NSAIDs	Mean	2.53	0.229
		Standard deviation	1.252	
		Minimum	1	
		Maximum	7	
Parity	Waiting 1 minute after paracervical block	Mean	2.90	0.388
		Standard deviation	2.123	
		Minimum	0	
		Maximum	9	
	Waiting 3 minutes after paracervical block	Mean	2.33	0.188
		Standard deviation	1.028	
		Minimum	0	
		Maximum	4	
	Control group	Mean	2.53	0.355
		Standard deviation	1.943	
		Minimum	0	
		Maximum	11	
	Waiting 60 minutes after taking oral NSAIDs	Mean	2.33	0.205
		Standard deviation	1.124	
		Minimum	1	
		Maximum	7	

NSAID — non-steroidal anti-inflammatory drugs

PCB is controversial and the optimal waiting time after PCB is unknown. There are two opinions for how the PCB works: it may have an infiltrative part, relying on distention, or it may work as a peripheral nerve block, requiring time to diffuse into the neurons to block pain. The distention action would be immediate; the blockage of pain transmission would need 1 to 3 minutes for the onset of action when prilocaine is used. Hall et. al. [12] concluded that the addition of PCB to general anaesthesia for first trimester abortion did not influence pre- and postoperative pain scores significantly or analgesic consumption. In a prospective, randomised-controlled study, no beneficial effect was found when a PCB was added to either systemic or local analgesics for pain control during and 30 min after hysterosalpingography [13]. Conversely, in a randomised, double-blind, placebo-controlled trial, significant pain reduction was achieved for both intraoperative and postoperative period with PCB or lidocaine spray during first- trimester surgical abortion [5]. Kalkat & Cartmill [14] demonstrated that impedance controlled endometrial ablation procedure for menorrhagia is acceptable for use in an outpatient setting under PCB with high acceptance and success rates. In a randomized controlled trial, a wait of 3 min after PCB was more effective in reducing pain than no waiting before a first-trimester surgical abortion, although the difference did not reach a significant level [15]. Phair et. al. [16] found that delay between PCB and intervention

Table 2. Demographic data — 2

Trial arms			Statistic	Standard error
Caesarean history	Waiting 1 minute after paracervical block	Mean	0.47	0.133
		Standard deviation	0.730	
		Minimum	0	
		Maximum	2	
	Waiting 3 minutes after paracervical block	Mean	0.60	0.170
		Standard deviation	0.932	
		Minimum	0	
		Maximum	3	
	Control group	Mean	0.67	0.168
		Standard deviation	0.922	
		Minimum	0	
		Maximum	3	
	Waiting 60 minutes after taking oral NSAIDs	Mean	0.73	0.191
		Standard deviation	1.048	
		Minimum	0	
		Maximum	3	
Day of menstruation	Waiting 1 minute after paracervical block	Mean	8.97	1.753
		Standard deviation	9.601	
		Minimum	0	
		Maximum	30	
	Waiting 3 minutes after paracervical block	Mean	9.50	1.262
		Standard deviation	6.912	
		Minimum	0	
		Maximum	26	
	Control group	Mean	6.67	1.367
		Standard deviation	7.489	
		Minimum	0	
		Maximum	23	
	Waiting 60 minutes after taking oral NSAIDs	Mean	6.87	1.087
		Standard deviation	5.952	
		Minimum	0	
		Maximum	20	

NSAID — non-steroidal anti-inflammatory drugs

does not have an impact on pain during first trimester elective abortion.

The current study was conducted to compare the effectiveness of pain control after a wait of 1 and 3 min following a PCB and oral intake of NSAİD 60 min before the procedure. The evaluation was made using NPRS 0 and NPRS 30 scores. In our study, the lowest NPRS 0 and NPRS 30 scores were in Group 2: Waiting 3 minute after paracervical block. Group 2 was followed by Group 1: Waiting 1 minute after paracervical block. However, the difference did not reach a statistically significant level. The study results suggest that the distention mechanism predominates because a waiting period produced no additional anaesthetic effect. Regardless of waiting times, maximum pain control was achieved in the paracervical block groups compared to control and oral NSAID groups during the procedure and 30 minutes after the procedure. We did not find a significant difference in pain scores between the intervention and control groups according to the menopausal status, contrary to previous studies [15, 16].

A randomized controlled trial showed that the administration of 550 mg of naproxen sodium 60 min before the procedure was equally effective as intrauterine lidocaine injection [2]. However, we did not find any significant difference in NPRS 0 and NPRS 30 scores between control group and Oral NSAIDs group.

Table 3. The Numeric Pain Rating Scale (NPRS) scores for each arm

Trial arms			Statistic	Standard error
NPRS 0	Waiting 1 minute after paracervical block	Mean	2.60	0.451
		Standard deviation	2.472	
		Minimum	0	
		Maximum	10	
	Waiting 3 minutes after paracervical block	Mean	1.60	0.317
		Standard deviation	1.734	
		Minimum	0	
		Maximum	6	
	Control group	Mean	5.30	0.384
		Standard deviation	2.103	
		Minimum	2	
		Maximum	10	
	Waiting 60 minutes after taking oral NSAIDs	Mean	5.63	0.364
		Standard deviation	1.991	
		Minimum	2	
		Maximum	10	
NPRS 30	Waiting 1 minute after paracervical block	Mean	0.80	0.162
		Standard deviation	0.887	
		Minimum	0	
		Maximum	3	
	Waiting 3 minutes after paracervical block	Mean	0.43	0.149
		Standard deviation	0.817	
		Minimum	0	
		Maximum	3	
	Control group	Mean	1.90	0.241
		Standard deviation	1.322	
		Minimum	0	
		Maximum	6	
	Waiting 60 minutes after taking oral NSAIDs	Mean	2.70	0.259
		Standard deviation	1.418	
		Minimum	1	
		Maximum	7	

NSAID — non-steroidal anti-inflammatory drugs

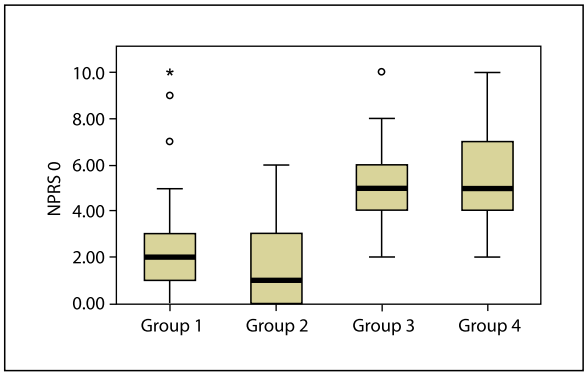

Figure 2. Numeric Pain Rating Scale (NPRS) 0 Scores in Groups

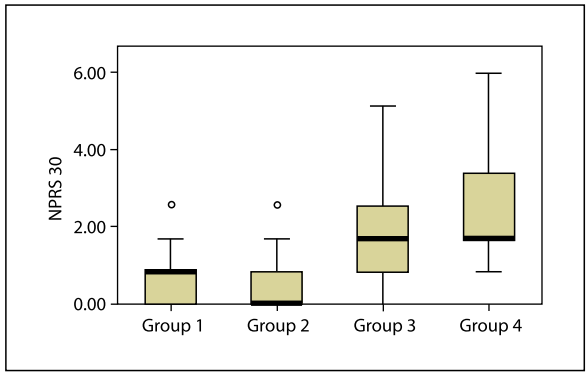

Figure 3. Numeric Pain Rating Scale (NPRS) 30 Scores in Groups

The strengths of this study are randomized controlled design and the use of the NPRS system for pain assessment. Further, the procedures were performed by the same physician to avoid differences between operators. The limitation of this trial was combining pre- and post-menopausal women, although it represented local demographics.

CONCLUSIONS

Paracervical block is an effective method for pain control for endometrial sampling procedures and superior to oral NSAIDs. Waiting 1 minute or 3 minutes after PCB were equally effective in terms of pain during endometrial biopsy with a low-pressure sampling device.

Conflict of interest

All authors declare no conflict of interest.

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Meta-analysis for the evaluation of perioperative enhanced recovery after gynaecological surgery

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ABSTRACT

Objectives: To systematically evaluate the effectiveness and safety of enhanced recovery after surgery (ERAS) in gynaecological surgery and provide a scientific basis for its clinical promotion and application in the Chinese population.

Material and methods: Systematic retrieval from CNKI, Wanfang, VIP database and other Chinese literature databases. Studies on ERAS application with a randomised controlled trial in gynaecological surgery were included in the present report. Outcome indicators: hospitalisation time, postoperative ambulation time, postoperative feeding time, postoperative exhaust time, postoperative defecation time, operation time, postoperative blood loss, postoperative morbidity, patient satisfaction, hospitalisation expenses, etc. The meta-analysis was performed using the Revman 5.3 software.

Results: A total of 24 studies were included in the analysis. The results showed that, compared with the traditional group, the ERAS group had a lower hospitalisation time (SMD = −1.67, 95% CI = −2.03 ~ −1.30, $p < 0.0001$), postoperative ambulation time (SMD = −4.16, 95% CI = −5.12 ~ −3.20, $p < 0.0001$), postoperative feeding time (SMD = −7.36, 95% CI = −9.67 ~ −5.05, $p < 0.0001$), postoperative exhaust time (SMD = −2.59, 95% CI = −3.15 ~ −2.03, $p < 0.0001$), postoperative defecation time (SMD = −2.23, 95% CI = −2.88 ~ −1.57, $p < 0.0001$), postoperative morbidity (OR = 0.22, 95% CI = 0.15 ~ 0.31, $p < 0.0001$) and hospitalisation expenses (SMD = −0.53, 95% CI = −0.78 ~ −0.28, $p < 0.0001$). The patient satisfaction was significantly improved (odds ratio = 8.11, 95% CI = 4.96 ~ 13.24, $p < 0.0001$), and there were no significant differences in intraoperative blood loss and operation time between the two groups.

Conclusions: The application of the ERAS protocol in gynaecological surgery significantly improves the effectiveness and safety of the procedure. Thus, it can be promoted and applied in clinical practice in China.

Key words: ERAS; gynaecological surgery; systematic review; meta-analysis; effect evaluation

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INTRODUCTION

With the development of medical science, surgery has undergone great changes in the past three decades. Traditional open surgery has rapidly developed into minimally invasive surgery dominated by using laparoscopy, single-hole surgery and da Vinci surgical robots. In addition, the management of the perioperative period has gradually shifted to Enhanced Recovery after Surgery (ERAS), which has achieved remarkable results in improving surgical quality, patient satisfaction and health economics [1].

ERAS was first proposed by Danish surgeon Kehlet in 1997, and it is widely used in surgical clinical practice [2]. This concept is a multi-mode optimisation of perioperative treatment based on evidence-based medicine evidence and multi-disciplinary cooperation. By reducing the stress response of the surgical stress level, it avoids the occurrence of serious surgical trauma and organ failure, ensures that patients with normal physiological function, and improves the operation quality as well as patient quality of life. With its promotion in China, the ERAS protocol has achieved good clinical application results in many surgical fields, such as breast, colorectal and gastrointestinal surgery.

As a special clinical department, gynaecology has different characteristics than other departments. Minimally invasive surgery has a significant clinical application effect in the field of gynaecology, which continues to develop and innovate. However, the understanding of the concept of ERAS in the perioperative period is relatively backward and one-sided. Therefore, it is very important to deeply

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understand the concept of ERAS and explore its application effect in gynaecological surgery.

Hence, this study uses the Meta-analysis method to expand the sample size and comprehensively analyse the effectiveness and safety of ERAS concept application in gynaecological surgery, providing a theoretical basis for the clinical practice and promotion of ERAS in the field of gynaecology.

MATERIAL AND METHODS

Retrieval strategy

In accordance with the Cochrane Handbook for Systematic Reviews [3], relevant pieces of literature dated up to September 2021 were systematically retrieved from CNKI, Wanfang and VIP databases. Key words: ERAS; enhanced Recovery after Surgery; gynaecological surgery, etc. In addition, literature that met the inclusion and exclusion criteria was obtained by reading relevant systematic evaluation articles.

Inclusion and exclusion criteria

Inclusion criteria:

1. The subjects were female patients who underwent gynaecological surgery;
2. the intervention measures were gynaecological operations on endometrial cancer, cervical cancer, endometriosis, uterine fibroids, ovarian cancer, gynaecological tumours, etc.;
3. the control group was treated with traditional or conventional strategies;
4. the outcome indicators included hospitalisation time, hospitalisation expenses, postoperative recovery time, and incidence of postoperative complications; and
5. the research types include randomised controlled trials (RCTs).

Exclusion criteria:

1. The research types were descriptive studies, systematic reviews, case reports and other non-original studies;
2. the control groups lacked traditional strategies; and
3. there were no relevant outcome indicators, or the literature was incomplete.

Literature screening and data extraction

Two researchers (Wu XF and Liu LL) independently screened the literature according to the criteria. In case of a disagreement between the two, a third researcher (Zhou F) was invited to discuss and reach a consensus. After literature screening, the data were extracted by two researchers; this included literature information, demographic characteristics of the subjects, surgical methods, related outcome indicators and research types.

Quality evaluation

RCT quality was assessed using the Cochrane bias risk assessment tool [4]. The evaluation items included random sequence generation, assignment concealment, blind method for researchers and subjects, blind method for results, completeness of outcome data, optional reporting of study results and other sources of bias. The evaluation grades of each item were divided into low risk, unknown risk and high risk. Finally, the literature quality was divided into A, B and C according to the evaluation results. Level A represented low deviation, i.e., four or more items that meet quality standards = low risk. Level B represented moderate bias, i.e., meeting two to three quality criteria = low risk. Level C represented high bias, i.e., the quality standard for one or more items = high risk.

Statistical analysis

The statistical analysis was conducted using the Revman 5.3 software. The odds ratio (OR) and weighted mean difference were used to indicate the effect of count data and measurement data, respectively. The 95% confidence interval (CI) was used to estimate effect range. The heterogeneity test used the combination of χ^2 test and I^2 to determine the size of heterogeneity. $I^2 < 50\%$ meant that the included studies were not homogeneous; these were analysed using a random effects model. If heterogeneity was large, subgroup analysis or sensitivity analysis were used. The funnel plot was used to evaluate the publication bias of the analysis if the number of included pieces of literature was ≥ 10.

RESULTS

Characteristics of included literature

Through the search of keywords, a total of 1352 articles were retrieved, and 601 repetitive articles were preliminarily excluded. A total of 479 articles were excluded by reading literature titles, abstracts, conference abstracts, animal experiments and case reports. After further reading the full text, there were 24 articles (excluding non-randomised controlled articles, unreported related outcome indicators, unmet inclusion criteria and incomplete data) [5–28]. The document retrieval flow chart is shown in Figure 1. "Flow chart of document retrieval".

In the 24 studies included in the present report, the ERAS concept was used for perioperative treatment in the experimental group (n = 1632), and the traditional or conventional concept was used for perioperative treatment in the control group (n = 1636). The basic characteristics of the included literature are shown in Table 1.

Quality evaluation results

According to the Cochrane bias risk tool, the quality level of the 24 studies included in this analysis was evaluated: 9 studies = A, 11 studies = B and 4 studies = C. Bias was mainly caused by the absence of a blind method

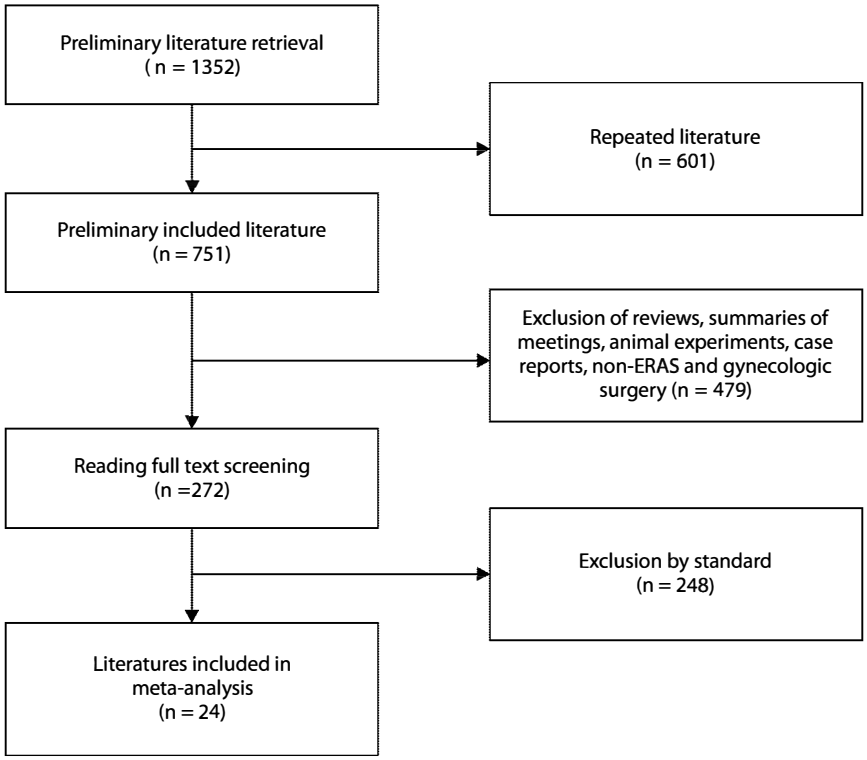

Figure 1. Flow chart of document retrieval

and group hiding. The detailed evaluation results are shown in Table 2.

Meta-analysis results

Hospitalisation time

The results of hospitalisation time were reported in 21 articles. The random-effects model Meta-analysis showed a significantly shorter hospitalisation time in the ERAS group than in the control group (combined effect: SMD = −1.67, 95% CI= −2.03 ~ −1.30, $p < 0.0001$). There was significant heterogeneity between the literature ($I^2 = 95\%$, $p < 0.0001$). The source of heterogeneity was not found using the sensitivity analysis and subgroup analysis, indicating that the results were relatively stable.

The publication bias analysis results showed that the left and right funnel plots were basically symmetrical (Fig. 2). There was no symmetry in certain pieces of literature, suggesting a certain publication bias; however, the risk was small.

Postoperative ambulation time

The results regarding the postoperative ambulation time (h) were reported in 14 articles. The random-effects model Meta-analysis showed a significantly earlier postoperative feeding time in the ERAS group than in the control group (combined effect: SMD = −4.16, 95% CI = −5.12 ~ −3.20, $p < 0.0001$). There was significant heterogeneity between the literature ($I^2 = 98\%$, $p < 0.0001$). The source of heterogeneity was not found using the sensitivity analysis and subgroup analysis, indicating that the results were relatively stable.

The publication bias analysis results showed that the left and right funnel plots were basically symmetrical, indicating that the possibility of publication bias was small (Fig. 3).

Postoperative feeding time

The results regarding the postoperative feeding time (h) were reported in 9 articles. The random effects model Meta-analysis showed a significantly earlier postoperative feeding time in the ERAS group than in the control group (combined effect: SMD = −7.36, 95% CI= −9.67 ~ −5.05, $p < 0.0001$). There was significant heterogeneity between the literature ($I^2 = 99\%$, $p < 0.0001$). The source of heterogeneity was not found using the sensitivity analysis and subgroup analysis, indicating that the results were relatively stable.

Postoperative exhaust time

The results regarding the postoperative exhaust time (h) were reported in 20 articles. The random-effects model Meta-analysis showed significantly earlier postoperative venting in the ERAS group than in the control group (combined effect: SMD = −2.59, 95% CI = −3.15 ~ −2.03, $p < 0.0001$).

Table 1. Basic characteristics of included literature

Author	Year	Sample size		Age		Disease	Modus operandi
		ERAS group	Control group	ERAS group	Control group		
Lian Guomei	2020	46	46	31.48 ± 6.95	32.51 ± 6.84	Cancer	Microtrauma
Zhao Wei	2019	40	40	50.24 ± 1.36	50.24 ± 1.36	Cancer	NR
Yue Fengxian	2018	85	85	39.72 ± 3.10	40.25 ± 3.06	NR	Laparoscope
Cai Bin	2020	100	100	50.14 ± 6.43	50.36 ± 6.29	Cancer	Laparoscope
Xu Jun	2020	53	53	45.37 ± 10.26	44.91 ± 11.22	NR	NR
Gong Guifang	2019	100	100	30.91 ± 2.51	30.88 ± 2.46	Cancer	Laparoscope
Wang Jinmei	2019	64	64	18–60	18–60	NR	Laparoscope
Fang Lingling	2021	60	60	34.8 ± 2.4	35.5 ± 2.2	NR	Laparoscope
Zhou Jingjing	2020	75	75	50.34 ± 4.34	49.89 ± 5.03	Cancer	Microtrauma
LV juping	2021	79	79	51.6 ± 10.7	50.2 ± 12.4	NR	Microtrauma
Liu Lanlan	2020	30	30	52.2 ± 5.7	52.5 ± 5.8	NR	Laparoscope
Chen Dongluan	2020	48	48	45.32 ± 4.97	44.69 ± 4.36	NR	Laparoscope
Yu Yamin	2020	80	80	34.3 ± 4.8	33.6 ± 5.1	NR	Laparoscope
Wang Jing	2018	82	83	47.15 ± 11.43	45.00 ± 10.81	NR	Laparoscope
Jing Wang	2018	52	52	44.07 ± 9.97	42.13 ± 10.12	NR	Laparoscope
Cheng Chuanxi	2017	83	83	43.63 ± 9.02	44.84 ± 8.88	NR	NR
Xiao Lihong	2019	51	51	42.69 ± 7.85	42.17 ± 7.69	NR	Pelvic surgery
Chu Boliang	2020	54	57	37.74 ± 11.45	38.98 ± 13.10	Tumour	Laparoscope
Huang Zhujuan	2012	53	53	37.5 ± 8.5	37.5 ± 8.5	NR	NR
Fan Yinghong	2019	50	50	55.7 ± 12.5	55.9 ± 12.6	Cancer	NR
Zhang Qun	2019	42	42	36 ± 8.5	36 ± 8.8	NR	Laparoscope
Zhi Binlin	2014	100	100	NR	NR	NR	NR
GUI Lingli	2017	55	55	43.5 ± 6.6	43.1 ± 6.5	NR	NR
Qiu Huajuan	2019	150	150	43.7 ± 10.4	42.9 ± 10.1	NR	NR

NR — not reported

There was significant heterogeneity between the literature ($I^2 = 97\%$, $p < 0.0001$). The source of heterogeneity was not found using the sensitivity analysis and subgroup analysis, indicating that the results were relatively stable.

In addition, the results of the analysis of subgroups who underwent minimally invasive gynaecologic surgery showed that there was no significant difference in postoperative exhaust time between the two groups (SMD = −3.04, 95% CI = −7.47 ~ 1.38). The publication bias analysis results showed that the left and right funnel plots were basically asymmetric, indicating a certain publication bias (Fig. 4).

Postoperative defecation time

The results regarding the postoperative defecation time (h) were reported in seven articles. The results of the random effects model Meta-analysis suggested a significantly earlier postoperative feeding time in the ERAS group than in the control group (combined effect: SMD = −2.23, 95% CI = −2.88 ~ −1.57, $p < 0.0001$). There was significant heterogeneity between the literature ($I^2 = 94\%$, $p < 0.0001$). The subgroup analysis showed that laparoscopy heterogeneity was significantly lower than combined heterogeneity ($I^2 = 0\%$) (combined effect: SMD = −1.86, 95% CI = −2.09 ~ −1.62, $p < 0.0001$).

Hospitalisation expenses

The results regarding the hospitalisation expenses (CNY 10,000) were reported in four articles. The random-effects model Meta-analysis showed significantly lower hospitalisation expenses in the ERAS group than in the control group (combined effect: SMD = −0.53, 95% CI = −0.78 ~ −0.28, $p < 0.0001$). The heterogeneity in the literature was acceptable ($I^2 = 54\%$, $p < 0.0001$).

Table 2. Quality evaluation of 24 studies included in the analysis

Author	Year	Random sequence	Blind grouping	Blinding researchers and subjects	Blinding the evaluation	Integrity of outcome data	Results of selective reporting	Other sources of bias
Lian Guomei	2020	Unknown risks	Unknown risks	Low risks	Low risks	Low risks	Low risks	Unknown risks
Zhao Wei	2019	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Low risks
Yue Fengxian	2018	Low risks	Unknown risks	High risks	High risks	Low risks	Unknown risks	Unknown risks
Cai Bin	2020	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Xu Jun	2020	Unknown risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Unknown risks
Gong Guifang	2019	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Wang Jinmei	2019	Unknown risks	High risks	High risks	Unknown risks	Low risks	Unknown risks	Unknown risks
Fang Lingling	2021	Unknown risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Unknown risks
Zhou Jingjing	2020	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Unknown risks
LV juping	2021	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Liu Lanlan	2020	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Chen Dongluan	2020	Unknown risks	Unknown risks	High risks	Unknown risks	Low risks	Low risks	Unknown risks
Yu Yamin	2020	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Wang Jing	2018	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Unknown risks
Jing Wang	2018	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Cheng Chuanxi	2017	Unknown risks	High risks	Unknown risks	Unknown risks	Low risks	Unknown risks	High risks
Xiao Lihong	2019	Unknown risks	High risks	Unknown risks	Unknown risks	Low risks	Low risks	Unknown risks
Chu Boliang	2020	Unknown risks	High risks	Unknown risks	Unknown risks	Low risks	Low risks	Unknown risks
Huang Zhujuan	2012	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Fan Yinghong	2019	Low risks	Unknown risks	Unknown risks	Unknown risks	Low risks	Low risks	Low risks
Zhang Qun	2019	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Unknown risks
Zhi Binlin	2014	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Unknown risks
GUI Lingli	2017	Unknown risks	Unknown risks	Unknown risks	High risks	Low risks	Low risks	Unknown risks
Qiu Huajuan	2019	Unknown risks	Unknown risks	High risks	High risks	Low risks	Low risks	Unknown risks

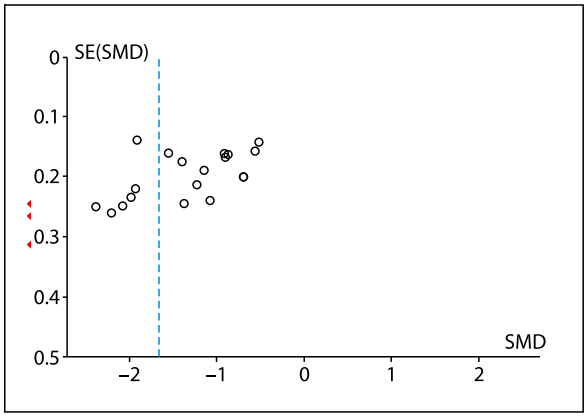

Figure 2. The funnel plot of LOS (d)

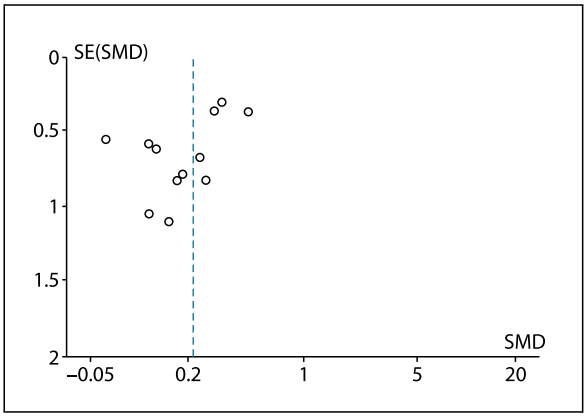

Figure 5. The funnel plot of postoperative morbidity

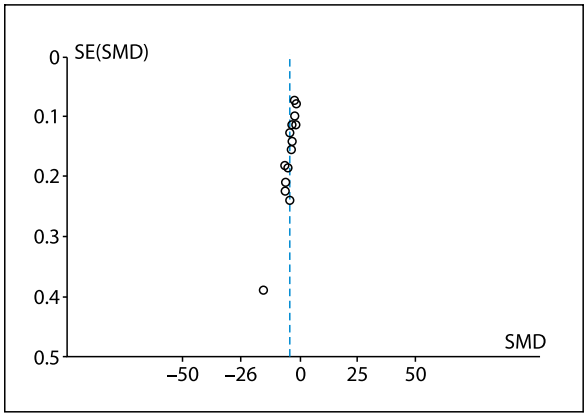

Figure 3. The funnel plot of postoperative ambulation time

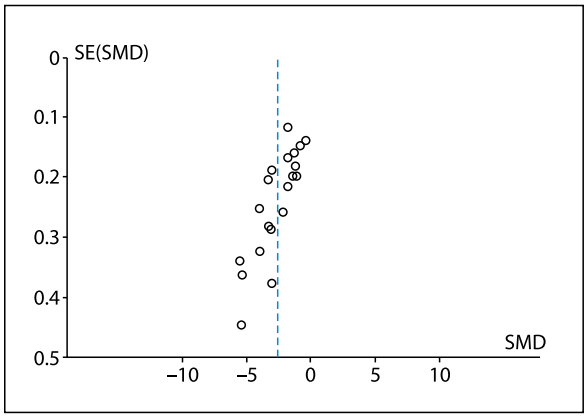

Figure 4. The funnel plot of postoperative exhaust time

Operation time

The results regarding the operation time (min) were reported in five articles. There was significant heterogeneity among the literatures (I^2 = 95%, p < 0.0001). Heterogeneity was significantly reduced after excluding one article after sensitivity analysis [27] (I^2 = 0%, p = 0.851). The meta-analysis results of the random effect model showed no significant difference in operation time between the ERAS group and the control group (combined effect: SMD = −0.07, 95% CI = −0.24 ~ 0.11, p = 0.462).

Intraoperative blood loss

The results regarding the postoperative bleeding volume (mL) were reported in three articles. The random-effects model Meta-analysis showed that the difference in intraoperative bleeding between the ERAS group and the control group was not statistically significant (combined effect: SMD = −1.43, 95% CI = −3.48 ~ 0.63, p = 0.173). There was significant heterogeneity between the literature (I^2 = 98%, p < 0.0001). The source of heterogeneity was not found using the sensitivity analysis, indicating that the results were relatively stable.

Postoperative morbidity

The results regarding postoperative morbidity incidence were reported in 12 articles, with low heterogeneity (I^2 = 19%) and good homogeneity. The fixed-effects model meta-analysis indicated a significantly lower rate of postoperative complications in the ERAS group than in the control group (combined effect: OR = 0.22, 95% CI = 0.15 ~ 0.31, p < 0.0001). The publication bias analysis results showed that the left and right funnel plots were basically symmetrical, indicating a low risk of publication bias (Fig. 5).

Patient satisfaction

The results regarding patient satisfaction incidence were reported in eight articles, with low heterogeneity (I^2 = 0%) and good homogeneity. The fixed effect model Meta-analysis showed that patient satisfaction was significantly higher in the ERAS group than in the control

group (combined effect: OR = 8.11, 95% CI = 4.96 ~ 13.24, p < 0.0001).

DISCUSSION

Improving the safety and effectiveness of surgery and accelerating the postoperative rehabilitation in patients have been the goals of modern medicine for a long time. Many original studies show that the ERAS concept has achieved remarkable results in perioperative nursing with its wide application in various surgical fields in China. At present, its application in gynaecological surgery is becoming more and more widely used.

Original research shows that, compared with traditional or conventional nursing measures, the ERAS concept has certain advantages in all perioperative period aspects. However, few studies systematically analyse all original evidence.

Therefore, the present study systematically analyses original evidence of the ERAS concept compared with traditional or conventional surgical concepts in gynaecological surgery in order to provide a solid theoretical basis for the promotion and application of the ERAS concept in the field of gynaecological surgery in China.

The results of the present study show that the ERAS concept can effectively improve surgery safety and effectiveness; significantly shorten the length of hospital stay and postoperative ambulation, feeding, exhaust and defecation times; reduce hospitalisation expenses and postoperative morbidity; and improve patient satisfaction.

However, no significant difference was found between the ERAS concept and the conventional or routine concept in terms of operative time and intraoperative blood loss. The research results are consistent with the result of previous systematic analyses [29, 30].

In contrast with traditional or conventional surgical concepts, the ERAS concept emphasises cancelling preoperative bowel preparation and reducing excessive consumption before surgery, thus accelerating patient recovery. In addition, the use of the ERAS concept in postoperative analgesia, early postoperative feeding and ambulation promotes patient recovery.

Studies have shown that effective analgesia can reduce postoperative stress response and intestinal paralysis, which is conducive to postoperative activity and eating habits of patients [31]. Early feeding after surgery plays an important role in patient intestinal function recovery [32]. A systematic evaluation of the effect of early feeding on gastrointestinal function showed that, compared with non-feeding after surgery, early feeding significantly shortened the patient hospitalisation time and gastrointestinal recovery time [33].

The recommendation of the ERAS guideline (2019) in the gynaecology/tumour field was reorganised. It was recommended that patients eat general food six hours before anaesthesia, receive a liquid diet 2 h before anaesthesia, and adopt target-directed liquid therapy during the operation.

An appropriate nutritional status should be maintained after operation, and a routine diet should be carried out within 24 h after operation to promote the rapid recovery of intestinal function and improve the operation quality [34]. The results of a study conducted by Relph et al. showed that the total cost of hospitalisation in the ERAS group decreased by 12.7%, saving approximately GBP 176.15 [35]. Furthermore, a retrospective analysis carried out by Pache et al. [36] found that the average total hospitalisation expenses of patients in the ERAS group saved $4381 compared with the traditional concept group. Thus, the ERAS concept can not only promote postoperative recovery, but also has economic benefits in health economics.

There are still several limitations to this study:

1. The heterogeneity of research and analysis is high. Considering that the heterogeneity is derived from research carried out by different institutions, there may be no unified standard in surgical skills and data recording; this can easily lead to bias.
2. It was difficult to implement the blind method in patients and testers included in the study due to disease and surgical method types.

CONCLUSIONS

In summary, the application of the ERAS concept in gynaecological surgery is safe and effective. However, there are still certain limitations to this study, and a large number of large-samples, high-quality and multi-centre RCTs are required to verify the application effect of the ERAS concept in the field of gynaecology.

Conflict of interest

All authors declare no conflict of interest.

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Endometrial regeneration in Asherman's syndrome and endometrial atrophy using Wharton's jelly-derived mesenchymal stem cells

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ABSTRACT

Objectives: Reconstruction of the endometrium in patients with endometrial atrophy and Asherman's syndrome using Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs).

Material and methods: Prospective pilot study, with the inclusion of two patients.

Results: After administration of WJ-MSCs into the uterine cavity, endometrial reconstruction was achieved in both patients. Pregnancy was achieved in one of them, after transfer of a frozen embryo, completed by delivery around the due date.

Conclusions: Endometrial atrophy and Asherman's syndrome, is one of the most frustrating clinical situations we face in assisted reproductive procedures. The use of Wharton's jelly-derived mesenchymal stem cells in restoring the normal function of the endometrium, could become an easy and accessible therapeutic medal, for this endometrial dysfunction, which is so difficult to treat.

Key words: endometrial regeneration; endometrial atrophy; Asherman's syndrome; stem cells; mesenchymal cells; procreation; Wharton's jelly

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INTRODUCTION

The ability to fertilize, develop a pregnancy and give birth to a 20-year-old healthy woman is relatively low and at 20% per cycle [1]. For a woman to become pregnant, not only the gametes (egg cell and sperm) are needed but also the appropriate environment in which the fertilized egg can grow. This environment is the endometrium [2].

To date, the exact mechanism by which the embryo is implanted in the endometrium is not known. The most popular theory is that this happens through signals sent from the embryo to the endometrium and *vice versa*, while suppressing the immune system [3]. A necessary element of this process is proper reactivity of the uterine mucous membrane, which we monitor by measuring its thickness in a transvaginal USG examination [4]. With the use of molecular diagnostics and the expression of 238 genes present on the surface of the endometrium, an individual assessment of the "implantation window" has become possible. Performing embryo transfer at the most optimal time for the embryo has become the basis for increasing the effectiveness of the *in vitro* fertilization procedure [5].

Lack of growth of the endometrium and, consequently, the lack of its reactivity results in repeated embryo implantation failure, a situation in which a properly formed and healthy embryo does not implant in the uterine cavity [6].

The existing methods of therapy involve:

- hormone replacement therapy (estrogen administration);
- improvement of vascular flow by phosphodiesterase type 5 inhibitors administration (Sildenafil) — endometrial scratching;
- controlled damage to the endometrium to stimulate its reactivity;
- surrogacy — transfer of the embryo into the body of another woman (a method prohibited in Poland).

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The methods described above did not give expected therapeutic effects and with variable luck they are propagated as the leading ways to stimulate endometrial growth [6, 7]. In case of iatrogenic damage to the endometrium, the standard treatment for years is hysteroscopic repair [8–10, 11–13]. Malformations within the uterus are one of the causes limiting fertility. It is estimated that they constitute about 5–10% of causes of female infertility [14–16]. In this group, between 2% to 20% of women suffer from Asherman's syndrome or intrauterine adhesions [7, 8]. The term "mesenchymal stem cells" (MSC — mesenchymal stromal cells) is defined as multipotent progenitor cells with the ability to differentiate and mature into cartilage, bone and fat cells [17]. They perform an auxiliary function for other stem cells in the production of connective tissue of particular organs and form the stroma of bone marrow for hematopoietic cells. They also have the ability to modulate functions of the immune system [18]. According to the classification introduced by the International Society for Cellular Therapy, MSC should fulfill three conditions: have the ability to grow *in vitro* in the adherent form, have certain surface antigen expression such as CD13, CD44, CD90, CD73, CD105 with lack of CD14, CD11b, CD79, CD34, CD45 and HLA-DR and be able to differentiate into bone, cartilage and fat tissue [19, 20].

Cells meeting criteria for MSC can be isolated from a number of tissues of both germinal and fetal origins as well as from adult donors [21–25]. In recent years, the most frequently used source of MSC obtained from adults has been bone marrow (BM-MSC — bone marrow derived mesenchymal stromal cells). However, the obtained cell populations were very heterogeneous, of which hematopoietic stem cells were the majority with much smaller percentage of BM-MSC. Recently, adipose-derived stem cells (ADSC) have been gaining popularity. These cells are much more homogeneous cell group than BM-MSC. In addition, the occurrence of MSC in the bone marrow has been demonstrated to be between 1:2,500 and 1:100,000, and ADSC in the adipose tissue population 1:50.

At the fetal stage of development, MSC are present in the blood of the fetus (umbilical cord blood), Wharton jelly, perivascular region, submucosa, umbilical cord, placenta and amniotic fluid. In contrasts, clinically used MSC taken from umbilical cord and Wharton jelly are characterized as easily collectable appropriate amounts of material without ethical issues.

WJ-MSC jelly meet the criteria described above: they are self-renewing and can differentiate into different tissues, not only bone, cartilage and fat, but also into striated muscle cells [26], cardiomyocytes [27], hepatocytes [28, 29], pancreatic Langerhans cells [30–32] and nerve cells [33, 34]. They can also take part in the regeneration of retinal structures [35]. They have human leukocyte antigens class I (HLA-I) on their surface but they do not have HLA class II surface antigens [36–38]. They induce immunosuppressive effect on lymphocytes and inhibit T cell proliferation [39]. The above-mentioned immunological properties of these cells result in both lack of reactions of the recipient's immune system to mesenchymal cell transplantation as well as lack of reaction of allogeneic mesenchymal cells to function in the recipient's tissue system. It is believed that stem cells act as a local tissue repair coordinator. The repair of damaged tissue occurs by regulating endogenous regenerative processes and not by replacing damaged tissue with de novo structures originated from mesenchymal cells [40]. Fetal MSC have greater expansion potential in *in vitro* culture than adult mesenchymal stem cells.

Many authors believe that this is a consequence of two passages of primary hematopoietic cells from the embryo to the placenta and back [41]. The first passage, between 4 and 12 days of embryogenesis, starts through a primitive umbilical cord and hematopoietic cells migrate from the yolk sac to the placenta. Then during the second migration, the MSC are passed in the opposite direction. They return from the placenta to the fetus: to the liver and then to the bone marrow [42]. Researchers believe that during this migration, the mesenchymal cells are trapped in Wharton jelly, where they stay from early embryogenesis throughout pregnancy until delivery. WJ-MSC can interact in the organism of the donor in three independent mechanisms: differentiation into different types of tissues, through the immunomodulatory effect and through the ability to regulate external effects occurring in the environment of MSC, through the secretion of appropriate cytokines and direct contact with other cells. According to recent scientific reports, the most important role of MSC is considered to be the local coordination of tissue repair. The result of interaction between MSC, other cells and tissue regeneration mediators, can possibly adapt MSC signaling to the changing situations. It has been shown, however, that the structures directly created by posterior cells derived from MSC are relatively rarely responsible for the repair of the damaged area [17].

MATERIAL AND METHODS

Our research project is based on the already obtained scientific knowledge, described in more than 300 publications on the use of MSC in human repair processes. Every organ of the human organism to a greater or lesser extent has the ability to regenerate. A logical observation is that the endometrial ability has such ability, since it exfoliates during menstruation with a regularity of about 28 days. A breakthrough in the field of regenerative medicine within the mucous membrane of the uterine cavity was a scientific report by Taylor [42] — describing the regeneration of en-

dometrial cells in patients after bone marrow transplantation, in which during preparation for donor bone marrow cell administration, the mucous cells of uterine cavity are being damaged iatrogenically and loss of its functionality. Based on this discovery, the possibilities of endometrium regeneration began to be studied. The first scientific report describing the use of autologous stem cells in a patient with Asherman's syndrome was published in 2011 [43]. After administration of MSC to the uterine cavity, the endometrium increased above 7 mm, which allowed embryo transfer and development of an intrauterine, single pregnancy. Recently Santamaria et al. [44] described the procedure for the administration of autologous stem cells derived from the bone marrow to the uterine spiral arteries in 16 patients with Asherman's syndrome and endometrial atrophy [44]. All patients underwent endometrial regeneration and four of them became spontaneously pregnant. Stem cells derived not only from peripheral blood or from bone marrow [44, 45] but also from menstrual blood can be used for regeneration of the endometrium [46].

Umbilical cord collection, cell isolation and culturing All Umbilical Cord (UC) samples were obtained after patients provided informed consent, ethical approval was given by Bioethical Committee. UC were collected after natural delivery as well as caesarian sections. Transport conditions were monitored and tissue was processed within 48 h of delivery. Umbilical cord fragments were washed in a sterile saline with Antibiotic-Antimycotic solution (Gibco). Then UC was dissected and blood vessels were removed. Wharton Jelly was minced into 2 mm scraps and placed into culture flasks covered with MSC Attachment Solution (Biological Industries) according to manufacturer's recommendations and grown in serum free medium for human mesenchymal stem cells NutriStem® XF (Biological Industries) with NutriStem ®XF Supplement Mix (Biological Industries) with the addition of Antibiotic-Antimycotic solution (Gibco). Culture was incubated at 37 C in 5% CO2 in the air. Tissue explants were removed after 2–3 weeks of the culture. Adherent cells were passaged upon reaching 90% confluence and reseeded at 1.2×104 cells/cm^2 for further expansion. After trypsinization with Tryple solution (Biological Industries) number of cells were evaluated. When the required number of cells was obtained, they were transferred into a freezing bag and resuspended in 5% solution of human serum albumin (CSL Behring) in the presence of 10% DMSO (WAK-Chemie link), cooled down with controlled rate freezer and then placed in the vapor phase of liquid nitrogen Viability assay Viability was determined based on the thawed reference sample and counted by the trypan blue exclusion in hemocytometer. Immunophenotyping of human umbilical cord MSC Characterization of human umbilical cord derived mesenchymal stem cells (hUC-MSC) was carried out with accordance of minimal criteria of mesenchymal stem cells described elsewhere (Dominici et al. [19]) by immunophenotyping using both MSC-positive and MSC-negative surface markers. Briefly, 60 to 80% confluent flasks of expanded MSC were trypsinized and then incubated with following antibodies in the dark for 30 minutes. Cells were stained with antibodies against: CD34 FITC, CD14 FITC, CD19 FITC, CD 45 FITC, HLA-DR FITC as a negative marker, CD 73 PE, CD90 PE, and CD105 PE. Then cells were acquired and analyzed using a BD FACS CALIBUR cytometer equipped with 488 nm argon-ion laser.

Study design

This first pilot research project was approved by the Bioethics Committee at Centre of Postgraduate Medical Education in Warsaw (67/PB/2016) and sponsored by the Polish Stem Cell Bank in Warsaw. Two patients treated for infertility were included in the study. The first with Asherman's syndrome due to previous curettage of the uterine cavity as a result of postnatal hemorrhage. The first patient was initially twice subjected to hysteroscopic treatment of cutting intrauterine adhesions and then treated with HRT. The patient three times underwent frozen embryo transfer, without getting pregnant. The second patient was diagnosed with endometrial atrophy due to a double resectoscopic dissection of the septum in the uterine cavity.

Both patients were qualified for the study and operated by the same medical team. During menstrual bleeding, both underwent uterine cavity curettage and followed by ultrasound –guided injection of WJ-MSC in a 1 ml saline suspension into the uterine cavity. In order to accelerate the regeneration and growth of the endometrium, both patients received hormonal supplementations within one month and underwent the procedure without any complications.

RESULTS

In the patient with Asherman's Syndrome, before the MSC procedure, the endometrium was uneven and its width was between 3 to 5 mm (Fig. 1). During the first uterine curettage, a material was obtained that was examined and described by the histopathologist: fragments of the endometrium with features of prolonged proliferation in the form of small polyps. Fragments of the basal endometrial layer were hormonally non-reactive. One month after WJ-MSC administration, an increase in endometrium up to 7.6 mm was observed in ultrasound examination (Fig. 2).

The procedure of obtaining material from the uterine cavity was performed again. Histopathological examination revealed the endometrium in the initial phase of secretion. According to the patient's report, there was no significant difference in the duration of menstrual bleeding or its abundance in relation to the situation before the MSC admin-

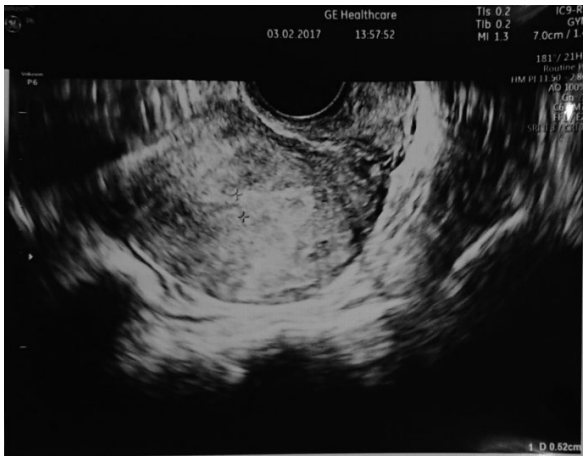

Figure 1. Endometrium of the first patient before the procedure

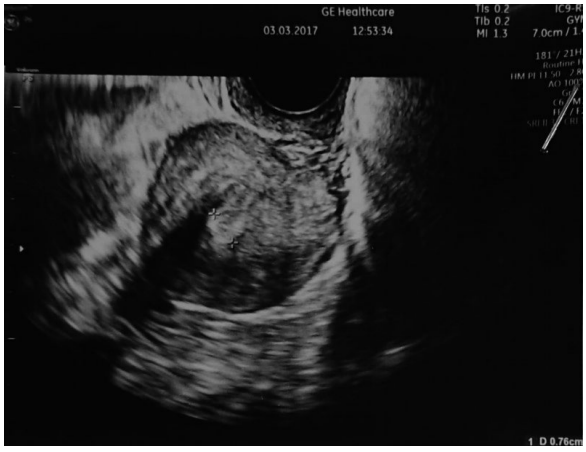

Figure 2. Endometrium of the first patient after MSC administration

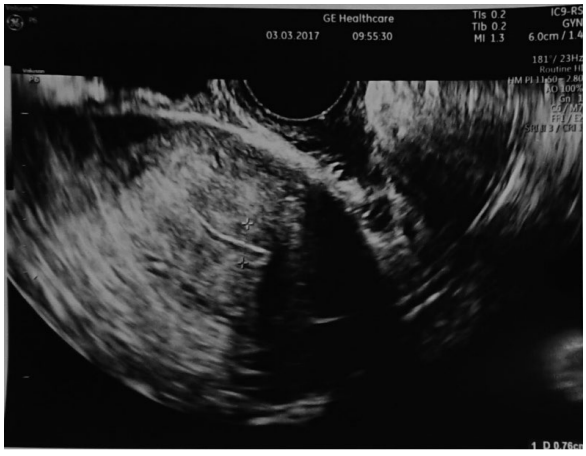

Figure 3. Endometrium of the second patient after MSC administration

istration. The patient re-joined the assisted reproductive treatment. As a result of the frozen embryo transfer, she became pregnant and delivered the baby with a Caesarean section at 34 weeks of pregnancy due to premature labor. The second patient had endometrium 3 mm wide before the MSC procedure in the control USG. In the histopathological examination of curetted material from the uterine cavity, a conclusion has been formed: endometrial fragments from basal layers were non-reactive hormonally. After one month of the MSC intrauterine administration the ultrasound examination showed an increase of endometrium width to 7.6–8 mm (Fig. 3).

In the material obtained from the re-curettage of the uterine cavity, the histopathologist identified fragments of the endometrium with features of prolonged proliferation. The patient reported that after the MSC administration, menstrual bleeding increased, and its abundance increased. The patient underwent assisted reproductive treatment and did not become pregnant due to the accompanying male factor.

DISCUSSION

Endometrial atrophy and Asherman's syndrome are diseases that prevent procreation for women who have been subjected to pre-operative obstetric or gynecological procedures. The patients we qualified for this first pilot study of the allogeneic Wharton's jelly-derived mesenchymal cells regenerative potential in the regeneration of the endometrium, had previously suffered iatrogenic endometrial trauma.

After MSC administration, endometrial hyperplasia was observed already in the first cycle, which was documented not only in the collected medical history of the menstruation but also in the change of endometrial thickness in transvaginal ultrasound examination, as well as histopathological examination of endometrial scrapings obtained before and after MSC administration. Particularly significant is the therapeutic effect obtained in the first patient who had previously performed three unsuccessful transfers of frozen embryos. After the endometrium regeneration and after the next frozen embryo transfer, she became pregnant and gave birth by the Caesarean section.

In the second patient, the intrauterine MSC administration caused permanent repair of the mucous membrane of uterine cavity, observed in the period of one and a half years from the medical procedure (the patient remains under constant care of our team).

Despite the fact that she did not become pregnant (the accompanying male factor of infertility), after each menstruation, the correct endometrial image is observed in the ultrasound examination, without the use of any hormonal therapy. The use of WJ-MSC seems to be a promising way to regenerate the mucosa of the uterine cavity. The use of allogeneic material in both patients did not cause any side effects. This safety was also confirmed by histopathological examination of the obtained uterine scrapings after MSC

administration. Therefore, it seems that the procedure of administrating allogeneic mesenchymal cells, in addition to allogeneic cells from bone marrow, peripheral blood or adipose tissue (not yet published), is a promising method of obtaining endometrial regeneration and may be an indispensable element of infertility treatment in the described groups of patients.

CONCLUSIONS

Endometrial atrophy and Asherman syndrome, is one of the most frustrating clinical situations we face in assisted reproductive procedures. The use of mesenchymal stem cells in restoring the normal function of the endometrium, could become an easy and accessible therapeutic medal, for this endometrial dysfunction, which is so difficult to treat.

Acknowledgments

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Conflict of interest

All authors declare no conflict of interest.

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Measurement of HE4 six months after first-line treatment as optimal time in identifying patients at high risk of progression advanced ovarian cancer

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ABSTRACT

Objectives: The objective of the study was to assess the usefulness of determining HE4 and CA125 in ovarian cancer patients, to indicate which of the measurements may be optimal in the prognosis, depending on the treatment scheme.

Material and methods: The concentrations of CA125 and HE4 were performed in 70 patients with advanced ovarian cancer during I-line therapy and after treatment. The subjects were divided based on the treatment scheme: group I - primary surgery and adjuvant chemotherapy, II- neoadjuvant therapy, and surgery.

Results: Multivariate analysis showed that HE4 levels six months after treatment was significantly higher in patients with disease progression. ROC analysis in the group of patients treated with neoadjuvant therapy showed that the cut-off values indicating relapse for HE4 and CA125 after six months of follow up, were > 90.4 pmol/L, > 25.6 IU/mL, respectively. In the group of patients not treated with neoadjuvant therapy, the cut-off points differentiating patients with progression were: HE4 > 79.1 pmol/L, CA125 > 30.7 IU/mL. We demonstrated significantly higher HE4 and CA125 at both 6- and 12-months follow-up in patients treated with neoadjuvant therapy. In both groups of patients, the cut-off points were lower than those proposed by the manufacturer of the kits.

Conclusions: Measurement of HE4 six months after treatment may be useful in identifying patients at high risk of progression, especially when CA125 levels may be non-specifically elevated. The cut-off values indicating relapse for HE4 and CA125 after six months of follow up may be lower than the normal range.

Key words: CA125; human epididymis 4; ovarian cancer; treatment monitoring; progression

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INTRODUCTION

Ovarian cancer is the second most frequent gynaecological neoplasm [1]. The results of ovarian cancer treatment are unsatisfactory, as most patients are diagnosed with clinically advanced cancer. However, the treatment outcomes depend not only on the severity of the disease, but also on a number of biological and molecular features of the tumour. Much also depends on the experience and skills of the treatment team, and the efficiency of the health care system in each country. Over the last 30 years, significant progress has been made in the treatment outcomes of this cancer and the 5-year survival rate has improved by approximately 15% [2]. Although up to 80% of patients with advanced ovarian cancer achieve remission after the treatment, 65% are diagnosed with recurrence in the first two years [3]. Overall, 75% of patients in stage III and IV, according to the FIGO classification, die of the cancer [4]. Surgical radicalism is one of the most important prognostic factors [5]. Compared to the group when infiltrative changes were left in patients after complete resection (*i.e.*, R0), the 5-year survival results are about 64% higher [6]. Other prognostic factors include family predisposition related mainly to mu-

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tations in the BRCA 1 and 2 genes, and Lynch syndrome, as well as infertility, childlessness, endometriosis, obesity, menopausal age and the use of talcum powder in cosmetics [7]. A mutation in the BRCA 1 gene increases the *in vivo* risk of ovarian cancer by 40–60%, and in BRCA 2 by 11–27% [8, 9]. The factors reducing the risk of this cancer are mainly oral contraceptives and having many children.

The most common and aggressive type of cancer is high grade serous ovarian cancer, which accounts for almost 70% of cases [10]. Almost 20% of cases of this type of cancer have a confirmed family predisposition related to mutations in the BRCA 1 or 2 genes [8]. The second type of serous ovarian cancer is low grade with a completely different clinical course and prognosis. This type is characterized by mutations in the following genes: *BRAF* and *KRAS* [11, 12]. It usually expresses both oestrogen and progesterone receptors, therefore hormone therapy may be effective. Other histopathological types include clear cell, mucous or endometrioid carcinoma.

The cytostatic treatment and maintenance therapy with the use of targeted procedures significantly changed the fate of patients. Still, surgery appears to be a very important stage of treatment. It is debated whether primary cytoreductive surgery or interval surgery after neoadjuvant treatment has an advantage in advanced cases. Disputes on this topic have not ended so far, therefore the results of clinical trials are still pending. The AGO-OVAR study clearly indicates the benefit of primary surgery [6]. However, the assessment of genetic and molecular factors by Riester et al. [13] showed that in certain constellations of molecular factors, radical cytoreduction may not be possible. Thus, starting with systemic treatment may be the most optimal manner.

Proper supervision of patients who have undergone treatment for ovarian cancer is extremely important. In addition to the clinical examination, the results of imaging examinations and monitoring of the value of CA125 and HE4 are also important. The results of these examinations can be used to predict the recurrence and thus allow for timely treatment. HE4 is a new test used in the monitoring of treatment results and follow-up after the therapy. The application of CA125 and HE4 is a very useful tool for the surveillance of patients with ovarian cancer, both during treatment and in post-treatment monitoring. This management is widely recommended by all Societies of Oncological Gynecology and Clinical Oncology.

Objectives

The aim of the study was to assess the usefulness of determining HE4 and CA125 during therapy and flow up of ovarian cancer patients, to indicate which of the time point may be optimal in the prognosis of the disease and determination cut-off points for the tumour markers differentiating patients with progression depending on the treatment scheme.

MATERIAL AND METHODS

We retrospectively analysed data from ovarian cancer patients' disease who were treated at the Gynecological Oncology Department, National Institute of Oncology in Warsaw, in 2017–2019. The study group consisted of 70 patients with epithelial ovarian cancer (EOC) stage FIGO III–IV, aged 40–84 years; median 61 years. In 37 patients (group I), primary surgical treatment was followed by standard systemic treatment, and in 33 patients (group II) neoadjuvant chemotherapy (NACT) was introduced prior to the surgery. All patients received the same cytostatic treatment — Carboplatin AUC 5 and Paclitaxel 175 mg/m^2 every three weeks. A total of six cycles in follow up treatment. For neoadjuvant chemotherapy group, 3/4 cycles before and three cycles after deferred cytoreduction surgery. FIGO stage IV patients additionally received Bevacizumab at a dose of 7.5 mg for 18 cycles. The markers were determined in the blood serum during treatment monitoring at the following time points:

- before treatment (collection 0),
- after surgery/NACT,
- after 3/4 CHTH/NACT courses,
- after 6 CHTH courses (the end of treatment),
- 6 months after the end of treatment,
- 12 months after the end of the first line of treatment.

A total of about 350 blood serum samples were collected for the study. The follow-up time was about 2.5 years. The clinical and pathological characteristics of the study group are presented in Table 1.

Tumor markers were determined in serum samples, stored in low-temperature freezers (–80°C). CA125 and HE4 determinations were performed in a total of 350 serum samples selected during treatment monitoring of 70 patients. CA125 and HE4 concentrations were determined by COBAS e601 system. The cut-off points for CA125 and HE4 were set according to the recommendations of the kit manufacturer. All methods were carried out in accordance with relevant guidelines and regulations.

Statistical calculations used the Statistica PL. 6.0 software for Windows. The Wilcoxon test and the Mann-Whitney U test were used to analyse the differences in variables within and between groups. The impact of clinico-pathological features and biochemical factors on DFS and OS was estimated in the univariate analyses according to the Kaplan-Meier method. Log-rank tests were used for comparisons and the Cox proportional hazards regression model was applied in the multivariate analyses. The diagnostic power of determined parameters was analysed using the MedCalc program. The analysis of the receiver operat-

Table 1. Clinicopathological characteristics of patients with EOC

Characteristics	*Group I n = 37		**Group II n = 33	
Age/years/range	40 - 76		30 - 80	
Median age	61		61	
	n	%	n	%
Menopausal status				
< 50/premenopausal	8/37	22	2/33	6
≥ 50/postmenopausa	29/37	78	31/33	94
Stage/FIGO				
3A-3B	34/37	92	20/33	61
4-4B	3/37	8	13/33	39
Histological Grade/G				
G3	34/37	92	29/33	88
Gx	3/37	8	4/33	12
Clinical status (6 months after treatment)				
Progression/P	10/35	29	14/29	48
Remission/R	20/35	57	14/29	48
Stabilization/SD	5/35	14	1/29	4

*patients primary surgical treatment; **neoadjuvant therapy

ing characteristic (ROC) was applied to determine our own cut-off points for the tested parameters depending on the clinical condition.

We performed a single center, retrospective, observational study according to the ethical standards of the Declaration of Helsinki. The samples were taken after informed consent form all the study participants.

Written informed consent was obtained from all patients before the treatment.

RESULTS

In our study, the concentrations of tumor markers were measured during monitoring and after treatment of patients. The subjects were divided based on the treatment regimen used in the first: group I - primary surgery and adjuvant CHTH, group II- NACT, and surgery.

The comparison of the levels of tumor markers in patients with EOC, depending on the treatment scheme

In both study groups, median concentrations of HE4 and CA125 before treatment were significantly above the cut-off points, but in patients qualified for NAT (group II) the values were several times higher, and for HE4 the differences were significant (p = 0.0004) (Fig. 1).

The comparison of the levels of markers in patients during monitoring with the treatment schedule showed that HE4 concentrations (p = 0.003) were significantly higher in

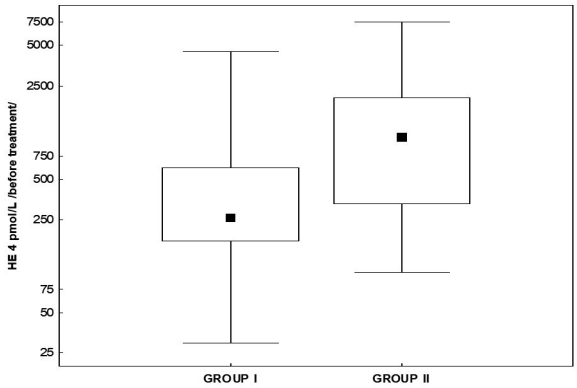

Figure 1. Distribution of HE4 concentrations and medians in patients before treatment, according to the treatment used

patients after NAT than in those after surgery, but no such differences were found for CA125 (Tab. 2).

In both groups of patients, a significant decrease in median markers was observed as a result of the treatment. It was demonstrated that in patients initially treated surgically (group I), the median concentrations of HE4 and CA125 decreased after the surgery by 67% and 66%, respectively, compared to the level before the treatment. In Group II, however, after using NACT, the decrease was much greater and amounted to HE4 by 84% and CA125 by 94%. In Group I, a similar decrease in medians of both markers, HE4 72% and CA125 96%, was observed only after the administration of six courses of adjuvant chemotherapy. In the first group, significantly lower concentrations of both markers, HE4 (p = 0.001) and CA125 (p = 0.003), were noted after surgery, compared to the concentrations before treatment, and significantly lower values of CA125 were reported in the sixth vs third course of CHTH. However, no such a relationship was found for HE4. Similarly, in group II: the concentrations of HE4 (p = 0.002) and CA125 (p = 0.0001) were significantly lower after NACT, compared to the concentrations before treatment. Such a relationship was also found when comparing the values of both markers after the first step of treatment (NACT) and the sixth CHTH course: HE4 (p = 0.009), CA125 (p = 0.021).

The comparison of the levels of markers measured 6 and 12 months after the first line of treatment showed significantly higher values of HE4 (p = 0.003; p = 0.005) and CA125 (p = 0.002; p = 0.003) in patients treated in the first step with NAT, compared with the concentrations observed in surgically treated patients (Tab. 2).

The concentrations of CA 125 and HE4, depending on the clinical status after treatment

In the next step of the study, the concentrations of tumor markers were analysed depending on the clinical condition, which was determined 6 and 12 months after the first line

Table 2. Median levels of CA 125 and HE4 in patients with EOC during follow-up, depending on the treatment scheme

Time points serum collection	Marker	Group I * n = 37	Group II** n = 33	P
		median	median	
Before treatment	CA 125 IU/mL	460.0	947.6	0.12
	HE4 pmol/L	256.5	1027	**0.0004**
After surgery/NACT	CA 125 IU/mL	156.3	61.5	0.32
	HE4 pmol/L	85.1	168.5	**0.003**
After 3/4 CHTH courses	CA 125 IU/mL	16.7	24.2	0.15
	HE4 pmol/L	85.3	110.9	0.051
After 6 CHTH courses	CA 125 IU/mL	16.4	34.9	0.057
	HE4 pmol/L	71.5	83.0	0.048
6 months after treatment	CA 125 IU/mL	10.4	79.4	**0.002**
	HE4 pmol/L	78.2	140.8	**0.003**
12 months after treatment	CA 125 IU/mL	15.8	75.9	**0.002**
	HE4 pmol/L	75.3	110.2	**0.005**

* patients primary surgical treatment; ** neoadjuvant therapy followed surgery

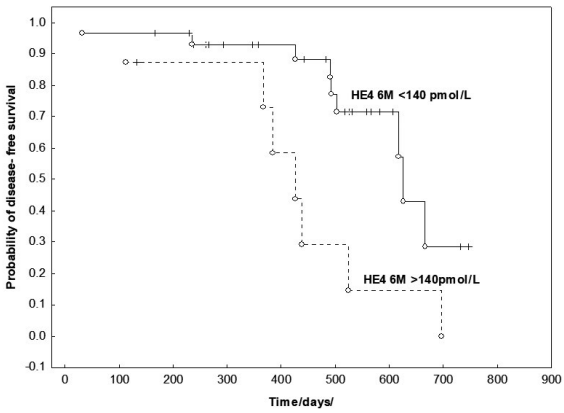

Figure 2. Probability of disease free survival to HE4 concentrations in patients 6 months after completion of first-line treatment

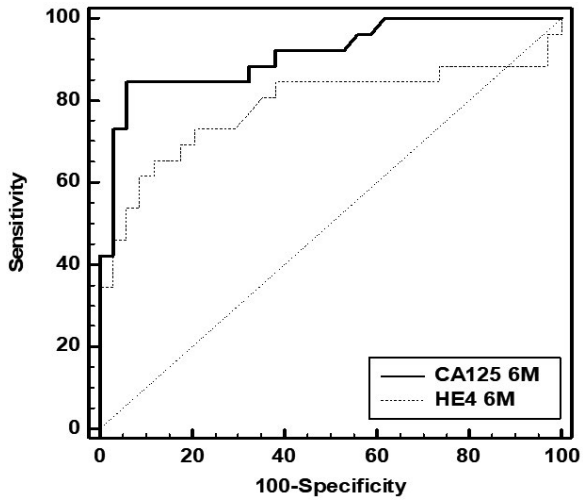

Figure 3. Receiving operation curve for HE4 and CA 125 concentrations determined 6 months after first-line treatment

of treatment. After six months of the follow-up, remission was observed in 62% and progression in 37% of the subjects; after 12 months, the percentage of patients with remission was lower (52%) and with progression higher (49%). As for the treatment method, remission was confirmed in a significantly higher percentage of patients treated surgically, both after 6 (72%) and 12 months (67%), compared to patients treated with NACT (53% and 33%). Five patients died during the follow-up, all of them were treated with neoadjuvant therapy (Group II). The comparison of the concentrations of both markers determined during post-treatment monitoring, both after 6 (CA125 and HE4 p = 0.0001) and 12 months (CA125 p = 0.0001; HE4 p = 0.003) showed significantly higher concentrations in patients with progression.

The univariate analysis demonstrated that only HE4 levels (p = 0.010) 6 months after the treatment (regardless of the regimen) were significantly higher in patients in whom at the end of the follow-up (about 2.5 years), progression was found and confirmed in the Cox multivariate analysis (HR = 2.74, p = 0.026) (Fig. 2).

The assessment of the diagnostic sensitivity of the tests and the analysis of ROC curves (progression vs remission) showed a greater AUC for CA125 at 6 (AUC = 0.913) and 12 (AUC = 0.844) months of the follow-up than for HE4 (AUC = 0.785; AUC = 0.739). These differences were statistically significant (p = 0.032) (Fig. 3).

Serum levels of CA 125 and HE4 in differentiating patients with progression

At the final step of the work, considering the method of treatment, cut-off points were determined for the markers

differentiating patients with progression. The ROC analysis conducted for the group of patients treated in the first surgically showed the cut-off point for HE4 concentrations measured after six months indicating a recurrence (> 79.1 pmol/L) (with sensitivity = 80% and specificity = 72.7%), and CA125 > 30.7 IU/mL (sensitivity = 80% and specificity = 100%). In the group of patients treated with NAT, the cut-off points for markers differentiating patients with progression were HE4 > 90.4 pmol/mL (sensitivity = 100%, specificity = 76.9%) and CA125 > 25.6 IU/mL (sensitivity = 100%, specificity = 86.7%). In both groups of patients, the cut-off points were lower than those proposed by the manufacturer of the kits.

DISCUSSION

Although tumor markers have a recognized position in laboratory oncological diagnostics (especially in oncological gynecology), they are still the subject of research and clinical evaluation in terms of usefulness in patients with malignancies. Determinations of serum markers in the clinical practice shows that they can provide important information regarding, among others, the assessment of sensitivity to the treatment, or the prognosis of disease [14–16].

A recently published meta-analysis proved that the sensitivity of HE4 is 0.86 (95% CI: 0.79–0.91) and specificity is 0.90 (95% CI: 0.49–0.99). The positive predictive value was 8.33 and the negative predictive value was 0.15 [17]. Studies emphasize that the monitoring of patients after treatment with HE4 may be more sensitive than with CA125 [18–20]. The value of CA125 determinations in treatment effect monitoring and post-treatment surveillance has been known and used for years. An increase in this marker, even by several months, may precede the clinical features of cancer progression [21].

In the literature, there is little research on the determination of serum markers in the treatment of patients. The subjects were divided based on the treatment regimen used in the first: group I — primary surgery and adjuvant CHTH, group II — NACT, and surgery. As a result of the treatment, in both groups of patients, a significant decrease was observed in the median concentration of markers, but the decrease was much greater after the use of NACT.

The analysis of the dynamics of changes in the concentrations of the markers during treatment monitoring revealed significant differences in the medians of both markers: in group I, significantly lower values of both markers after surgery only CA125 after the sixth course of adjuvant CHTH. Such relationships were not found for HE4 concentrations. It was similar in group II, significantly lower concentrations of CA125 and HE4 after NACT and then after the sixth course of adjuvant therapy. Chudecka et al. [18] demonstrated significantly lower HE4 values after the neoadjuvant therapy.

The analysis of the concentrations of tumor markers in the groups, I vs II, showed that patients who received NACT in the first step of treatment (group II) had significantly higher values of both HE4 and CA125. The analyses confirm the fact that after the surgical removal of the tumor, the concentrations of both markers are much lower, and their decrease is much greater than in patients after NACT. Therefore, we have shown that the surgical treatment used in the first stage has a greater impact on the reduction of CA125 and HE4 levels compared to neoadjuvant therapy, which may have a prognostic value. Other researchers found that a decrease or even normalization of HE4 levels during the first-line therapy of ovarian cancer may have a beneficial effect on PFS and OS [18].

In the next step of our research, the concentrations of neoplastic markers were analysed depending on the clinical condition assessed at the end of the follow-up. Remission (CR) after 6 and 12 months of the follow-up was confirmed in a much higher percentage of patients treated surgically than in those after NACT.

EOC is, in a way, a chronic disease characterized by relapses and, finally, resistance to treatment [16, 22]. Hence, it is very important to determine the importance of the markers in assessing the response to treatment. Although the literature contains many studies on CA125 and HE4 in ovarian cancer, only a few concern the evaluation of the clinical usefulness of markers in treatment monitoring, and thus focus on the analysis of their concentrations not only before treatment, but also during therapy and follow-up. Therefore, the most important issue of our work was to indicate the marker and determine the time of blood collection during treatment monitoring as important in the prognosis of the disease. We showed that, regardless of the regimen, the elevated HE4 levels six months after treatment, are a prognostic factor for EOC recurrence, but we did not observe such relationships for CA125. Other authors have demonstrated that HE4 levels after cytoreductive surgery are an independent prognostic factor for PFS in both low and high-stage patients during the first-line treatment of EOC [18, 20]. Analysing the concentrations of these markers, Ying et al. [23] found that HE4 was a better predictor of cancer recurrence than CA125 in patients initially operated on in the FIGO III/IV stage. Other researchers have shown a relationship between changes in the concentrations of both markers determined during monitoring and the prognosis of the recurrence of ovarian cancer in operated patients [16, 20].

There are works in which the authors attempt to set their own cut-off points for marker concentrations, differentiating patients in terms of predictive or prognostic value. The cut-off points are very different, and to a large extent depend on the clinical advancement of the study group,

and are most often determined before treatment [22, 23]. In our study, it was important to identify our own cut-off points for HE4 and CA125 (taken 6 months after treatment), which indicated progression, depending on the first-line treatment regimen. The very high sensitivity and specificity of both markers should be emphasized with these cut-off values in the assessment of the clinical status. In both study groups, the cut-off points indicating a relapse of the process were lower than the manufacturer's recommended reagent kits. Thus, it was confirmed that an increase in the concentration of markers during treatment monitoring (especially 6 months after treatment), even below the so-called norm, is most often associated with disease progression, which should be confirmed by imaging examinations.

CONCLUSIONS

In summary, significant changes in the concentrations of both markers during treatment, irrespective of the regimen, correlate with the clinical state, indicate their usefulness in monitoring the response to treatment, with the primary surgery having a greater impact on the decrease of the concentration of the markers. Measurement of HE4 six months after treatment may be useful in identifying patients at high risk of progression, especially when CA125 levels may be significantly nonspecifically elevated (e.g., recurrent ascites). Our own cut-off points for HE4 and CA125 concentrations determined 6 months after treatment may be helpful in differentiating patients with progression, without visible changes in imaging examinations.

Conflict of interest

All authors declare no conflicts of interests related to this article.

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The association between objectively-measured physical activity during pregnancy and the risk of cesarean delivery: a prospective study

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ABSTARCT

Objectives: To evaluate the association between physical activity (PA) and risk of cesarean delivery.

Material and methods: 197 singleton pregnant women recruited in this study. Participants were divided into vaginal and cesarean delivery group. PA based objectively monitoring between the two groups was compared. Logistic regression was used to analyze the association between PA and cesarean delivery.

Results: Moderate PA (MPA) of cesarean delivery group was less in the first (21.5 vs 27.5 min/day; p = 0.006) and second trimester (19.4 vs 26.8 min/day; p = 0.001). Light PA of cesarean delivery group was less (195.9 vs 217.3 min/day; p = 0.006) with more sedentary time (551.7 vs 529.1 min/day; p = 0.041) in the third trimester. Increased risk of cesarean delivery was noted in cases with MPA < 37.8 min/day compared to MPA ≥ 37.8 min/day (aOR 2.62; 95% CI 1.09 to 6.32; p = 0.031) in the first trimester. MPA < 17.9 min/day in the second trimester increased the risk of cesarean delivery (aOR 3.01; 95% CI 1.57 to 5.75; p = 0.001) compared to MPA ≥ 17.9 min/day.

Conclusions: MPA in the first two trimesters were associated with the risk of cesarean section. Women should increase MPA from early pregnancy.

Key words: delivery; physical activity; pregnancy; risk

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INTRODUCTION

Cesarean delivery increased the risk of placenta previa and placenta accreta in second pregnancy, and affected the long-term prognosis of both mother and child [1]. The rate of cesarean delivery has increased from 28.8% in 2008 to 34.9% in 2014 in China [2]. With the implementation of the two-child policy, the multipara and elderly and high-risk women as well as the rate of cesarean delivery had increased [3]. Therefore, interventions aim to reduce the rate of cesarean delivery, especially in primipara¸ should be explored.

Physical activity (PA) is one of the modifiable factors that may improve pregnancy outcomes. PA during pregnancy has beneficial on mother and fetus[4]. But the influence of PA on the risk of cesarean delivery was controversial. A meta-analysis showed that PA during pregnancy and nutritional intervention could reduce the risk of cesarean section [5]. However, research from Karabulut et al. showed that moderate PA during pregnancy has no effect on delivery methods [6]. In addition, few studies have compared the PA differences between vaginal delivery and cesarean delivery during pregnancy.

Few studies explored the effect of objectively PA on the mode of delivery. Therefore, we aim to analyze the difference of objectively measured PA between vaginal delivery and cesarean delivery and explore the relationship between PA and cesarean delivery.

MATERIAL AND METHODS

This data were secondary analysis from a prospective study. Singleton pregnant women at 10–14 weeks of gestation with no contraindications were recruited in the First Affiliated Hospital of Sun Yat-Sen University from March

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2018 to March 2020. The inclusion criteria included first-time participation at 10–14 weeks; age 18 to 40 years; single live fetus without malformation; primipara or the second child pregnant women, and no hypertension, diabetes, or heart disease. The exclusion criteria included twin or multiple pregnancies; the third or more child pregnancy; cervical insufficiency; threatened abortion; placenta previa; chronic diseases such as chronic kidney disease, mental disorders; and refusal to participate in the study. Participants were divided into vaginal delivery group or cesarean delivery group based on delivery outcome. This study was approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-Sen University (2017–296) and followed the tenets of the Declaration of Helsinki and the Good Clinical Practice. All participants had signed informed consent before participating in the study.

The sample size was calculated according to a small-to-moderate correlation (approximately 0.20) with an 80% statistical power and using two-tailed probability level of 5%. The sample size was 213 considering 10% potential lost rate in follow-up.

Data collection

Demographic data including maternal age, parity, way of conception, marital status, history of cesarean section, education level (high school and below, university and above), work status (full-time job, other), annual family income (annually income < 10000 CNY/person as low income, ≥ 10000 CNY/person as high income), weight before pregnancy (kg), height (cm), and smoking status were collected at the first visit. Delivery data including gestation age, mode of delivery (vaginal delivery or cesarean delivery) and birth weight (kg) were collected via the electronic medical record system. And pre-pregnancy body mass index (pre-pregnancy BMI, unit: kg/m^2) was calculated.

PA measurement

A triaxial accelerometer (Actigraph GT3X plus, Actigraph Inc., Florida, USA) was used for evaluation of PA of the three trimesters. Participants were asked to wear accelerometer during the first (10–14 weeks), second (20–24 weeks), and third trimesters (30–34 weeks) for seven consecutive days (including five weekdays and two weekend days) which recorded as T1, T2 and T3. Elastic belt was used to secure accelerometer to right hip for whole day and removed while sleeping and watering activities. Wearing accelerometer more than 10 hours each day was considered as an effective day, and effective days more than five days (at least including four weekdays and one weekend day) were considered as valid data. Accelerometer data were downloaded and analyzed using the Actilife 6.13.3 software (Actigraph Inc., Pensacola, FL, USA). Accelerometer data were integrated through Actilife 6.13.3 software and output as acceleration count (counts per minute, CPM). Zero count recorded by accelerometer for 60 minutes or more was considered as not worn and excluded from the analysis. The cut-off point of Freedom was used to divide PA into four categories: sedentary time (ST) (< 100 CPM), light physical activity (LPA) (100–1951 CPM), moderate physical activity (MPA) (1952–5724 CPM), and vigorous physical activity (VPA) (≥ 5725 CPM) [7].

Participants were divided into vaginal delivery group and cesarean delivery group. The differences of PA between the two groups in the three trimesters were compared, and the effects of PA on the mode of delivery were analyzed.

Statistical analysis

The SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Categorical variables were expressed as values or percentages and compared using a chi square test or Fisher's exact test. Continuous variables with normal distribution were expressed as mean ± SD (standard deviation, SD) and analyzed using an independent t-test. Receiver operator characteristic curve (ROC curve) was used to determine the cut-off point of PA on the incidence of cesarean delivery. The PA cut-off points with a significance value of $p < 0.05$ were classified according to ROC analysis. Binary logistic regression was performed to analyze the association between the cut-off points of PA and the mode of delivery. The model was adjusted for age, pre-pregnancy BMI, history of cesarean section and way of conception and expressed as adjusted odds rate (aOR) and 95% confidence interval (95% CI). AP value of < 0.05 was considered statistically significant.

RESULTS

There were 228 cases recruited in this study. There were 31 participants withdrew (16 in first trimester, four in second trimester, and 11 in third trimester). Finally, 197 pregnant women participated in the study (Fig. 1). There were 120 cases (60.9%) in vaginal delivery group and 77 (39.1%) in cesarean delivery group. The proportion of cases ≥ 35 years in cesarean delivery group was higher than vaginal delivery group (14.3% vs 4.2%; $p = 0.011$). There were 139 primipara (70.6%) and 58 multipara (29.4%). There was no difference in BMI before pregnancy, education, income, smoking, and parity between the two groups (Tab. 1).

The differences of PA between the two groups of the three trimesters were presented in Table 2. MPA was longer in vaginal delivery group than cesarean delivery group in the first and second trimester (27.5 vs 21.5 min/day, $p = 0.006$; 26.8 vs 19.4 min/day, $p = 0.001$). There were no differences in LPA and ST between the two groups in the first and the second trimester; however, LPA was longer in vagi-

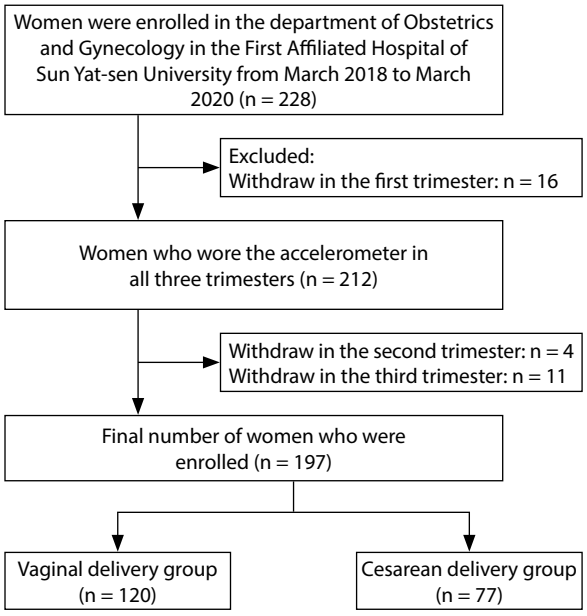

Figure 1. Flow chart of the enrollment process

Table 1. Demographic characteristics of the participants

Characteristics	Vaginal delivery (n = 120)	Cesarean delivery (n = 77)	p
Age [years]			
< 35	115 (95.8%)	66 (85.7%)	
≥ 35	5 (4.2%)	11 (14.3%)	0.011
Pre-pregnancy BMI [kg/m^2]			
< 18.5	29 (24.2%)	13 (16.9%)	
18.5–24.9	85 (70.8%)	54 (70.1%)	0.088
≥ 25.0	6 (5.0%)	10 (13.0%)	
Education			
Less than high school	9 (7.5%)	9 (11.7%)	0.319
Graduate and above	111 (92.5%)	68 (88.3%)	
Income			
Low	66 (55.0%)	36 (46.8%)	0.258
High	54 (45.0%)	41 (53.2%)	
Smoking before pregnancy			
Yes	4 (3.3%)	1 (1.3%)	0.650
No	116 (96.7%)	76 (98.7%)	
Conception way			
IVF	13 (10.8%)	16 (20.8%)	0.055
Normal	107 (89.2%)	61 (79.2%)	
Parity			
0	85 (70.8%)	52 (67.5%)	0.623
1	35 (29.2%)	25 (32.5%)	

Categorical variables were expressed as values or percentages and compared with a chi square test or Fisher's exact test; BMI — body mass index; IVF — *in vitro* fertilization

Table 2. Differences in PA between vaginal delivery and cesarean delivery group

PA		Vaginal delivery (n = 120)	Cesarean delivery (n = 77)	p
T1	**ST (min/d)**	**546.3 ± 77.7**	555.0 ± 72.8	0.429
	LPA (min/d)	213.6 ± 64.7	203.9 + 55.7	0.280
	MPA (min/d)	27.5 ± 17.6	21.5 ± 13.2	**0.006**
T2	ST (min/d)	537.3 ± 74.0	541.9 ± 68.5	0.661
	LPA (min/d)	214.6 ± 65.2	206.7 ± 49.5	0.336
	MPA (min/d)	26.8 ± 18.4	19.4 ± 12.3	**0.001**
T3	ST (min/d)	529.1 ± 76.5	551.7 ± 73.7	**0.041**
	LPA (min/d)	217.3 ± 60.6	195.9 ± 46.8	**0.006**
	MPA (min/d)	23.3 ± 16.5	19.4 ± 14.2	0.088

Continuous variables with normal distribution were expressed as mean standard deviation and analyzed with an independent t-test; LPA — light physical activity; MPA — moderate physical activity; PA — physical activity; SD — standard deviation; ST — sedentary time; T1 — first trimester; T2 — second trimester; T3 — third trimester

nal delivery group than cesarean delivery group (217.3 vs 195.9 min/day; p = 0.006) and ST was lesser in vaginal delivery group than cesarean group in the third trimester (529.1 vs 551.7 min/day; p = 0.041). VPA in the two groups was rare in all three trimesters (< 0.1 min/day) and was not comparable between both groups.

Table 3 shows the ROC analysis results of PA cut-off points on the rate of cesarean delivery. The PA cut-off points for the rate of cesarean delivery were analyzed using ROC analysis with vaginal delivery as the state variable. The cut-off point of MPA in the first trimester was 37.8 min/day [area under the curve (AUC) = 0.59; p = 0.024] and second trimester was 17.9 min/day (AUC = 0.63, p = 0.002). The cut-off point of LPA in the third trimester was 201.0 min/day (AUC = 0.61; p = 0.009) and that of ST in the third trimester was 552.6 min/day (AUC = 0.59; p = 0.035). ST and LPA in the first and second trimesters did not have cut-off points (p > 0.05).

Table 4 illustrates the influence of PA on the risk of cesarean delivery. Considering vaginal delivery group as reference, after adjusting for age, pre-pregnancy BMI, parity and history of cesarean section, binary logistic regression analysis showed that women with MPA < 37.8 min/day increased the risk of cesarean delivery compared to cases with MPA ≥37.8 min/day in first trimester (aOR 2.62; 95% CI 1.09 to 6.32; p = 0.031). Compared to women with MPA ≥ 17.9 min/day, participants with MPA < 17.9 min/day in the second trimester increased the risk of cesarean delivery (aOR 3.01; 95% CI 1.57 to 5.75; p = 0.001). ST and LPA during pregnancy did not increase or decrease the risk of cesarean delivery (p > 0.05).

Table 3. The receiver operator characteristic curve analysis of physical activity cut-off on cesarean section rate

Trimester	T1			T2			T3		
PA	ST	LPA	MPA	ST	LPA	MPA	ST	LPA	MPA
AUC	0.49	0.54	0.59	0.48	0.51	0.63	0.59	0.61	0.57
P value	0.872	0.324	0.024	0.662	0.74	0.002	0.035	0.009	0.087
95% CI									
Lower	0.41	0.46	0.51	0.40	0.43	0.55	0.52	0.53	0.49
Upper	0.58	0.62	0.67	0.57	0.6	0.70	0.66	0.68	0.66

AUC — area under curve; CI — confidence index; LPA — light physical activity; MPA — moderate physical activity; PA — physical activity; ST — sedentary time; T1 — the first trimester; T2 — the second trimester; T3 — the third trimester

Table 4. Binary logistics regression analysis of PA in the three trimesters on the risk of cesarean delivery

Trimesters	PA	aOR*	95% CI	P-value
T1	ST	1.00	0.99–1.01	0.780
	LPA	1.00	0.99–1.01	0.813
	MPA < 37.8 min/d[b]	2.62	1.09–6.32	0.031
T2	ST	1.00	0.99–1.01	0.690
	LPA	1.00	0.99–1.01	0.527
	MPA < 17.9 min/d[c]	3.01	1.57–5.75	0.001
T3	ST ≥ 552.6 min/d[d]	1.53	0.79–2.96	0.207
	LPA < 201.0 min/d[e]	1.78	0.87–3.62	0.112
	MPA	0.99	0.97–1.01	0.219

* adjusted age, pre-pregnancy BMI, way of conception and parity; [b]MPA ≥ 37.8 min/d in the first trimester as reference; [c]MPA ≥ 17.9 min/d in the second trimester as reference; [d]ST < 552.6 min/d in the third trimester as reference; [e]PA ≥ 201.0 min/d in the third trimester as reference; CI — confidence index; LPA — light physical activity; MPA — moderate physical activity; OR — odds rate; PA — physical activity; ST — sedentary time

DISCUSSION

Lack of PA is known to be a risk factor for many adverse outcomes. This study analyzes the impact of PA on delivery model of singleton pregnant women, which is of great significance to pregnant women's physical and mental health and safe delivery.

There were few studies on the difference between vaginal and cesarean delivery of the three trimesters. A prospective study from Baena-García et al. used triaxial accelerometer to monitor PA for seven consecutive days in the second trimester of 94 cases and results showed that ST was shorter while the LPA and MPA were longer in vaginal delivery group compared to the cesarean delivery group, but no difference (ST 503.3 vs 542.2 min/day; LPA 2800.0 vs 2617.1 min/week and MPA 255.4 vs 237.8 min/week, $p > 0.100$) [8]. The researchers only assessed PA and ST in the second trimester; their results could not represent the entire pregnancy. A prospective study by Ko et al. using questionnaire to evaluate PA of 150 primipara and showed that total PA in spontaneous delivery group was longer than unplanned cesarean group (38.76 vs 29.60 hour/week; $p = 0.04$) [9]. However, the authors used questionnaire to evaluate PA and did not analysis the differences of different types of PA between the two groups. Our results showed that MPA was longer in the vaginal delivery group than in the cesarean delivery group in the first and second trimesters (27.5 vs 21.5 min/day, $p = 0.006$; 26.8 vs 19.4 min/day, $p = 0.001$, respectively). LPA in the third trimester was longer in the vaginal delivery group than the cesarean group (217.3 vs 195.9 min/day; $p = 0.006$). These results are different from those of other studies. Our results showed that there was no significant difference in ST between the two groups in the first two trimesters. However, ST in the third trimester was shorter in the vaginal delivery group than in the cesarean group (529.1 vs 551.7 min/day; $p = 0.041$). These results indicate that increasing PA in the first two trimesters and decreasing ST in the third trimester could be helpful in increasing the chances of vaginal delivery.

A few studies have explored the effect of PA on the mode of delivery. Sanda et al. conducted a study involving 606 cases divided into intervention group and control group. The results showed that there was no difference in the rate of cesarean delivery between both groups (2.7% vs 2.4%; $p > 0.05$) [10]. In a study by Ferreira et al., women in the exercise group engaged in PA three times a week for two hours each, from 12–15 weeks of gestation until delivery.

No difference in the rate of cesarean delivery was observed between the exercise and control groups (27.3% vs 32.1%; p = 0.418) [11]. However, a meta-analysis from Domenjoz et al. revealed that PA during pregnancy can lower the risk of cesarean delivery (relative risk 0.85; 95% CI 0.73–0.99) [12]. These studies only established intervention measures but did not assess levels of PA in the intervention groups, and they did not compare the difference of PA between control group and intervention group. Tinius et al. utilized telephone interviews to review PA during pregnancy in 96 obese cases and divided them into active and inactive groups. No difference was observed in the rate of cesarean delivery between the two groups (25.0% vs 31.2%; p = 0.46) [13]. The authors only included obese women. Another study by Koushkie Jahromi et al. using questionnaire to assess PA of 132 women in the third trimester, and showed no difference in the rate of cesarean delivery between exercise and no exercise groups during pregnancy (28.71% vs 30.64%; p = 0.594) [14]. A study by Russo et al. which used questionnaire to evaluate PA of 1313 cases showed that ST in the second and third trimesters increased the risk of cesarean delivery (OR = 1.54, p = 0.05) [15]. A study by Takami et al. used questionnaire to assess PA of 92796 cases during the second and third trimester and noted that the risk of cesarean delivery was higher in the low PA group (OR = 1.07; P = 0.007) [16]. However, Bovbjerg et al. conducted questionnaire via telephone to assess PA of 1205 women at 17–22 weeks and 27–30 weeks of gestation. Their results showed that only total PA at 27–30 weeks was associated with risk of cesarean delivery (β = –0.07, p = 0.049); there was no correlation between moderate-to-vigorous PA and risk of cesarean delivery(p > 0.05) [17]. These studies often used questionnaire to assess PA, with low accuracy and recall bias. Research from Mizgier et al. used triaxial accelerometer to monitor PA of the second trimester of 57 cases. They found no difference in the rate of cesarean delivery between women with MPA > 21.38 min/day and those with MPA < 21.38 min/day (20.69% vs 21.43%; p > 0.05) [18]. But the sample size was small, and they only compared the rate of cesarean delivery. Our study also used triaxial accelerometer to assess PA, but we followed the participants for the three trimesters, and results showed that women with less MPA in the first trimester (aOR 2.62, p = 0.031) and in the second trimester increased the risk of cesarean delivery (aOR 3.01, p = 0.001). These findings indicated that increasing MPA in the first two trimesters would decrease the risk of cesarean section and increase the rate of vaginal delivery. Considering the negative effects of cesarean delivery on maternal and fetal health, this finding had relevant clinical and public health significance. We speculated that increasing MPA from the early pregnancy could reduce the risk of cesarean delivery, mostly because higher MPA would control excessive weight gain during pregnancy and reduce the risk of gestational diabetes mellitus and macrosomia. But our results showed that ST, LPA and MPA of the third trimester were not associated with cesarean section. This indicated that women only decreased ST and increased PA in the third trimester would not help to change the risk of cesarean delivery.

There were some limitations. First, subgroups of pre-pregnancy BMI were not analyzed because of the sample size, but we adjusted for pre-pregnancy BMI attempted to decrease bias. Second, primipara group were not analyzed separately, but we adjusted for parity and history of cesarean section to decrease the bias. Third, all women were from urban areas, the results maybe not be suitable to rural people.

CONCLUSIONS

Less MPA in the first two trimesters were associated with higher risk of cesarean delivery. Pregnant women should target to increase moderate PA from early pregnancy.

Authors' contributions

Hanqing Chen and Wai-Kit Ming contributed to the concept of this article and the analysis and interpretation of data. Hanqing Chen has composed the article draft. Casper J. P. Zhang provided guidance on data processing and revised the manuscript. Wai-Kit Ming and Zilian Wang raised the idea of this article and contributed to the study design and manuscript revision.

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Conflict of interest

The authors declare that they have no conflict of interests to disclose.

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Does predelivery body mass index really matter in pregnancy?

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ABSTRACT

Objectives: The aim of the study was to compare the perinatal outcome between the normal weight, overweight and obese pregnant women who delivered in the third-level center of reference. Moreover, the objective was to analyze the usefulness of predelivery body mass index (BMI) in prediction of preterm delivery, prolonged second stage of labor, instrumental vaginal delivery, cesarean section, fetal macrosomia, dystocia and newborn acidosis.

Material and methods: The retrospective study included 2104 patients, divided into three groups, with BMI between 18.5 and 24.9; 25.0 and 29.9; higher than or equal 30.0 kg/m^2, respectively. The data were assessed from the medical history.

Results: The predelivery obesity increases the risk of cesarean section (aOR 1.63), macrosomia (aOR 8.89) and dystocia (aOR 3.40) in comparison to normal weight women. Moreover, the obese females had three times greater risk of having a macrosomic child (aOR 3.57) and 1.5 times greater risk of cesarean section (aOR 1.52) than overweight group. The role of predelivery BMI in the prediction of cesarean delivery (AUC 0.550; sensitivity 0.39; specificity 0.71, $p < 0.001$, cut-off value 28.7 kg/m^2), macrosomia (AUC 0.714; sensitivity 0.66; specificity 0.70; $p < 0.001$, cut-off value 29.0 kg/m^2) and dystocia (AUC 0.658; sensitivity 0.77; specificity 0.53, $p < 0.001$, cut-off value 27.0 kg/m^2) was significant.

Conclusions: The predelivery obesity increases the risk of cesarean section, macrosomia and shoulder dystocia and is a useful parameter in the prediction of perinatal outcomes. The establishing cut-off value for predelivery BMI was the lowest in prediction of shoulder dystocia.

Key words: predelivery body mass index; perinatal outcomes; maternal obesity; maternal overweight; newborn weight; cesarean section

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INTRODUCTION

According to the World Health Organization (WHO) report, the worldwide prevalence of obesity nearly tripled between 1975 and 2016 and it is considered a global epidemic [1]. Approximately 27% and 37% of reproductive-aged women in the United States are overweight and obese, respectively [2–4]. One of the main reasons for so high widespread, which has influenced the economic condition of health system worldwide, is the modern lifestyle related to an unbalanced calorie intake and insufficient physical activity [5]. That exposes most fetuses to maternal overnutrition and high-fat diet during the key period of *in utero* development [6]. Despite the overweight and obesity rates are lower in Europe, the trend is also increasing, suggesting occurrence of maternal obesity above 20% in six European countries [7]. Moreover, about 50–60% of overweight and obese pregnant women gain on weight more than Institute of Medicine recommends, what leads to the postpartum weight retention, later cardiovascular diseases and inappropriate body mass index (BMI) in future pregnancies [8].

Several studies underline the influence of high maternal BMI in pregestational or gestational period on obstetric and post-partum complications, such as gestational diabetes mellitus (GDM) [9, 10], pregnancy induced hypertension (PIH), preeclampsia (PE) [11, 12], cesarean sections [13],

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urinary and genital tract infections, post-partum wound infection, chorioamnionitis [14–16], venous thromboembolism [17], breastfeeding difficulties [18] and depression [19].

Despite many perinatal and post-partum consequences, the maternal obesity impacts also fetal programming and effects in offspring disorders [20]. It increases the neonatal morbidity because of preterm deliveries and congenital anomalies [8] but also in future life offspring are themselves at higher risk of obesity and cardiometabolic morbidity [21, 22]. As mentioned above, maternal obesity increases the risk of GDM, what correlates positively with adiposity in both young and adult offspring. Saucedo et al. found that maternal adipokines and inflammatory cytokines secreted from adipose tissue in GDM, such as C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-alpha), may regulate fetal growth [23].

The long-term consequences of prenatal and lactational exposure to maternal excessive weight and inappropriate nutrition are psychiatric and neurodevelopmental offspring's diseases which include cognitive impairment, autism spectrum disorders, attention deficit hyperactivity disorder, cerebral palsy, anxiety, depression, schizophrenia and eating disorders [24]. Furthermore, the maternal obesity affects the maturation and development of the newborn immune system increasing the susceptibility to pathogens, changing the vaccines response and resulting in immunopathological disturbances, such as development of asthma or allergy [25].

Objectives

The aim of this study was to compare the perinatal outcome between the normal weight, overweight and obese pregnant women, who delivered in the third-level reference center. Moreover, the objective was to evaluate predelivery BMI in prediction of preterm delivery, prolonged 2nd stage of labor, instrumental vaginal delivery, cesarean section, fetal macrosomia, dystocia, and newborn acidosis. Furthermore, the risk of preterm labor, cesarean delivery, macrosomia, and dystocia was assessed.

MATERIAL AND METHODS

The retrospective study included 2104 women, who gave birth to a child in the Obstetrics and Gynecology Hospital of Medical Sciences University in Poznan. The patients were divided according to predelivery BMI into three age-matched groups, as follows: the I group consisted of 614 women with BMI between 18.5 and 24.9, the II group included 964 patients with BMI ranged 25.0 to 29.9 and the III group was composed of 526 females with BMI equal or greater than 30.0 kg/m^2. The BMI was measured in the antepartum period, what constitutes some limitation of the study due to the lack of established norms for BMI in pregnancy. The gravidity and parity were comparable between groups.

The characteristics of study groups are presented in the Table 1. The data were collected from medical history. The analyzed parameters included age, predelivery BMI,

Table 1. The general characteristics of study groups

Characteristics	Group I (n = 614)	Group II (n = 964)	Group III (n = 526)	p
Age [years] (Mean ± SD)	30 ± 5	31 ± 5	31 ± 5	* ns ** ns *** ns
Predelivery BMI [kg/m^2]	23.3 ± 1.3	27.2 ± 1.4	33.5 ± 3.5	* **< 0.01** ** **< 0.01** *** **< 0.01**
Gravidity (n) (Median, Min-Max)	2 (1–7)	2 (1–11)	2 (1–8)	* ns ** ns *** ns
Parity (n) (Median, Min-Max)	0 (0–6)	0 (0–9)	1 (0–5)	* ns ** ns *** ns
PIH [%]	1.1	2.3	11.6	* **< 0.05** ** **< 0.0001** *** **< 0.0001**
GDM [%]	6.0	7.6	8.8	* ns ** ns *** ns
FGR [%]	3.3	2.3	2.9	* ns ** ns *** ns

SD — standard deviation; ns — not significant; BMI — body mass index; PIH — pregnancy induced hypertension; GDM — gestational diabetes mellitus; FGR — fetal growth restriction

pregnancy complications such as PIH, GDM, fetal growth restriction (FGR), the term and mode of delivery, duration of the 1st and the 2nd stage of labor, perinatal blood loss, perinatal hemorrhage, perineal incision and rupture, newborn weight, Apgar score, umbilical venous and artery pH and base excess (BE). The gestational age was set according to the last menstrual period or ultrasound examination from the 1st trimester of pregnancy. The preterm delivery was diagnosed between 22 and 36 + 6-weeks' gestation. The prolonged second stage of labor was defined as: for nulliparous women > 3 h with epidural anesthesia or > 2 h without it, for multiparous women > 2 h with epidural anesthesia or > 1 h without it. The postpartum hemorrhage was determined as blood loss of equal or more than 500 mL during spontaneous or assisted vaginal delivery and equal or more than 1000 mL during cesarean section. The macrosomia was diagnosed as the birth weight equal or above 4500 g. The newborn acidosis was diagnosed as umbilical arterial pH below 7.2.

The statistical analysis was performed in GraphPad InStat 3, Statistica StatSoft 13.1, MedCalc 19.5.3 and PQStat 1.8.0. The Kruskal-Wallis test (nonparametric ANOVA) with subsequently Dunn's Multiple Comparisons was used to analyze the results in interval and ordinal scale. Mean (M) and standard deviation (SD) were used to describe the interval variables, while Median, Minimum (Min), Maximum (Max) value referred to ordinal data. The results in nominal scale were analyzed using the Chi-square test and presented in percentages. The regression analysis was described using coefficient of multiple correlation (R) and adjusted coefficient of determination (R^2_{adj}). The unadjusted odds ratios (ORs) and adjusted odds ratios (aOR) were calculated using logistic regression and the Wald Chi-square test. The 95% confidence interval (95% CI) was designated to estimate the precision of the ORs and aORs. The usefulness of predelivery BMI in the prognosis of preterm delivery, prolonged second stage of labor, instrumental vaginal delivery, cesarean section, fetal macrosomia, dystocia, and newborn acidosis was specified with receiver operating curve (ROC). The prediction analysis concerned area under curve (AUC), sensitivity, specificity, and cut-off value. The significance level for all calculations was assumed as p-value below 0.05.

RESULTS

The normal weight women delivered significantly earlier comparing to overweight (38.3 ± 2.6 vs 38.7 ± 2.4 weeks, $p < 0.001$), and obese females (38.3 ± 2.6 vs 38.7 ± 2.2 weeks, $p < 0.001$). The frequency of preterm labor was statistically higher among normal weight group comparing to the overweight one (11.4 vs 6.9%, $p < 0.01$) (Tab. 2).

The spontaneous vaginal delivery occurred significantly more often in group I comparing to III (60.3 vs 47.5%, $p < 0.0001$), and in group II comparing to III (57.1 vs 47.5%, $p < 0.001$). The incidence of instrumental vaginal deliveries did not differ between groups. Cesarean sections were observed significantly more often in the obese group compared to normal weight (42.6 vs 32.4%, $p < 0.001$), and overweight females (42.6 vs 32.4%, $p < 0.0001$) (Tab. 2).

The first stage of labor lasted significantly longer in overweight women compared to the obese ones (4.6 ± 4.0 vs 4.1 ± 4.3 hours, $p < 0.05$). The second stage of labor was also longer in group II when compared to the I (36 ± 34 vs. 33 ± 34 minutes, $p < 0.05$) (Tab. 2).

In the obese patients, greater perinatal blood loss was noticed compared to the normal weight women (356 ± 154 vs 321 ± 132, $p < 0.001$). The incidence of postpartum hemorrhage, perineal incision and perineal rupture was comparable between groups (Tab. 2).

The newborns of obese mothers had significantly higher birth weight compared to overweight (3451 ± 648 vs 3383 ± 560 g, $p < 0.05$), and normal weight group (3451 ± 648 vs 3197 ± 616 g, $p < 0.001$). Also, overweight women gave birth to larger children compared to females with normal predelivery BMI (3383 ± 560 vs 3197 ± 616 g, $p < 0.001$) (Tab. 3). In regression analysis, the birth weight was dependent on predelivery BMI and gestational age at delivery ($R = 0.65$, $R^2_{adj} = 0.43$, $p < 0.000001$).

The frequency of 5-minute Apgar score < 7 points was comparable in all analyzed groups. The newborns of normal weight mothers more often showed the 1-minute Apgar < 7 points than those from overweight group (6.0 vs 3.9%, $p < 0.05$). Though, the offspring of normal weight women had higher arterial pH (7.26 ± 0.09 vs 7.25 ± 0.08, $p < 0.05$) compared to overweight group. Furthermore, the newborns of normal weight females had significantly higher venous (7.34 ± 0.08 vs 7.33 ± 0.09, $p < 0.01$), and arterial pH (7.26 ± 0.09 vs 7.25 ± 0.08, $p < 0.05$) compared to the group with obesity. The umbilical venous or arterial BE, and the incidence of newborn acidosis did not differ between groups (Tab. 3).

The overweight (aOR = 0.55; $p < 0.01$) females, in the multivariable logistic regression model controlling for PIH and FGR, had nearly 40% reduced chance of preterm delivery, if we compared to normal weight group. Significant difference in occurrence of preterm delivery was also observed between the group III and I. The obese women were approximately 40% less likely to deliver prematurely (aOR = 0.59; $p < 0.05$) (Tab. 4). After adjustment for gestational age at delivery, PIH and FGR, the logistic regression analysis revealed that predelivery BMI ≥ 30.0 kg/m^2 was independent predictive factor for cesarean delivery com-

Table 2. Comparison of labor outcomes between study groups

Parameters	Group I (n = 614)	Group II (n = 964)	Group III (n = 526)	p
Gestational age at delivery [weeks]	38.3 ± 2.6	38.7 ± 2.4	38.7 ± 2.2	* **< 0.001** ** **< 0.001** *** ns
Preterm delivery [%]	11.4	6.9	8.6	* **< 0.01** ** ns *** ns
Spontaneous vaginal delivery [%]	60.3	57.1	47.5	* ns ** **< 0.0001** *** **< 0.001**
Instrumental vaginal delivery [%]	9.3	12.1	10.8	* ns ** ns *** ns
Cesarean section [%]	32.4	32.4	42.6	* ns ** **< 0.001** *** **< 0.0001**
1st stage of labor [hours]	4.1 ± 3.6	4.6 ± 4.0	4.1 ± 4.3	* ns ** ns *** **< 0.05**
2nd stage of labor [min]	33 ± 32	36 ± 34	33 ± 32	* **< 0.05** ** ns *** ns
Prolonged 2nd stage of labor [%]#	2.4	2.8	3.3	* ns ** ns *** ns
Perineal incision [%]#	63.6	67.5	62.2	* ns ** ns *** ns
Perineal rupture [%]#	6.0	9.2	9.8	* ns ** ns *** ns
Blood loss [mL]	321 ± 132	342 ± 161	356 ± 154	* ns ** **< 0.001** *** ns
Postpartum hemorrhage [%]	5.1	6.9	5.7	* ns ** ns *** ns

*comparison between groups I–II; **comparison between groups I–III; ***comparison between groups II–III; #it refers to vaginal deliveries

pared to overweight (aOR = 1.52, $p < 0.001$), and normal BMI (aOR = 1.63, $p < 0.001$) (Tab. 4). Moreover, the logistic regression, after correction for gestational age at delivery, showed that obese females were nearly 9-fold (aOR = 8.89, $p < 0.01$) more likely to deliver a macrosomic newborn, when we compared to normal weight women, and about 3.5-fold (aOR = 3.57, $p < 0.01$) more probable than overweight ones (Tab. 4). The predelivery obesity was also associated, after adjustment for gestational age at delivery and macrosomia, with more than 3-fold (aOR = 3.40, $p < 0.05$) higher chance of shoulder dystocia than normal predelivery BMI group (Tab. 4).

The role of predelivery BMI in the prediction of cesarean delivery (AUC 0.550; sensitivity 0.39; specificity 0.71, $p < 0.001$, cut-off value 28.7 kg/m^2) (Fig. 1), fetal macrosomia (AUC 0.714; sensitivity 0.66; specificity 0.70; $p < 0.001$, cut-off value 29.0 kg/m^2) (Fig. 2), and shoulder dystocia (AUC 0.658; sensitivity 0.77; specificity 0.53, $p < 0.001$, cut-off value 27.0 kg/m^2) (Fig. 3) was found. The predelivery BMI was not useful in the prognosis of preterm delivery, prolonged second stage of labor, instrumental vaginal delivery, and newborn acidosis.

DISCUSSION

Although the relationship between perinatal outcome, and prepregnancy BMI or gestational weight gain has been defined, only a few studies analyzed the impact of maternal BMI in the antenatal period on labor, and neonatal outcome.

Table 3. Comparison of neonatal outcomes between study groups

Parameters	Group I (n = 614]	Group II (n = 964]	Group III (n = 526]	p
Birth weight [g]	3197 ± 616	3383 ± 560	3451 ± 648	* **< 0.001** ** **< 0.001** *** **< 0.05**
1-minute Apgar < 7 points [%]	6.0	3.9	4.4	* **< 0.05** ** ns *** ns
5-minute Apgar < 7 points [%]	3.8	2.3	2.5	* ns ** ns *** ns
Umbilical venous pH	7.34 ± 0.08	7.33 ± 0.08	7.33 ± 0.09	* ns ** **< 0.01** *** ns
Umbilical venous BE	–3.67 ± 3.04	–3.99 ± 3.00	–3.87 ± 2.88	* ns ** ns *** ns
Umbilical arterial pH	7.26 ± 0.09	7.25 ± 0.08	7.25 ± 0.08	* **< 0.05** ** **< 0.05** *** ns
Umbilical arterial BE	–4.15 ± 3.37	–4.35 ± 3.35	–4.23 ± 3.49	* ns ** ns *** ns
Newborn acidosis [%]	23.9	28.2	25.7	* ns ** ns *** ns

* comparison between groups I–II; ** comparison between groups I–III; *** comparison between groups II–III; BE — base excess

Table 4. The association between predelivery BMI and perinatal outcomes expressed as the ORs and aORs for obese and overweight women

Parameters	III vs II		III vs I		II vs I	
	OR (95% CI) p-value	aOR (95% CI) p-value	OR (95% CI) p-value	aOR (95% CI) p-value	OR (95% CI) p-value	aOR (95% CI) p-value
Preterm delivery	1.25 (0.85–1.86) ns	1.08 (0.71–1.64) ns	0.73 (0.49–1.08) ns	0.59 (0.39–0.91) **< 0.05**	0.58 (0.41–0.82) **< 0.01**	0.55 (0.38–0.78) **< 0.01**
Cesarean section	1.55 (1.24–1.93) **< 0.0001**	1.52 (1.21–1.90) **< 0.001**	1.55 (1.22–1.97) **< 0.001**	1.63 (1.26–2.10) **< 0.001**	0.99 (0.80–1.24) ns	1.07 (0.85–1.34) ns
Macrosomia	2.89 (1.35–6.23) **< 0.01**	3.57 (1.57–8.09) **< 0.01**	6.80 (1.98–23.34) **< 0.01**	8.89 (2.03–38.82) **< 0.01**	2.35 (0.65–8.46) ns	2.49 (0.53–11.59) ns
Dystocia	1.62 (0.78–3.34) ns	1.80 (0.82–3.96) ns	4.17 (1.36–12.75) **< 0.05**	3.40 (1.08–10.62) **< 0.05**	2.57 (0.86–7.74) ns	1.88 (0.60–5.81) ns

Models controlling for: preterm delivery — PIH, FGR; cesarean section — gestational age at delivery, PIH, FGR; macrosomia — gestational age at delivery; dystocia — gestational age at delivery, macrosomia; OR — odds ratios; CI — confidence interval; aOR — adjusted odds ratios

In addition, some researchers focused mainly on the super obesity defined as the BMI > 50 kg/m^2 or weight > 140 kg at any point of pregnancy in 20 or more weeks of gestation [26–28].

We proved the role of predelivery BMI in the prediction of cesarean section but the sensitivity was very low (39%) and specificity was 71%. The cut-off value was set as BMI 28.7 kg/m^2. Morais et al. [29] observed higher risk of cesar-

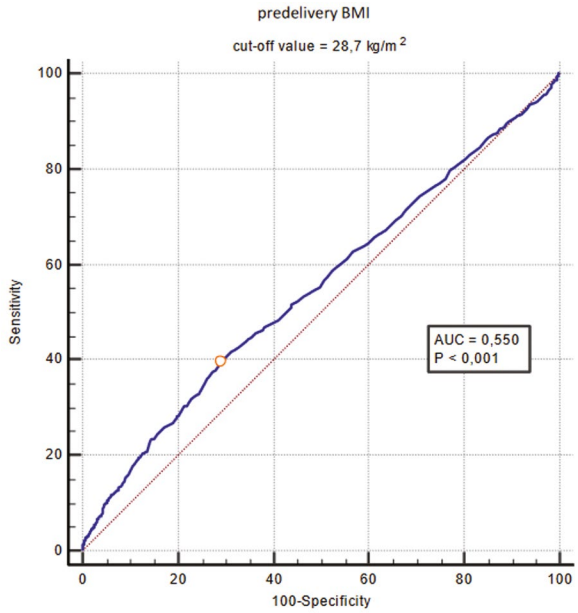

Figure 1. Predelivery body mass index in the prediction of cesarean section; AUC — area under curve

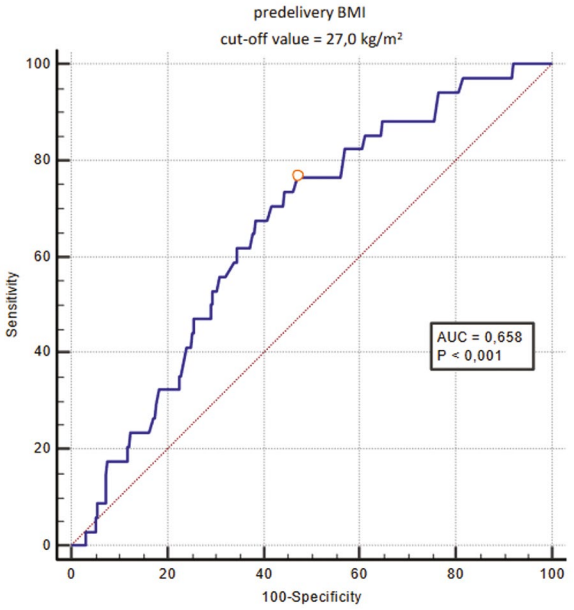

Figure 3. Predelivery body mass index in the prediction of shoulder dystocia; AUC — area under curve

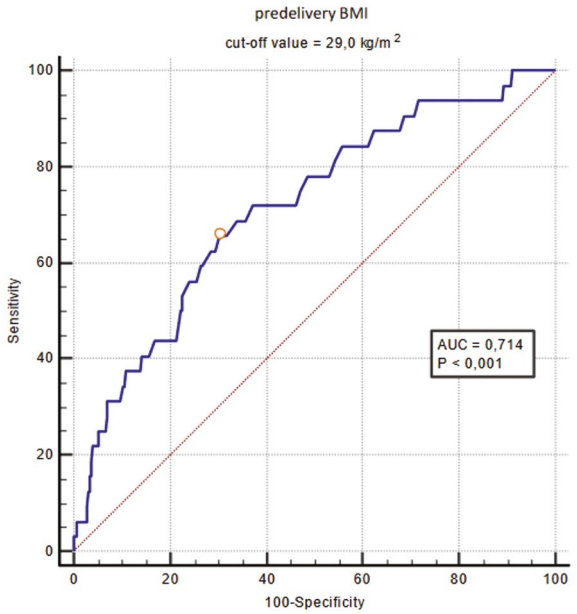

Figure 2. Predelivery body mass index in the prediction of fetal macrosomia (birth weight ≥ 4500 g); AUC — area under curve

ean section (OR = 1.97) in women, who were classified as normal weight on the first prenatal visit, and until the last prenatal examination, increased in the BMI classification. Also, firstly overweight patients, who significantly gained on weight during pregnancy and became obese, had a higher chance of cesarean delivery (OR = 2.28) comparing to constantly overweight women [29]. In our study, obesity raised the chance of cesarean delivery nearly 1.5-fold comparing to as well overweight (aOR = 1.52) or normal weight women (aOR = 1.63). Arora et al. [30] also noticed the association between predelivery BMI, and higher risk of cesarean section. The authors emphasized that high prepregnancy BMI or high gestational weight gain stronger affected the risk of cesarean delivery. Sullivan et al. [27] observed, in extremely obese women, the higher risk of obstetric complications (aOR = 2.42), including higher frequency of cesarean delivery (51.6 vs 31.7%) comparing to the representative group for general population in Australia and New Zealand. The McCall's et al. [26] international collaborative study proved the significantly higher chance of cesarean section in the group of women with super obesity (aOR = 2.77). Alanis et al. [28] presented similar results, and observed the highest rate of cesarean delivery among extremely obese patients comparing to less obese women (56.0 vs 30.9%, aOR = 2.86).

Neonatal macrosomia is diagnosed in about 10% of all pregnancies, what is much higher incidence than in our study (1.5%). In the literature there are several definitions of macrosomia. Birth weight of 4000–4500 g or greater than 90th percentile for gestational age (with correction for neonatal sex, and ethnicity) is presented. This can lead to discrepancies in the assessment of the frequency of this pathology. Moreover, several studies refer to the frequency of the prenatally diagnosed macrosomia, but this condition must be obligatory confirmed after delivery [31]. The obese, and overweight women delivered newborns of higher birth weight, which was associated with predelivery BMI and gestational age at delivery. Our research revealed the useful-

ness of predelivery BMI in the prognosis of fetal macrosomia with sensitivity of 66%, and specificity of 70%. The cut-off value was set as maternal BMI equal 29.0 kg/m^2. Asplund et al. reported the incidence of macrosomic newborns about 15.6%. 86.2% of these mothers had equal or greater than 25% increase in BMI during pregnancy. These raises of maternal BMI revealed sensitivity of 86.2% and specificity of 93.6% in prediction of macrosomia. After adjustment for maternal age, race, parity, and gravidity, these women had more than 200 times (aOR = 219.3) higher probability for delivering macrosomic newborn [32]. Swank et al. [33] reported that women with moderate and excessive BMI changes in pregnancy had aOR of 1.66 and 3.21, accordingly, for macrosomia in comparison to females with minimal BMI change. The study of Sullivan et al. [27] observed that extremely obese patients delivered newborns with birth weight equal or more than 4500 g significantly more often than normal weight women (9.8 vs 0.8%). McCall's et al. [26] international collaborative study proved the increased risk for fetal macrosomia in superobese women. Furthermore, Alanis et al. [28] observed that extreme obesity increased 3.5- and 2-fold the risk of delivering the large for gestational age (LGA) infant compared to non-obese patients and less obese women, respectively. In comparison, our study revealed, after adjustment for gestational age at delivery, about 3.5-fold (aOR = 3.57), and nearly 9-fold (aOR = 8.89) higher chance of postanal macrosomia in obese women comparing to overweight and normal weight females, accordingly. Morais et. al. [29] classified pregnant women on the first and last prenatal visit as low weight, adequate weight, overweight, and obese. The researchers reported that patients whose BMI acquired an increase in the classification, according to the Atalah curve, from the first until the last prenatal visit, had a higher chance of delivering LGA newborn (OR = 2.88). Also, women with firstly adequate BMI, who increased in their classification, were nearly four times more likely to give a birth to a macrosomic infant (OR = 4.13). Furthermore, overweight women, who evolved an increase of their BMI, had raised odd ratio of fetal macrosomia (OR = 12.54) than those with stagnant BMI [29].

To our knowledge, until now no study evaluated the association between predelivery BMI, and shoulder dystocia. Our results proved the usefulness of maternal BMI in the prognosis of dystocia with sensitivity of 77%, and specificity of 53%. The cut-off value for predelivery BMI was set as 27.0 kg/m^2. The obese women were, after adjustment for term of delivery and macrosomia, about 3-fold more likely to experience obstructed labor (aOR = 3.40) than patients with normal BMI. Through the probability of prognosis both macrosomia and shoulder dystocia, the predelivery BMI can be helpful parameter in the prediction of that adverse perinatal outcomes, and making the decision about the most favorable mode of delivery.

The only adverse perinatal outcome, in our study, with higher frequency in normal weight women comparing to overweight females was preterm delivery (11.4 vs 6.9%). Both obese (aOR = 0.59, $p < 0.05$) and overweight (aOR = 0.55, $p < 0.01$) mothers had approximately 40% lower chance of preterm delivery, using regression model adjusted for PIH and FGR diagnosis. The trend of higher incidence for preterm birth among normal weight women was similar to Alanis et al. results (25.0 vs 16.6%) [28].

Although both the first and second stage of labor lasted longer in overweight mothers than in obese and normal weight patients, respectively, the proportion of prolonged second labor stage was comparable between groups. The I group had higher percentage of 1-minute Apgar score below 7 points than the II one. Moreover, there was no significant difference in diagnosis of 5-minute Apgar below 7 points between groups. These results are similar to Alanis et al. observations [28].

We observed higher frequency of PIH among overweight and obese mothers comparing to normal weight women. Interestingly, nearly similar incidence of GDM and FGR in all groups was noticed. The newborns of obese mothers had lower umbilical venous and arterial pH with similar incidence of newborn acidosis compared to normal weight and overweight group, respectively. Finally, we did not observe the differences in frequency of instrumental vaginal delivery, perineal incision, perineal rupture, postpartum hemorrhage, umbilical venous or arterial BE between groups. To our knowledge, until now, there are no studies in the literature assessing perinatal outcome in relations to predelivery BMI. Future studies, considering the predelivery obesity grading, and the long-term consequences of inappropriate maternal BMI, are needed.

CONCLUSIONS

The predelivery BMI is a useful parameter in the prediction of cesarean section, macrosomia, and shoulder dystocia. The establishing cut-off value for predelivery BMI was the lowest in prediction of shoulder dystocia.

The obese women are at higher risk of cesarean section comparing to overweight, and normal weight women. Predelivery obesity increases the chance of macrosomia compared to normal weight women, and to overweight females. Moreover, the obese patients are more likely to experience shoulder dystocia comparing to normal weight group.

The antenatal obesity is associated with higher perinatal blood loss, slightly lower umbilical venous and arterial pH in comparison to women with appropriate BMI. Moreover,

the overweight mothers present significantly lower umbilical arterial pH than normal weight females. Furthermore, predelivery obesity and overweight are related to lower risk of preterm delivery than normal BMI. Additionally, the first and the second stage of labor last longer among overweight women comparing to obese, and normal weight mothers, respectively.

Awareness, that obesity and overweight are major risk factors for many obstetrical complications, imposes clinicians to implement the unique management, including special recommendations for prenatal care.

Conflict of interest

The authors declare no conflict of interest

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Genetic variants of progesterone receptor in etiology of preterm delivery

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ABSTRACT

Objectives: Preterm delivery (PTD) accounts for around 11% of pregnancies worldwide. Unfortunately, no diagnostic indicator, specific mechanism or genetic predisposition has yet been identified. One of the hypotheses suggest local or functional progesterone decrease as a potential reason for preterm uterine contractions leading to preterm delivery. It is believed that any change in progesterone receptor DNA may be crucial for higher risk of preterm delivery due to abnormal response to prostaglandins, normally inhibited by properly built progesterone. The aim of this study was to determine whether there is an association between progesterone gene polymorphisms (PROGINS and +331G/A) and preterm birth.

Material and methods: A total of 230 women were enrolled, including 115 cases of preterm deliveries (between 22 and 36 weeks of gestation) and 115 healthy mothers of full-term infants. Genomic DNA was isolated from the blood sample. Polymerase chain reaction (PCR) amplification was carried out in a final volume of 25 µL. Genotyping was assayed by PCR. Statistical analysis of the results was conducted with $p < 0.05$ accepted as statistically significant.

Results: For both PROGINS (Alu ins/del) and +331G/A (rs10895068) polymorphisms were equally frequent in case and control group. The prevalence of PGR alleles in both groups was also comparable.

Conclusions: The results of our study showed no association between progesterone gene polymorphisms (PROGINS and +331G/A) and risk of preterm delivery. Identifying mechanisms to prolong the length of gestation, particularly in women at risk for preterm delivery, will improve both maternal and fetal outcomes.

Key words: genetic polymorphism; preterm delivery; progesterone receptor gene

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INTRODUCTION

Preterm delivery (PTD), defined as birth occurring prior to 37 weeks of gestation, is one of the greatest challenges in modern perinatal medicine, accounting for 11% of pregnancies worldwide [1]. Preterm births concerns about 75% of perinatal morbidity which ranks PTD as fifth in the leading causes of disease burden over time [2, 3]. Unfortunately, effective diagnostic indicators of PTD and effective treatment are yet to be identified. To date no specific mechanisms leading to PTD or genetic predisposition has been discovered and accumulating evidence suggests multiple attributable causes [4].

Studies indicate that prophylactic administration of progesterone, beginning at 16 to 20 weeks of gestation and continued to delivery or 36 weeks of gestation, decreases significantly the incidence of preterm birth in high risk pregnant women [5]. Considering that the levels of circulating progesterone levels are not decreasing with

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the onset of human labor, presumably local or functional progesterone decrease is of significance in pathogenesis of preterm birth [6].

The physiological effects of progesterone are mainly controlled by binding to specific progesterone receptors (PGR) or by changing the expression level of the PGR [7]. Polymorphic variants of human progesterone receptor have been described [8–11]. "PROGINS", firstly described as a 306bp insertion in intron G of the T2 allele of the PGR [12], is found to be linked to other polymorphisms [G to T substitution in exon 4, causing a Valine to Leucine change (V660L) and a C to T substitution in exon 5 (H770H)] and therefore have been referred in literature as PROGINS complex [13]. It has been suggested that its polymorphism results in abnormal transcription of progesterone receptor gene [14]. Other widely described polymorphism, known as +331G/A, is of more functional significance and is able to affect the ratio of two main progesterone receptor isoforms: A and B [15].

The role of progesterone in early pregnancy is critical to its maintenance until the placenta takes over its function. However, the role of progesterone in later stages of pregnancy remains unclear. One of the hypotheses proposed that progesterone may be responsible for limiting the production of stimulatory prostaglandins and inhibiting the expression of contraction-associated protein genes and prostaglandin receptors within the myometrium [16, 17]. Therefore, it is believed that any change in PGR DNA may be crucial for higher risk of preterm delivery due to abnormal response to prostaglandins, normally inhibited by properly built progesterone.

The aim of this study was to determine whether there is an association between progesterone gene polymorphisms (PROGINS and +331G/A) and preterm birth.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Bioethics Committee of Poznan University of Medical Sciences, Poland (No: 490/21). All participants were voluntary recruited and provided written informed consent before taking part in this research.

Patient recruitment

A total of 230 women were enrolled, including 115 cases of preterm pregnancy termination (between 22 and 36 weeks of gestation from the date of the last menstrual period) and 115 healthy mothers of full-term infants who born in the Division of Perinatology and Women's Diseases of University of Medical Sciences in Poznan, Poland between February 2015 and April 2020. Each patient donated a one-time 10 ml of the whole blood drawn into a K_2EDTA tube for genetic testing. Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. Exclusion criteria included: maternal age of less than 18 years, history of drug abuse, pregnancy acquired through assisted reproduction techniques, past terminations of pregnancy, hypertension, preeclampsia, bleedings during present pregnancy, preterm premature rupture of membranes, intrauterine infection, multiple pregnancy, detected fetal or placental abnormalities, cholestasis of pregnancy, cervical insufficiency. Controls were recruited during a routine check-up in uncomplicated pregnancy with a history of a previous at least two healthy gestations. All subjects were obliged to sign a written consent to participate in the study. Medical records of the participants were obtained for more detailed information about the patients.

Genotyping

Genotyping was performed in the Molecular Biology Laboratory of Poznan Medical Science University. Genomic DNA was isolated from the blood sample, using a Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentrations were determined by spectrophotometric measurement of absorbance at 260 nm and the purities were calculated by A260/A280 ratio using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). and the DNA was stored at –20°C until analyzed. PCR amplification was carried out using DNA Engine Dyad Thermocycler (Bio-Rad Laboratories, Inc.) in a final volume of 25µL. Genotyping was assayed by polymerase chain reaction (PCR) using isolated genomic DNA, deionized water, 200 mM dNTP Mix (Thermo Fisher Scientific, USA), 0.25 µM forward and reverse primers (TiBMolBiol, Germany), 1U of Taq DNA polymerase and respective buffer (DreamTaq Green DNA polymerase, Thermo Fisher Scientific, USA). For each analyzed polymorphism, 10% of the total samples were randomly retested with 100% concordance.

Alu insertion/deletion variant (PROGINS)

The following primers were used: 5'-GGC AGA AAG CAA AAT AAA AAG A-3' in the forward direction and 5'-AAA GTA TTT TCT TGC TAA ATG TC-3' in the reverse direction, as reported by Lancaster et al. [18], the DNA was amplified by initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. The PCR products of the PGR gene were visualized on a 2% agarose gel with Midori Green staining (Nippon Genetics, Europe GmbH). Alleles for this variant are named traditionally as described by Rowe et al. [12]. The *T1* allele was defined as the absence an *Alu* insertion in intron G and generated a 149 base pair fragment in agarose gel, while the polymorphic allele (allele *T2*) generated a 455-base pair.

Table 1. Demographic and clinical characteristics of the study population

Characteristics	PTD (n = 115)	Controls (n = 115)	p
Maternal age, years	29.18 (5.14)	30.14 (4.60)	0.14
Gestational age at delivery, weeks extremely preterm, n, % very preterm, n, %	33.65 (2.74) 5 (4.35) 18 (15.65)	38.90 (1.09) — —	< 0.001
Systolic blood pressure [mmHg]	110.83 (15.63)	107.26 (11.28)	0.049
Diastolic blood pressure [mmHg]	70.17 (11.12)	67.00 (8.35)	0.02
Pre-pregnancy BMI [kg/m^2]	21.43 (2.89)	20.71 (1.76)	0.02
Post-pregnancy BMI [kg/m^2]	26.15 (3.66)	25.87 (2.38)	0.50
Caesarean section, n (%)	39 (33.91)	24 (20.87)	0.041
Primipara, n (%)	44 (38.26)	0 (0.00)	—
Birth weight [g]	2375.30 (714.36)	3473.130 (403.79)	< 0.001
Placenta weight [g]	552.01 (149.32)	626.80 (112.03)	< 0.001
Infant male, n (%)	65 (56.52)	74 (64.35)	0.281
1-min Apgar score	7.83 (2.68)	9.86 (0.54)	< 0.001
5-min Apgar score	8.97 (1.44)	9.98 (0.13)	< 0.001

mean ± SD; p — Two Sample t-test; p^1 — Pearson's Chi-squared test; BMI — body mass index; PTD — preterm delivery

+331G>A (rs10895068)

The *PGR* gene rs10895068 variant were detected by using PCR–RFLP technique as previously described by Li et al. [19]. Primers with the following sequences were used for the PCR reaction: forward primer 5′-CAC TAC TGG GAT CTG AGA TC-3′ and reverse primer 5′-CAC AAG TCC GGC ACT TGA GT-3′ PCR conditions consisted of an initial denaturation at 95°C for 10 minutes, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s with a final extension at 72°C for 10 minutes. The 262 base pair PCR products were digested with *BspL*I (*Nla*IV) (Thermo Scientific, USA) in a volume of 20 µL at 37°C overnight. Products were electrophoresed in a 2.5% agarose gel with Midori Green (Nippon Genetics, Europe GmbH) and visualized through the UV transilluminator. A band of 156, 56, 50 bp represented *GG* (wild type) genotype; two bands of 212 bp and 50 bp represented *AA* (polymorphic).

Statistical methods

All analyses were performed using R version 4.0.3 [20] and R package "SNPassoc." [21]. Continuous variables without skewness were estimated via means ± standard deviation (SD) and compared with the student's t tests. Categorical variables were used through frequency counts and percentage. Data were tested for goodness of fit between observed and expected genotype frequencies according to Hardy–Weinberg equilibrium. Associations between the genotypes and the susceptibility of PTD were assessed via odds ratio (OR) with 95% confidence interval (CI) by logistic regression analyses. The most appropriate inheritance model was selected based on Akaike information criteria (AIC) and was adjusted by pre-pregnancy BMI. Linkage disequilibrium between loci was assessed by Haploview version 4.0 (Daly Lab at the Broad Institute, Cambridge, MA, USA). Statistical significance was defined as a p value < 0.05 with a two-tailed test.

RESULTS

Characteristics of the study population

A hospital-based case-control study was conducted, involving 115 women with preterm birth and 115 controls. Cases and controls were evenly matched by age. Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. Preterm women were divided into three groups based on their gestational age: extremely preterm (less than 28 weeks), very preterm (28 to 31 + 6 weeks) and moderate to late preterm (32 to 36 + 6 weeks). The characteristics of the cases and controls are summarized in Table 1. The majority of the PTD women gave birth moderate to late preterm (80.00%) followed by very preterm (15.65%) and extremely (4.35%). The mean ages of the PTD patients and full-term controls were 29.18 (5.14) and 30.14 (4.60) years, respectively (p = 0.14). Cases were more probably to have higher blood pressures, pre-pregnancy BMI and more likely to have caesarian section then controls. All the control patients were multiparous, while this was the first delivery for 38.26% of the PTD women. Interestingly, the women from the study group had a higher BMI at the end of pregnancy (without statistical significance), even though their pregnancies were on average shorter than in the control group [33.65 (2.74) weeks vs 38.90 (1.09) in controls, p < 0.001].

Table 2. Prevalence of PGR alleles in cases and controls

Variants	Alleles	PTD n = 230		Controls n = 230		p
		MAF n (frequency)	HWE p	MAF n (frequency)	HWE p	
Ins/del	*T1 > T2*	39 (0.169)	0.69	40 (0.173)	0.94	0.90
Rs10895068	*G > A*	14 (0.061)	0.79	13 (0.056)	0.54	0.84

HWE — Hardy-Weinberg equilibrium; χ^2 p — Pearson's Chi-squared test; PTD — preterm delivery

Table 3. Genotype distributions of the studied polymorphisms in cases and controls, and their risk prediction for preterm delivery under three genetic models of inheritance

Polymorphism	Controls (n = 115)	PTD (n = 115)	Crude OR (95%CI)	p-value	AIC	Adjusted OR (95% CI)	p value	AIC
Ins/del								
T1T1	79 (68.7)	78 (67.8)	1.00			1.00		
T1T2	32 (27.8)	35 (30.4)	1.11 (0.62–1.96)	0.66	324.0	1.06 (0.59–1.90)	0.61	320.6
T2T2	4 (3.5)	2 (1.7)	0.51 (0.09–2.84)			0.44 (0.08–2.56)		
Dominant	36 (31.3)	37 (32.2)	1.04 (0.60–1.81)	0.89	322.8	0.99 (0.56–1.74)	0.98	319.6
Recessive	111 (96.5)	113 (98.3)	0.49 (0.09–2.74)	0.40	322.2	0.43 (0.07–2.49)	0.33	318.6
+331G > A (rs10895068)								
GG	103 (89.6)	101 (87.8)	1.00			1.00		
GA	11 (9.6)	14 (12.2)	1.30 (0.56–2.99)	0.67	323.1	1.40 (0.60-3.26)	0.43	319.9
AA	1 (0.9)	0 (0.0)	—			—		
Dominant	12 (10.4)	14 (12.2)	1.19 (0.52–2.70)	0.68	322.7	1.30 (0.57–2.98)	0.53	319.2
Recessive	114 (99.1)	115 (100.0)	—	1.00	321.5	—	0.30	318.5

AIC — Akaike information criteria, adjusted for pre-pregnancy BMI; CI — confidence interval; OR — odds ratio; PTD — preterm delivery

When analyzing the clinical data of newborns from the studied groups, statistically significant differences were observed between the Apgar scores, newborn weights and placental weights. There were no differences in the frequency distributions of newborns gender between the PTD women and the controls (p = 0.28).

PGR gene polymorphisms and the susceptibility of PTD

As shown in Table 2, the genotype distributions of PGR polymorphisms in the case and control groups were in Hardy-Weinberg equilibrium (p > 0.05). However, there were no differences in allele frequencies between the women with preterm delivery and controls. A frequency of minor alleles in the cases (*T2* — 16.9% and *A* — 6.1%) were similar to the frequency observed in controls (17.3% and 5.6% respectively).

The genotype distributions of the PGR variants (ins/del and rs10895068) in PTD women and controls are presented in Table 3. There were no differences in frequency of each of the three genotypes, neither for ins/del (T1T1: 68.7% vs 67.8%, T1T2: 27.8% vs 30.4%, T2T2: 3.5% vs 1.7%) nor rs10895068 (GG: 89.6% vs 87.8%. GA: 9.6 vs 12.2%, AA: 0.9% vs 0.0%). Moreover, no significant differences were observed for these two polymorphisms in dominant or recessive models even after adjusting for pre-pregnancy BMI.

An analysis of the linkage disequilibrium was also performed by using Haploview software. Analyzed variants of the *PGR* gene (ins/del and rs10895068) are in a linkage equilibrium with $D' = 1.0$ and $r^2 = 0.01$.

Stratified analysis of PGR polymorphisms and the risk of PTD

We carried out stratified analysis to assess the relationship between the *PGR* variants and the risk of PTD by the subtypes distinguished according to the week of pregnancy termination (Tab. 4). As the group of women whose pregnancy ended before 28 weeks (extremely preterm delivery, EPTD) was very small (n = 5), we connected it with the group very preterm delivery (VPTD). Both crude and adjusted subgroup analyses revealed no significant differences for genotype frequencies of studied variants in any genetic models.

Effect of PGR ins/del and +331G > A genotypes on week of pregnancy termination

We found no significant difference in week of pregnancy termination before and after adjusted for pre-pregnancy

Table 4. Observed genotype for progesterone gene variants among women with early (extremely and very) preterm and late preterm births

	Genotypes/ /models	Controls (n = 115) n (%)	E+VPTD (n = 23) n (%)	MPTD (n = 92) n (%)	Controls vs E+VPTD		Controls vs MPTD		E+VPTD vs MPTD	
					OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Ins/del	*T1T1*	79 (68.7)	16 (69.6)	62 (67.4)	1.00	1.00	1.00	0.80	1.00	1.00
	T1T2	32 (27.8)	7 (30.4)	28 (30.4)	1.08 (0.41–2.87)		1.11 (0.61–2.04)		1.03 (0.38–2.79)	
	T2T2	4 (3.5)	0 (0.0)	2 (2.2)	—		0.64 (0.11–3.59)		—	
	Dominant	36 (31.3)	7 (30.4)	30 (32.6)	0.96 (0.36–2.54)	0.93	1.06 (0.59-1.91)	0.84	1.11 (0.51–2.97)	0.84
	Recessive	111 (96.5)	23 (100.0)	90 (97.8)	—	1.00	0.62 (0.11-3.44)	0.57	—	1.00
+331G>A rs10895068	*GG*	103 (89.6)	21 (91.3)	80 (87.0)	1.00	1.00	1.00	0.65	1.00	0.55
	GA	11 (9.6)	2 (8.7)	12 (13.0)	0.89 (0.18-4.32)		1.40 (0.59–3.35)		1.58 (0.33–7.59)	
	AA	1 (0.9)	0 (0.0)	0 (0.0)	—		—		—	
	Dominant	12 (10.4)	2 (8.7)	12 (13.0)	0.82 (0.17–3.92)	0.80	1.29 (0.55–3.02)	0.56	—	—
	Recessive	114 (99.1)	23 (100.0)	92 (100.0)	—	1.00	—	1.00	—	—

p-value — logistic regression adjusted for pre-pregnancy BMI; EPTD — extremely preterm delivery; VPTD — very preterm delivery; MPTD — moderately preterm delivery

Table 5. Week of pregnancy termination (mean ± SD) according to PGR genotypes in PTD and control subjects

Variant	Genotypes/ /models	PTD			Controls		
		mean (SD)	p	p[1]	mean ± SD	p	p[1]
ins/del	*T1T1*	33.67 (2.99)	0.69	0.72	38.94 (1.08)	0.78	0.77
	T1T2	33.71 (2.18)			38.78 (1.16)		
	T2T2	32.00 (0.00)			39.00 (0.82)		
	Dominant	33.62 (2.15)	0.93	0.96	38.81 (1.12)	0.55	0.53
	Recessive	33.68 (2.76)	0.39	0.42	38.89 (1.10)	0.85	0.85
+331G>A rs10895068	*GG*	33.55 (2.78)	0.31	0.32	38.88 (1.09)	0.60	0.57
	GA	34.36 (2.41)			38.91 (1.14)		
	AA	—			40.00 (0.00)		
	Dominant	—	—	—	39.00 (1.13)	0.73	0.70
	Recessive	—	—	—	38.89 (1.09)	0.31	0.29

p[1] value adjusted for pre-pregnancy BMI

BMI among the genotypes of study polymorphisms in either the patients or the control group (Tab. 5).

DISCUSSION

The hypothesis of the present study assumed that genetic variations in the progesterone receptor gene would be more frequent in PTD in comparison to the control group. Progesterone is responsible for the maintenance of the pregnancy thus any deviations in both progesterone receptor and progesterone itself might be responsible for loss of its physiological function and in consequence result in induction of preterm uterine contractions [16].

A recent metanalysis and systematic review considering association of estrogen and progesterone receptor with recurrent pregnancy loss (RPL) proved an association between those two determinants [22]. The authors evaluated six studies from years 1993–2011 which investigated the PR polymorphism, from which two presented positive association [23, 24], whereas the other four did not suggest the risk. However, the meta-analysis showed that PROGINS polymorphism had no impact on higher rate of RPL.

Present research focuses mostly on impact of progesterone receptor polymorphisms on cancer risk. A few authors proved that PROGINS polymorphism might be

associated with an increased risk of ovarian cancer [11, 25–27] and endometrial carcinoma [28], however the results are not consistent and further studies are required. Similarly, the progesterone receptor gene +331 G/A polymorphism might increase breast cancer risk [8, 15]. A recent metanalysis suggested that this effect appears to be more prominent in American rather than in Europeans and Australians [9].

Until present four different studies investigated the association between progesterone gene receptor and preterm delivery. In Portuguese population Oliveira et al. on a group of 114 women did not prove that the presence of PROGINS polymorphism constitute a risk factor for premature birth [29]. Similarly, Gouyang et al. found that maternal carriage of minor alleles of +331G/A, +770C/T, and +600G/T single nucleotide polymorphisms in the PR gene is not associated with spontaneous preterm birth. Interestingly, the carriage of +770T and +660T was confirmed to be linked with preterm birth in women with a low body mass index (< 18.5 kg/m2) [30]. On the contrary a recent study by Tiwari et al. showed that the distribution of the PROGINS mutation was higher in preterm delivery cases compared to controls but the increase was not statistically significant ($p = 0.09$). Even if divided into three different cohorts (extremely — less than 28 weeks, very — between 28 and 32 weeks and moderately preterm — between 32 and 37 weeks) and analysed against term deliveries, the presence of PR mutation was not associated with a higher risk of preterm birth. However, it was significantly related to negative pregnancy outcomes (intrauterine death) in moderately preterm group ($p = 0.03$) and lower baby birth weight in both term and preterm cases ($p = 0.04$) [31]. Another interesting study focused on evaluation of both fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth [32]. Ehn et al. [32] suggested that genetic variation in the PGR gene of either the mother or the fetus may trigger preterm delivery. Another study conducted to determine whether increase frequency of mutant alleles of the progesterone receptor gene was associated with preterm birth in a population of Hispanic women proved that PROGINS and +331G/A polymorphisms are not associated with preterm birth [33]. The findings of the present study on Polish women are similar to those on Hispanic population, which suggests that the genetic variations of those specific locus in the PGR gene do not contribute to this pathology.

Limitations of the study include relatively small sample size and single-center character. Moreover, the study examined the association of only a few PGR variants while possibly the presence of additional PGR polymorphisms may influence the risk of PTD. Lastly, the study population was only Polish women therefore the results cannot be generalized to other nationalities and ethnicities. We recognize that further studies are necessary to determine whether progesterone gene polymorphisms are associated with preterm birth in a more diverse and bigger group.

CONCLUSIONS

In conclusion, the results of our study showed no association between progesterone gene polymorphisms (PROGINS and +331G/A) and risk of preterm delivery. Identifying mechanisms to prolong the length of gestation, particularly in women at risk for preterm delivery, will improve both maternal and fetal outcomes.

Conflict of interest

The authors have no conflict of interest to disclosure. The manuscript has not been and will not be submitted to any other journal while it is under consideration by the *Polish Archives of Internal Medicine*. Also, there are no prior publications or submissions with any overlapping information.

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Prenatal diagnosis of chromosome 3q25.32 and 12p11.22p11.1 microduplication with a favorable outcome

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Chromosomal abnormalities, including microdeletions and microduplications, have long been associated with abnormal developmental outcomes [1]. Recently, chromosomal microarray analysis has been introduced into routine practice for clinical diagnosis of chromosome imbalances, allowing for the identification of chromosome imbalances smaller than 5 Mb [2]. As a result, numerous copy number variations have been identified and their clinical significance needs to be clarified. Here we report the first inherited 3q25.32 and 12p11.22p11.1 microduplications with a favorable outcome.

A 39-year-old gravida-1-para-0 mother underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Her husband was 38 years old. There was no family history of birth defects or genetic diseases. Before amniocentesis, the expectant mother had undergone first trimester ultrasonography scanning and the results showed low risk (At 12 weeks of gestation, crown-rump length 55 mm, nuchal translucency 0.9 mm, nasal bone 2.8 mm and fetal heart rate 150 bpm). Conventional karyotyping revealed a normal karyotype of 46, XX (Fig. 1). Chromosomal microarray analysis (CMA) on uncultured amniocytes using the Affymetrix SNP 6.0 platform (Affymetrix, Inc., Santa Clara, CA) identified a 539 kb duplication, arr [hg19] 3q25.32 (158051049-158589843) x3 inherited from the mother and a 6.0 Mb duplication, arr [hg19] 12p11.22p11.1 (28833490-34835641) x3 from the father (Fig. 2). Both parents received a comprehensive physical examination, and the results were normal. Prenatal ultrasound showed no dysmorphisms or intrauterine growth restriction (IUGR) in the fetus. After genetic counseling, the parents decided to continue the pregnancy. At 39 weeks of gestation, a 3150 g phenotypically normal female baby was delivered vaginally. The infant was phenotypically normal at the 18-month checkup.

For both microduplications, we searched the Database of Genomic Variants (DGV, http://dgv.tcag.ca/) for the presence of these DGVs in the control population and found no previous reports. We also searched several clinical databases including DECIPHER (https://decipher.sanger.ac.uk) and ClinGen (https://clinicalgenome.org) and found no case with the exact duplications.

The microduplication in the region of 3q25.32 is 539 kb in size and includes five genes: RSRC1, MLF1, GFM1, LXN, and RARRES1. RARRES1 is a tumor suppressor gene associated with fatty acid metabolism, stem cell differentiation and is the most methylated loci in multiple cancers [3, 4]. Methylation at LXN and RARRES1 was highly correlated [5]. Increasing methylation was associated with decreased expression of both genes and worse clinical features [5].

The microduplication in the region of 12p11.22p11.1 is 6 Mb in size and includes 15 genes: FAR2, ERGIC2, TMTC1, IPO8, CAPRIN2, DDX11, FAM60A, ETFBKMT, H3F3C, BICD1, FGD4, DNM1L, YARS2, PKP2 and ALG10. There is no report supporting the triplosensitivity of these genes. Duplications in the region of 12p11 are associated with diverse phenotypes, ranging from normal phenotypes to severe physical defects in different organ systems [6]. In our case, the infant

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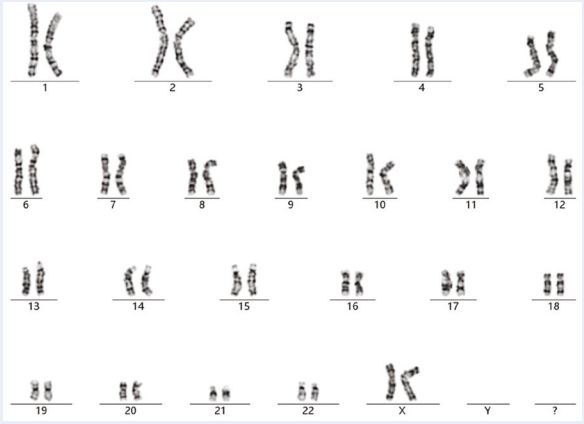

Figure 1. Karyotype of 46, XX

Figure 2. CMA analysis revealed a 539 kb duplication on chromosome arr [hg19] 3q25.32 (158051049-158589843) x3 and a 6.0 Mb duplication on chromosome arr [hg19] 12p11.22p11.1 (28833490-34835641) x3

is phenotypically normal at birth and develops normally in the first 18 months of life. The father who carries the same duplication was phenotypically normal.

To summarize, we present a case of inherited 3q25.32 and 12p11.22p11.1 microduplications with a favorable outcome. Our case presents evidence that these novel microduplications can be associated with a favorable outcome.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of Wuhan Blood Center. All patient guardians gave informed consent to this study.

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