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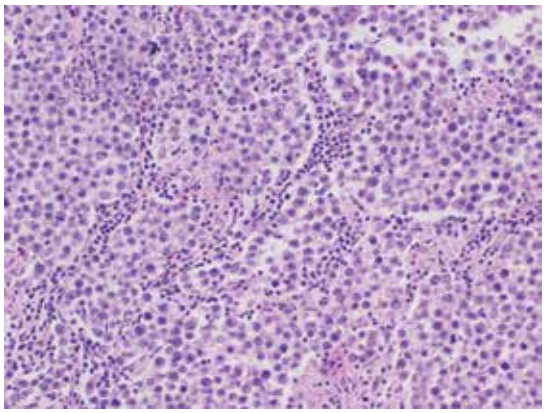

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Depression, anxiety and self-esteem in adolescent girls with polycystic ovary syndrome

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ABSTRACT

Objectives: Objective of the study was to evaluate the depression, anxiety and perceived stress level in adolescent girls with diagnosed polycystic ovary syndrome (PCOS), as well as to assess their body and self-esteem and its impact on emotional status.

Material and methods: In 27 adolescent girls with confirmed diagnosis of PCOS (study group) as well as 27 healthy, regularly menstruating, age and BMI matched girls (control group) Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale-10 (PSS-10), Rosenberg Self-Esteem Scale (RSES) and Body-Esteem Scale (BES) containing three subscales (sexual attractiveness, weight concern, physical condition) were performed.

Results: There were no significant differences between PCOS group and control group in depression and PSS-10 scores, but the anxiety score was significantly higher in control than in PCOS group (9.6 ± 3.0 vs 7.3 ± 3.9, $p = 0.02$). Moreover, in BES subscales' scores there were no significant differences between the groups, whereas RSES score was significantly higher in PCOS group (25.0 ± 7.1 vs 28.3 ± 4.6, $p = 0.04$). In PCOS group anxiety score was related to PSS-10 score ($r = 0.56$, $p = 0.005$). Moreover, we found that obesity was negatively related to anxiety ($r_y = -0.4$, $p = 0.04$), depression ($r_y = -0.48$, $p = 0.02$), PSS-10 ($r_y = -0.59$, $p = 0.004$) and physical condition scores ($r_y = -0.44$, $p = 0.04$). In girls with PCOS the more severe depression the worse weight control ($r_y = -0.56$, $p = 0.04$).

Conclusions: We conclude that in adolescent girls PCOS is not related to anxiety and depressive symptoms as well as poor self-esteem.

Key words: adolescent girls; polycystic ovary syndrome; depression; anxiety; self-esteem

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in adult women, with characteristic features of anovulation, clinical and biochemical hyperandrogenism. It is a chronic condition leading to the occurrence of many relevant concerns, such as fear of infertility, obesity and its metabolic complications and finally unfeminine, unattractive appearance due to hirsutism, acne or alopecia. PCOS begins in adolescent years, however in adolescent girls, clinical features of the syndrome could be less pronounced or be on the border with adolescent physiology. Nevertheless, in adolescents, quality of life seems to be affected by similar signs and symptoms of PCOS as in adult women: excessive weight, clinical hyperandrogenism, menstrual disturbances and infertility [1].

There are many studies on psychological well-being, emotional problems and stress that arose from clinical features, comorbidities and consequences of PCOS in adult women [2, 3]. Cooney et al. [2] in a meta-analysis showed that women with PCOS have significantly higher risk of moderate and severe depressive and anxiety symptoms, independently of obesity. Moreover, in women with PCOS increased risk of conversion to depression with time is observed [3]. Their perceived stress in higher that in healthy women. The concerns about the emotional status led to recommendation for screening for depression and anxiety in all PCOS women, contained in guidelines of the assessment and management of PCOS published by the Androgen Excess and PCOS Society [4].

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In today's culture physical attractiveness is believed to be one of the main determinants of the success and physical features characteristic for PCOS, such as excessive body weight, hirsutism and acne, are remote from the beauty standards [5]. They can lead to dissatisfaction with the appearance and lower self-esteem. In adult women PCOS is related to greater body dissatisfaction [6]. Moreover Bazarganipour et al., [7] shoved that PCOS women with amenorrhea had lover level of self-esteem and infertility was negatively associated with both self-esteem and body satisfaction. Also in adolescents some researches showed that visible symptoms of androgen excess influence self-esteem and provoke discomfort [8].

The data about the psychological well-being of adolescent girls with PCOS are less numerous, inconsistent and sometimes even conflicting. Considering the duration of the disease, the severity and type of the symptoms, as well as the specificity of lifetime period, the psychological and emotional consequences could be different than in adult patients, associated with different factors. The aim of the study was to evaluate the depression, anxiety and perceived stress level in adolescent girls with diagnosed PCOS, as well as to assess their body and self-esteem and its impact on emotional status.

MATERIAL AND METHODS

We recruited 54 adolescent girls admitted to the Department of Pediatric Endocrinology due to suspicion of hirsutism and/or menstrual disturbances. Twenty-seven adolescent girls with confirmed diagnosis of PCOS, prior to treatment introduction, were included into the study group and 27 healthy, regularly menstruating, non-hirsute, age and BMI matched girls who voluntarily agreed to participate into the study were included into the control group. PCOS was diagnosed according to the criteria proposed by Ibanez et al [9]. A diagnosis of PCOS was made when both criteria were present: menstrual disturbances (oligomenorrhea, secondary amenorrhea) and clinical or biochemical hyperandrogenism. Oligomenorrhea was defined as menstrual cycles longer than 45 days in the last six months (or less than 6 menstrual cycles during the last year) and secondary amenorrhea as a lack of menstruation in the last three months. Hirsutism, diagnosed if the modified Ferriman-Gallwey score was ≥ 8, was considered as a sign of clinical hyperandrogenism. Biochemical hyperandrogenism was identified by testosterone level ≥ 55 ng/mL [10]. The exclusion criteria were other hormonal disturbances (abnormal thyroid function, hyperprolactinaemia, congenital adrenal hyperplasia) and former serious psychiatric disease.

The study was conducted according to Helsinki declaration and approved by the Ethic Committee of The Medical University of Silesia (KNW/0022/KB1/79/12). Informed consent was obtained from each participant and their parent/guardian.

In all participants somatic development data (weight, height) were measured, hirsutism score was assessed by the same observer (AZ). BMI was calculated and obesity was diagnosed in participants with BMI exceeded 97th percentile. Serum level of androstenedione (A), testosterone (T), 17-hydroxyprogesterone (17OHP), dehydroepiandrosterone-sulfate (DHEAS) and estradiol (E_2) were measured during follicular phase of menstrual cycle (2–5 day of the cycle) or after 3 months from last menstruation. Serum levels of T, DHEAS, and E_2 were measured using chemiluminescent immunoassay by Immulite 2000 analyzer (DPC, USA). 17OHP and A were measured by enzyme-linked immunosorbent assay (DRG Diagnostics GmbH, Germany).

Psychological assessment

Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale-10 (PSS-10), Rosenberg Self-Esteem Scale (RSES) and Body-Esteem Scale (BES) were performed in all patients and controls. The questionnaires were anonymous and independently answered during unlimited time. The average time of completion was about 20 minutes.

HADS is a questionnaire validated for Polish adolescents, consisted with seven questions dedicated to the assessment of anxiety, and seven to assess depression symptoms [11]. Scores of 8 or above are considered abnormal, characteristic for mild (score of 8-10), moderate (score of 11–14) and severe (score above 15) anxiety/depression symptoms.

PSS-10 is a questionnaire consisting of 10 items measuring the perception of stress in the last month, developed by Cohen et al. in 1983 year [12], validated for Polish population by Juczynski and Oginska-Bulik [13]. It measures the degree to which situation in one's life are appraised as stressful.

RSES is diagnostic 10-item tool assessing global self-worth, developed by Rosenberg in 1965 year. It uses four grade Likert scale from strongly agree to strongly disagree [14].

BES is 35-item questionnaire assessing the body image in three subscales: 1) sexual attractiveness — related to the body parts those which appearance cannot generally be changed through exercise but only through cosmetics; 2) weight concern — related to body parts that can be physically altered through exercise or control of food intake; 3) physical condition — related to stamina, strength and agility. It was developed by Franzoi and Shields in 1984 year and validated for Polish population by Lipowska and Lipowski [15, 16]. It uses 5-point Likert scale where 1 means "I have strong negative feelings" and 5 — "I have strong positive feelings".

The statistical analysis was performed using Statistica 12,0 PL. The quantitative variables were described with

mean (SD) and qualitative variables were defined by frequency (%). To evaluate the normality of variables Shapiro-Wilk test was used. We used χ^2 test for qualitative data comparison of groups, and independent sample t-Student test and U Mann-Whitney test for quantitative data comparison of groups, as appropriate. Correlation analysis was performed using Pearson correlation coefficient for normally distributed samples, Spearman correlation coefficient for non-normally distributed data, and Gamma correlation for non-normally distributed data with many tide ranks. P value < 0.05 was considered statistically significant. To evaluate variables effecting the strength of the HADS scales, standard multiple regression analysis was performed with β coefficient interpretation regarding the strength and direction of the relationship between variables.

RESULTS

Twenty-seven adolescent girls with PCOS aged 13.6–17.9 years, as well as 27 healthy, regularly menstruated girls aged 14.3–18.0 years were involved into the study. The groups were age and BMI matched so the chronological age, BMI and BMI z-score did not differ significantly between the groups. Clinical and hormonal characteristics are presented in Table 1. The menstrual disturbances were present in all girls from the study group and in all girls from the control group the menstrual cycles were regular. Obesity was seen in nine girls from the study group (33.3%) and 13 from the control group (48%) (p = 0.2). The biochemical and/or clinical hyperandrogenism were present in all girls from the study group. Hirsutism was present in 10 subjects with PCOS (38.5%). However, most of the girls presented mild hirsutism (8–15 points in Ferriman-Gallwey scale) and only in one girl the excessive hair growth was assessed at 16 points. Biochemical hyperandrogenism was found significantly more often in girls from the study group than from the control group [15 girls (57.7%) vs 5 girls (23.8%), p = 0.02].

There were no significant differences in the depression score and PSS-10 score between the groups (p > 0.05) (Tab. 2). But anxiety score was significantly higher in the control group than in study group (p = 0.02). Moderate and severe anxiety was found in five (18.5%) girls with PCOS and in 14 (51.8%) girls from the control group (p = 0.01), whereas moderate and high depression was present in one girl from the study group (3.7%) and in two girls from the control group (7.2%) (p > 0.05) (Fig. 1). There were no significant differences between PCOS and the control group for all subscales of BES. Whereas RSES score was significantly higher in PCOS than in the control group (p = 0.04).

In the study group we found significant positive correlation between anxiety score and PSS-10 score (r = 0.56, p = 0.005) and negative with RSES score (r = –0.44, p = 0.03). PSS-10 score correlated negatively with RSES score (r = –0.58, p = 0.003). Surprisingly, depression score and PSS-10 score correlated negatively with BMI (r = –0.38, p = 0.049; r = –0.43, p = 0.03, respectively), whereas RSES score correlated positively with BMI (r = 0.53, p = 0.004). In the study group obesity was negatively related to anxiety score (r_y = –0.4, p = 0.04), depression score (r_y = –0.48, p = 0.02) and PSS-10 score (r_y = –0.59, p = 0.004) as well as physical condition score (r_y = –0.44, p = 0.04). Moreover, we found that the more severe depression, the worse weight control (r_y = –0.56,

Table 1. Clinical and hormonal characteristics of adolescent girls with polycystic ovary syndrome (PCOS) and control group of healthy girls

	Girls with PCOS (n = 27)	Control group (n = 27)	p
Chronological age [years]	16.7 ± 1.2	16.1 ± 1.1	NS
Gynaecological age [months]	52.5 ± 19.4	40.7 ± 16.9	NS
Cycle duration [days]	108.6 ± 60.8	29.7 ± 4.8	< 0.001
BMI z-score	1.1 ± 0.9	1.0 ± 1.0	NS
Ferriman-Gallwey score	5.2 ± 4.4	3.3 ± 2.9	NS
Testosterone [ng/dL]	61.2 ± 23.2	45.0 ± 14.2	0.009
Androstenedione [ng/mL]	4.6 ± 1.3	3.3 ± 0.8	< 0.001
DHEAS [µg/dL]	312.7 ± 134.5	266.2 ± 88.0	NS
17OHprogesteron [ng/mL]	2.4 ± 1.5	2.4 ± 1.8	NS
Estradiol [pmol/L]	155.0 ± 56.0	107.0 ± 80.4	0.052

PCOS — polycystic ovary syndrome; BMI — body mass index; DHEAS — dehydroepiandrosterone-sulfate; NS — nonsignificant

Table 2. Psychological assessment of adolescent girls with polycystic ovary syndrome (PCOS) and control group of healthy girls

	Girls with PCOS (n = 27)	Control group (n = 27)	p
Hospital Anxiety and Depression Scale			
anxiety [scores]	7.3 ± 3.9	9.6 ± 3.0	0.02
depression [scores]	4.2 ± 2.9	5.1 ± 3.4	NS
Perceived Stress Scale — 10 [scores]	22.0 ± 3.4	22.7 ± 3.3	NS
Rosenberg Self-Esteem Scale [scores]	28.3 ± 4.6	25.0 ± 7.1	0.04
Body Self Esteem			
sexual attractiveness [score]	44.3 ± 5.2	44.0 ± 9.6	NS
weight concern [score]	28.1 ± 8.0	25.3 ± 10.5	NS
physical condition [scores]	30.7 ± 5.2	30.0 ± 6.5	NS

PCOS — polycystic ovary syndrome; NS — nonsignificant

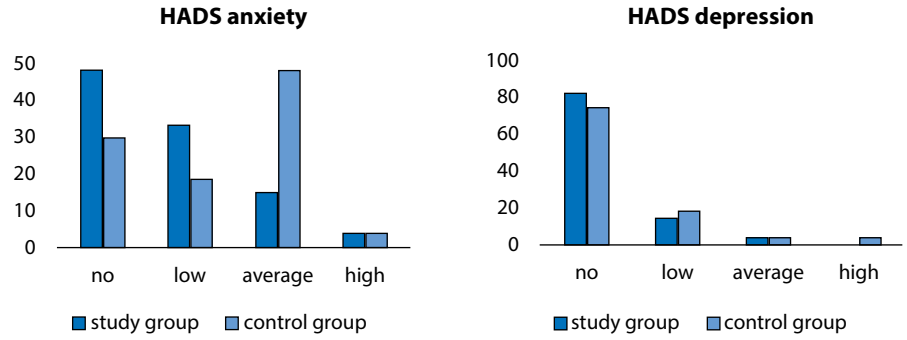

Figure 1. Distribution of no, low, average and high anxiety and depression symptoms in the study and control groups; Scores below 8 is characteristic for no anxiety/depression symptoms, 8–10 scores for mild, 11–14 score for moderate and > 15 score for severe anxiety/depression symptoms; HADS — Hospital Anxiety and Depression Scale

$p = 0.04$). We found no significant relationship between depression, anxiety, PSS-10, RSES and BES subscales scores and clinical and biochemical hyperandrogenism, as well as severity of clinical phenotype of PCOS.

In multiple regression analysis, PSS-10, RSES, sexual attractiveness, weight concern, physical condition scores, BMI, hirsutism scores were included in the analysis as scale variables. The anxiety score was significantly associated with the PSS-10 score ($\beta = 0.33$, $p = 0.05$) in the control group but not in the study group. In examining depression score we found significant association with weight concern score in the control group ($\beta = 0.72$, $p = 0.01$) but not in the study group.

DISCUSSION

The study was designed to estimate the depression and anxiety occurrence in adolescent girls with PCOS as well as to assess their self-esteem. We tried to study the relationship of depression, anxiety and self-esteem with clinical and biochemical features of hyperandrogenism. The anxiety score was higher in control than in PCOS group, but we did not find any differences between the groups in depression and PSS-10 scores. Researches showed that there is significant relationship between depression and anxiety and PCOS in adult women [17, 18]. The prevalence of depression and anxiety symptoms in women with PCOS is 29–50% and 57% respectively [19, 20]. Moreover, the symptoms are related to hyperandrogenism, insulin resistance and in some research to obesity [20]. However, such relationships are not so obvious in adolescent girls with PCOS. The studies on psychological well-being in PCOS girls are on many fields scarce and conflicting. Quality of life in adolescent girls with PCOS by some researchers is assessed as negatively influenced by the syndrome [21], whereas in other studies no relationship was found [22]. Ghazeeni et al. [23], showed no significant difference with anxiety and depression status between the adolescent girls with PCOS and healthy age-matched girls. On the other hand, Eneksis et al. [24], found higher level of anxiety and depression in PCOS than in controls. However, their study group was older than our PCOS group, so the persistence of the clinical symptoms could last longer. And as showed Guidi et al. [25], significantly higher anxiety and depression, impaired well-being and quality of life was found among adolescents with hirsutism. However, in their research the hirsutism score was self-estimated, meaning strongly subjective assessment. Neither menstrual irregularity, nor PCOS was associated with higher psychological distress. They concluded that among late adolescents and young women more important is concern about physical appearance then reproductive problems [25]. In our study we did not find the difference in depression score between the groups, whereas anxiety score was significantly higher in control group than in study group, significantly more girls from control group experienced moderate and severe anxiety. There could be several reasons underlying this lack of relationship. First, some symptoms of PCOS occurring during adolescence overlap findings that are considered physiological, normal during puberty [26]. Irregular menses occurring in many girls especially in the first years after menarche should not be treated as symptoms of PCOS, as well acne, which is typical physiological finding during puberty. So, these features could be perceived by adolescent girls as not pathological. Also, the main clinical presentation of hyperandrogenism, hirsutism could be mild in adolescent years, increases its severity with age. The severity of hirsutism in studied girls with PCOS was also not very high, only in one girl from study group her excessive hair growth was assessed at more than 15 score in Ferriman-Gallwey scale. Most of the girls from the study group have mild hirsutism, which could be not very relevant to emotional distress.

The reason for lack of anxiety and depression symptoms in our study group could be short duration of the symptoms, and there is also the suggestion that the predictor of the depression in women with PCOS is the age [26]. It is

postulated that not clinical hyperandrogenism per se but the chronic character of clinical features of PCOS could be associated with increased risk of depression and anxiety disturbances. In adult women the symptoms of menstrual irregularities and clinical hyperandrogenism last for many years with unsatisfactory results of treatment, leading to increase in emotional and psychological distress. And finally, very important concern which arises from the age and period of life of young adult woman is infertility.

The lack of difference between depression score with higher anxiety in control group could be also related to the control group characteristic. We involved into the control group adolescents with regular cycles without clinical hyperandrogenism, however they were referred to our clinic for evaluation due to a suspicion of hormonal disorders. All endocrine diseases were excluded in this group of girls however it seems that not real, objective physical signs of endocrine disturbances are important for the level of depression or anxiety but the subjective perception of the complains. Similar relationship we saw in our study group in respect to BMI. BMI correlated negatively with depression score and PSS-10 and positively with RSES, whereas more severe depression was related to the worse weight control. So, the issue is not a real weight status but how it is perceived. The similar depression score and lower anxiety could be related to the social and medical support that PCOS subjects get from the family and professionals as well from the fact that the cause of their symptoms was discovered. In the control group, with strong subjective beliefs of hormonal disturbances, the higher anxiety can be related to lack of symptoms interpretation despite of staying in hospital. The regression analysis, showing in the control group significant influence of stress on anxiety score as well as weight concern on depression score, seems to confirm these arguments.

Body image is a mental picture of one's body and it is highly subjective [7]. Usually, low self-esteem is associated with anxiety, depression, whereas high self-esteem may serve as a protection in coping with new or chronic disease [7]. In our study we also found significant negative relationship between anxiety and PSS-10 score and RSES score. However, the results about relationship of hyperandrogenism with self-esteem are conflicting [27]. According to Bazarganipour et al. [7] adult women with PCOS have poorer self-esteem and self-image satisfaction because of clinical symptoms as menstrual disorders, obesity and clinical hyperandrogenism (acne, alopecia, hirsutism). They found that women with PCOS with menstrual disorders had the greater body dissatisfaction, linking it especially with the fertility concern [7]. However, they examined women from Iran, where strong social pressure to have a child soon after marriage is present. In Poland, like in other European countries, there is a tendency to postpone the maternity. According to Eurostat, in recent years the average age of a Polish woman at the birth of the first child is 27.3 years [28]. So, the concern about fertility is also postpone in time. Keegan et al. [27] did not report the difference in self-esteem between women with and without hirsutism. In adolescents with PCOS, Coban et al. [22] showed no significant difference compared with control group. They also did not find any relationship between RSES score and clinical symptoms of hyperandrogenism [22]. In our study RSES score was even higher in PCOS group than in control one. Drosdzol et al. [29] showed that in adolescents with hirsutism RSES score was similar as in healthy peers. These results refer to RSES, which is very reliable but general tool that does not measure items of self-esteem related to physical appearance. That is why we decided to use also BES to assess body self-esteem in the three areas: sexual attractiveness, weight concern and physical condition. And we found also lack of significant differences in all three BES subscales scores between the groups, leading to the conclusion that the body self-esteem related to sexual attractiveness, weight concern and physical condition is not influenced by PCOS in adolescent girls. To the similar conclusion arrived also Annagur et al., [30], finding that higher body satisfaction was present in PCOS women than in healthy control.

Previous research suggested that excessive body weight gain during adolescence may be related to the depression, negative mood state and poor self-esteem [31]. Surprisingly, we found negative relationship between BMI and obesity and depression, anxiety and PSS-10 scores, meaning that the higher weight the lower level of depression, anxiety and perceived stress. It contrasts with other research in adults and adolescents [17, 18]. Moreover, RSES score significantly correlated with BMI. However, in our previous research we showed that in adolescent girls with clinical hyperandrogenism and obesity, BMI z-score was positively related to social competences [32]. And the group with strongest inclinations to withdrawing from social relations was the non-hirsute, non-obese subgroup. It could be due to that excessive body weight is no longer the feature that stigmatize anybody, especially if the whole family is obese or overweight and increasing amounts of peers have excessive body weight. Possible explanation is also the subjective, positive self-assessment of own body image which could neutralize negative self-assessment [33]. Obesity, lasting from early childhood could lead also to the feeling of "getting used" to this image of the body, does not influence body self-esteem. The data about the influence of obesity on psychological distress are no so clear. In a meta-analysis, Rankin et al., [31] tried to clarify the relationship between excessive weight in children and depression and anxiety. They found that depression could be related to obesity in childhood, whereas the data about relation to anxiety disor-

ders are not so obvious. However, study findings analyzed in this meta-analysis vary — in couple of them, obese children were found to be significantly more likely to experience depression. In other studies depression risk was only modestly greater than in general population or the association was weak [31]. Moreover, three meta-analysis focusing on emotional distress and mental disorder in PCOS women suggested that BMI and hirsutism have a small or moderate effect on anxiety and depression [2, 34, 35].

CONCLUSIONS

We conclude that in adolescent girls PCOS is not related to anxiety and depressive symptoms as well as poor self-esteem. However, considering the possible relationship between PCOS and emotional distress in adult women, due to long lasting, chronic medical condition, patients with PCOS should be monitored for emotional and psychological problems from adolescence to adulthood.

The study limitation is a relatively small number of recruited patients. Increasing the size of the study group might demonstrate more reliable estimates for several independent variables as well determine the ability to detect further subtle relationships.

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Conflict of interest

The authors declare no conflict of interest.

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Evaluation of the presence of SARS-CoV-2 in the vaginal fluid of reproductive-aged women

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ABSTRACT

Objectives: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is mainly transmitted through respiration and direct contact. The status of the infection in the female genital system is still unknown. The study aimed to evaluate whether SARS-CoV-2 is present in the vaginal fluid of women with COVID-19 infection in reproductive period.

Material and methods: Women who were between the ages of 18–50 years and clinically confirmed to have COVID-19 infection at our hospital between 20 April–31 May 2020 were included in the study. Women who were in their menstrual cycle during the study and who had a known cervical intraepithelial lesion and/or cancer, sexually transmitted disease and history and/or symptoms of vaginitis were excluded from the study. In patients in whom no pathology was detected during the examination, a sample was taken from the vaginal fluid for PCR by using Dacron tip swab. Analysis was performed with Genesig Real-Time PCR COVID-19 kit (Primer Design, England).

Results: Eighteen women who were in reproductive period and diagnosed with severe COVID-19 pneumonia were included in the study. The mean age of the patients was 38.16 ± 8.54. None of the patients were in their menopause period. The clinical symptoms of these women were similar to those of confirmed severe COVID-19 cases. SARS-CoV-2 was found to be negative in the samples taken from the vaginal fluid in all patients.

Conclusions: SARS-CoV-2 virus was not detected in the vaginal fluid of the patients who tested positive for COVID-19 in reproductive period.

Key words: coronavirus; SARS-CoV-2; vaginal fluid

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INTRODUCTION

Viral pneumonia and severe acute respiratory symptom observed in coronavirus disease (COVID-19) have spread between many countries in a short period of time and has affected the whole world, causing a pandemic to be declared. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which is the pathogen of the coronavirus disease, is a highly contagious virus, and it is mainly transmitted through the respiratory droplets of people in the immediate vicinity and through direct contact [1]. The virus especially affects the upper and lower respiratory systems in human body. It can lead to fever, cough, chest pain, dyspnea, fatigue, and muscle pains [2]. In addition, it has also been known to cause abdominal pain and diarrhea by affecting the gastrointestinal system, which can lead to acute cardiac destruction, arrhythmia and shock symptoms in the cardiovascular system, as well as mental fog and confusion in the central nervous system, and patients facing mortality resulting from multiple organ failure [3]. However, it is still not clearly determined whether it creates infection in the female genital system. There are few studies conducted in the literature on the presence of SARS-CoV-2 virus in body fluids. It has been observed that very few women in post-menopausal period were included in the studies investigating the virus in the vaginal fluid [4].

Objectives

Determining this virus in the genital systems of women during the reproductive period is important in that it may help reporting especially the risk of transmission through sexual intercourse and from mother to baby during delivery. The purpose of the study was to investigate whether

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there are SARS-CoV-2 virus symptoms in the vaginal fluids of women who are sexually active and in the reproductive period.

MATERIAL AND METHODS

Study group and ethical approval

In this cross-sectional prospective study, sexually active menstruating women between the ages of 18–50 years who were hospitalized in the COVID-19 services of Sakarya University Training and Research Hospital, which was transformed into a pandemic hospital where COVID-19 patients were treated and followed up, in Sakarya Province of Turkey. Patients who were clinically confirmed to have COVID-19 infection (whose swap sample taken from the throat tested positive in PCR test) between 20 April–31 May 2020 were included in the study. Patients who were in their menstrual cycle during the study, who had a diagnosed cervical intraepithelial lesion and/or cancer, sexually transmitted disease, and vaginitis history and symptoms were excluded from the study. All patients were informed about the study, and after obtaining their verbal/written approval, they were evaluated in lithotomy position. Ethical approval for the study was taken from Sakarya University Medical Faculty Ethical Committee (Project ID:16214662/050.01.04/83) (April 13, 2020).

Taking sample from the vaginal fluid

Within 0–2 days of hospitalization, samples were taken from the vaginal fluids for PCR test by using Dacron (Polyester) tip swab from patients in whom no pathology was observed in the examination made after insertion of vaginal speculum in lithotomy position on the examination table. The swab sample was taken from the vaginal fluid by applying the swab on the anterior, posterior and lateral walls once, and touching the posterior fornix once. All samples taken were placed into screw-capped sealed vessels which contained 3 mL of viral transport medium. All samples were kept at 2–8°C in refrigerator right after taking the samples and were transported quickly to the laboratory. The samples were sent to the laboratory by complying with infection protection and control procedures and cold chain rules in triple transportation system.

Nucleic acid isolation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

After the samples were accepted at the microbiology laboratory, all samples were studied in biosecurity cabin in level 3 negative pressure room.

After the vaginal fluid samples were well vortexed, 400 µL was removed and loaded onto BioRobot EZ1 (Qiagen, Germany) device, and 60 µL elution was taken. Total nucleic acid isolation was performed with EZ1 Virus Mini Kit v2.0 (Qiagen, Germany) in accordance with the company's recommendations.

Table 1. COVID-19 RT-PCR temperature and duration adjustments

Stages		Temperature	Duration
Reverse transcription		55°C	10 minutes
Enzyme activation		95°C	2 minutes
X50 Cycle	Denaturation	95°C	10 seconds
	Binding and Stretching	60°C	60 seconds

For RT-PCR study, a total mixture with a volume of 20 µL made up of 10 µL master mix, 2 µL primary, and 8 µL RNA template was formed, and reaction was realized with a total of 20 µL reaction volume in the durations and at the temperatures given in Table 1.

At the end of the reaction, the curves whose Cycle Threshold (CT) value was lower than 45 and were observed to be sigmoidal were interpreted as positive for SARS-CoV-2 RNA.

RESULTS

Eighteen women in reproductive period with severe COVID-19 pneumonia were included in the study. The mean age of the patients was 38.16 ± 8.54. None of the patients were in their menstrual cycle. The clinical symptoms of these patients were comparable to those of confirmed COVID-19 patients. The most common symptoms were fever (100% n = 18) and cough (66.6% n = 12). Other symptoms included sore throat (44.4% n = 8), fatigue (27.7% n = 5), and diarrhea (16.7% n = 3). Two patients complained of dyspnea, and no patients complained about urinary tract symptoms. While no chronic diseases were observed in the patients, hypertension (n = 5) and diabetes (n = 1) were present in some patients. Computer tomography scans of the patents were observed to be consistent with typical viral pneumonia. The swab samples taken from the throats of the patients at the time of hospitalization tested positive in PCR. PCR tests of these patients repeated after 24 hours also tested positive. ICU treatment was not required for any of the patients. For all patients, treatment with antiviral (oseltamivir/favipiravir) and antibacterial (azithromycin and hydroxychloroquine) drugs were initiated. Baseline characteristics of the 18 patients infected with SARS-CoV-2 are presented in Table 2. The samples taken from the vaginal fluid tested negative for SARS-CoV-2 in all patients.

DISCUSSION

In our study, viral RNA was not determined in the vaginal fluids of the COVID-19 positive women during the reproductive period with a mean age of 38.16 ± 8.54. The virus was

Table 2. Baseline characteristics of the patients infected with SARS-CoV-2	
Age (mean ± SD)	38.16 ± 8.54
Fever (n, %)	18 (100%)
Cough (n, %)	12 (66.6%)
Sore throat (n, %)	8 (44.4%)
Fatigue (n, %)	5 (27.7%)
Diarrhea (n, %)	3 (16.7%)
Hypertension (n, %)	5 (27.7%)
Diabetes Mellitus (n, %)	1 (5.5%)
Samples	1.33 (0–2 days)
Discharge	7.33 (5–12 days)

not detected in the vaginal fluid in the studies conducted on this issue in the literature as well. What makes our study different is that the patients included in the study were younger and in the reproductive period. In a similar study in which 12 pregnant women with a mean age of 32 ± 7.9 were included, the virus could not be shown in the vagina through isolation methods [5].

In a study carried out by Qui et al. [4], SARS-CoV-2 was investigated with PCR in the vaginal fluid samples of 10 patients aged between 50–80 in their postmenopausal period who tested positive for COVID-19, and no virus was found. Moreover, in a study in which 35 patients, 28 of whom were in the postmenopausal period, were included, SARS-CoV-2 similarly could not be detected in the vaginal fluids [6]. According to International Menopause Society (IMS), it has been reported that within 4–5 years following menopause, urogenital atrophy and vaginal dryness develop in most women [7]. Atrophy and dryness that develop in the vaginal epithelium as a result of hormonal changes in menopause may have caused lack of material, and PCR test results may have proved negative in these studies.

SAR-CoV-2 is an enveloped RNA virus. It has been demonstrated that SARS-CoV-2 enters the body by binding to angiotensin-converting enzyme 2 receptors (ACE2) expressed in vascular endothelial membrane in many organs such as lungs, gastrointestinal system, kidney and testicles by spike proteins, and causes infection [8]. These ACE2 receptors have been shown to be non-existing in the vaginal epithelium [9]. This could be the reason why neither our study nor other studies could detect the virus in the vagina.

The potential for a patient to spread the infection requires the virus to be present in different body fluids. In the literature, the virus has been isolated in the urine, feces, nasopharyngeal swab sample and blood [10]. In a study conducted, it has been shown that although the clinical symptoms of the patents improved and PCR tested negative for the swab sample taken from the throat, the virus was found to be present in the samples taken in later periods and to continue spreading [11]. In our study, the samples were taken in early period and the virus could not be isolated in the vaginal fluid. Accordingly, since the invasion duration of the virus in the tissues is not known, the presence of the virus should be evaluated by taking samples from the vaginal fluid in later periods.

The limitations of our study are that the number of patients included in the study was low, the patients were not evaluated together with their sexual partners, the patients did not have serious diseases that required intensive care though they tested positive in PCR, and the vaginal fluid samples were taken in the first days of hospitalization (0–2 days) at the beginning of the treatment. There is a need for further studies in which vaginal samples taken in later periods from women who were clinically confirmed to be infected with COVID-19 through PCR are compared.

CONCLUSIONS

In our study, SARS-CoV-2 virus could not be detected in the vaginal fluids of female patients during the reproductive period who tested positive for COVID-19 infection. Our results are important in that they show that the lower genital system in infected women in reproductive period is not a mode of transmission for SAR-CoV-2. In addition, our results can provide a guidance for the choice of delivery method in infected pregnant women in the management of SARS-CoV-2. Randomized controlled studies with large samples are needed regarding this issue.

Conflict of interest

The authors declare no conflict of interest.

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Pilot study of testing a clinical tool for pelvic physical examination in patients with vulvodynia

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ABSTRACT

Objectives: Vulvodynia diagnosis is based on medical history and physical examination. The study is aimed to evaluate the clinical usefulness of a pelvic floor physical examination (VAMP protocol) for vulvodynia diagnosis, applied during gynecological examination, proposed as educational and diagnostic tool.

Material and methods: Pelvic physical examinations were performed for 650 non-pregnant female patients. A study group of 449 cases met the vulvodynia diagnostic criteria (120 with provoked, 104 with spontaneous, and 121 with mixed subtype) and were compared with those of 201 healthy individuals. Four anatomical regions were examined: the vulva (V) and anus (A) with a cotton swab, the internal pelvic muscles (M) with a digital examination of the levator ani, and the paraurethral (P) area with digital pressure. Only the maximum pain score for a given area was recorded, using a Numerical Rating Scale. The four anatomical regions were recorded under the VAMP acronym.

Results: Differences in mean scores VAMP protocol were statistically between vulvodynia and comparison group for V = 6.48 vs 0.98; M = 6.29 vs 1.05; and P = 6.89 vs 1.33, with exception of A = 0.03 vs 0.08. Patient age, weight, way of delivery, other concomitant diseases (*e.g.*, dysuria, anal and bowel symptoms), vulvodynia subtype, and pain duration did not influence VAMP scores in patients with vulvodynia and comparison group.

Conclusions: Pelvic examination according to VAPM protocol can be applied in vulvar pain patients for diagnostic purposes. Besides of vulvodynia symptoms any other analyzed variables did not influence on scores of VAMP protocols. We found that cut-off score ≥ 3 even in one of V, M or P component of VAMP protocol can be considered as diagnostic criterium for vulvodynia. Component A (anus area) was not useful for vulvodynia diagnosis.

Key words: gynecological exam; pelvic examination; pelvic floor muscles; vulvar pain; vulvodynia

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INTRODUCTION

Vulvodynia (Vd) is a form of chronic vulvar pain and other discomfort that persists for more than three months in the absence of any evident vulvovaginal pathology, in accordance with the criteria outlined in the recent consensus statement [1]. Chronic vulvar pain is most commonly described as pain perceived superficially in areas of the vulva, anus, perineum, or vagina [2–4]. The International Society for the Study of Vulvovaginal Disease (ISSVD) described Vd as vulvar discomfort, reported as pain, irritation, pruritus, sickness, and oversensitivity, which are often overlooked [1, 2]. Remission and relapse are common, although consecutive recurrences can present varying symptoms that can be confusing for the patient and healthcare practitioners.

The classification of vulvodynia is based on the site of the pain; whether it is generalized, localized, or mixed; whether it is provoked (pain on touching, pressure, vaginal penetration) or spontaneous (for no apparent reason), or mixed; whether the onset is primary or secondary; and the temporal pattern (whether the pain is intermittent, persistent, constant, immediate, or delayed) [1, 2] .

In the general population, Vd affects 8.3–16% of adult women [2, 5, 6], and the most prevalent form is provoked Vd, which presents in 12% of women and can cause superficial dyspareunia. Spontaneous Vd is less common (6–7% of Vd) [5].

Vd is classified as a functional pain syndrome that is mediated by pelvic floor muscles (PFM) dysfunction in the

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form of an overactive, hypertonic/non-relaxing muscle state that then potentially gives rise to pain [3, 5–11]. Overactive muscles have been suspected in 80–90% of patients with Vd and provide the indication for pelvic manual and general physiotherapy [12, 13]. A psychologic predisposition to chronic functional pain syndromes has been previously identified [14], and emotional stress can contribute to myofascial disorders. The manual palpation of muscles and points of tenderness (trigger points) provides an effective real-time method for identifying the source of pain in Vd [9, 15–17], although there is no standardized protocol for clinical practice [18]. From the literature, several key points should be maintained for pelvic physical exam requirements in patients with chronic pain [18]. The Numeric Rating Scale (NRS) is a preferred scale for patient self-perceived pain ratings [9, 19–21], graded during the test using a numeric scale of 0 to 10, with 0 representing "no pain at all" and 10 suggesting "the worst possible pain".

Diagnosis of Vd is based on medical history (reported symptoms, duration of pain or other discomfort), vulvar examination with exclusion of other specific disorders as a cause of pain, like identified infection (*e.g.*, candidiasis, herpes), inflammation (*e.g.*, lichen simplex chronicus, sclerosus, lichen planus), neoplasia (eg, Paget disease, vulvar cancer), or a neurologic disorder (*e.g.*, herpes neuralgia) [1]. Consecutive step in Vd diagnosis is composed of hymenal remnant base pain on pressure as a cotton swab testing [9, 15]. Vestibular cotton swab test remains PFM referred pain anatomical area, what is sufficient for Vd diagnosis [22]. However, examinations of additional anatomical areas can give additional, accurate insight to increase internal consistency in PFM overactive status assessment in Vd women [15–17]. Examination of pelvic muscles is recommended but rarely conducted. Currently no standardized protocol exists [9, 15, 17, 18].

The Integrated Pain Mapping and Assessment Protocol (IMAP) was developed for research purposes as referred pain diagnostic maps by Jantos [13, 19], and includes 54 relevant and irrelevant, external and internal points of examination. Shortened version of IMAP, which focuses on the vulva, anus, internal pelvic muscles, and paraurethral regions was elaborated, proposed as the part of gynecological examination, recorded as VAMP acronym and evaluated in this study as a simplified clinical pain mapping modality for diagnostic and educational purposes. The study is aimed to evaluate the clinical usefulness of a pelvic floor physical examination (VAMP protocol) for vulvodynia diagnosis, applied during gynecological examination, proposed as educational and diagnostic tool.

MATERIAL AND METHODS

The study characteristics were extracted from the medical records at outpatient clinic for women, with a focus on vulvar diseases. The study was approved by the appropriate IRB. We included patients with Vd who were seen between January 2016 and July 2018. The described diagnostic method has been applied as a routine practice at the center since 2014. The comparative data was obtained from a group of healthy women between February 2019 and June 2019. Every patient in both groups were diagnosed by the study authors. A total of 650 women were included in the study, 449 of who met the diagnostic criteria for Vd, as a study group, and 201 women were assigned to the control group. The included participants were nonpregnant adults, with no serious general diseases and urogenital surgery performed previously.

Vd was diagnosed as idiopathic vulvar pain (raw or vague) or other discomfort as burning sensation, stabbing, pressure, and pulling pain, also as the pruritus or dryness, discomfort, and oversensitivity. The symptoms sustained at least for three months, induced by vaginal penetration (provoked), without specific trigger (spontaneous) or both (mixed Vd subtype) [1, 2]. A general medical and gynecological history were recorded for every case, and a physical exam was carried out. Four anatomical regions were examined: the vulva (V) and anus (A) with a cotton swab test, the internal pelvic muscles (M) with a digital examination of the levator ani, and the paraurethral (P) area with digital pressure (Fig. 1). In every patient their VAMP scores were determined as a part of a gynecological exam, for any visible or detectable pathology exclusion. In the litothomy position vaginal speculum insertion, for gynecological purposes, was permitted to take place before the internal exam (when accepted or possible).

For the VAMP protocol, the following steps were followed consecutively (as outlined in Fig. 1):

- The Numerical Pain Rating Scale (NRS) was explained, through which the patients were asked to rate each examined point, if painful.
- Verbal consent should be taken after an explanation.
- A cotton swab test was performed by applying gentle pressure to five points within the vestibular base of the hymenal remnant (following a vestibular clock from the two to ten position) using a dry swob. The pressure was adjusted to a level tolerated by the patient. Only the maximum NRS rating was noted (V).
- A similar cotton swab test was then used for two points around the anus, with similar pressure as that used for the vulva, and the NRS maximum rating was noted (A).
- The insertion of one lubricated, gloved index finger, for a bimanual transvaginal or rectal examination was performed.
- The palmar side of the index finger was rotated backward to palpate the pelvic floor muscles. The finger was moved laterally along the length of the rectum, using the single sites mid-muscle belly technique, from the

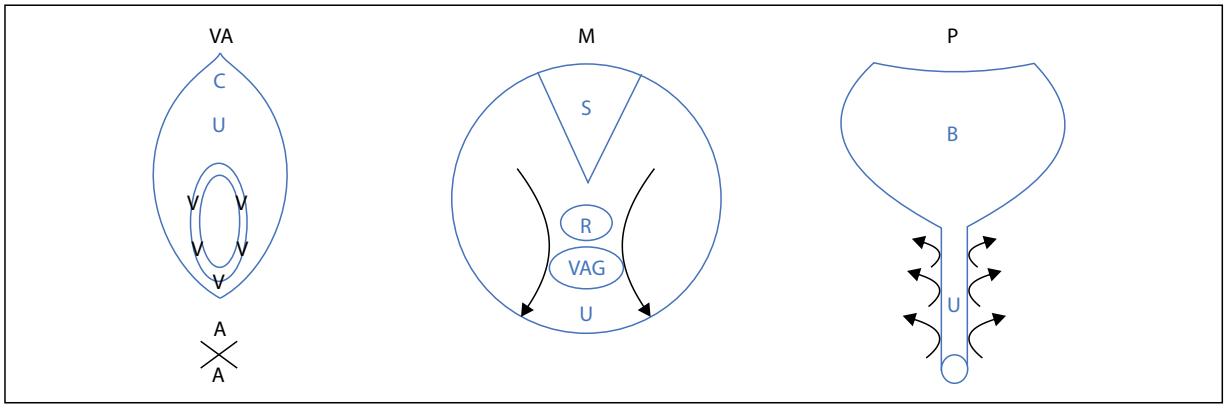

Figure 1. VAMP protocol diagram; V — vulva 5 points cotton swab pressure; A — anus 2 points cotton swab pressure; C — clitoris; U — urethra; S — sacrum; R — rectum; VAG — vagina; B — bladder; arrows — internal digital examination

inside to the outside on both the right and the left side. A bilateral sliding motion was performed, with marked pressure applied to the muscles until the patient's accepted pain threshold was reached. The maximum NRS rating was noted (M) in the medical records.

- For paraurethral area examination, the palmar side of the index finger was rotated upward, laterally to the urethra, and similarly to the point of urethra detachment from the pubic bone. The movement was performed from the outside to the inside of the pubis, lengthwise, on both the right and left sides. The pressure was increased with particular attention to patient pain tolerance, and the maximum NRS rating was noted (P).

The physical exam results were recorded under the VAMP acronym (*e.g.*, VAMP 3048), which reflects the maximum NRS ratings in the four areas: vulva, anus, muscles, and paraurethra. The pelvic physical examination added approximately two minutes to the gynecological examination. The VAMP scores were evaluated in relation with patient age, body weight, and deliveries in both groups, in relation to pelvic diaphragm comorbidities and type of pain felt by the study group.

Statistical analyses were performed using Statistica v 9.1, provided by Statsoft Polska, with population parameters (mean and standard deviation), $\chi 2$ test, one-sided t-test, Spearman Correlation and Mann-Whitney U test for critical value $p = 0.05$ application.

RESULTS

Among the 650 women who were included in this study, 449 (69.1%) women comprised the Vd group and 201 (30.9%) comprised the control group. According the Vd subtype differentiation [1], half of the Vd cohort suffered from mixed Vd (50.1%, 225 women), followed by provoked Vd (26.7%, 120 women) and spontaneous Vd (23.2%, 104 women).

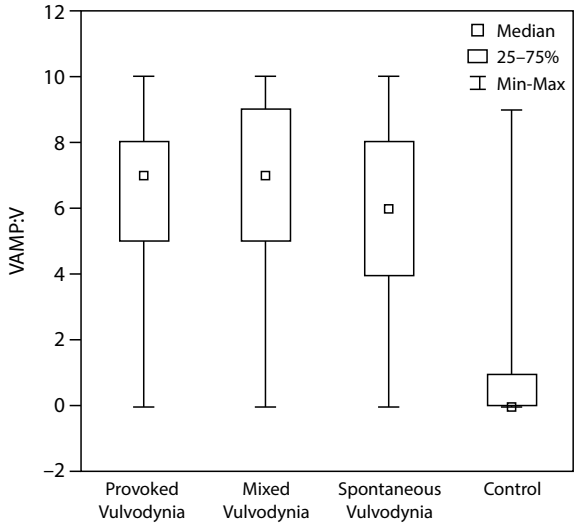

Figure 2. VAMP protocol score: vulvar pain (V) in the three vulvodynia subgroups and the control group

The individuals in the study and the control group differed slightly in age (34.7 years vs 37.6 years old) and body weight (61.3 kg vs 66.0 kg) ($Z = -3.256$; $p < 0.001$).

Differences in the VAMP protocol scores were identified between the Vd and the control comparisons in features V (vulva) (6.48 ± 2.64 vs 0.98 ± 1.96), M (muscles) (6.29 ± 2.55 vs 1.05 ± 1.90), and P (paraurethral area) (6.89 ± 2.37 vs 1.33 ± 2.17), with $p < 0.001$ for each of above values. The Vd and comparative cohort did not differ in the A (anus) item (0.03 ± 0.37 vs 0.08 ± 0.57, respectively, $p = 0.11$). There were also no differences in the VAMP scores between the three vulvodynia subtypes (provoked, mixed, and spontaneous) (Fig. 2–4).

Regression analysis showed no differences in the VAMP ratings in relation to age, body weight, or cesarean delivery. Patients who underwent vaginal deliveries presented with

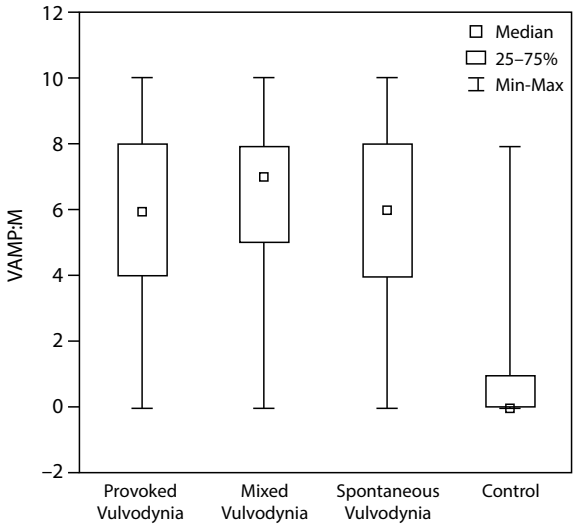

Figure 3. VAMP protocol score: muscle pain (M) in the three vulvodynia subgroups and the control group

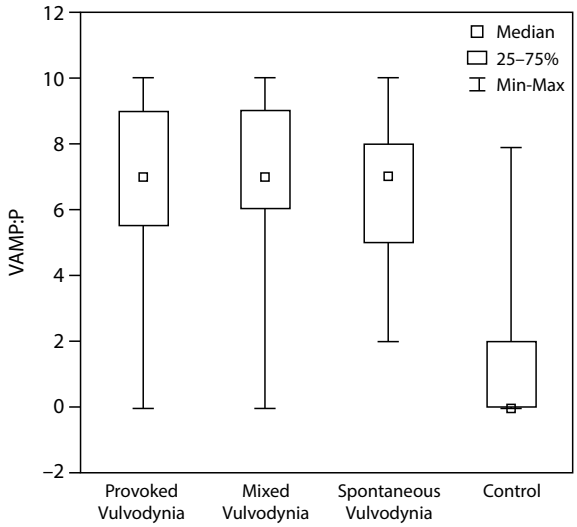

Figure 4. VAMP protocol score: paraurethral area pain (P) in the three vulvodynia subgroups and the control group

Table 1. VAMP protocol score correlation with age, body weight, and vaginal and cesarean delivery in the study (Vd) and control group, based on the regression analysis

VAMP Feature	V	M	P
Vulvodynia	p ≤ 0.001***	p ≤ 0.001 ***	p ≤ 0.001 ***
Age	p = 0.481 (NS)	p = 0.179 (NS)	p = 0.689 (NS)
Body weigh	p = 0.475 (NS)	p = 0.493 (NS)	p = 0.256 (NS)
Vaginal delivery	p = 0.036 *	p = 0.310 (NS)	p = 0.256 (NS)
Cesarean delivery	p = 0.737 (NS)	p = 0.500 (NS)	p = 0.968 (NS)

V — vulva; M — muscles; P — paraurethral area; NS — not significant

lower V scores (Tab. 1), and this was the only significant correlation, other than Vd diagnosis, in all of the groups.

On the basis of statistical data, VAMP protocol score cut-off (NRS on pressure) was defined according to consecutive features: V, score 2; A, not relevant; M, score 3; and P, score 3, which differed between the study and the healthy control, comparison group. Based on the ROC curve analysis, there were no differences between VAMP features in the study cohort (pV-M = 0.959, pV-P = 0.647, pM-P = 0.539).

Women with Vd showed stable P scores (R Spearman = 0.074; p = 0.126) that were independent of the duration of pain, whereas V scores (R Spearman = 0.136; p = 0.005) and M scores (R Spearman = 0.104; p = 0.032) increased with persistent symptoms, although the correlation was very weak.

Type of pain and discomfort were divided in Vd women: 1, pain without specific description (raw or vague) in 34.5% (155 women); 2, burning sensation in 50.1% (225 women); 3, stabbing, pressure, and pulling pain in 10.9% (49 women); 4, pruritus in 42.1% (189 women); and 5, dryness, discomfort, and oversensitivity in 23.2% (104 women), in the study cohort. When compared with the VAMP features, the highest V scores (Z = –4.01; p < 0.001), M scores (Z = –3.51; p < 0.001), and P scores (Z = –3.66; p < 0.001) were observed in relation to pain (description 1). In the remaining symptoms (descriptions 2, 3, 4) VMP scores were insignificant for Vd group (range from Z = –0.27 , p = 0.7 to Z = 0.1, p = 0.92). Lower M scores (Z = 2.30; p = 0.021) were associated with dryness, discomfort, and vulvar oversensitivity (description 5).

No differences in VAMP protocol scores were found in relation to pelvic diaphragm symptoms coexisting with Vd (Tab. 2).

DISCUSSION

In our study cohort, half of the women with Vd presented a mixed (both spontaneous and provoked symptoms) Vd subtype, 25% presented provoked, and 25% presented spontaneous. This differs from previous reports in the literature, where provoked Vd has been reported as the most prevalent subtype (64.8%), followed by spontaneous (20.3%) and mixed Vd (14.9%) [2]. The differences may be attributed to the detailed patient history that was taken, as even mild

Table 2. Mann-Whitney U test comparison of VAMP protocol values with comorbidities in the study (Vd) and control group

Symptoms	% cohort	Nr of woman	V	M	P
Vd and dysuria	35.2	158	p = 0.124 (NS)	p = 0.660 (NS)	p = 0.083 (NS)
Vd and bowel/ anal symptoms	42.8	192	p = 0.702 (NS)	p = 0.330 (NS)	p = 0.218 (NS)
Dysuria in control	13.1	24	p = 0.217 (NS)	p = 0.063 (NS)	p = 0.098 (NS)
Bowel/anal symptoms in control	38.2	76	p = 0.744 (NS)	p = 0.107 (NS)	p = 0.805 (NS)

Vd — vulvodynia; V — vulva; M — muscles; P — paraurethral area; NS — not significant

recurrent vulvar itch, irritation, or discomfort (*e.g.*, the dryness or oversensitivity found in one fourth of women with Vd) were considered as spontaneous symptoms in patients with provoked Vd, which satisfied mixed Vd subtype criteria.

Vague or raw pain symptoms were found in one third of the patients with Vd, although different unpleasant vulvar sensations (stabbing, pulling, pressure, and irritation) were equally found in the study group. The most frequent descriptor of vulvar pain was a burning vulvar sensation (half of the women with Vd), followed by vulvar itch (42.1%). Mistakenly, these items are often not considered as pain by healthcare professionals or the affected patients. Vd diagnostic criteria, including history taking and physical (gynecological and pelvic) examination, have to be introduced for such patients with recurrent vulvar symptoms.

In the process of diagnosing patients with Vd, a few consecutive steps have to be undertaken [3, 6, 9, 13], in agreement with the study protocol. PFM status examinations were used as a diagnostic tool for every patient with Vd and were proven useful in distinguishing approximately 80–90% of patients who benefit from pelvic manual and general physiotherapy, indicated for spontaneous and provoked Vd with PFM dysfunction [23–25]. The main feature of overactive PFM is pain upon touching the vulva (referred pain) and deep muscles upon internal (vaginal or rectal) access [15]. Thus far, no physical pelvic examinations for pelvic floor myofascial pain syndrome assessments have been standardized [18]. Women with prior vaginal delivery presented less vestibulum sensitivity on pressure (lower V score in both study and control group). Regarding to vulvar pain, vulvar score (V) and paraurethral pain on pressure (P) score were higher in patients with vague, raw pain than in those with other pain descriptions. This could be because certain types of pain *per se* might be more troublesome for patients, and ischemic pain is often described as stabbing, burning, heavy, exhausting and results in lower pain thresholds that are consistent with peripheral sensitization [26].

Since the IMAP research and publications in 2015 [13, 19], where three maps were proposed for pelvic examinations in patients with Vd, a short, modified version (the VAMP protocol) has entered clinical practice and research in outpatient clinic in diagnosing chronic vulvar pain.

Pain on pressure in VAMP protocol scores were highly significant in patients with Vd, according three features: V, M and P. This reflects the importance of the three particular referred trigger points for PFM overactive dysfunction. The paraurethral area has to be emphasized as a diagnostic anatomical region.

The anal sphincter area did not experience pain on touch, as noticed in previous publications [19], in both groups, and thereby was excluded from further statistical analyses. Even though the anal cotton swab pressure test (A feature in VAMP) was advised by the study authors, to concentrate the physician's attention on that particular area and to identify the presence of serious pain sources such as anal fissure, lichen simplex or hemorrhoids, especially "red flags" such as anal cancer, should not to be overlooked. The second reason why the anal area should be tested is to objective the overestimation of all VAMP protocol scores due to catastrophism or intentional patient's pain simulation, describing as painful area on pressure.

The relevant level of PFM pain on examination (as the M feature in the present study) is consistent with other reports that used a cut-off pain score of 3, where pain ≤ 3 was considered not clinically significant and pain scores >3 were deemed significant [27]. In another publication, pelvic floor myofascial pain was determined by transvaginal palpation of the bilateral obturator internus and levator ani muscles, and, in NRS, it was categorized as none (0), mild (1–3), moderate (4–6), or severe (7–10) for each site [25]. Scores ≥ 3 (in NRS on examination) on at least one area (V ≥ 3, M ≥ 3, or P ≥ 3) confirmed PFM overactive dysfunction in this study. The three VAMP examination areas can give a broader overactive PFM status estimation than the cotton swab test alone, to improve reliability, as internal consistency of Vd assessment method.

Based on the systematic review of the pelvic physical examination techniques used to assess pelvic floor myofascial pain in women, no specific technique has been standardized yet, although the key points have been emphasized [18]. Our search for professional pelvic floor muscles dysfunction assessments that are commonly used to validate patient-reported pelvic symptoms identified the Pelvic Floor Distress

Inventory-20, Pelvic Floor Impact Questionnaire, and Pelvic Organ Prolapse Incontinence Sexual Questionnaire are considered as recommended materials [11, 16]. The PFM status diagnostic methods that are useful for clinical practice are mostly dedicated to underactive PFM dysfunction assessment [11, 16]. Overactive PFM dysfunction estimation methods based on physical examination require simplification, description for clinical and educational purposes and further validation.

The study limitation concerns the examination according to VAMP protocol were performed unblinded without a calibrated method of applied pressure, which results from the simplification of the diagnosis protocol for clinical practice. Another weakness of presented method is subjective character of results reported by the patients during examination, although perceived pain is always considered as subjective.

The consciousness of women to PFM problems have grown with time, along with the demand for healthcare professionals in the chronic vulvar pain diagnosis and treatment field. The study provides more detailed information to explain vulvodynia management complex issue, method for gathering data on sensitive and neglected woman health problem. The examination according to VAMP protocol data collection is not time consuming, performed as a part of bimanual exam of woman with persistent vulvar pain. According to the literature and authors experience, the confirmation of an overactive PFM status is crucial for further management through which manual therapy and biofeedback can be applied [10, 13, 18]. Our study suggests that VAMP protocol examination can be useful clinical tool for diagnostic and educational purposes. There is a need for perspective, multicenter studies to confirm VAMP protocol usefulness among physicians dealing with Vd patients.

CONCLUSIONS

Considering the high prevalence of pelvic floor myofascial pain in Vd patients a routine assessment for pelvic floor dysfunction should be followed for all patients who present for chronic vulvar and pelvic pain [9, 25]. Pelvic examination according to VAPM protocol can be applied in vulvar pain patients for diagnostic purposes. Besides vulvodynia symptoms, no other analyzed variables had influence on scores of VAMP protocol. We found the cut-off score ≥ 3, even in one of V, M or P component of VAMP protocol, can be considered as diagnostic criterium for Vd with PFM overactivity. Component A (anus area) was not useful for Vd diagnosis, although remains useful for educational purposes. The VAMP schedule is proposed to be a simple exam protocol that consists of a cotton swab test of the vestibulum, anus touch (although the anus does not experience pain upon applying pressure), pelvic floor muscles evaluation by "finger both sides with pressure motions" and finally, the application of pressure to both sides of the periurethral area. The procedure can be performed during a gynecological exam for Vd diagnosis in chronic vulvar pain women.

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Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication.

Ethics approval

IRB approval KE-0254/111/2020, Medical University of Lublin, Poland

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Evaluation of the effect of Birth Preparation Program on birth satisfaction with „Salmon's Item List" scale

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ABSTRACT

Objectives: The aim of this study is to investigate the effects of a birth preparation program on birth satisfaction.

Material and methods: This cross-sectional study was conducted with patients who applied to our hospital between January 2018 and January 2019. A total of 164 pregnant women (Study Group) who applied for the birth preparation program and completed all training in our hospital and 152 pregnant women who did not apply for the birth preparation program and who did not know about such training (Control Group) were included in the study. Demographical data and obstetric parameters of the groups were recorded. All patients were evaluated with the Visual Analog Scale and Salmon's Item List scale 48 hours after the delivery. The scores of both groups were compared.

Results: There were no significant differences between the groups in terms of age, gravida, parity, gestational week of birth, the birth weight of infants, and 5th-minute APGAR scores. It was found that the Visual Analog Scale scores of the Control Group were significantly higher than in the Study Group. The Salmon's Item List scores of the Study Group were significantly higher than those of the patients in the Control Group (< 0.01).

Conclusions: The birth preparation program increases satisfaction during labor and decreases the traumas that may occur in the following births and increase comfort in the postpartum period. For this reason, such programs must be applied commonly to ensure that women can face both the birth and postpartum processes comfortably.

Key words: childbirth satisfaction; Salmon's Item List score; birth preparation; labor pain; antenatal education

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INTRODUCTION

Birth preparation programs, which have been applied routinely since the beginning of the 20th Century [1], are called under various names in several countries like birth preparation program, Expectant Parent Classes, Antenatal Parenthood Education, Antenatal Education, Childbirth Classes and Antenatal Classes. A significant percentage of pregnant women participate in birth preparation programs worldwide. The United States, the UK, Canada, Mexico, Brazil, Finland, Germany, Australia, Japan, Turkey, and China are among countries that actively implement these programs [2].

In most developed countries, birth preparation programs are planned to inform, train, support, and help parents to cope with the challenges that will appear in the maternity process and childbirth. Pregnancy and childbirth are among the most special and important experiences in women's lives. Regardless of the way a mother gives birth, it is important to support mothers to have a positive birth experience because it affects their self-esteem and mother-baby interaction. Satisfaction in the pregnancy process and labor action is the indicator of the quality of the medical institution and is also effective on women's health and the health of the newborn [3]. After a dissatisfied and traumatic birth, depression, post-traumatic stress disorder, less breastfeeding of the newborn, apathy to the newborn, and bad sex life can be observed [4].

Birth preparation programs have become an integral part of healthcare services, and constitute an important component of prenatal care. The contents of birth preparation programs vary among countries; however, the common target in all these programs is to prepare parents for childbirth and parenting [2].

Studies conducted on antenatal education are limited in number and have yielded conflicting results. Although

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it was reported in previous studies that birth preparation program contributes to increasing women's satisfaction during labor, provides their active participation in this process, and also ensures that women face less psychological problems during the postpartum process [5–7], there are also other publications arguing that these training do not have any effects [8, 9].

Two wide-series literature reviews were published recently on measuring birth satisfaction. Various questionnaires used to assess birth satisfaction were compared in these two compilations, and it was reported that various questionnaires could be used for measuring birth satisfaction [10, 11]. In this study, the "Salmon's Item List (SIL)" questionnaire was preferred to evaluate the satisfaction during childbirth. Because, as the questionnaire consists only of adjectives, it is also quite easy for postpartum women to answer. SIL was developed in 1992 [12] and was designed to measure women's feelings about birth and their birth experiences.

The purpose of the present study was to investigate the effect of the Birth Preparation Programs on birth satisfaction

MATERIAL AND METHODS

This cross-sectional study was conducted with patients who applied to the second clinic of Kırıkhan State Hospital, Hatay, Turkey between January 2018 and January 2019. The study was approved by the ethics committee (Ethics Committee Decision No: 2019/15). Informed consent was given by all participants before their enrolment into our study. A total of 164 pregnant women (Study Group), who applied for birth preparation programs, which was actively run in our hospital, who completed all relevant training in our hospital, and who gave birth in our hospital; and 152 pregnant women, who did not apply for birth preparation programs previously, who did not know about such training and who gave birth in our hospital (Control Group), were included in the study. Those who delivered their babies with cesarean section upon the request of the mother, those who gave birth before the 34th week of pregnancy, who had chromosomal and structural malformations in the fetus, those who gave intrauterine stillbirth, those with postpartum bleeding and severe preeclampsia, and those with additional diseases due to pregnancy were excluded from the study. Also, pregnant women who did not want to participate in the study or who did not complete our birth preparation program were not included in the Study Group. The gestational weeks of all included patients were calculated according to the last menstrual dates. In pregnant women who did not know their last menstrual dates, the gestational week was calculated by using the fetal crown-rump length measurement in the first trimester. The patients in both groups gave birth in the same maternity rooms and under the same conditions. Episiotomy was performed routinely for all nulliparous patients. No episiotomy was performed for multiparous patients. No patients received analgesics before birth.

The age, gravida, parity, birth shape, birth weight of the baby, 5th-minute APGAR scores, birth weeks, education and income status, and professions of all patients were recorded; and both groups were compared with each other. All patients were evaluated 48 hours after birth for estimating how much pain they suffered and for evaluating their pain severity with the Visual Analog Scale. The patients in both groups were also evaluated in terms of birth satisfaction by applying the SIL scale face-to-face 48 hours after the delivery. The Visual Analog Scale (VAS) values and SIL scores of both groups were also compared.

Birth preparation program

Our hospital has a birth preparation program class that was approved by the Ministry of Health of the Republic of Turkey in November 2018. Pregnant women between 16––20 weeks are informed about the program, and those who wish to participate in it are enrolled. All pregnant women, who have their first pregnancy, multiparous and have given normal birth previously, or who have the previous form of delivery as cesarean section, can participate in this program. Each participant is subjected to a three-hour training session once a month for four months. These sessions are provided to a group of up to 10 women. There is a projection system in the training room, and pregnant women are informed with a presentation about the pregnancy process, birth, and postpartum period. Besides, there are also whiteboards, educational models, Pilates balls, and yoga mats in the training room. A psychologist, a physiotherapist, a dietitian, an obstetrician, a child development specialist, and two midwives participate in the program. The main themes in each session are summarized in Table 1. A certificate is given to the participants who complete all sessions at the end of the program. The program is free. There is no obligation to participate and participation is voluntary.

Salmon's Item List (SIL)

In this study, the form of SIL that was translated from the English version was used [12]. The themes measured in the scale are the feelings of women regarding their labor and delivery experiences. There are 20 items that comprise contrasting adjective pairs (*i.e.* fulfilled-not fulfilled, easy-not easy, etc.). The SIL scale is shown in Table 2. Each item was rated between 1 and 7 points according to the satisfaction status; 2nd, 3rd, 4th, 5th, 7th, 8th, 9th, 10th, 12th, 13th, 14th, 17th, 18th, and 20th. The points given to the questions were subtracted from 8 points (in this way, the lowest point was 1, the highest was 7 points), and all points were averaged. This average value was then multiplied by 20,

Table 1. Headings of the courses in Birth Preparation Programs

Session 1	Structure of female and male reproductive organs, and the formation of pregnancy
	Maternal changes during pregnancy and recommendations
	Intrauterine fetal development and growth
Session 2	Labor preparation plan
	Stages of labor
	Drug-free methods used in coping with labor pains
	Caesarian birth
	After 20th gestational week (3-Session Training) • Breathing exercises during pregnancy (Practice) • Massage techniques (Practice) • Exercise during pregnancy (Practice) • Birth yoga (Practice) • How to bathe the infant? • Clothing the infant • Massage to the infant • Delivery room and maternity ward trip
Session 3	Postpartum period
	Nutrition in the postpartum period
	Breast milk and breastfeeding
	Care of the newborn

and 20 was subtracted from the resulting value. As a result, the calculated values ranged from the lowest 0 points to the highest 120 points. Although SIL was actively used in many countries, the German Salmon's Item List (SIL-Ger) version was published in 2001 by Stadlmayr et al., and the (SIL-Ger) computational system was used in this study [13]. SIL-Ger scores ≥ 70 suggested satisfactory experience; however, < 70 scores were considered to be an unsatisfactory experience [13, 14].

Visual Analog Score (VAS)

The meaning of the numbers ranging from 0 to 10 on a 10-cm line was explained to the patients. No pain at all was scored with 0, the most severe pain felt in life was scored with 10 points, and moderate pain was scored with five points. The patients were asked to mark their pain on this 10-cm line according to these explanations.

Statistical analysis

The Statistical Package for Social Sciences 20.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analyses. The distribution of the data was evaluated with the Kolmogorov-Smirnov test. Descriptive statistical methods (mean, standard deviation) were used in the evaluation of normally distributed data, and the independent t-test was used to compare paired groups. If the distribution of variables was not normal, the Mann-Whitney U-test was used. The results were evaluated at a $p < 0.05$ significance level.

Table 2. Salmon's Item List

Items	Original English version	
1	Disappointed	Not disappointed
2	Fulfilled	Not fulfilled
3	Enthusiastic	Not enthusiastic
4	Satisfied	Not satisfied
5	Delighted	Not delighted
6	Depressed	Not depressed
7	Happy	Not happy
8	Excited	Not excited
9	Good experience	Bad experience
10	Coped well	Did not cope well
11	Cheated	Not cheated
12	In control	Not under control
13	Enjoyable	Not enjoyable
14	Relaxed	Not relaxed
15	Anxious	Not anxious
16	Painful	Not painful
17	Easy	Not easy
18	Time going fast	Time going slowly
19	Exhausted	Not exhausted
20	Confident	Not confident

RESULTS

A total of 990 pregnant women applied to the birth preparation program of our hospital between January 2018 and January 2019; and 180 of these pregnant women completed all three sessions, received the participation certificate, and gave birth in our hospital. Since 12 of the 180 pregnant women gave birth before 34 weeks, two had fetal abnormalities, one had an intrauterine stillbirth, and one had postpartum atony, 16 pregnant women were excluded from the study, and a total of 164 pregnant women were included in the Study Group. The Control Group included 152 pregnant women who gave birth in our hospital between the same dates and did not apply for any birth preparation program during pregnancy, which means that 316 patients were included in the study in total. The demographic data of both groups are listed in Table 3.

The mean age of the Study Group was 26.3 ± 0.9 and that of the Control Group was 25.4 ± 1.2. No significant differences were detected between the groups. There were no significant differences between the groups in terms of gravida, parity, gestational weeks of birth, birth weight of the infant, and 5th-minute APGAR scores. In both groups, pregnant women who had never received education at schools were at the largest number (the study group 19.5%, and Control Group 23%). Most of the groups had housewives (the Study Group 48.1%, and Control Group 48%).

Table 3. Comparison of demographical data and obstetric parameters of the groups

	Study Group (n: 164)	Control Group (n: 152)	P
Age (mean ± SD)	26.3 ± 0.9	25.4 ± 1.2	0.53
Gravida	1.8 ± 1.1	1.9 ± 0.9	0.42
Parity (mean ± SD)	1.4 ± 0.2	1.3 ± 0.5	0.33
Gestational age at delivery (week ± SD)	38.2 ± 0.6	38.6 ± 0.5	0.25
Delivery method (n, %)			
• Cesarean section (n)	16 (9.7)	14 (9.2)	0.62
• Vaginal birth (n)	148 (90.2)	138 (90.7)	0.53
Birth weight (g ± SD)	3375 ± 230	3220 ± 150	0.48
5th-minute APGAR score (mean ± SD)	9.0 ± 0.5	9.2 ± 0.5	0.52
Education (n, %)			
• None	32 (19.5)	35 (23)	N/A
• Primary	19 (11.5)	12 (7.8)	
• Secondary	40 (24.3)	34 (22.3)	
• High	38 (23.1)	41 (26.9)	
• University	32 (19.5)	29 (19)	
• Master's degree	3 (1.8)	1 (0.6)	
Profession (n, %)			
• Housewife	79 (48.1)	73 (48)	N/A
• Unemployed	12 (7.3)	15 (28.8)	
• Part-time employee	29 (17.6)	22 (14.4)	
• Full-time employee	24 (14.6)	25 (16.4)	
• Other	20 (12.1)	17 (11.1)	
Income (n, %)			
• Subsistence wage	48 (29.2)	52 (34.2)	N/A
• Middle	75 (45.7)	72 (47.3)	
• High	41 (25)	28 (18.4)	

SD — standard deviation; N/A — not applicable

Table 4. Comparison of Visual Analog Scale and Salmon's Item List scores of the groups

	Study Group (n: 164)	Control Group (n: 152)	P
VAS score (mean ± SD)	7.0 ± 0.6	9.1 ± 0.5	< 0.01
SIL-Ger score (mean ± SD)	108.5 ± 4.2	79 ± 3.5	< 0.01
SIL-Ger score ≥70 (n, %)	149 (90.8%)	42 (27.6%)	< 0.01

VAS — Visual Analog Scale; SIL — Salmon's Item List

Table 5. Comparison of those with Salmon's Item List score ≥ 70 and < 70

	SIL-Ger score ≥ 70 (n: 191)	SIL-Ger score < 70 (n: 125)	P
Rate of participation in the program	149 (78%)	15 (12%)	< 0.001
Age (mean ± SD)	25.4 ± 0.7	23.2 ± 0.2	0.06
Gravida	1.5 ± 2.1	1.6 ± 0.7	0.42
Parity (mean ± SD)	1.5 ± 0.4	1.4 ± 0.1	0.18
Gestational age at delivery (week ± SD)	37.6 ± 0.4	38.0 ± 0.6	0.14
Education (n, %)			
• None	25 (13)	42 (27.6)	0.02
• Primary	14 (7.3)	17 (13.6)	0.62
• Secondary	47 (24.6)	27 (21.6)	0.43
• High	52 (27.2)	27 (21.6)	0.003
• University	42 (21.9)	19 (15.2)	< 0.001
• Master's degree	4 (2)	0 (0)	< 0.001
Birth weight (g ± SD)	3082 ± 120	3320 ± 312	0.48
5th-minute APGAR score (mean ± SD)	9.3 ± 1.5	8 ± 0.3	< 0.001
VAS score (mean ± SD)	7.21 ± 0.33	9.33 ± 0.41	< 0.001

SIL — Salmon's Item List; SD — standard deviation; VAS — Visual Analog Scale

The VAS and SIL scores of the groups are listed in Table 4. The VAS scores of the pregnant women in the Control Group were found to be significantly higher than those in the Study Group (9.1 ± 0.5, 7.0 ± 0.6, respectively; $p < 0.01$). Also, the SIL scores of the Study Group were significantly higher than the patients in the Control Group ($p < 0.01$). A total of 90.8% of the patients who participated in the Study Group had SIL scores above 70, and only 27.6% of the Control Group had SIL scores above 70. The demographic data of the pregnant women who had SIL scores more than 70 and those under 70 are listed in Table 5. In this respect, there were no differences between the groups in terms of age, gravida, parity, week of delivery, birth weight of the infant. However, the 5th-minute APGAR scores of the pregnant women with SIL scores above 70 were significantly higher than those with lower than 70 ($p < 0.001$). Also, the number of pregnant women who had high school degrees, who were university graduates, and who had master's degree in the group with SIL scores more than 70 (27.2%, 21.9%, 2%) was significantly higher than those below SIL score 70 ($p = 0.003$, $p < 0.001$, $p < 0.001$, respectively); and in the group with SIL score below 70, those with none school education at all were significantly more ($p = 0.02$). Again, VAS scores were detected to be lower in the group with SIL scores above 70 compared to those with SIL score below 70 ($p < 0.001$). The rate of those with SIL scores over 70 in participating in the birth preparation program was significantly higher than those with SIL score lower than 70 (78%, 12%, respectively; $p < 0.001$).

DISCUSSION

It was found in the present study that the birth satisfaction rates of pregnant women who participated in the birth preparation program, which lasted four months and which

was a 12-hour program, were higher than those who did not participate in these programs. It was also found that the postpartum VAS scores of the pregnant women who participated in this training program were lower.

Several studies were conducted to examine the relation of satisfaction during pregnancy and during delivery with various demographic data like age, parity, birth type or educational level, and profession [14–16]. As a result of these studies, no full consensus was reached, and contradictions emerged. Although studies are reporting that birth satisfaction is related to the educational level, age, and income status [14], there are also some studies arguing that there are no associations between satisfaction and demographic data [16]. Among the patients who had SIL scores more than 70, the number of those who had a high school, university, and master's degrees was higher at significant levels compared to those with SIL scores below 70. It was also determined that 78% of the patients with SIL scores above 70 participated in the birth preparation program. In other words, according to our study, higher education levels might be effective in high satisfaction rates during delivery.

One of the most important parameters determining satisfaction during childbirth is a pain because it is known that birth is one of the most painful processes in human life [17]. It was reported in a previous study that pain perception was associated with low SIL scores [14]. In our study, it was found that postpartum VAS scores were lower and SIL scores were significantly higher in women who participated in the birth preparation program. However, contrary to this, a systematic review speculated that the satisfaction of birth experiences was not related to the elimination of pain, but was related with how much the expectations of the pregnant woman were met, how the attitudes and behaviors of the healthcare employees who served her were, and how good their communication was [18]. It is possible that the SIL results and satisfaction levels were high in our study since the pregnant women, who participated in the birth preparation program, were told what they could face during this process and how to control the process. In other words, the active participation of the pregnant women in this process and the fact that the birth becomes controlled in this way might have caused that the pain was felt less and the satisfaction was higher. It was found in a systematic review conducted in 2008 that antenatal training was effective for pregnant women in terms of active participation in the delivery process, and had the potential to make conscious decisions [19].

The duration and contents of antenatal training vary among countries or according to healthcare organizations that provide obstetrics services. Different training programs are varying between up to one year or one-day training. Although there are arguments regarding the effects of these different periods on antenatal training, it was reported that long-term birth preparation programs might be useful during the labor process because they include additional training like exercises, yoga, Pilates, pelvic floor training, breathing techniques, and stretching movements, etc. [5, 7]. A long-term birth preparation program was implemented in our study. Practical breathing exercises, pelvic floor exercises, and yoga training were also offered in this training. The total SIL scores of the pregnant women, who participated in this training, were found to be significantly higher compared to those who did not participate in this program (108.5 ± 4.2, 79 ± 3.5, respectively). Similarly, the VAS scores were also found to be lower (7.0 ± 0.6, 9.1 ± 0.5, respectively). Also, when fetal results were evaluated, it was found that the APGAR scores of the infants of the pregnant women with SIL scores more than 70 were higher than those with SIL scores lower than 70 (9.3 ± 1.5, 8 ± 0.3, respectively). In this context, it is possible to argue that our birth preparation program was effective because 78% of the patients with SIL scores above 70 participated in antenatal training.

It was reported that antenatal training improved the rate of breastfeeding and provided women with the necessary information about breastfeeding [20, 21]. However, no evaluations were made in our study regarding breastfeeding. Although publications are arguing that antenatal training increases vaginal birth rates [22, 23], several others do not support these data [2, 24]. In a study including 1193 cases that received and that did not receive antenatal training, it was reported that pregnant women who received training admitted to the hospital while they were actively in delivery action and that they needed epidural anesthesia less. Besides, cesarean section rates were found to be similar among groups [25]. In another study that compared 197 nulliparous women who received antenatal training and who did not receive, it was found that there were no significant differences between birth type and pain scores [9]. In our study, the effect of antenatal training on the birth type was not investigated. However, the cesarean section rate was 9.7% in the group that received training and 9.2% in the group that did not receive any training. The reason why no comparisons were made regarding the birth type in our study was that not only nulliparous patients but also patients who gave vaginal birth previously and who had a cesarean section. In other words, participation in the antenatal training was designed independently from the way of birth. For this reason, the effect of antenatal training on the birth type was not investigated.

There were some limitations to the present study. First of all, the study was conducted in single-centered fashion, and since it was a survey study, patients' educational status, literacy, perception levels of the questions or socio-demographic data might have affected the answers to the ques-

tions. Another limitation was that it was not the case in the study like including only nulliparous, only multiparous, or only cesarean patients in it. All patients who applied and completed antenatal training were included in the study regardless of their birth types. For this reason, the effect of antenatal training on the birth type could not be investigated. Also, although there is breastfeeding training in the birth preparation program, no evaluations regarding breastfeeding were made in the present study. However, the strength of our study was that unlike the studies conducted on birth satisfaction, the SIL scale was used in this study, which increased the reliability of the answers because the content of the questionnaire is easy. Also, the number of patients in the study was not less compared to other studies.

CONCLUSIONS

In conclusion, birth preparation programs help pregnant women to engage in the process actively and become part of the team. Such programs also increase the satisfaction during delivery and the comfort of the postpartum period and reduce the traumas that might occur in the following births. For this reason, such programs must be disseminated to ensure that women can feel comfortable during both the birth process and the postpartum process.

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Conflict of interest

The authors report no conflict of interest.

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Maternal body mass index and external cephalic version success rate — are they related?

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ABSTRACT

Objectives: External cephalic version (ECV) is a useful method helping to reduce the incidence of planned caesarean deliveries for fetal malpresentation. There is an effort to look for the best predictors for a successful ECV, the effect of maternal weight is still unclear.

The aim of our study is to determine maternal body mass index (BMI) in association with the ECV success rate and the risk of complications.

Material and methods: A retrospective observational cohort study in 981 women after the 36th week of gestation with a fetus in a breech presentation who had undergone an ECV attempt. We evaluated the success rate and complications of ECV in association with BMI categories according to the WHO classification of obesity.

Results: ECV was successful in 478 cases (48.7%). In the category of overweight patients (BMI > 25; n = 484), ECV was successful in 51% and unsuccessful in 49% (p = 0.28) of cases. In obese patients (BMI > 30; n = 187), ECV was successful in 44.8% and unsuccessful in 55.2% (p = 0.28) of cases. The effect of BMI on the success rate of ECV for the category of overweight and obesity was not proven by statistical analysis. Serious complications occurred in seven cases in similar numbers in all three subgroups according to BMI.

Conclusions: BMI in the categories of overweight and obesity is not a factor influencing the success rate and risk of complications of ECV. These results can be helpful when consulting pregnant women the chance of successful ECV.

Key words: external version; breech presentation; pregnancy; maternal obesity; body mass index

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INTRODUCTION

One of the methods allowing reduction of the incidence of planned caesarean sections for the breech presentation of the fetus is the external cephalic version (ECV), the overall success rate of the method is about 50% [1]. External cephalic version is a safe procedure that is not associated with a higher rate of perinatal complications [1–3]. Despite this, the fear for complications may be the cause of the mother's concerns about the procedure — about 25% of women reject ECV and more than 40% feel a fear of pain and are worried about fetal safety [4, 5]. Due to this there is an effort to select patients who would be more or less suitable for the procedure.

A large number of factors potentially influencing the success rate of the ECV are still being the subject of discussion. Although there are a lot of prediction models and scoring systems that should help increase the success rate of the procedure they are, unfortunately, rather inconsistent [6]. Increasing parity, posterior placenta, amount of amniotic fluid, the position of parts of the fetus are the most frequently cited positive predictors of successful external version. Other factors, such as estimated fetal weight, palpation of the fetal head, fundal height and uterine tension, are questionable [6–12]. Maternal weight is also one of these factors under discussion [9, 13, 14]. Overweight and obesity are a great obstetric issue, especially as the prevalence of

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overweight women and pre-pregnancy obesity has been increasing rapidly over the last decade [15]. Overweight itself represents a significant risk of comorbidities in pregnancy and in overweight women, caesarean section is associated with a higher level of complications [16, 17].

Objectives

Attempting successful ECV could reduce the incidence of potential complications due to surgical procedure. Our retrospective study aimed to determine BMI at the time of the ECV as an independent factor of the success rate of the procedure.

MATERIAL AND METHODS

A retrospective observational cohort study was conducted at the Department of Obstetrics and Gynaecology, Masaryk University, University Hospital Brno, Czech Republic. We collected data for ECV performed between January 2003 and December 2019.

A total of 992 women who underwent ECV were recruited. Eleven participants were excluded because we failed to obtain their complete data and 981 ECV cases were further analysed. We assessed the weight of all patients at the time of the procedure and according to this, we divided all patients into four groups according to WHO classification of obesity (*i.e.* BMI < 25 — normal weight, BMI ≥ 25 — overweight, BMI ≥ 35 — obesity, BMI ≥ 40 — morbid obesity). All ECV included in the study were performed by three experienced obstetricians. All participants in the study were ≥ 36 + 0 weeks of gestation, signed a declaration of informed consent to the procedure.

Our standardized clinical protocol for ECV was always respected — exclusion criteria for ECV were ruptured membranes, vaginal bleeding, uterine abnormality, contraindications of vaginal delivery, signs of intrauterine fetal distress and fetal malformations [18]. Prior to ECV, vaginal examination and cardiotocography (CTG) was performed, an ultrasound was used to determine the estimated fetal weight (EFW), amount of amniotic fluid, placental location, and type of breech presentation. The maximum time limit for ECV was ten minutes. The intravenous tocolytic agent (Hexoprenaline 10 ug + 100 mL of 0,9% NaCl) was applied and no analgesia used in all attempts. The ultrasound for monitoring the fetal position, fetal heart rate and placental status was used at all time during the procedure. After each ECV a CTG of a minimum length of 45 minutes was recorded and another one was done within two hours. If physiologically well, the patient was discharged (after an ultrasound check and a CTG) in most cases, on the following day.

Characteristics of the study population and obstetric factors are listed in Table 1 — other recorded factors were the success rate of external cephalic version and mode of delivery. In addition, serious complications associated with ECV (bleeding, placental abruption, intrauterine fetal distress with the need for an emergency caesarean section within 24 hours, intrauterine fetal death) were carefully noted.

The primary outcome was to determine the effect of BMI on the success rate of ECV. The secondary outcome was to define complications of ECV related to BMI.

Data was collected and analysed using statistical analysis. Continuous values were expressed as medians and ranges of the parameter and evaluated for all three BMI groups by the Kruskal-Wallis test. The Mann-Whitney test and Bonferroni correction were used for post hoc analysis of the pairwise comparison of BMI groups. Categorical parameters were compared using the Chi-square test for all three BMI groups and Fisher's exact test with Bonferroni correction was utilized in a pairwise comparison. The logistic regression model with a stepwise selection method was built for the multivariable analysis. The odds ratio (OR) statistic was used for the evaluation of parameters in the multivariable analysis. A p-value lower than 0.05 was considered to be significant. Statistical analyses were performed in MedCalc Statistical Software version 19.2.6 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020).

RESULTS

From the total number of 981 women, the external cephalic version was successful in 478 cases (48.7%). Concerning the baseline characteristics of the study group — parity, placental localization and type of breech were not significantly different in all BMI groups. There was an inter-group difference in maternal age and estimated fetal weight of the fetus ($p = 0.004$ for age, $p < 0.001$ for the EFW) (Tab. 1).

To the subgroup of women with a BMI ≥ 30, six women with a BMI ≥ 40 (morbidly obese) were added - due to the low number of participants, it was not possible to evaluate them separately. Of these six cases, ECV was successful in two cases, one woman after successful ECV delivered vaginally and five women by caesarean section.

Using univariate analysis of BMI as a continuous factor, there was no statistically significant effect on the success rate of the ECV ($p = 0.45$). Concerning the fact that it is clinically useful testing the BMI by WHO categories, a univariate analysis was performed for standard categories. This analysis also did not show a significant impact of BMI on the success rate of the ECV ($p = 0.28$) (Tab. 1).

In multivariate analysis, the category of BMI ≥ 30 is significantly important ($p = 0.012$), but the resulting odds ratio (OR = 1.58) demonstrates only a small effect of this BMI category on the success rate of ECV (Fig. 1) [19, 20]. Further testing revealed that, after the separation of six cases of morbid obesity (BMI ≥ 40), all BMI categories in the multivariate analysis are also of no significance.

Table 1. Characteristics of the study population — three groups of the BMI (data of the BMI ≥ 40 group included in the BMI ≥ 30 group)

Variables	Total (n = 981)	BMI < 25 (n = 310)	BMI ≥ 25 (n = 484)	BMI ≥ 30 (n = 187)	p-value
Maternal age [years]					
Median	31	30	31	31	**0.004**
Range	17–44	17–42	20–44	22–44	
Parity					
Nulliparous	638 (65.0%)	207 (66.8%)	314 (64.9%)	117 (62.6%)	0.63
Multiparous	343 (35.0%)	103 (33.2%)	170 (35.1%)	70 (37.4%)	
Gestational age at ECV [weeks]					
Median	38	38	38	38	0.07
Range	35–41	35–41	35–41	35–41	
Type of breech					
Frank	724 (73.8%)	234 (75.5%)	354 (73.1%)	136 (72.7%)	0.71
Non-Frank	257 (26.2%)	76 (24.5%)	130 (26.9%)	51 (27.3%)	
Estimated Fetal weight [grams]					
median	2900	2800	2900	3000	**< 0.001**
range	1900–4200	2000–3840	1900–3900	2200–4200	
Amniotic fluid amount (MVP in mm)					
< 40 mm	152 (15.6%)	54 (17.5%)	74 (15.4%)	24 (12.8%)	0.41
≥ 40 mm	823 (84.4%)	255 (82.5%)	408 (84.6%)	163 (87.2%)	
Placental location†					
Anterior wall	381 (39.0%)	107 (34.5%)	199 (41.5%)	75 (40.3%)	0.14
Non-anterior wall	595 (61.0%)	203 (65.5%)	281 (58.5%)	111 (59.7%)	
Outcome of ECV					
Success	478 (48.7%)	148 (47.7%)	247 (51.0%)	83 (44.4%)	0.28
Failure	503 (51.3%)	162 (52.3%)	237 (49.0%)	104 (55.6%)	
Delivery mode‡					
Spontaneous vaginal	566 (57.9%)	193 (62.9%)	284 (58.7%)	89 (47.6%)	0.003
Caesarean section	412 (42.1%)	114 (37.1%)	200 (41.3%)	98 (52.4%)	
Delivery mode (successful ECV only)§	477 (48.6%)				
Spontaneous vaginal	385 (80.7%)	129 (87.8%)	199 (80.6%)	57 (68.7%)	0.002
Caesarean section	92 (19.3%)	18 (12.2%)	48 (19.4%)	26 (31.3%)	
Serious complications					
Yes	7 (0.7%)	1 (0.3%)	3 (0.6%)	3 (1.6%)	
No	974 (99.3%)	309 (99.7%)	481 (99.4%)	184 (98.4%)	

BMI — body mass index; ECV — external cephalic version; MVP — Maximum Vertical Pocket; † Missing information of placental location in five cases (0.5%); ‡Missing information of the delivery mode in three cases (0.3%); §Delivery mode of successful external cephalic versions only

Women after successful ECV had a vaginal delivery in 80.7% (385/477) versus 36.1% (181/501) after an unsuccessful ECV. After successful ECV in the category of normal weight (BMI < 25), 87.8% patients delivered vaginally, in overweight patients (BMI ≥ 25) 80.6% succeeded in spontaneous labour and in the category of the obesity (BMI ≥ 30), 68.7% of the patients had vaginal delivery (Tab. 1).

Another finding is the relationship between increasing age and BMI, but using continual analysis, we prove this correlation negligible.

We noted serious complications with the need for an emergency caesarean section in seven cases (0.7%), these complications occurred in similar numbers in all three subgroups according to BMI (Tab. 1). Two caesarean sections were performed due to vaginal bleeding immediately after ECV, in one case, vaginal bleeding occurred 36 hours after ECV and placental abruption was confirmed. The four other caesarean sections were performed as a result of pathological CTG after an ECV. In six of seven cases, the physiological pH values from the umbilical artery were confirmed, the Apgar score and postnatal status of the newborn were physiological in all seven cases.

DISCUSSION

The overall success rate of ECV in our study group reached 48,7% and represents a stable success rate of ECV at our department, consistent with the literature [1, 6]. In the Czech Republic, it is strongly recommended that ECV is performed from the 36 weeks + 0 days of gestation [18].

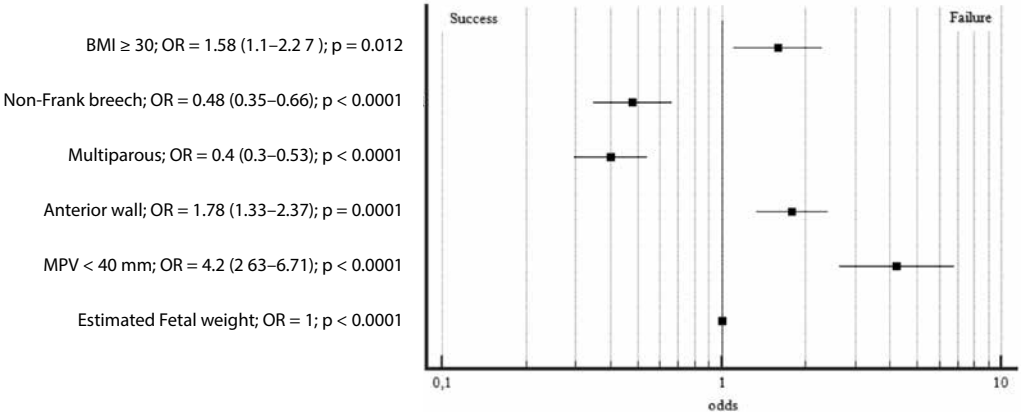

Figure 1. Multivariate odds ratios (OR) with confidence variables for the success rate of external cephalic version (ECV) based on different parameters and category body mass index (BMI) ≥ 30

When attempting an external cephalic version in an overweight woman, it can be assumed that the thicker the abdominal wall is, the more difficult is the handling and turning of the fetus so the success rate is lower [8]. Measuring the thickness of the abdominal wall objectively is difficult in practice, the body mass index assessment still seems to be the simplest and most appropriate method for estimating body status, excluding inter- and intra-observer disagreement [9]. There are numerous studies predicting the success rate of ECV due to variable factors, but just a few studies dealing with the relationship between success rate and maternal weight in particular. Chaudhary et al. has evaluated a large group of 51 002 ECV and he showed a slightly worse success rate in morbidly obese patients (BMI > 40) [21]. Holman et al. in a group of 135 patients didn't prove the relation [22]. Other studies showed that maternal BMI can affect the success rate [5, 14] or not [8–10, 23, 24] but the size of these groups ranged between 67 and 250 patients. In our group of 981 patients who underwent ECV we showed there to be no significant difference in the success rate of ECV in the categories of overweight and obesity. We have also demonstrated that higher BMI values are associated with increasing fetal weight (established as EFW) — this fact is already proven by many studies; obese women have a higher chance of the delivery of macrosomic or higher weight infant [29–30].

Another interesting finding is the relationship between increasing age and BMI. In the study of Lin et al. [28] women over 40 years of age had a higher incidence of overweight. Veghari et al. [29] also showed a coincidence between maternal age, birth weight and BMI. We hypothesized that this is because of the hormonal changes during pregnancy and age-related changes in body fat distribution and metabolism (lower secretion of growth hormone and responsiveness to TSH, higher leptin resistance) [28]. Using continual analysis, the correlation was negligible which means that this dependency is not so strong as to affect the final results. Apart of maternal age and estimated fetal weight, other factors (parity, gestational age at ECV, type of breech, amniotic fluid amount and placental location) are independent.

We have also confirmed that higher BMI values are significantly associated with a decrease in the success rate of vaginal births in the group of successful ECV (87.8%, 80.6% and 68.7% in the subgroups of BMI < 25, BMI ≥ 25 and BMI ≥ 30) (Tab. 1). This can be well justified by the negative effect of obesity on vaginal births — the rate of caesarean delivery increased with a rise in maternal weight regardless of ECV outcome. There are some potential factors which may be contributing to lower rates of vaginal delivery after successful ECV among obese women: a higher rate of labour dystocia probably due to an adverse effect on uterine contractility, more frequent macrosomia and also a different deposition of soft tissue within the maternal pelvis [25–27, 30].

In our study we demonstrated that the complications rate is low and stable even if BMI increases.

The weakness of our study is a small group of morbidly obese women amounting of only six patients so we didn't evaluate this group separately. It can be assumed that in the group of morbidly obese the success rate is lower. Chaudhary et al. prove this hypothesis on a group of 2,128 morbidly obese women [OR 0.756 (0.691–0.827)] [21] (but morbid obesity in his study in the USA represents 4.2% vs 0.6% in our group).

Another disadvantage of the study may be the fact, that the assessment of being overweight during pregnancy as body mass index may not be perfectly accurate. It is possible to think about the different weight gain of the fetus, the amount of amniotic fluid, swelling. Currently, we don't have an easier and more precise method for the evaluation of maternal obesity.

The strength of our study is the size of the group and respected standardized clinical protocol for ECV and a stable team of performing, experienced obstetricians.

CONCLUSIONS

On a large sample using retrospective analysis, we ruled out BMI as an independent factor influencing the success of external cephalic version — a relationship between pregnant women with their BMI in the category overweight and obese and the success rate of ECV was not shown. While counselling women about the probability of successful ECV, weight parameters should not be considered a contraindication to the procedure — moreover, this method should be offered to overweight patients in particular, which could reduce the incidence of potential complications associated with the surgical procedure of caesarean section. External version is a safe procedure helping to reduce caesarean delivery rates.

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Conflicts of interest

The authors report no conflicts of interest.

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Vaginal dinoprostone and misoprostol are equally safe in labour induction at term whereas dinoprostone is less efficacious for cervical ripening and shortening the time of labour

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ABSTRACT

Objectives: The aims of the study is to analyze the effectiveness and safety of the use of intravaginal inserts with prostaglandin analogues: dinoprostone and misoprostol, in the labor induction.

Material and methods: Pregnant women (177), with use of dinoprostone (n = 69) or misoprostol (n = 108) for labor induction were analyzed.

Results: The length of time of delivery differed significantly between primiparous and multiparous women and depended on the type of prostaglandin. The incidence of cesarean sections did not differ significantly in analysed groups. The risk of failed induction was over two-fold higher in the dinoprostone group as compared to misoprostol. A statistically significant longer duration of the first and second stage of labor was observed in primiparous compared to multiparous women as well as differences of cervical ripening were observed. There was no statistically significant relationship between the occurrence of hyperstimulation and worsening the newborns condition determined after delivery.

Conclusions: Vaginal dinoprostone and misoprostol are equally safe in labor induction at term whereas dinoprostone is less efficacious for cervical ripening and shortening the time of labor. There was no advantage of any of the prostaglandins used in increasing the risk of having a child in a worse condition and increasing the percentage of caesarean sections.

Key words: dinoprostone; misoprostol; labor induction

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INTRODUCTION

Labor induction is one of the most common procedures carried in obstetrics, and its main goal is to reduce the perinatal risk of a pregnant woman or her newborn through earlier pregnancy termination [1]. The development of perinatal surveillance techniques and popularization of ultrasound diagnostic methods in perinatology significantly contributed to the more frequent decisions about these procedures. Labor induction is usually preceded by biochemical process leading to a multitude of changes of the cervix, referred as softening and repining [2, 3]. Of the many available possibilities, the intravaginal administration of prostaglandins (PG) is one of the most used methods [1]. About 10% of pregnant women undergo induction of labor preceded by ripening of the cervix and acceleration of its maturation with the use of PGE2 prostaglandins or their analogues [3]. The response of the cervix to prostaglandins differs in women and depends on its condition at the time of drug administration, week of gestation and individual characteristics. The overall risk of ineffective preparation of the cervix within 12–24 hours of labor induction following the administration of vaginal prostaglandins (vPG) is 21.6% [4]. The cervical repining process begins several weeks before the onset of delivery, when an increase in the concentration

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of proinflammatory myeloid and lymphoid phenotypes related cytokines, chemotaxis regulators and factors inducing oxidative stress is observed [5, 6]. In turn, the lack of collagen cross-linked degradation together with the reduction of cell nuclei density in the stroma and the depletion of macrophage resources suggests blocking the cervical ripening process [2]. Macrophages, as myeloid-derived immune cells, play an important regulatory role in the cervical tissue. Macrophages also have ability to produce nitric oxide (NO) and prostaglandins. Reduction in NO and PG synthesis inhibits the cervix maturation process. The inflammatory response process is particularly visible during the dilatation phase of the cervix, where an increase in inducible oxide synthetase (iNOS) is observed. Increased iNOS concentration enhances vasodilation, a mechanism facilitating tissue perfusion during labor and leukocyte inflow [7]. The presence of pro-inflammatory cells in the cervix in the antenatal period enables the action of paracrine factors (cytokines) influencing changes in the structure of the extracellular matrix. According to current research data, the cervical remodeling process is completed before delivery. Fibrous collagen from the stroma extracellular matrix is replaced by less cross-linked collagen [2]. The rearrangement and realignment of collagen fibrils as well as glycosaminoglycan composition changes are first components of labor induction. High endogenous PG concentration in the cervix is due to increased cyclooxygenase-2 (COX-2) activity, detected particularly before delivery. Contrary to COX-2, a decrease of 15-prostaglandin dehydrogenase (15-PGDH) has been observed, the role of which is to inactivate PG during and before the cervix maturation process [3, 8]. Excessively active 15-PGDH is therefore associated with the ineffective action of both endogenous and exogenous PG [3].

Exogenous PG, dinoprostone (PGE2 analog) and misoprostol (PGE1 analog), acting through receptors, stimulate signaling processes in various cells of the cervix [3].

MATERIAL AND METHODS

Out of the group of 800 women giving birth in the second half of 2019 in the Department of Perinatology, Obstetrics and Gynecology, 177 (22,1%) pregnant women were qualified for the observational study, who were between 36 + 3 and 42 + 1 weeks of gestation (Tab. 1). Among the analyzed group of patients, 141 (79,7%) were primiparous, and 36 (20,3%) multiparous women. All examined women had an unfavorable cervix for labor, determined by Bishop's score from 0 to 6 points (Mean 2,3) and required intravaginal administration of vPG in the form of an insert: Misoprostol (Misodel) at a dose of 200 μg (n = 108) or Dinoprostone (Cervidil) at a dose of 10 mg (n = 69) [reference group (vM) or study group(vD), respectively]. Exclusion study criteria were contraindications to the use of vPG: previous caesarean section or scarred uteri, abnormal placenta implantation, non-cephalic presentation, fetal distress syndrome, cephalopelvic disproportion. Women with premature rupture of membranes have been also excluded from the study analysis.

Table 1. Demographic characteristics of study population

Parameter		vM (n = 108)	vD (n = 69)	p
Age [years]		23.8 ± 4.10	24.79 ± 4.94	N.S.
Maternal weight [kg]		68.2 ± 9.11	70.1 ± 5.18	N.S.
BMI [kg/m^2]	< 30.0 (n = 166)	103 (95.4%)	63 (91.3%)	N.S.
	≥ 30.0 (n = 11)	5 (4.6%)	6 (8.7%)	
Gestational age [week] (range)		39.8 C1.2 (36.3–42.1)	39.6 ± 1.1 (37.1–41.6)	N.S.
Parity	Primipara (n = 36)	23 (21.3%)	13 (18.8%)	N.S.
	Multipara (n = 141)	85 (78.7%)	56 (81.2%)	N.S.
Birthweight [g] (range)		3444 ± 509 (1660–4575)	3518 ± 400 (2590–4580)	N.S.

*p < 0.05; vM — reference group; vD — study group; N.S. — not significant

The indications for labour induction were based on A and B grade recommendations and consistent in all the pregnant women (Tab. 2).

Following administration of vPG, the continuous evaluation of the fetus has been performed with cardiotocography in all women. At the start of regular uterine contractions (more than three uterine contractions in the period of 10 minutes), the vPG has been retrieved and the patient was transferred to the delivery unit. In cases of fetal distress syndrome, fetal life-threatening signs, uterine tachysystole with fetal heart rate involvement or other indications for urgent termination of pregnancy, a caesarean section has been immediately performed.

The study was approved by the ethical committee of Pomeranian Medical University and informed consent was obtained from all individual participants included in the study.

Statistical analysis

All continuous variables were checked for the normality of the distribution using the Kolmogorov-Smirnow test. The Student's or Mann-Whitney's t-tests were used to analyze statistical differences in the scores of two or more groups. To gain information about the relationship between the dependent and independent variables, the analysis of variance (ANOVA) or Kruskal-Wallis test was used. The $\chi 2$ Pearson test or Fisher's exact test was used to study the statistical relationships between discontinuous variables and determine if there are nonrandom associations between two categorical variables. In order to estimate the risk of pathology depending on various factors, a logistic regression model was used. The results were described by giving the relative risk (OR) along with the 95% confidence intervals and the

Table 2. Indications for labor induction in the analyzed group of women.

Indication	vM (n = 108) n (%)	vD (n = 69) n (%)	TOTAL (n = 177) n (%)
Gestational diabetes mellitus Primipara (n = 21) Multipara (n = 22)	18 (16.7) 15 3	25 (36.2) 6 19	43 (24.3)
Post term pregnancy Primipara (n = 3) Multipara (n = 31)	22 (20.4) 2 20	12 (17.4) 1 11	34 (19.2)
Oligohydramnion Primipara Multipara (n = 20)	14 (13) 0 14	6 (8.7) 0 6	20 (11.3)
Gestational hypertension Primipara (n = 4) Multipara (n = 10)	8 (7.4) 2 6	6 (8.7) 2 4	14 (7.9)
Decreased fetal movements or Suspicious CTG trace* Primipara Multipara (n = 14)	14 (13) 0 14	0	14 (7.9)
Pregestational diabetes mellitus Primipara (n = 4) Multipara (n = 8)	8 (7.4) 0 8	4 (5.8) 4 0	12 (6.8)
Fetal growth restriction Primipara (n = 4) Multipara (n = 2)	6 (5.6) 4 2	0	6 (3.4)
Others Primipara Multipara (n = 34)	18 (16.7) 0 18	16 (23.2) 0 16	34 (19.2)

*Intrapartum care: NICE guideline CG190 (February 2017); vM — reference group; vD — study group

probability. The probability in this model was calculated with the $\chi 2$ Pearson test or with Fisher's two-sided test. The ROC (Receiver Operating Characteristic Curves) analysis was used to estimate the sensitivity and specificity for individual ranges of the continuous variable values. The results were described by specifying the area under the curve, the probability p, and the coordinates of the ROC curves. Statistically significant differences in all tests were considered those for which the probability $p < 0.05$. Statistical analyzes were performed using the statistical program STATA 11, license number 30110532736.

RESULTS

Our study analysis has demonstrated that the length of time from vPG administration to the onset of regular uterine contractions depended on the type of drug administered and differed significantly between primiparous and multiparous women. In primiparous women who received vM, the mean time to uterine contractions was statistically significantly shorter ($p = 0.0173$) compared to the vD group and amounted to 18.16 ± 24.04 h vs 22.73 ± 36.43 h, respectively. On the other hand, in the group of multiparous women, the time to uterine contractions, both after vM and vD administration, did not differ significantly (Fig. 1).

In the group of all examined patients, 108 (61.02%) women gave birth by vaginal delivery, while 69 (38.98%) had a caesarean section. When analyzing the method of termination of pregnancy, the percentage of caesarean sections did not differ significantly ($p = 0.506$) in both groups of women receiving vD 29 (42.03%) and vM 40 (37.04%), which proves that both drugs show comparable influence on the effectiveness of vaginal delivery. The most frequent indications for caesarean section were: failure to progress in labor 25 (14.12%), fetal distress syndrome 19 (10.73%), cervical dystocia 15 (8.47%), fetal pelvic disproportion 4 (2.26%), fetal malposition 2 (1.13%), Indications for operative vaginal delivery (forceps or vacuum) were made in 7 (3.95%) women. The risk assessment of the presence of several factors in the group of women treated with vD compared to those treated with vM is presented in Table 3.

The risk of failed induction was over two-fold higher in the vD group as compared to vM and this relationship was statistically significant. In those cases, further medical procedures to increase the uterine contractibility have been required: oxytocin infusion or amniotomy. In the vD multipara group, the induction failure was more frequent than in the vM multipara group (25% vs 12,9%, respectively) (Tab. 4).

Compared to vM, the group of women receiving vD had also higher risk tendency of caesarean section or vaginal operative delivery, but this relationship was statistically insignificant. There was an over two-fold higher risk of failure to progress with vD compared to vM and this relationship was at the limit of statistical significance. In turn, the risk of a successful pre-induction was reduced (OR = 0.23) in the vD group compared to the vM group (Tab. 5).

In the group of women treated with both vM and vD, a statistically significant longer duration of the first stage of labor ($p = 0.0028$) was observed in primiparous women (vM 5.93 h; vD 6.33 h) compared to multiparous women (vM 3.34 h; vD 3.49 h) (Fig. 2).

Similar relationships were observed in the second stage of labor. In the group of women treated with both vM and vD, a statistically significantly longer duration of the first stage of labor ($p = 0.0001$) was observed in primiparous women (vM 0.81 h; vD 1.06 h) compared to multiparous women (vM 0.26 h; vD 0.34 h) (Fig. 3).

In order to determine the cut-off value of continuous variables with respect to the drug (vD, vM), the ROC analysis was used. In primiparous women with a duration of labor ≥ 22.28 h, the sensitivity for vD was 69%. In turn, only 36%

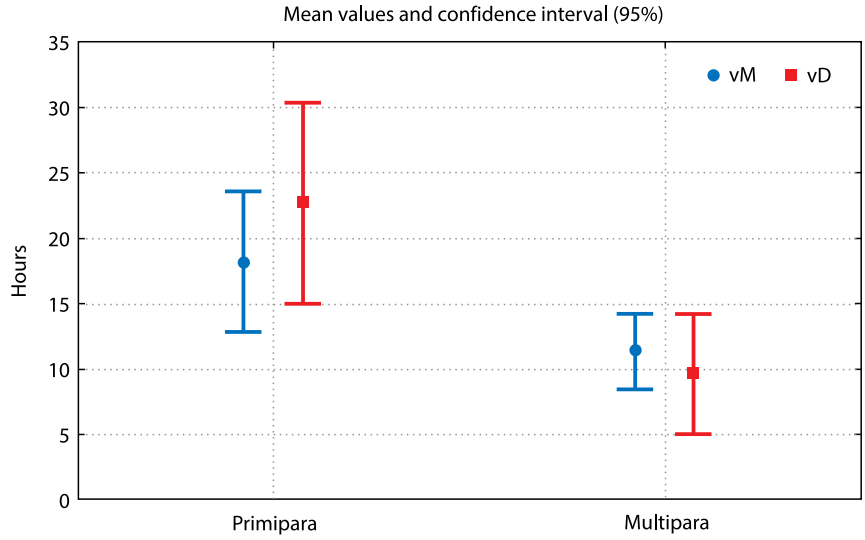

Figure 1. Mean time since the vaginal prostaglandins administration to the beginning of labour in primipara and multipara groups; vD — study group; vM — reference group

Table 3. Determination of confidence interval for odds ratio as a measure of association between the presence of risk factors and their occurrence in the study group compared to reference group, calculated using the $\chi 2$ Pearson's test or Fisher's two-sided exact test

Dependent Variable	Risk factors	OR	[95%]	CI	p
Occurrence in the vD group compared to vM grouzp	Induction of labour followed by amniotomy	2.18	1.11	4.27	0.023
	Induction of labour followed by oxitocin infusion	2.61	1.36	5.01	0.004
	Successful preinduction followed by vaginal birth	0.23	0.00	0.93	0.042
	Caesarean section	1.23	0.66	2.28	N.S.
	Vaginal operative delivery	1.18	0.26	5.45	N.S.
	Cephalopelvic disproportion.	1.58	0.22	11.50	N.S.
	Failure to progress in labour	2.24	0.95	5.28	0.064
	Fetal distress	0.90	0.34	2.42	N.S.
	Cervical dystocia	0.54	0.17	1.78	N.S.

vM — reference group; vD — study group; N.S. — not significant

Table 4. The rate of failed labor induction in reference and study groups

		Multipara n (%)	Primipara n (%)	p
vM	Normal labor progress (n = 97)	74 (87.1)	23 (100)	N.S.
	Failure to progress (n = 11)	11 (12.9)	0	
vD	Normal labor progress (n = 55)	42 (75)	13 (100)	0.04
	Failure to progress (n = 14)	14 (25)	0	

vM — reference group; vD — study group; N.S. — not significant

of respondents who received vM had a delivery time ≥ 22.28 h (Specificity). For primiparous women, vD vs vM has a statistically significantly increased risk of prolonging the period of labor (first and second stage of labor time prolongation).

Statistically significant differences in cervical ripening were observed on the Bishop Score after administration of both vM and vD in primiparous and multiparous groups (Tab. 6), which proves that both drugs show high effectiveness in the area of the cervical tissue. In the group of primiparous women where a pregnancy was terminated by caesarean section, significantly lower Bishop score values were observed in comparison with vaginal deliveries, which indicates that the effectiveness of vaginal labor depends on the cervical ripening. This relationship was not observed in multiparous women.

There were no significant differences between analyzed groups of women for postpartum hemorrhage and the rate was 18.5% (n = 20) and 20.3% (n = 14) for vM and vD respectively. There was also no statistically significant correlation between the newborns' birth condition in the Apgar score (3rd and 5th min.) on the degree of cervical ripening. The babies' condition after birth has not been also related to the type of drug administered, duration of the I and II stage of labour, and the time from drug administration to delivery. Uterine hyperstimulation, understood as uterine contraction followed by pathological CTG recordings, occurred in 14 (7.91%) women: 10 in vM and 4 in vD and the difference was not statistically significant (p = 0.69) in compared groups of patients. There was also no statistically significant relationship between the occurrence of hyperstimulation and parity, and its occurrence was not associated with worsening of newborns 'condition determined at the 3rd and 5th minutes of Apgar (p = 0.33) after delivery.

Table 5. Relationships between vPG and cervical repining according to the Bishop score (BS) in the studied groups of women

Para	vPG	BS	n	The mean ± SD BS difference before and after vPG administration	p
Primipara	vM	< 4 4–9 > 9	18 52 12	0.78 ± 0.81 3.60 ± 2.34 8.33 ± 3.34	0.0001
	vD	< 4 4–9 > 9	21 27 8	0.67 ± 0.97 3.89 ± 2.17 7.75 ± 2.12	0.0001
Multipara	vM	< 4 4–9 > 9	1 13 9	0 3.31 ± 2.29 9.11 ± 3.82	0.0004
	vD	< 4 4–9 > 9	1 5 5	0 4.40 ± 2.61 7.80 ± 1.48	0.0177

vD — study group; vM — reference group

DISCUSSION

The mean percentage of induced labour is in the range of about 20–25 percent, which signifies that every fifth pregnant woman is qualified to pregnancy termination before the onset of spontaneous uterine contractions [9]. In our material, this percentage was similar and did not differ from the data of other authors. Although the fetal-maternal indications for labour induction are well defined, there is still no clear consensus as to which of the available methods should be used. In our material, one of the most common indications for induction of labour was post-term pregnancy, which is consistent with the reports of other authors [10]. Mostly, pharmacological (prostaglandin analogs), mechanical (eg Foley catheter) or simultaneously both methods are commonly used for labour induction [11].

In the case of labor induction, a particularly important element for the successful delivery is cervical ripening, a dynamic process accelerated by both endogenous and exogenous prostaglandins [3, 4, 11]. It seems that the ripening of the cervix determines the quality and duration of labour as well as the need for additional stimulation of uterine contractions with oxytocin and the use of analgesics. Prostaglandins may be applied in the form of a gel to the cervical canal, vaginal insert or orally/ buccally titrated tablets. The most used are propstaglandin E1 E2 analogs:

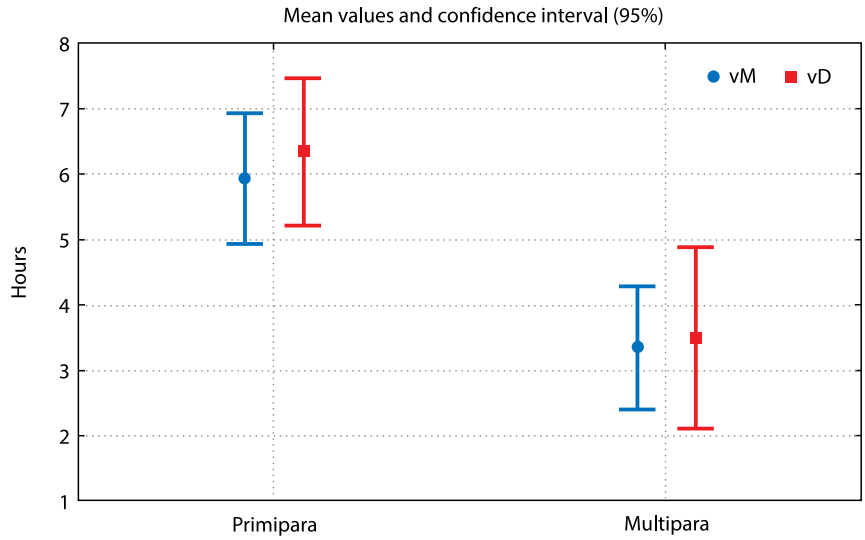

Figure 2. Mean time of the 1st stage of labour in primipara and multipara groups; vD — study group; vM — reference group

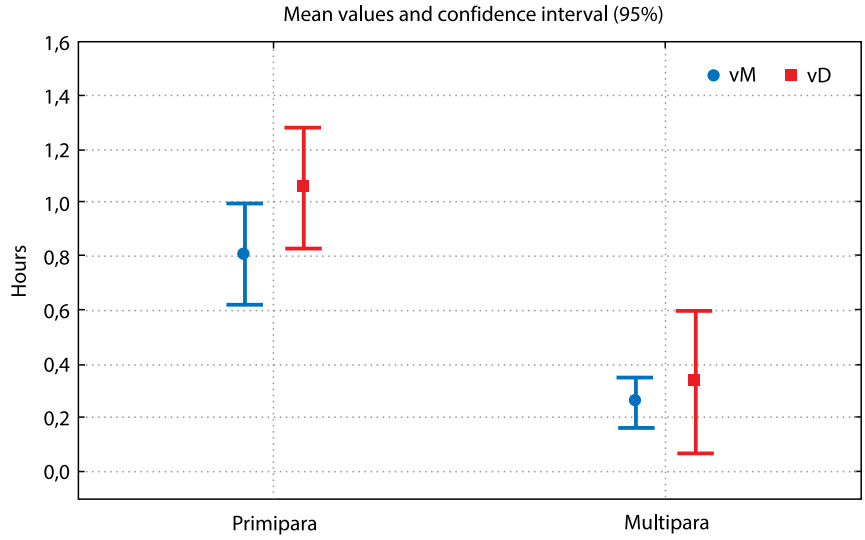

Figure 3. Mean time of the 2nd stage of labour in primipara and multipara groups; vD — study group; vM — reference group

Table 6. Relationships between the mode of delivery and cervical repining according to the Bishop score (BS) in the studied groups of women

vPG	Mode of delivery	N	The mean ± SD BS difference before and after vPG administration	p
vM	VB	46	4.41 ± 3.33	
	CC	36	2.72 ± 2.76	0.0163
vD	VB	28	4.04 ± 3.00	
	CC	28	2.43 ± 2.77	0.0420

vPG — vaginal prostaglandins; VB — vaginal birth; CC — caesarean section; vD — study group; vM — reference group

misoprostol and dinoprostone respectively [12]. Both drugs, apart from inducing uterine contractions, play a critical role in cervical ripening and softening [3, 9, 11]. Studies by Melamed et al. [13] proved that after prostaglandin E2 administration in a dose of 3 mg, an effective cervical ripening with favourable cervix to delivery was not achieved in 25% of pregnant women. The cervical ripening is a complex biochemical reaction, the mechanism of action of which is still not fully understood. An inflammatory process probably plays an important role, accompanied by an increase in the immune activity of cells and a change in their functions [14]. Yoshida et al. [15] found in an animal model that the softening phase of cervical remodeling is related to the decline of collagen cross-link density. At the same time, increased hydration of cervical cells was observed. This process may be modulated by changes in sex hormone levels leading to the activation of immune cells. In our study, a positive correlation was observed between the cervical ripening expressed with the Bishop scale and vaginally administrated prostaglandins. Our results, however, showed different dynamics of the action of two compared drugs. Misoprostol has shown greater effectiveness compared to dinoprostone, which is consistent with studies by other authors [16–18]. Its higher efficiency is manifested by a significant shortening of the delivery time, both in the first and the second stage of labor [18]. In consequence of a shortened stay in the maternity ward with accelerated delivery, the risk of vaginal operative delivery and cervical dystocia is reduced, as our research has also shown. It was calculated that vPG (especially vM versus vD) shortened the mean reduction in bed hours by nine and six hours in primiparous and multiparous women, respectively [18]. According to reports by other authors, shortened labour time reduces the risk of maternal infection and the need for antibiotics use [19]. In our studies, the mean time from vPG administration to the onset of labour differed significantly between primiparous and multiparous women. In the studies by Shmid et al. [17], similar statistically significant relationships were obtained: 14.9 hours of primiparous women and 11.8 hours of multiparous women.

According to the research literature, the use of dinorpostone compared to misoprostol is more often associated with the need to stimulate uterine contractions with oxytocin [16, 20, 21], although some authors report no significant differences between these two prostaglandins use [22]. In our studies, oxytocin was used more than twice as often in the group of women induced with dinoprostone.

We found no correlation between the caesarean section rate and the type of vPG administered during labour induction. In a study by Schmidt et al. [17], caesarean section was performed in 31.1% of induced pregnancies (110/354), more often in primiparous women. In our study, the percentage of

caesarean sections was slightly higher (38.9%) and the most common indications for its performance were abnormal CTG recordings, no progression of labour or cervical dystocia.

There are reports that tachysystole and uterine hyperstimulation are more common in women induced with misoprostol compared to dinoprostone [22]. In a study by Wing et al. [21], tachysystole was present in 13.3% of women induced by misprostol (vaginal 200 mcg) vs 4.0% induced by dionoprostone (vaginal 10 mg). On the other hand, in the studies by Yehia et al. [20], no statistical differences were observed in the occurrence of uterine hyperstimulation in the groups of women undergoing induction with misoprostol and dinoprostone, which may be due to differences in the form and dose of drugs used: titrated oral misoprostol (20 mcg every 2 hours) and vaginal dinoprostone (3 mg). Other studies compared oral misoprostol (50mcg every 4 hours), vaginal misoprostol (25–50mcg every 6 hours) with vaginal gel dinoprostone [23] or vaginal insert dinoprostone (10 mg) [9]. In all the studied groups, the authors did not find statistically significant differences in the occurrence of tachysystole and uterine hyperstimulation. Our studies have also shown no differences in the percentage of uterine hyperstimulation in the studied groups of women. A randomized clinical trial is currently in progress regarding efficacy and safety of administering oral misoprostol by titration compared to vaginal misoprostol and dinoprostone for cervical ripening and induction of labor [24]. Most studies reports do not show any increased risk of side effects of misoprostol compared to dinoprostone, as well as worse birth condition of the newborns, longer stay on NICU admission and higher neonatal mortality after induction of labor with misoprostol or dinoprostone [20, 22, 23, 25]. In randomized studies of PLOS ONE — the median birth status of newborns on the Apgar scale at the first and fifth minutes of life did not differ significantly in the groups of mothers induced with prostaglandin analogues: oral or vaginal misoprostol and vaginal dinoprostone [23]. The use of exogenous prostaglandins does not affect the birth condition of newborns, regardless of the exposure time and the time of prostaglandin removal [25]. Similar results were obtained in our study, where the condition of newborns did not differ significantly in the studied groups.

Our research shows that both prostaglandins used, vM and vD are effective during labour induction and show a similar safety profile, which means that the use of pharmacological methods for labour induction may be a safe treatment alternative to other methods.

CONCLUSIONS

Vaginal dinoprostone and misoprostol are equally safe in labour induction at term whereas dinoprostone is less efficacious for cervical ripening and shortening the time of labour. There was no advantage of any of the prostaglandins used in increasing the risk of having a child in a worse condition and increasing the percentage of caesarean sections.

Conflict of interest

The authors declare no conflict of interest.

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The influence of vaginal progesterone on Uterine Artery Pulsatility Index

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ABSTRACT

Objectives: Uterine artery Doppler is frequently used in the first trimester and it is one of the more effective measurement methods in the prediction of preeclampsia and intrauterine growth restriction (IUGR). Progesterone is a hormone that is used quite frequently in various indications in obstetrics and gynecologic practice. We aimed to investigate the influence of progesterone on the uterine artery Doppler pulsatility index (PI) at 11–14 gestational weeks.

Material and methods: This study is a retrospective case-control study conducted in Selcuk University Faculty of Medicine between January and December 2019. Uterine artery Doppler PI values of patients using progesterone were compared with PI values of patients not using progesterone. Uterine artery PI was measured two times, left and right. Then the mean PI was calculated. All measurements were made by two operators and by the same ultrasonography machine

Results: A total of 288 patients, 140 patients using progesterone and 148 patients not using progesterone were included in the study. Demographic characteristics were similar between the groups ($p > 0.05$). There were no significant differences between the groups in the right and left uterine artery PI values. There was no significant difference for average uterine artery PI between the groups ($p < 0.05$).

Conclusions: Progesterone has no significant influence on uterine artery PI. However, more prospective studies in which all potential confounding factors are considered including serum progesterone levels are needed for this subject.

Key words: uterine artery; preeclampsia; progesterone; pulsatility index

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INTRODUCTION

Many functional and structural uterine vascular system changes occur in pregnancy. The basis of the changes in the uterine artery is the trophoblastic invasion of the spiral arteries, and these changes provide the fetus with a greater blood supply [1]. Incorrect changes in the spiral arteries may cause insufficient uteroplacental perfusion and this may lead to some complications, such as intrauterine growth restriction (IUGR), preeclampsia, spontaneous preterm delivery, and premature rupture of membranes [1, 2].

Preeclampsia is an important life-threatening disease for both the mother and fetus. The pathogenesis of this disease occurs as a result of a defect in the trophoblastic invasion of the placenta in the first trimester of pregnancy [2]. Preeclampsia is the most important cause of maternal and fetal morbidity and mortality in both developed and developing countries [3]. Unfortunately, there is still no effective treatment other than delivery. Due to the increased fetal morbidity and mortality, especially in preterm pregnancies, preeclampsia prediction is very important. Many ultrasonographic and biochemical evaluations of preeclampsia predictions have been defined in the literature [4–6]. Uterine artery Doppler is a well-known diagnostic method for the prediction of preeclampsia [6, 7]. The uterine artery Doppler pulsatility index (PI) is used for the prediction of preeclampsia during the 11^{th}–14^{th} gestational weeks. Many studies indicate that preeclampsia development is more frequent in women with high uterine artery PI [6–10]. External factors affecting PI values of uterine artery Doppler have been investigated previously in the literature. The effects of parameters such as patient position and emptiness/fullness of bladder were explored [11, 12]. The relationship between progesterone use and uterine artery Doppler at the 11^{th}–14^{th} gestational weeks has not been specifically

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investigated in the literature. Progesterone is one of the main hormones in pregnancy, and it is used very often in obstetric practice. Therefore, we aimed to investigate the effect of progesterone use on uterine artery Doppler PI at the 11^{th}–14^{th} gestational weeks.

MATERIAL AND METHODS

This study is a retrospective case-control study conducted in Selcuk University Faculty of Medicine between January and December 2019. It was approved by the local ethics committee and conducted in accordance with the principles of the Helsinki Declaration (Reg.No:2020/022).

The patients between 18–45 years old and who had singleton pregnancy at 11 + 0 to 13 + 6 weeks of gestation with a normal fetus, without having systemic disease and treatment except vitamins, and using vaginal micronized progesterone for more than one week were included into the study. The exclusion criteria were the presence of hypertension, diabetes, vasculopathy, preeclampsia history, multiple pregnancies, usage of aspirin, and anticoagulant drug. Uterine artery Doppler PI values of progesterone users were compared with PI values of patients who were not using progesterone.

Uterine artery Doppler measurement

In our clinic, first trimester uterine artery Doppler is measured at 11–14 gestational weeks. The midsagittal section of the uterus and cervical canal is determined using transabdominal ultrasound. After the internal cervical canal is recognized, the uterine artery is determined by shifting the probe to both paracervical areas. During the measurement, it is acceptable to have 100–150 cc of urine in the bladder. When three similar consecutive waveforms are obtained, the right and left uterine artery PI values are recorded. It was measured two times, left and right. Then the mean PI was calculated. All measurements were made by two operators (NM and EÇ) and by the same ultrasonography machine. (LOGIQ F8, General Electric Co.; Milwaukee, WI, USA). 95th percentile of the mean UA PI of the present study lied similar within the ranges recorded by Gómez et al. [13].

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software package (IBM Corp., Armonk, NY, USA). A histogram curve and Shapiro–Wilk analysis were used to check for normal distribution. Descriptive statistics are expressed in mean ± standard deviation (SD), median (min-max), and number, where appropriate. Data were analyzed using the Mann–Whitney U test, Student's t-test, and Pearson's chi-squared test. P values of < 0.05 were considered statistically significant.

RESULTS

A total of 288 patients, 140 progesterone using patients and 148 patients not using progesterone were included in the study. All of the patients using progesterone in our study were patients using drugs due to the threat of miscarriage. There was no significant difference for age between the groups (mean 28.7 ± 5.0 vs 28.8 ± 5.01; p = 0.885). Gestational age was no significant difference between the groups [median 12 (11–13) vs 12 (11–13); p = 0.224]. Gravida and parity were similar between the groups (p > 0.05). There were no significant differences between the groups in terms of the right and left uterine artery PI values (mean 1.70 ± 0.46 vs 1.81 ± 0.51; p = 0.062, and mean 1.72 ± 0.52 vs 1.73 ± 0.40; p = 0.843; respectively). There was no significant difference for average uterine artery PI between the groups (mean 1.71 ± 0.40 vs 1.77 ± 0.37; p = 0.184). In five patients, the average uterine artery PI value was found to be greater than the 95^{th} percentile (p > 0.05) (Tab. 1).

DISCUSSION

Progesterone is a hormone that has a very important function both in the regulation of the menstrual cycle and in pregnancy. Progesterone is also one of the most commonly prescribed drugs during pregnancy. Uterine artery Doppler, measured between 11–14 weeks, has also been used recently in the prediction of preeclampsia. However, the interaction of these two conditions has not been specifically investigated in the literature. In our study, we found that the use of progesterone had no effect on bilateral uterine artery PI.

The uterine artery is one of the main vessels of the uterus and fetus. During pregnancy, the spiral arteries are exposed to remodeling for providing enough blood supply to the

Table 1. Demographic and uterine artery Doppler data according to the use of progesterone

Variables	Progesterone use		p values
	No n = 140	Yes n = 148	
Age	28.7 ± 5.0	28.8 ± 5.01	0.885*
Gestational age	12 (11–13)	12 (11–13)	0.224*
Gravida	2 (1–6)	2 (1–6)	0.740⁺
Parity	1 (0–4)	0 (0–5)	0.899⁺
RUA.PI	1.70 ± 0.46	1.81 ± 0.51	0.062*
LUA.PI	1.72 ± 0.52	1.73 ± 0.40	0.843*
AUA.PI	1.71 ± 0.40	1.77 ± 0.37	0.184*
AUA.PI > 95^{th} centile	4 (2.9%)	1 (0.7%)	0.157**

Data are given as mean (± SD), number (%) or median (minimum-maximum) where appropriate; *Independent Simple t-test; ⁺Mann–Whitney U test; **Pearson's chi-squared test; PI — pulsatility index; RUA.PI — right uterine artery PI; LUA.PI — left uterine artery PI; AUA.PI — average uterine artery PI

fetus. Makikalio et al. demonstrated that in uncomplicated pregnancies, a progressive decrease began in the spiral artery PI at the fifth week gestation [14]. The impaired remodeling process can result in further pregnancy complications such as IUGR or pregnancy-induced hypertension. There are many studies claiming that these changes in the uterine artery can be used to predict preeclampsia [6, 7]. In a meta-analysis of Velauthan et al. on about 55,000 pregnant women, they found that first-trimester uterine artery Doppler was a useful tool for predicting early-onset pre-eclampsia and other adverse pregnancy outcomes [15]. In the present study, we investigated whether a common agent such as progesterone had a confounding effect on uterine artery Doppler. We found no effect of progesterone use on uterine artery Doppler PI.

Progesterone is used quite frequently in various indications in obstetrics and gynecologic practice. Although the effect of progesterone on the uterine artery in non-pregnant women is known, there is limited and conflicting information in the literature regarding its effect on pregnant women. Micronized progesterone is frequently used during pregnancy, it has a direct effect on the uterus and has higher concentrations in the uterine tissue. In addition, its vaginal form is superior to the oral form; although it reaches a faster plasma peak level in oral intake, more constant drug concentrations are observed in vaginal use [16, 17]. Progesterone is effectively used for the prevention of threatened miscarriage, preterm labor, preterm birth in women with a short cervix, luteal deficiency, and recurrent miscarriage [18–23].

Czajkowski et al. [24] found that vaginal progesterone administration resulted in a decrease in spiral artery PI in early pregnancy complicated by threatened abortion. In addition, they found that vaginal progesterone treatment was associated with a significant decrease in the spiral artery PI after 2 weeks' and 4 weeks' treatment. In a study conducted by Jamal et al. [25] on pregnant women at 18–20 weeks of gestation, it was found that the use of progesterone and aspirin had a significant effect on uterine artery PI, and they found that vaginal progesterone use suppressed the resistance of uterine artery. Maged et al. [26] found that the use of progesterone for one week in patients in the third trimester had no significant effects on uterine artery PI. In this study, our hypothesis was that progesterone use might be a confounding factor on the uterine artery PI while predicting preeclampsia in the first trimester, but it was shown that progesterone use had no significant effect on uterine artery Doppler.

The retrospective design, unknown serum progesterone levels, and lack of long-term follow-up outcomes are limitations of this study. The strength of this study is that the effect of progesterone, a hormone commonly used at 11–14 weeks of gestation, on uterine artery Doppler, has not been investigated previously.

CONCLUSIONS

In conclusion, progesterone has no significant effect on uterine artery PI at 11–14 weeks of gestation. However, prospective studies are needed for this issue, where all potential confounding effects are considered, such as serum progesterone levels.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Financial disclosure

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Informed consent

Informed consent was obtained from all individual participants included in the study.

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Association between idiopathic recurrent pregnancy loss and genetic polymorphisms in cytokine and matrix metalloproteinase genes

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ABSTRACT

Objectives: Recurrent reproductive loss (RPL) is a global health issue affecting a significant number of women. Approximately half of miscarriages have an unexplained etiology. Familial aggregation and twins studies prove that some cases of the RPL could have a genetic background. Recent evidences suggest that cytokines (*e.g.* IL-6, TNF alpha or TGF beta) and matrix metalloproteinases (MMP) are important for maintenance of pregnancy. Single gene polymorphisms (SNP), affecting these proteins production or their function may predispose to the loss of the pregnancy. The aim of this study was to evaluate the association between the following polymorphisms of *IL6* (rs1800795), *TNF* (rs1800629), *TGFB1* (rs1800471), *MMP1* (rs1799750), *MMP2* (rs2285053 and rs243865), *MMP3* (rs35068180), *MMP9* (rs3918242) and the recurrent pregnancy loss in polish population.

Material and methods: Study subjects comprised of 67 patients with a history of recurrent pregnancy loss (≥ 2 miscarriages in history) and 75 controls. The distribution of genotypes for selected polymorphisms were determined by RFLP-PCR.

Results: Maternal genotypes *GG TNF*, or *5A/5A MMP3* may be associated with the recurrent pregnancy loss. No association between the *IL6*, *TGFB1*, *MMP1*, *MMP2*, or *MMP9* studied polymorphisms and the predisposition to miscarriage was found.

Conclusions: This study demonstrated a possible association between rs1800629 *TNF*, rs35068180 *MMP3* polymorphisms and recurrent pregnancy loss.

Key words: recurrent pregnancy loss (RPL); genetic polymorphisms; cytokines; matrix metalloproteinases (MMP)

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INTRODUCTION

Recurrent pregnancy loss (RPL) is a reproductive disorder, which affects approximately 1–5% of couples [1, 2]. Miscarriages occur in 10–15% and even up to 30% of pregnancies [3, 4]. Repeatability of recurrent reproductive wastage in a certain number of couples shows that the phenomenon is not random and urges to define a cause. Genetic variation may have a high impact on reproductive failure and thus delineating of specific genetic factors is of great importance for genetic counselling.

RPL is traditionally defined as the occurrence of three or more (≥ 3) consecutive pregnancy losses before 20 weeks of gestation. However, due to the growing problem of infertility, global and European scientific societies including the American Society of Reproductive Medicine (ASRM) and European Society of Human Reproduction (ESRE), has recently redefined RPL as two or more pregnancy losses [5, 6]. The aetiology of the disease comprises of different factors, such as autoimmune diseases (20%), endocrinological disorders (17–20%), uterine alterations (10–15%), genetic factors such as chromosome abnormalities in the parents (2–5%) and infections (0.5–5%) [7]. Nevertheless, approximately 50% of RPL cases remain unexplained and defined as idiopathic [3, 4].

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Familial predisposition to RPL is described [8, 9]. Clinical data suggest from two to sevenfold increased prevalence of RPL among first-degree blood relatives compared to the general population [9]. Population-based register studies showed that overall frequency of miscarriage among the siblings of idiopathic RPL is approximately doubled compared to general population [10, 11]. A genome-wide linkage scan using sibling pairs with idiopathic RPL confirmed heterogeneity of contributing genetic factors [11, 12]. Moreover, some studies emphasize that not only maternally, but also paternally inherited genetic factors might influence the miscarriages [12–14].

A successful pregnancy is the result of a number of processes including implantation, decidual tissue and vessel remodeling and maternal-fetus immune tolerance [15]. Each of these phenomena may be genetically determined. Recent research results show that cytokines and extracellular matrix metalloproteinases (MMPs) are important determinants of the opening of the implantation window and the proper invasion of trophoblast into the uterine wall and maintenance of pregnancy [16–19].

The maintenance of pregnancy depends on the balance between Th1 and Th2 cells [20]. It was proven that domination of the anti-inflammatory Th2 cytokine pattern is associated with gestational success/normal pregnancy, whereas a pro-inflammatory Th1 cytokine profile is related to the pregnancy failure [20].

Communication between trophoblastic and decidual cells is mediated by cytokines *e.g.* IL6, TNFα, TGFβ [21]. The cytokines production undergo the genetic control, and the genetic polymorphisms might influence the modulation of their expression therefore it may be at least partially responsible for the incidence of unexplained recurrent pregnancy losses [22].

Metalloproteinases belong to a large family of zinc-dependent endopeptidases that include collagenases (MMP1, MMP8, and MMP13), gelatinases (MMP2, MMP9), stromelysins (MMP3, MMP10), matrilysins (MMP7, MMP26), and transmembrane metalloproteinases types I and II. MMPs are crucial regulators of vascular and uterine remodeling and are involved in spiral artery formation and adhesion [23].

Changes in the nucleotide sequences in the binding sites of transcription factors or transcription repressors alter the regulation of MMP gene expression and the level of their protein products. The imbalance in the MMPs level disurbes the process of implantation and placenation [24].

Objectives

The aim of this study is to evaluate the association between the following polymorphisms: rs1800795 (*IL6* gene), rs1800629 (*TNF* gene), rs1800471 (*TGFB1* gene), rs1799750 (*MMP1* gene), rs2285053 and rs243865 (*MMP2* gene), rs35068180 (*MMP3* gene), rs3918242 (*MMP9* gene) and the recurrent pregnancy loss in case and control groups.

MATERIAL AND METHODS

Blood samples were collected from 67 women with a history of two or more consecutive spontaneous abortions (mean age of 32.85 ± 5.53 years old) and 75 healthy women who had a history of successful pregnancy (mean age of 35.11 ± 3.98 years old). Case groups were enrolled between September 2016 and June 2018 in Department of Genetics, Polish Mother's Memorial Hospital Research Institute in Łódź and Department of Obstetrics and Perinatology, Jagiellonian Medical University of Cracow, Poland.

All members of the study and control groups were Caucasians and residents of Poland, with no immunological diseases, weight disorders [obesity body mass index (BMI) < 30 kg/m^2], hypertension, diabetes or coagulation disorders.

The study was positively evaluated by the Bioethics Committee at the Polish Mother's Memorial Hospital Research Institute in Łódź. All participants were informed of the study protocol and completed a consent form before participating to the study.

DNA extraction

Peripheral venous blood samples (3–5 mL) from patients with RPL and controls were collected into EDTA-coated vacutainers. Genomic DNA was isolated from peripheral blood leukocytes by standard procedures using a commercially available kits Blood Mini Kit and Genomic Midi AX (A&A Biotechnology, Poland). The concentration and quality of the DNA were examined by optical density in a spectrophotometer NanoDrop 2000 (ThermoFisher Scientific, USA).

Genotyping

The above-mentioned polymorphic variants were genotyped by polymerase chain reaction — restriction fragment length polymorphism (PCR-RFLP). PCR reaction conditions was optimized for each polymorphism. Characteristic of the polymorphisms and the specific primer sequences are shown in Table 1.

Statistical Analyses

Data analysis was performed using Statistica v12 (StatSoft, Tulsa, OK). The Hardy–Weinberg equilibrium was tested in control group. Comparisons of variables with a categorizing distribution were made using the *chi*^{2} test or the Yates corrected *chi*^{2} test or the Fisher bilateral test. Variables relevant for single-factor comparisons were introduced to the regression model. To multifactor analysis (logistic regression) those parameters were introduced, which in univariate analyses obtained the significance

Table 1. Primer sequences, annealing temperatures, and restriction enzymes of the polymorphisms

SNP-ID	Polymorphism	Primer sequences 5′→3′	Annealing temperature	Restriction enzyme	Restriction products (bp)
MMP1 rs1799750	-16071G>2G	F: GAGTATATCTGCCACTCCTTGAC R: CTTGGATTGATTTGAGATAAGTCATA	53°C	AluI	*G1/G1*-288 *G1/G2*-262, 288 *G2/G2*-262
MMP2 rs2285053	-735C>T	F: GGTGGGTGCTTCCTTTAACATG R: GTAAAATGAGGCTGAGACCTGC	60°C	HinfI	*CC*-247 *CT*-203, 247 *TT*-203
MMP2 rs243865	-1306C>T	F: CTTCCTAGGCTGGTCCTTACTG R: GCTGAGACCTGAAGAGCCA	56°C	BstXI	*CC*-194 *CT*-170, 194 *TT*-170
MMP3 rs35068180	-11715A>6A	F: CATTCCTTTGATGGGGGGAAAGA R: GAAGGAATTAGAGCTGCCACAGC	60°C	Tth111I	*6A/6A*-194 *6A/5A*-170, 194 *5A/5A*-170
MMP9 rs3918242	-1562C>T	F:GCAGATCACTTGAGTCAGAAGTTC R: GGGAAAAACCTGCTAACAACTC	63°C	SphI	*CC*-286 *CT*-188, 286 *T T*-188
IL6 rs1800795	-174G>C	F: GTCAAGACATGCCAAAGTGCT R: GAGGGGCTGATTGGAAACC	60°C	NlaIII	*GG*-173, 11 *GC*-173, 122, 51, 11 *CC*-122, 51, 11
TNF *alpha* rs1800629	-308G>A	F: GGCAATAGGTTTTGAGGGCCA R: CCTTCTGTCTCGGTTTCTTCTCC	60°C	NcoI	*GG*-177, 19 *GA*-197, 177, 19 *AA*-197, 19
TGFB1 rs1800471	915C>G Arg25Pro	F: CACACCAGCCCTGTTCGC R: CTTCCGCTTCACCAGCTCCAT	65°C	BglI	*CC*-142, 103, 60 *CG*-163, 142, 103, 60 *GG*-163, 142

level $p < 0.15$. Multivariate analysis was performed with backward stepwise logistic regression. Results for which p was < 0.05 were considered statistically significant. For allele carriers and genotypes whose frequencies differed between groups, the odds ratio (OR) was calculated with a 95% confidence interval (CI).

RESULTS

The distribution of genotypes and alleles for the eight investigated polymorphisms and deviation from Hardy–Weinberg equilibrium in RPL cases and in control group are shown in Table 2.

Among the 8 polymorphisms, only 3: rs1800629 (*TNF* gene), rs243865 (*MMP2* gene) and rs35068180 (*MMP3* gene) demonstrated a significant association with risk of recurrent pregnancy loss. Significant statistically relevant maternal genotypes were included in the multivariable analysis. The results of this analysis are presented in Table 3.

These analyses revealed that *GG* homozygosity in *TNF* rs1800629 increases over 2.5 times the risk of RPL (OR = 2.56, 95% CI: 1.23–5.32; p = 0,0002). We also observed *MMP3* rs35068180 homozygosity *5A/5A* decreases the risk of RPL 0.24-fold (OR = 0.24, 95% CI: 0.11–0.52).

Based on univariate analysis, the statistical significance of the data for the analysis of the rs243865 polymorphic variant of the *MMP2* gene was not confirmed.

No associations between occurrence of recurrent pregnancy loss and the distribution of genotypes or alleles of studied *IL6, TGFB1, MMP1, MMP9* and *MMP2* rs2285053 gene polymorphisms were observed.

DISCUSSION

The regulation of cytokine and metalloproteinase secretion in the maternal-fetal interface plays a pivotal role in the process of trophoblast invasion and placentation.

The present study examines whether the occurrence of eight single-nucleotide polymorphisms (SNPs) in the *IL6, TNF, TGFB1, MMP1, MMP2, MMP3* and *MMP9* genes is related to the recurrent pregnancy loss. We observed that the three following polymorphisms: *TNF* rs1800629, *MMP2* rs243865 and *MMP3* rs35068180 are associated with the recurrent pregnancy loss.

Tumor necrosis factor α (TNF alpha) is a cytokine associated with the regulation of a wide spectrum of biological processes, including inflammation, cell proliferation and apoptosis. This multifunctional proinflammatory cytokine is produced mainly by the active monocytes and macrophages and by other cells (adipocytes, keratinocytes, fibroblasts, neutrophils, mast cells and some lymphocytes).

The *TNF* gene is located on chromosome 6p21.33. Changes in the nucleotide sequence of the gene in its promoter part are very important because the expression of the *TNF*

Table 2. Distribution of genotypes between cases and controls in compliance with Hardy-Weinberg law

Polymorphism SNP-ID	Genotypes alleles	Cases n = 67	Controls n = 75	pHWE	p
***MMP1* rs1799750**	1G/1G	21	22	0.05	0.794
	1G/2G	26	29		0.986
	2G/2G	20	24		0.782
	2G	66	77		0.849
	1G	68	73		0.849
***MMP2* rs2285053**	CC	51	60	0.233	0.576
	CT	16	13		0.339
	TT	0	2		0.526
	T	16	17		0.812
	C	118	133		0.812
***MMP2* rs243865**	CC	7	1	0.000	0.044
	CT	60	70		0.593
	TT	0	4		0.162
	T	60	78		0.428
	C	74	72		0.428
***MMP3* rs35068180**	5A/5A	16	43	0.000	0.00005
	5A/6A	34	15		0.0001
	6A/6A	17	17		0.706
	5A	66	101		0.002
	6A	68	49		0.002
***MMP9* rs3918242**	CC	49	58	0.268	0.562
	CT	14	17		0.799
	TT	4	0		0.101
	T	22	17		0.576
	C	112	133		0.576
***IL6* rs1800795**	CC	17	22	0.662	0.597
	CG	37	39		0.700
	GG	13	14		0.911
	G	63	67		0.743
	C	71	83		0.743
***TNF alpha* rs1800629**	GG	46	33	0.003	0.003
	GA	20	41		0.003
	AA	1	1		0.526
	A	22	43		0.014
	G	112	107		0.014
***TGFB1* rs1800471**	CC	56	65	0.536	0.779
	CG	10	10		0.975
	GG	1	0		0.954
	G	12	10		0.494
	C	122	140		0.494

For p value analysis, the chi2 or Yates' corrected chi2 tests were used; HWE — Hardy–Weinberg equilibrium

Table 3. Logistic regression results determining the chance of a miscarriage

Parameter	OR	95% CI	p
5A/5A MMP3	0.24	0.11 – 0.52	0.0002
GG TNF alpha	2.56	1.23 – 5.32	0.011

OR — odds ratio; CI — confidence interval

gene is mainly regulated at the transcription level. The *TNF* gene polymorphism of greatest interest is the transition of the guanine into the adenine at the position of –308. This polymorphism identified as rs1800629 is associated with increased expression of *TNF*, probably by changing the binding efficiency of transcription factor AP 2 [25].

Among the three polymorphisms in cytokine genes investigated in this study, only *TNF* rs1800629 was found to be significantly associated with an increased risk of recurrent pregnancy loss. The analysis shows that patients with *GG* genotype for rs1800629 polymorphism have a higher risk of miscarriage according to controls (OR = 2.56, 95% CI: 1.23–5.32).

A meta-analysis performed by a group from Iran showed a correlation of the rs1800629 polymorphism of the *TNF* gene promoter region with an increased risk of reproductive failure [6]. Moreover, it suggests that the investigated polymorphic variant is more significant in Asians than in Caucasians.

One of the purposes of this study was also to verify if the occurrence of five SNPs in the matrix metalloproteinase genes is related to the miscarriage predisposition.

Gelatinases A (MMP2) and B (MMP9) digests collagen type IV and V, elastin and other extracellular matrix proteins (ECM), which indicates their important role in the metabolism of vessel basement membrane.

MMP2 is encoded by the matrix metalloproteinase 2 gene (*MMP2*) located on chromosome 16q12.2. Two polymorphic variants of this gene rs2285053 and rs243865 are located in the promoter region and are responsible for regulating the expression of the *MMP2* gene and thus may affect the amount of synthesized protein. The presence of thymidine at positions –735 and –1306 in the *MMP2* gene promoter region prevents binding to the transcriptional factor Sp-1, thus reducing the activity of the *MMP2* promoter [26].

We did not find an association between the studied polymorphism of *MMP2* gene (rs2285053) and risk of recurrent pregnancy loss. These observations were similar to the results presented by Li et al., and. Behforouz et al. [19, 23]. However, not all studies are in consistency with results of the present study. Pereza et al., indicated that polymorphism rs2285053 was associated with recurrent miscarriage risk [27].

Results of our study referring to rs243865 *MMP2* gene polymorphism after univariate analysis did not identify any significant relationship for genotype nor for allele distribution between cases and control. They agrees with the results of Behforouz et al. [23], Pereza et al. [27], and Ramu et al. [28]. In contrast Li et al. [19], showed that the polymorphism rs243865 were significantly associated with an increased susceptibility to recurrent miscarriages.

Stromielizines, which include MMP3 (stromielizine-1), digest basement membrane collagen, proteoglycans and extracellular matrix glycoproteins. The stromielzine-1 gene is located on chromosome 11q22.3. The polymorphism rs35068180 of the *MMP3* gene occurs at –1171 in the promoter region and is associated with increased transcription and local expression of the *MMP3* gene [29]. This common polymorphism, which was identified by Ye et al. [30], in 1996, has one allele with a sequence of six adenosine (*6A*) and another five adenosine (*5A*). In vitro studies have shown that the *5A* allele is associated with a higher expression of the *MMP3* gene compared to the *6A* allele [29, 30].

Our results show that the maternal polymorphism rs35068180 *MMP3* gene occur significantly more frequently in RPL cases. Multifactorial analysis showed that patients with the *5A/5A* genotype for the rs35068180 polymorphism of the *MMP3* gene are about 0.24 less likely to experience a miscarriage OR (95% CI) = 0.24 (0.11–0.52). This relationship may result from the fact that *5A* allele carriers, associated with higher transcriptional activity of *MMP3* gene, are characterized by higher degradation of the extracellular matrix. As a result, *5A* allele predisposes to the successful of implantation and consequently reduces the risk of pregnancy loss.

Our findings confirm the results of Behforouz et al. [23]. They have found a significant association between rs35068180 polymorphism of *MMP3* gene and the pregnancy loss. The findings of this study are in keeping with the reports of Balci and Özdemir [31].

CONCLUSIONS

In the present study, we have for the first time investigated an association between recurrent pregnancy loss and the profile of eight selected single nucleotide polymorphisms in cytokine and metalloproteinase genes in women in the polish population.

In conclusion, this work has demonstrated an association between *TNF* rs1800629 and *MMP3* rs35068180 gene polymorphisms and recurrent pregnancy loss. Our results show that the maternal *GG TNF* and *5A/5A MMP3* gene genotypes occur significantly more frequently in cases with repeated miscarriages.

Limitations of the Study

The study included a relatively small number of patients, and the findings need to be confirmed in a larger population.

Conflicts of Interest

The authors declare no conflict of interests.

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Perinatal complications associated with neuraxial blocks

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ABSTRACT

Regional techniques are the gold standard of obstetric anaesthesia. In both vaginal and Caesarean section deliveries, neuraxial blocks are the most frequently used methods for relieving pain. Although it provides excellent analgesia, regional anaesthesia is associated with certain adverse side effects and possible complications. In this narrative review, we bring together all available data and create a catalogue of complications resulting from the use of perinatal neuraxial anaesthesia which we divide according to their severity and the duration of their impact on patients' health. We focus on complications that have significant or long-term consequences. Even though their incidence is low at 1:1600 neuraxial anaesthetics performed, we believe that better understanding of the possible severe problems that can result from regional anaesthesia procedures would enhance the overall safety of patients during labour, delivery, and the postpartum period. Despite the pivotal role neuraxial techniques play in providing anaesthesia for parturients, there is a lack of good quality studies on the incidence of complications. We believe that a thorough assessment of the occurrence of complications should be carried out by analysing data from nationwide medical databases. By analysing the adverse side effects, both qualitatively and quantitatively, we think it possible to further improve the quality of patient care.

Key words: complications; adverse effects; neuraxial anaesthesia; obstetrical anaesthesia; epidural analgesia; spinal anaesthesia

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INTRODUCTION

Childbirth is one of the most painful events in the lives of the vast majority of women. Among the many currently available pain relief methods, neuraxial techniques — including subarachnoid blocks, epidural blocks, and combined spinal-epidural (CSE) blocks — are considered the gold standards [1]. The difference between the anatomical placement of an epidural and spinal block is showed in Figure 1. Although these techniques are well-known and have been commonly used in anaesthesia since the 19th century, their prevalence in obstetrics varies widely according to organisational and financial factors, local medical policies, and sometimes even the individual preferences of the anaesthesiologist. Generally, epidurals are the most prevalent form of anaesthesia during vaginal delivery. One of the biggest advantages of this method is the possibility of utilizing the epidural catheter that is already in place to induce anaesthesia in the event of an emergency Caesarean section. In cases of Caesarean sections performed without an attempt of a vaginal delivery, spinal blocks are most commonly used. There is a lack of good quality data regarding how often neuraxial anaesthesia is used for pain relief during childbirth. In 2019 it is estimated that around 140 million births took place worldwide. The literature shows extreme variation between different countries in the use of neuraxial labour analgesia, ranging from 0% to over 80% [2]. This variation results not only from a country income but also from cultural and social factors. In Poland, unfortunately, there are no up-to-date scientific data on the prevalence of different methods of alleviating labour pain.

The provision of high-quality services requires in-depth knowledge of anaesthetic techniques and systematic oversight of the number of complications related to neuraxial techniques. An analysis carried out by the American Society for Obstetric Anaesthesia and Perinatology found that serious adverse events occur in one out of every 1636 central blocks among obstetrics patients [3]. It is difficult, however, to determine unequivocally whether a given complication is

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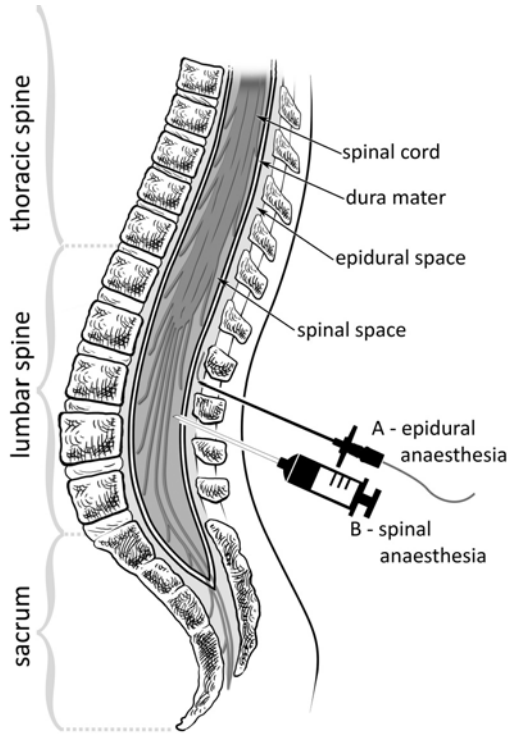

Figure 1. Anatomical placement of neuraxial blocks; A — epidural anaesthesia; B — spinal anaesthesia

causally linked to the regional technique used. Nevertheless, this does not explain the lack or marginalisation of analysis of the incidence and causes of adverse events resulting from obstetric anaesthesia procedures. This following narrative review aims to qualitatively and quantitatively describe the undesirable effects and complications of central blocks in patients in the perinatal period [4].

The literature does not precisely define the term "complication". The inconsistent use of the various terms — complications, adverse effects, side effects, sequelae — significantly impedes efforts to precisely define "complications". Semantically, these terms differ from each other, yet they are not uniformly or consistently employed in scientific literature. The term "side effect" deserves to be explained more clearly, as it refers to an effect secondary to that which is initially intended from the given medication or procedure. Therefore, side effects are anticipated additional consequences that may be either positive or negative for the patient's health [1]. For the purpose of our review, we have considered any deterioration of a patient's health for which a cause-and-effect relationship to the performance of a regional block can be demonstrated as a complication.

We searched MEDLINE, Google Scholar and Scopus using queries based on keywords such as "obstetric anaesthesia", "labour anaesthesia", "neuraxial anaesthesia", "adverse effects", and "complications". Initially, we reviewed the abstracts of 854 scholarly articles. Then, the papers were analysed for complications related to anaesthesia in obstetrics. After a thorough analysis, we considered eight articles to be especially applicable to our review [3, 5–11].

Due to the large number of possible complications, we organised them according to the degree and duration of their impact on a woman's health. The highly subjective nature of this division should be emphasised, as it is not always valid for every individual case. Short-lived complications with a low potential for harm are presented in Table 1. Among the many complications falling into the category of "side effects", the most common is hypotension, which is an expected side effect of a neuraxial block. It is caused by a blockade of sympathetic neurons and will occur in varying degrees of severity in most patients. In extreme cases, it may affect organ perfusion in the mother or may impair maternal–fetal blood flow. In most cases, hypotension is transient and easily treatable and does not cause any long-term consequences.

FEVER

A meta-analysis published in 2018 in the Cochrane Database of Systematic Reviews showed that the use of epidural anaesthesia doubles the risk of temperatures above 38°C compared to women who received opioids to relieve labour pain [12]. The incidence of this complication varies widely, between 1% and 33%, from study to study and is most commonly observed among primiparas [15]. Randomized studies contradict the previously observed correlation between the frequency of fever and prolonged epidural catheterization. However, the aetiology of the association of elevated body temperature with neuraxial blocks is unclear. Possible causes include the systemic inflammatory reaction induced by the neuraxial block, as well as reduced heat loss since anaesthesia reduces the perspiration and hyperventilation that would otherwise be caused by pain [1]. However, it should not be forgotten that fever in patients during labour may be indicative of an intra-amniotic infection. In addition, some side effects of neuraxial anaesthesia, such as motor block, may prolong labour and thus contribute indirectly to the risk of developing chorioamnionitis. Due to the lack of tests that can clearly differentiate between fever resulting from a neuraxial block and fever that is symptomatic of a serious

Table 1. Complications with low impact on women's health

Hypotension — spinal anaesthesia	25–71.25% [3]
Hypotension — epidural anaesthesia	8–30.7% [12]
Pruritus	1.3–85% [13]
Nausea and vomiting	3.2–34% [13]
Urinary retention	0.006–3.4% [10]
Shivering	36–71% [14]

infection, it is necessary to remain vigilant and — after performing a physical examination and excluding other sources of fever — consider implementing antibiotic therapy.

INSUFFICIENT PAIN RELIEF OR FAILED NEURAXIAL BLOCK

A neuraxial block is considered ineffective if it fails to provide satisfactory pain reduction or anaesthesia, or when the anaesthesia is insufficient to perform the surgery and an alternative method is necessary. Studies analysing the causes of neuraxial anaesthesia failure implicate emergency Caesarean sections and high BMI values as well as the obstetric histories of patients and the choices of anaesthesia techniques as possible causes [16]. With epidural and CSE blocks used for analgesia during labour and vaginal delivery, the failure rate ranges from 12% to 23% [16, 17]. In these cases, considering the individual case of each patient, we may offer the parturient a reinsertion of the epidural catheter or another pain relief method. For Caesarean sections, insufficient anaesthesia is usually the result of converting a labour analgesia epidural into a surgical anaesthetic block using the same catheter that had been previously sited in the labour suite. This occurs in 7% to 23% of cases, up to half of which may require conversion to general anaesthesia according to some studies [16]. Spinal anaesthesia is characterised by a considerably lower incidence of inadequate blockade (2.7–5%) and the need for general anaesthesia (1.2–2%) [7].

The use of ultrasonography for placing neuraxial blocks seems to be of significant benefit. Clinical observations show significantly lower risks of incomplete blocks and failures of epidural anaesthesia in patients for whom ultrasound visualisation was used during neuraxial anaesthesia placement [18]. This technique is particularly helpful in patients with anatomical abnormalities.

MOTOR BLOCK

There has been a lack of good quality research on the incidence of motor blocks. Neuraxial techniques used for vaginal delivery aim to switch off the conduction of pain impulses in the nervous pathways without simultaneously impairing motor function. This is often referred to as a "walking epidural", as it allows the patient to move freely. Excessive, undesirable motor block may inhibit activity in the first stage, as well as hinder and lengthen the second stage of labour. As a result, it may increase the number of deliveries that require the use of forceps or a vacuum [1]. The incidence of motor block is largely correlated to the anaesthesiologist's experience in obstetric anaesthesia. Although there is some individual variability in reactions to drugs, in the majority of cases, we can avoid motor block and other complications, such as surgical delivery, through the use of low concentrations of local anaesthetics in combination with opioid drugs [12].

We can use the patient-controlled epidural anaesthesia (PCEA) system, which enables the patient to administer pre-programmed drug doses using an infusion pump, in hopes of better controlling the level of anaesthesia. However, the literature does not conclusively establish the superiority of this method over physician-administered boluses of local anaesthetic.

POST DURAL PUNCTURE HEADACHE

Post dural puncture headache is defined as pain occurring within five days after the lumbar puncture and resulting from cerebrospinal fluid leakage. According to the International Classification of Headache Disorders (ICHD-3), it is commonly accompanied by symptoms such as neck stiffness and subjective hearing loss [19]. In most cases, symptoms appear during the first 72 hours after the procedure, but in rare cases, they can develop up to 14 days later. In differential diagnosis, we must consider pre-eclampsia or eclampsia, migraine, meningitis, and even CNS bleeding or venous sinus thrombosis. According to the diagnostic criteria provided in the ICHD-3, patients should be diagnosed with post dural puncture headache only after competing diagnoses have been ruled out. One prospective cohort study showed that up to 40% of women experience headache during the postpartum period, with less than five percent of cases being post dural puncture headache [20]. The incidence of this complication is around one in 114 neuraxial blocks, but it varies greatly depending on the type of neuraxial technique [3]. In particular, it is often associated with epidural anaesthesia during which an unintentional dural puncture (UDP) may occur, increasing the risk of post dural puncture headache to over 50% [21]. Post dural puncture headache results significantly less frequently from spinal blocks. The management of this condition depends on the severity of the symptoms. In some cases, fluid therapy, administration of nonsteroidal anti-inflammatory drugs, caffeine, and bed rest are sufficient. In cases where this conservative management fails, the treatment of choice is an autologous blood patch — a procedure in which a low volume of patients own blood is injected into their epidural space to create a seal to stop a leak of cerebrospinal fluid — which is about 80% effective [3].

HIGH BLOCK

The high block is one of the most serious complications in obstetric anaesthesiology. It occurs in one out of every 4,336 neuraxial blocks performed when the area of the sympathetic, sensory, and motor block reaches the level of the cervical segments of the spinal cord [3]. Its symptoms depend on the extent of the blocked area of the nervous sys-

tem and include nausea, dizziness, and respiratory distress up to loss of consciousness or cardiac arrest [10]. The patient may sometimes need to be intubated and mechanically ventilated. If there are no further consequences of respiratory or circulatory failure, the patient will most likely recover once the effects of local anaesthetics have subsided. The actual mechanism of this complication is not well-understood, but the anaesthesiologist's choice of technique or the type of the anaesthetic drug used may play a role [3].

For epidural anaesthesia, it is assumed that the dose of the drug, which may be as high as 20 mL, will be administered to the subarachnoid space instead of to the epidural space. High block may occur during both the *one bolus* technique and the continuous technique using a catheter in the epidural space to administer successive doses of local anaesthetics. Because an epidural catheter may spontaneously move to the subarachnoid space at any given time after it is placed, utmost vigilance is essential during the administration of subsequent doses of drugs [3].

RESPIRATORY DEPRESSION

Respiratory depression due to neuraxial block is assumed to be an extremely rare, extremely dangerous complication of neuraxial anaesthesia. The available data do not document the incidence of this complication in women in the perinatal period. The most frequently cited cause of respiratory depression is the administration of opioid drugs. Studies carried out on non-obstetric patients associated neuraxial anaesthesia with a 0.01–7% risk of respiratory depression [22]. The severity of the depressive effect depends on the dose, the route of administration, and the pharmacokinetics of the drug itself. The onset of symptoms can occur from a few minutes to several hours after the neuraxial block. Therefore, it is necessary to carefully assess the patient's status at each stage of anaesthesia and in the period immediately after its completion; The American Society of Anesthesiologists recommends monitoring the respiratory performance values for at least 24 hours after epidural or subarachnoid administration of morphine and for at least two hours after analogous administration of fentanyl or sufentanil [23]. Respiratory disorders may also occur as a result of excessive block and be related to the paralysis of the respiratory muscles or respiratory centre, as described above.

SERIOUS NEUROLOGICAL DISORDERS

Both pregnancy and childbirth, as well as anaesthesia itself, can be directly linked to the development of neurological dysfunctions. These most often manifest as sensory deficits in the lower extremities and buttocks or as deficits in motor function. This damage may occur in several forms, the most important of which include direct nerve damage; injury due to compression, which itself is caused by a haematoma or spinal canal abscess; and chemical damage caused by the administered drug. Although neuraxial anaesthesia procedures are an undeniable intervention in the patient's nervous system, they are not the most common cause of these symptoms. The risk of serious neurological injury resulting from neuraxial anaesthesia varies from 1:35,923 to 1:237,000 blocks performed [3]. Obstetric injuries, also called intrinsic maternal obstetric palsies, are more commonly caused by compression of the nerves or the blood vessels supplying them by the child passing through the birth canal; inappropriate position of the patient, especially in the second stage of labour; or direct injury during instrumental delivery [9]. The association of nerve damage with the performance of neuraxial blocks can only be confirmed by the occurrence of atypical neurological symptoms, such as pain and paraesthesia, during the procedure; these symptoms most often occur in the area of the neurological deficit observed after childbirth [5]. One of the most frequently described injuries is Cauda Equina Syndrome (CES), which consists of injury to the L5–S5 nerve roots and is associated with numbness of the skin in the perineal region, weakness of the lower limbs, and sphincter muscle dysfunction [5]. The damage is most often not permanent, and the symptoms tend to spontaneously resolve, but it may take several months to fully recover. Cases of permanent damage due to central blockade have been reported very infrequently (0.2–1.2:100000) [6].

DEEP INFECTION

Serious infection is a rare complication of neuraxial block, but its occurrence may threaten the patient's life. It manifests in the form of meningitis or spinal canal abscess and occurs in 1:62,866 to 1:145,000 neuraxial blocks performed [8]. Most often, meningitis is a complication of procedures during which the dura was punctured, whether intentionally or not, while spinal canal abscesses usually result from the use of epidurals. Research implicates Viridians streptococci as the pathogen responsible for almost 50% of iatrogenic cases of meningitis [24]. It is thought that the bacteria probably originate from the upper respiratory tract of the person performing the procedure, which highlights the importance of maintaining proper aseptic protocols. The risk of developing meningitis is also greater in patients with bacteriemia, which is more common in women who, among other things, have previously struggled with genitourinary infection or who required manual extraction of the placenta. In such cases, the pathogen causing meningitis may be B-group Streptococcus [25]. A diagnosis of meningitis should be considered in cases of the presentation of symptoms such as fever, severe headache with accompanying nausea and vomiting, neck stiffness, and

other neurological symptoms. Although the most common symptom of spinal canal abscess is back pain, this is a frequent complaint among women in the postpartum period and is often directly related to pregnancy or labour and delivery itself. If, however, the back pain is accompanied by symptoms of neurological deficiency — particularly sphincter insufficiency — and fever, this classic triad of symptoms points instead to an infection within the central nervous system. The risk of abscess is greater in patients who receive prolonged maintenance of an epidural catheter or who have a history of diabetes or immunodeficiency [5]. Magnetic resonance imaging (MRI) with gadolinium contrast is the diagnostic tool of choice. In some cases, surgical intervention is required, which increases the chances of regaining lost motor functions. Both in the case of meningitis and of spinal canal abscess, it is necessary to monitor inflammation marker levels, perform appropriate culture tests, and implement adequate antimicrobial therapy.

SPINAL EPIDURAL HEMATOMA

A haemorrhage into the central nervous system is a rare but serious complication that occurs less frequently in obstetric patients, at a rate of 1:168,000–1:251,469, than in other populations [5]. This complication is significantly more common in women suffering from coagulation disorders or taking anticoagulant or antiplatelet medications. Nowadays, many patients take acetylsalicylic acid and low-molecular-weight heparins for the prevention of obstetric complications. Early withdrawal of treatment with these drugs is therefore recommended in patients who wish to receive epidural analgesia for labour and delivery or who are scheduled for a Caesarean section. In addition, pregnancy predisposes women to idiopathic thrombocytopenic purpura (ITP) or low platelet count during HELLP syndrome. Platelet counts below 80,000/mm^3, regardless of the cause, are considered by most anaesthesiology specialists to be a contraindication for the use of neuraxial anaesthesia techniques [9]. The symptoms' progression usually depends on how rapidly the hematoma forms. The most common symptoms include sensory deprivation, motor dysfunction of the lower limbs, and sphincter function disorders. If a spinal haematoma is suspected, a spinal MRI is necessary, and a neurosurgical intervention may be required, in which case the surgery should be performed as soon as possible to minimise long-term neurological deficits.

SUDDEN CARDIAC ARREST

Sudden cardiac arrest in pregnant women is rare and carries a 60% survival rate [26]. According to studies conducted in the U.S., cardiac arrest occurred approximately once in every 12,000 hospitalizations related to childbirth [27]. In the Serious Complication Repository Project of the Society for Obstetric Anesthesia and Perinatology, of nearly 250,000 births, sudden cardiac arrest occurred in 43 cases, 88% of which were Caesarean section deliveries. The most common causes included haemorrhage, amniotic fluid embolism, and pre-existing cardiac conditions [3]. In the same study, the incidence of sudden cardiac arrest because of anaesthesia was estimated at 1:128,398 [3]. The results of the Royal College of Anaesthetists' 3rd National Audit Project (NAP3), which did not describe a single case of cardiac arrest in a group of seven hundred thousand patients, suggest an extremely low risk of cardiac arrest due to the use of neuraxial anaesthesia in the perinatal period [6]. The U.K.'s Cardiac Arrest in Pregnancy Study (CAPS), analysing all cases of cardiac arrest among pregnant women in which basic life support measures were started, found that almost 25% of them were related to anaesthesia [26]. Of these 17 cases of sudden cardiac arrest linked with anaesthetic management, as many as 10 of them resulted from high block from neuraxial anaesthesia [26]. Despite significant differences in data on the incidence of sudden cardiac arrest related to anaesthesia, in all cases reported in the aforementioned studies, resuscitation was successful, and the patients survived.

LOCAL ANAESTHETIC SYSTEMIC TOXICITY

Local anaesthetic (LA) medication acts by blocking sodium channels and thus stopping the conduction of nerve impulses. Such an important interference in the human body may cause serious consequences, especially in the cardiovascular and nervous systems. There is a linear relationship between the concentration of LAs in the blood serum and the severity of symptoms. The initial symptoms are cardiac dysrhythmias and pathognomy changes in sensation within the mouth and tongue, with patients often reporting a metallic taste. As the blood serum concentration of LA increases, the patient may experience seizures, heart dysrhythmias that result in cardiac failure, and finally, loss of consciousness and cardiac arrest. Due to the potentially fatal consequences of local anaesthetic systemic toxicity (LAST), the guidelines for performing obstetric anaesthesia recommend equipping the labour and delivery ward not only with a cardiopulmonary resuscitation kit, but also a 20% lipid emulsion available for immediate administration. This emulsion is intended to bind to LA and thus reduce its blood serum concentration [28]. If the patient experiences any symptoms that might be indicative of LAST, the LA infusion should be immediately discontinued, the resuscitation team should be called in, and 100% oxygen should be administered to the patient with instructions to breathe deeply.

In the perinatal period, the most common cause of toxicity symptoms is the unintentional injection of LA into a venous vessel in the epidural space. This complication is less common with spinal anaesthesia due to the much smaller

dose of LA used. It is worth noting that, even if the technique is properly performed, we can never be certain that the catheter is properly positioned in the epidural space. It should also be stressed that even a catheter correctly inserted in the epidural space may be displaced during labour. To minimise the risk of intravascular or subarachnoid administration of the medication, a test dose — *i.e.*, a small amount of LA given before the full intended dose — should always be used [1]. This complication is observed in 1:2,500 epidural anaesthetics performed in the general population; however, the incidence of this complication in the obstetric population has not been determined [28]. It seems that, in the obstetric population, LAST is not a common complication, as low-concentration solutions of LAs are administered, and the overall dose is not high.

SUMMARY

The widespread availability of pain relief in the perinatal period represents a significant milestone in the development of human civilisation. The improvement and increasingly widespread use of regional anaesthesia techniques in obstetric anaesthesiology has led to a reduction in perinatal mortality associated with anaesthesia. In order to maintain the quality and increase the safety of medical procedures, it is essential to understand the complications involved. While the catalogue of possible complications of regional anaesthesia techniques is well-known, the lack of good quality studies assessing their quantitative distribution is striking. This is also reflected in the very high dispersion of the incidence of specific complications in the analyses carried out by different authors. The frequency of adverse events may also be distorted due to the use of very small, unrepresentative samples in many studies. These problems may cause us to underestimate importance of some of complications and to overestimate the importance of others. On the other hand, some of the variability in the incidence of particular complications is related to local variations in the model of perinatal care and the resulting use of neuraxial blocks in obstetric patients. The literature also highlights the impact of the experience and skills of anaesthesiologists on the number of complications. This thesis can be applied to all areas of medicine; however, the great diversity and ever-changing dynamics of perinatal clinical situations — coupled with significant psychological considerations related to caring for patients in such a unique moment of life as the birth of a child — make obstetrics a unique area of medicine. We believe that the solution we should pursue is to create a system of reporting all complications into a single medical database. Only the analysis of such data would allow a reliable assessment of the quality of the procedures currently being performed and give us the grounds for making binding recommendations. It should be noted that not only are the legal regulations — which are a recognised standard in all high-income countries — an essential element here, but most importantly, a "culture of sharing failures" is important — a combination of respect for all patients' rights and, at the same time, respect for the rights and dignity of healthcare professionals. One of the prerequisites for spreading this culture is that medical errors needn't be penalised. Lastly, it should be stressed that serious complications of neuraxial anaesthesia techniques are very rare compared with those in other areas of medicine. Neuraxial anaesthesia is one of the safest medical techniques.

Conflict of interest

None.

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A comprehensive use of ultrasound examination in infertility workup

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ABSTRACT

Considering the growing availability of ultrasound diagnostic methods in gynecology, its role in the infertility setting is increasing. In this review, we present an up-to-date ultrasound based diagnostic scheme in infertility workup comprising the evaluation of ovarian anatomy and function, uterine exploration, as well as tubal patency. The possibility of performing the vast majority of infertility diagnostics by ultrasound in the ambulatory settings is not only attractive and beneficial to patients, but also to health care system. Thus, it is vital for gynecologists to implement modern non-invasive ultrasound modalities in their everyday practice.

Key words: infertility; reproduction; ultrasound; antral follicle count; sonohysterography; HyCoSy; HyFoSy; fertility scan

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INTRODUCTION

Imaging diagnostics is the essential part of contemporary medicine. The ultrasound-based examination plays a special role in gynecology and its use has been rising, particularly in infertility workup. Increasingly, ultrasound examinations allow to get a diagnosis without introducing invasive procedure. Patients are properly qualified to have invasive procedures and, owing to modern ultrasound modalities, hysteroscopy and laparoscopy can be applied rather for treatment than simply for diagnosing [1].

In this literature review, we aimed to present an up-to-date ultrasound based diagnostic scheme in infertility workup based on records concerning this field published in English in Pubmed/MEDLINE database from January 2010 to November 2020.

Ultrasound assessment of the ovaries

Besides hormonal methods, cycle monitoring and counting antral follicles by ultrasound are accepted, complementary methods to evaluate ovarian reserve and function [2]. They are offered to women of reproductive age for various reasons such as in subfertility and ovulatory dysfunction, in infertility and assisted reproduction workup or in predicting the risk of menopause.

The antral follicle count (AFC) includes a total number of antral follicles seen in both ovaries measuring 2 to 10 mm responsive to follicle-stimulating hormone (FSH) that can be recruited to maturation. Follicles > 10 mm are referred to as 'dominant' ones. Total AFC (follicles from both ovaries) is used frequently in assisted reproduction centers to predict ovarian response to gonadotropin stimulation, whereas the follicle number per ovary (FNPO) is more useful in gynecological practice to assess functional ovarian reserve [3]. The suggestion of how to interpret follicle count according to Consensus Opinion by Coelho Neto et al. [3] is presented in Table 1.

It has been suggested to examine AFC in the early follicular phase of the menstrual cycle, whereas an ultrasound between

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Table 1. Suggestion of how to interpret follicle count according to Consensus Opinion adapted from Coelho Neto et al. [3]

Nomenclature	Total AFC	Interpretation for ovarian stimulation
Very low number of recruitable follicles	0–4	Very high risk of poor response to ovarian stimulation
Low number of recruitable follicles	5–8	High risk of poor response to ovarian stimulation
Normal number of recruitable follicles	9–19	Normal response to ovarian stimulation expected
Large number of recruitable follicles	≥ 20	High risk of excessive ovarian response to ovarian stimulation
Nomenclature	**FNPO**	**Interpretation in clinical practice**
Low follicle count	1–3	Low ovarian reserve
Normal follicle count	4–24	Normal ovarian reserve
High follicle count	≥ 25	Polycystic pattern

AFC — antral follicle count; FNPO — follicle number per ovary

days 10 to 12 determines whether a 'good' dominant follicle is present and shows the endometrial response to follicular development [1]. Scans should be transvaginal (TVS) and with a minimum frequency of 7 MHz. Hormonal contraceptives and gonadotropin-releasing hormone agonists may reduce the quantity of follicles seen on an ultrasound, AFC is therefore preferentially measured during a natural cycle or after two to three months without hormone use. The observer-dependence is believed to be a disadvantage of this technique. However, accuracy increases with the operator's skill [3, 4].

Both two-dimensional (2D) and three-dimensional (3D) ultrasounds may be employed to perform AFC. On a 2D ultrasound, follicles are counted using either real-time imaging or stored cine-loops. When using a 3D ultrasound, the most common technique is to count the follicles manually in the multi-planar mode. However, there are likewise rendered modes to perform it semi-automatically, for example sonography-based automated volume calculation (sonoAVC™ by GE Healthcare, United States or syngo® Auto Follicle by Siemens Healthcare GmbH, Erlagen, Germany) [4] (Fig. 1A–B). Further research should include the reproducibility of new volume modalities available for follicle count.

Currently, anti-Mullerian Hormone (AMH) is considered as the most reliable marker for ovarian reserve and it is also recommended by Polish Society of Reproductive Medicine and Embryology (PTMRiE) in basic female fertility assessment [5]. It has been shown that there is a strong positive correlation between serum AMH level and AFC. The use of AMH combined with AFC may improve ovarian reserve evaluation [6].

Ultrasound assessment of the uterine cavity

The assessment of the uterine cavity is another routine examination performed in patients with subfertility and infertility. In the past, laparoscopy (to assess the outer shape of the uterus) with hysteroscopy (to assess the cavity) were the gold test in diagnosis of congenital uterine anomalies, however, now considering the evidence ultrasound-based techniques seem to play a crucial role [7].

Basic 2D ultrasounds should be employed as a screening tool to assess the uterine cavity and secondary uterine pathologies such as polyps, myomas, uterine adhesions, or adenomyosis [8]. A 2D transvaginal ultrasound performed by an expert in the field, with standardized evaluation of the uterus scans in mid-sagittal and transverse plane, was observed to be highly accurate (84–90.6%) in the differentiation of arcuate, bicornuate, and septate uteri compared to laparoscopy with hysteroscopy [8]. Compared with conventional 2D ultrasounds, 3D volume imaging has a higher diagnostic accuracy in detecting uterine anomalies (97.1–100%) [7].

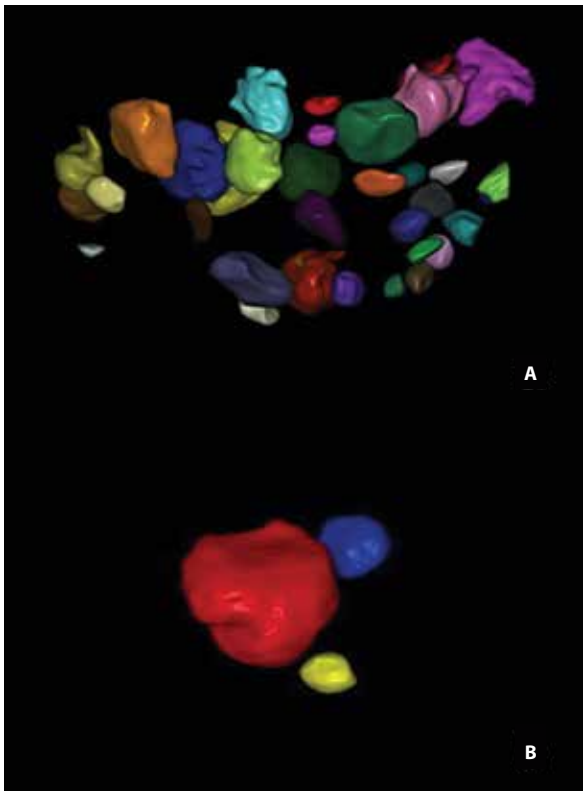

Figure 1. Ovaries visualised with sonoAVC; **A.** Ovary with several follicles; **B.** Ovary with few follicles

Coronal view, which is pivotal for the diagnosis, is rendered from a 3D dataset acquired, either on the ultrasound machine or on the personal computer. The reproducibility of 3D ultrasound is high, that is why it is recommended to be the first diagnostic step in the assessment of the uterine cavity [8, 9]. To obtain good quality images with clear margins between endometrium and myometrium, the exam should be performed in the second phase of the menstrual cycle [10]. A 3D TVS combined with Power Doppler also has value for the differential diagnosis of endometrial lesions among infertile women. Endometrial thickness and volume were larger among women with endometrial polyps and hyperplasia, whereas endometrial vascularization index, flow index, and vascularization flow index were lower among women with intrauterine adhesions [11].

According to The European Society of Human Reproduction and Embryology (ESHRE) and European Society for Gynaecological Endoscopy (ESGE) consensus on diagnosis of female genital anomalies magnetic resonance imaging (MRI) or eventually endoscopic evaluation are recommended for the subgroup of patients with suspected complex anomalies, diagnostic dilemmas or in case of poor quality of ultrasound visualization [9]. MRI is advised as a first line diagnostic procedure in the case of adolescents [9].

Sonohysterography

Sonohysterography or saline infusion sonohysterography (SIS) is minimally invasive, outpatient and low-cost method to visualize the endometrial cavity in more detail than is possible with routine transvaginal ultrasounds. Once the uterine cavity is filled with sterile fluid (*e.g.*, saline infusion), a real-time 2D scanning of the uterine cavity is completed. Additional techniques, such as 3D ultrasound, may be used for acquiring coronal view [12] (Fig. 2A). There are also novel, worth mentioning modalities assessing uterine cavity volume and shape using automatic volume calculation software [13] (Fig. 2B). The main indication for 3D-SIS is verification of doubtful 3D-TVS images [8]. Moreover, 2D and 3D-SIS are useful in the diagnosis of diseases closely related to infertility, in particular myomas, endometrial polyps and Asherman's syndrome [14, 15]. It allows the preoperative evaluation of benign intracavitary lesions [16]. Sonohysterography also plays an important role in secondary fertility investigation due to cesarian scar pregnancy. Recent studies revealed that 3D-SIS is superior in evaluation of the residual myometrial thickness and niche width providing better characterization of the scar niche [17]. It is suggested that uterine niches should always be assessed by SIS, because assessment of niche morphology is commonly dependent on the presence of natural fluids in a niche, which is highly changeable during the menstrual cycle. Using only 2D TVS may underestimate the prevalence of scar pregnancy defect [18].

According to Ludwin et al. [8], 3D-SIS is the only ultrasound method which provides results consistent with hysteroscopy performed with laparoscopy, considered as the gold standard, in the differential diagnosis of septate, bicornuate and arcuate uteri. SIS showed also significantly higher accuracy (100.0%) compared to diagnostic hysteroscopy without laparoscopy (80.7%) in the differential diagnosis of the aforementioned pathologies [19]. It seems that 3D-SIS should be preferred for a final differential diagnosis of the most frequent uterine anomalies if these conditions are not accompanied by other medical indications. The authors emphasize, that hysteroscopy without laparoscopy, which is often performed in these cases, is a suboptimal, poorly reproducible method to differentiate septate and bicornuate uterus, because the outer shape of the uterus cannot be verified [8]. PTMRiE recommends performing diagnostic laparoscopy in patients suspected of pelvic lesions or having risk factors for tubal occlusion, so that the patient could benefit from the surgery [5]. According to Polish Society of Gynecologists and Obstetricians guidelines regarding hysteroscopy, it should be offered routinely in case of intracavitary lesions detected on ultrasound, abnormal uterine bleeding or recurrent miscarriages. As far as uterine cavity assessed on ultrasound is normal, hysteroscopy should not be used as a first line screening tool in infertility workup or before in vitro fertilization procedure [20].

Ultrasound methods of tubal patency investigation

Hysterosalpingo-contrast-sonography (HyCoSy) was introduced as an alternative to hysterosalpingography (HSG) for outpatient tubal assessment. It overcomes such major drawbacks as hospitalization, radiation exposure and the use of iodinated contrast media.

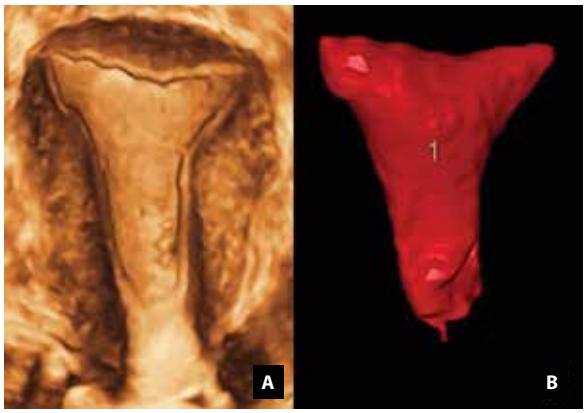

Figure 2. A. 3D sonohysterography; **B.** Uterine volume estimation using SonoHysteroAVC technique V = 2.5 mL

The most accessible contrast is saline, or a mixture of air and saline administered simultaneously or alternately. Subsequently, more hyperechoic contrast media were introduced, *e.g.*, Echovist® (galactose microparticles; Schering, AG, Berlin, Germany), SonoVue® (sulfur hexafluoride; Bracco, Milan, Italy) However, their use is limited because of a high cost or no license for an intrafallopian tube. In 2007, a micro-bubble contrast agent known as ExEm Foam® (GynaecologIQ, Deft, The Netherlands), containing hydroxyethylcellulose and glycerol was launched [21]. Owing to good quality sonograms obtained and acceptable price, hysterosalpingo-foam-sonography (HyFoSy) has become widely adopted in infertility office and ambulatory settings [22].

According to the National Institute for Health and Clinical Excellence (NICE), HyCoSy may be as effective as HSG for diagnosing fallopian tube occlusion, and both appear to have high sensitivity and specificity compared with laparoscopy [23]. NICE, ESHRE, and PTMRiE recommend that it should be offered to women with no comorbidities suggesting pelvic pathology, such as pelvic inflammatory disease, previous ectopic pregnancy or endometriosis [5, 23, 24]. Regarding different contrast media, it seems that high negative predictive value (99.5%) of air/saline-HyCoSy suggests that this procedure can be implemented as a screening examination. Nevertheless, HyCoSy requires greater experience and is observer-dependent, since the window for visualizing the passage of contrast through the tubes is short [25]. Rapid movements of the probe are necessary for tracing the circuitous or distant tubes in different planes. HyCoSy with more hyperechoic contrasts, e.g., HyFoSy, may be an alternative as the foam fills slowly, the tube and remains stable for at least 5–7 minutes. In addition to this, the use of hyperechoic contrast media does not require a learning period as observed in series for air/saline-HyCoSy [26]. HyFoSy having a significantly higher positive predictive value (30.4% air/saline HyCoSy vs 48% HyFoSy), is suggested a second-step technique in the event of *e.g.*, inconclusive examination, occlusion suspicion or poor images quality [27]. Additional scanning using high-definition flow Doppler further improves the accuracy of HyFoSy. According to research of Ludwin et al. (2017) [27], it was the only method (with the accuracy of 95.8%) that did not differ significantly concerning accuracy from laparoscopy with dye chromotubation as the reference method. According to Chinese data 4D-HyCosy represents also highly useful method for diagnosing tubal patency [28, 29]. Though in recent meta-analysis its diagnostic performance is similar to 2D-HyCoSy [30]. Certainly, future prospective studies comparing both technics in the same set of patients will give more precise answer. Finally, laparoscopy is dedicated to patients when hysterosalpingography remains inconclusive or as a first line diagnostic tool when pelvic pathology influencing the tubes patency is suspected [5].

A tubal patency exam should be carried in the preovulatory phase. The eventual antibiotic prophylaxis is left to examiner's decision as there are still no guidelines, nor randomized controlled trials addressing this issue [31]. HyCoSy was reported to be associated with very low risk of infections. In fact, post-procedural infections were recorded in 0.95% of patients undergoing HyCoSy and were absent in HyFoSy studies [32]. An echogenic medium is injected transcervically using a balloon catheter (diameter 5–8 French) or non-balloon dedicated cervical applicator [21]. After confirming the correct placement of the catheter, contrast is slowly injected into the endometrial cavity and in meantime its flow is observed in 2D transverse plane from uterine horn, through each tube until peritoneal spill is visualized (Fig. 3A–B). Additional scanning using high-definition flow Doppler is beneficial, as it improves the accuracy of the exam. The advantage of hyperechoic contrasts is maintaining echogenicity for few minutes; therefore, it allows 3D volume acquisition and visualization of the tubal course in the coronal view (Fig. 4A–B).

In a randomized controlled trial by Dreyer, HyFoSy turned out to be a less painful and less time-consuming tubal patency test compared with HSG [32]. Prophylactic analgesia is unnecessary, however, it is nevertheless considered, indomethacin trans-rectally and paracetamol or codeine orally seems to be effective [33, 34]. Lately, intrauterine lidocaine flushing before HyFoSy has been proven

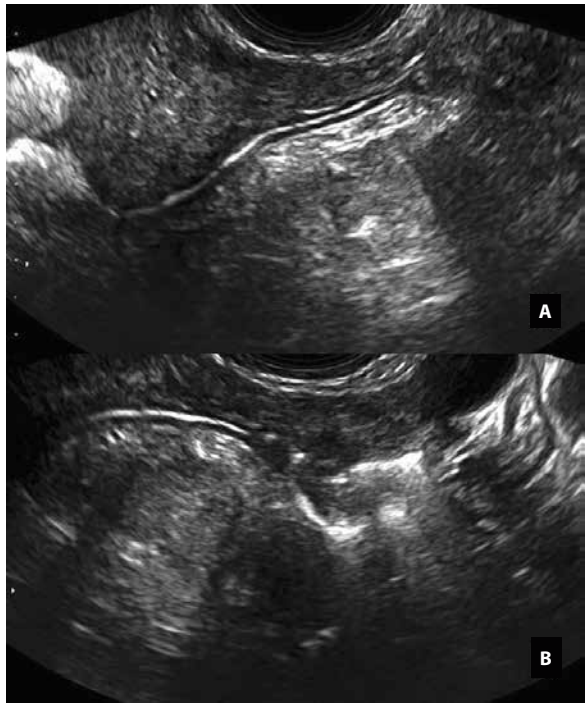

Figure 3. 2D HyFoSy; **A.** Foam visible in interstitial part, isthmus and ampulla of the left tube; **B.** Foam visible in infundibulum and then spilling into peritoneum

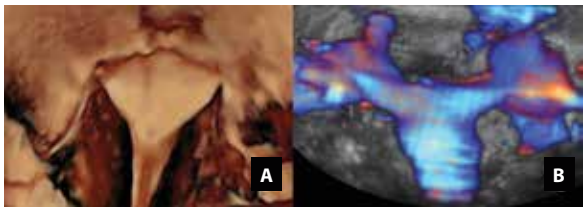

Figure 4. Offline renedered HyFoSy images; **A.** HD-live 3D HyFoSy; **B.** HD flow Doppler 3D HyFoSy

to decrease the pain during the procedure [35]. In a study of 632 women who underwent HyCoSy, no late complications were observe. Only 6.48% of the patient population experienced severe pelvic pain and 4.11% showed mild vasovagal reactions [36]. Even lower percentage of complications — 0.32%, including vasovagal reactions and mild urinary infection, were reported after HyFoSy. The median visual analogue scale (VAS) score for perception of pain was 2 (range 0–10), 1.9% of women reported severe pain. Recently, two cases of foam intravasation were reported in the literature [37, 38]. In 2019, U. S. Food and Drug Administration (FDA) approved HyFoSy for the detection of fallopian tube patency in women with known or suspected infertility [39].

So far, only observational studies investigating the chance of subsequent pregnancy after HyCoSy and HyFoSy, are available. Cautious conclusions that fertility enhancing effect may exist were drawn [40]. More is known about the effect of HSG [41]. A recent meta-analysis showed that tubal flushing using oil-based contrast medium compared with water-based contrast medium and no intervention, probably increases clinical pregnancy rates within six months after randomization and may increase subsequent live-birth rates. However, the authors stated that evidence on fertility outcomes beyond six months is inadequate to draw firm conclusions [41]. This year first research on oil-based contrast Lipiodol® (Guerbet LLC, Princeton, New Jersey, USA), previously used in HSG, utilization in HyCoSy was published. According to Zen et al., sonographic visualization of the agitated Lipiodol is similar or better than that of agitated saline [42]. We are waiting for the results of a large, randomized study — the FOAM study, which is currently ongoing. The researchers want to compare the effectiveness and costs of management guided by HyFoSy or by HSG. The primary outcome is ongoing pregnancy leading to live birth within 12 months after randomization [43].

FERTILITY SCAN

In 2011 Hrehorcak and Nargund described the idea of 'one-stop' fertility assessment [1]. The concept is focused on the investigation both anatomy and function of the ovaries and the uterus, as well as the tubes during one visit in infertility workup between days 10–12 of a regular cycle. To yield the best results the equipment should be of high resolution with sensitive color and spectral Doppler modalities and preferably 3D facilities. It is carried out in one place, saving the couple and the professional valuable time and is 66% less expensive. It offers a quick, one hour diagnosis in comparison to 18 weeks, on average, of standard multi-visit workup [1]. Lately, fertility scan including sonohysterography and HyFoSy, called Fertiliscan©, has been proposed [44].

SUMMARY

A diagnostic strategy has to be safe, noninvasive, well tolerated and possibly at a low cost for the health care system. For these reasons, ultrasound fertility assessment is an accurate choice for the first line in the infertility workup and its use has been rising.

Performing fertility scan, it is advised to count AFC manually using any of the following techniques: real-time 2D TVS, 2D cine-loops, or 3D TVS datasets. The 2D ultrasound is used as a screening tool to assess the uterine cavity. The 3D-TVS and 3D-SIS are recommended as an optimal diagnostic tool in women suspected to have a uterine anomaly. HyCoSy and HyFoSy has proved to be a safe and well tolerated outpatient procedure in the assessment of tubal patency. Air/saline-HyCoSy is considered a screening examination, whereas HyFoSy is suitable for a second-step technique. Further research should concern the diagnostic value of new ultrasound modalities, *e.g.*, 4D-HyCoSy.

The new concepts of 'one-stop' fertility scan, combining all tests in one, seems to be extremely beneficial to infertile couples regarding their fertility potential and stress. One stop fertility diagnosis has high demands on the clinic logistic, high quality ultrasound equipment and clinics ultrasound expertise. Above all, we believe that this is the right direction for the future infertility diagnostics improvement.

Conflict of interest

The authors declare no conflict of interest.

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Vitamin D3 and its receptor in selected obstetrical complications

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ABSTRACT

Vitamin D3 (VD3) and its steroidal nuclear receptor are necessary for proper development of a pregnancy. They play a key role in implantation, modulate the mother's immune response to the developing fetus, influence the final development of a placenta, and regulate blood pressure and glucose tolerance. VD3 deficiency can lead to the occurrence of obstetric complications such as recurrent miscarriages, preeclampsia, intrauterine growth restriction, gestational diabetes and preterm labor. VD3 deficiency is a common phenomenon across the globe; because of the higher demand placed on their bodies, pregnant women are more likely to develop VD3 deficiency. During pregnancy, VD3 supplementation is a safe method of treatment without risk of side effects or intoxication. To obtain the greatest efficacy, VD3 supplementation should start at the pregnancy planning stage, under control of the VD3 serum concentration, which should exceed 30 ng/mL (75 nmol/L); this is to start the positive effect of the optimal VD3 concentration from the beginning of a pregnancy.

Key words: vitamin D; VDR; pregnancy complications

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INTRODUCTION

Vitamin D (VD) has two main forms: Vitamin D2 (VD2), produced by plants, and VD3, produced under influence of UVB radiation in animal skin. They both have the same biological action and vary only in their side-chain structure. Because of its pleiotropic actions, VD3 plays an important role in the human body. The discovery of VD3 was a result of the investigation on the cause of rickets at the beginning of the 20th century. In recent years available data suggests a connection between low levels of VD3 and the development of cancers, and cardiovascular and autoimmune diseases [1].

VD3 is synthesised from 7-dehydrocholesterol in the cortical layer of the epidermis under the influence of 290––315 nm wavelength UVB radiation. Many factors such as geographic location, season, air pollution, skin colour, and age affect VD3 synthesis. The largest amount of UVB radiation able to induce synthesis of VD3 occurs around the equator, and it decreases when approaching the poles. In Poland, VD3 synthesis is almost impossible from October to March, and in the remaining months, 60% of the UVB radiation able to induce synthesis of VD3 occurs between 11 AM and 3 PM. VD3 is a secosteroid prohormone which undergoes activation by 25-hydroxylation and 1α-hydroxylation, respectively, in the liver and the kidneys as well as in other tissues. The active form of VD3, 1.25(OH)D3, is known as calcitriol. Serum VD3 level is measured based on the 25(OH)D3 concentration because it has a half-life 1000 times higher than 1.25(OH)D3. Calcitriol affects target cells after binding with the specific steroidal VD receptor (VDR). After binding with calcitriol, VDR creates a heterodimer with retinoid receptor X (RXR) which binds with VD responsive elements on DNA (VDRE), affecting transcription of many genes [2].

It is accepted that the minimal 25(OH)D3 serum level which enables optimal functioning of the human body is 30 ng/mL (75 nmol/L). This concentration was based on the dependence of the VD3 and parathormone concentration, intestinal calcium absorption and bone density measurements. VD3 deficiency can affect about 30% of the world's population. In Poland, the study of Płudowski et al. [3], from

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2014 showed that almost 90% of the investigated population had a 25(OH)D3 level below the optimal 30 ng/mL level.

VD3 influences pregnancy development from the time of implantation. An increase in calcitriol concentration is observed from 10–12 weeks of gestation and grows two- to threefold until the end of pregnancy [4]. A large part of this increase is due to the expression of 1α-hydroxylase in the chorion and placenta [5]. Calcitriol helps in the transformation of endometrial cells into decidual cells, promotes a human trophoblast invasion in vitro, and increases the expression of HOXA10, a gene necessary in the implantation process, and marrow differentiation in early gestation [6]. The expression of VDR and CYP27B1 in the chorion and placenta is greatest in the first and second trimesters, which proves the high demand for VD3 at the beginning of the pregnancy [7]. In the maintenance of a proper pregnancy, a state of transient immunosuppression is necessary, so the mother's immune system does not reject foreign antigens of the embryo and the fetus. VD3 modulates actions of the immune system by regulating synthesis of cytokines and inhibiting proliferation of pro-inflammatory cells. Calcitriol directly influences the naive CD4+ lymphocytes, inhibiting their differentiation into Th1 lymphocytes (synthesis of pro-inflammatory cytokines such as IFNγ), while promoting differentiation into Th2 lymphocytes (synthesis of anti-inflammatory such as IL-4, IL-5, IL-10). VD3 also inhibits lymphocyte B synthesis and production of immunoglobulin type G. In a human syncytiotrophoblast, VD3 regulates, in an autocrine manner, synthesis of human chorionic gonadotropin (hCG), human placental lactogen (hPL), estradiol, and progesterone. These hormones help pregnancy to develop and stimulate growth and development of the placenta [8, 9].

VD3 AND RECURRENT PREGNANCY LOSS

In recent years, the importance of impaired function of the immune system in the physiopathology of recurrent pregnancy loss (RPL) is being increasingly recognised. There is elevation of NK CD56+ cells as a percentage of total peripheral lymphocytes in the serum of women with recurrent pregnancy loss [10]. Also, there is a strong immunological response of Th1 lymphocytes, which produce pro-inflammatory cytokines and increase cytotoxicity of NK cells [11].

VD3, because of its immunomodulatory potential, can exert a positive effect on implantation and development of early gestation. A meta-analysis from 2018, which included 2700 women (11 studies), assessed the influence of VD3 concentration on assisted reproductive treatment. In this study, VD3 concentration higher than 30 ng/mL was associated with greater chances of getting a positive pregnancy test, clinical pregnancy and giving birth to a healthy newborn. Therefore, investigators highlight the possible positive effects of VD3 supplementation in groups of women who undergo assisted reproductive treatment [12]. The study of Ota et al. [13], conducted on a group of 133 women with a history of three or more pregnancy losses, has shown that 47.7% of women had VD3 deficiency, which was correlated with increased cell immunity and autoimmunization. Similar results were obtained by Chen et al. [14], in a group of 99 women with a history of recurrent pregnancy loss, when VD3 deficiency was measured in 64.6% of the cases and was associated with an adverse immunological profile: increased concentration of the T helper lymphocytes synthesizing TNF α, lymphocytes CD19+ and NK cells. Moreover, in this study, supplementation of calcitriol (0.5 ug) for two months was reversing an adverse immunological profile. Expression of VDR in the endometrium of women with recurrent pregnancy loss was also analysed. The study of Tavakoli et al. did not show any difference in the expression of VDR in the endometrium of the women with recurrent pregnancy loss in comparison to the healthy controls [15]. In two other studies, chorion expression of VDR was lower in women with a history of recurrent pregnancy loss in comparison to healthy controls [16, 17]. The European Society of Human Reproduction and Embryology, in recommendations from 2018 regarding recurrent pregnancy loss, advises prophylactic VD3 supplementation, although it does not advise routine determination of serum concentration of 25(OH)D3 [18].

Although there is no randomised control trial (RCT) on the effectiveness of VD3 supplementation in the prevention of miscarriages, most data suggest that it has positive effect of immunological profile, which promotes implantation and development of early gestation.

VD3 and preeclampsia

In Scandinavia, more frequent occurrence of preeclampsia (PE) in the winter months was observed, this later being proved in many population studies. This correlation was explained by the local lower skin synthesis of VD3 in the winter months [19]. Nowadays, many observational studies and meta-analyses are showing that low maternal VD3 serum concentration is related with greater risk of PE. In a meta-analysis from 2018 which included 23 studies, a serum level of 25(OH)D3 below 20 ng/mL was associated with greater risk of PE [20]. VD3 can also affect blood pressure by inhibiting renin synthesis by the kidney capsular apparatus which was shown in the *in vitro* study [21]. Growth and development of the placenta is affected by VD3, and its deficiency is associated with PE, simultaneously in PE, placental synthesis of VD3 is decreased. Trophoblasts isolated from women with PE had only $1/10^{th}$ the activity of 1α hydroxylase in comparison to the trophoblasts isolated from healthy controls [22]. VDR expression is also signifi-

cantly lower in placentas from women with PE [23]. There were several studies on the effects of VD3 supplementation on PE occurrence. In a meta-analysis conducted by Fogacci et al. [24] in 2019, supplementation of VD3 significantly reduced the risk of PE OR (0.37, 95% CI: 0.26, 0.52). A Cochrane meta-analysis, also from 2019, showed that VD3 only supplementation decreases the risk of PE (RR 0.48, four studies conducted on a group of 499 women) [25]. Most data suggest that VD3 reduces the risk of PE in the general population. Royal College of Obstetricians and Gynaecologists for women with high risk of PE advises intake of at least 800 IU daily combined with calcium [26].

VD3 and fetal growth

VD deficiency can disturb the process of formation and growth of the placenta, which can subsequently lead to intrauterine growth restriction (IUGR). In placentas from pregnancies complicated by IUGR, lower expression of VDR was observed [27]. Studies focused on proving the correlation between VD3 deficiency and higher IUGR occurrence have not found clear results. Some, like a study from 2019 conducted in Iran on a group of 812 women [28], or a multicenter study conducted on a group of 2,146 women from the USA [29], have shown that VD3 deficiency was associated with lower body weight in the newborns — a meta-analysis from 2013 showed such a correlation [30]. However, a study conducted in Norway in a group of 712 women from different ethnic groups showed that VD3 is not an independent factor affecting differences between them in anthropometric parameters of the newborns and birth weight [31]. There is also inconsistency in data about the effects of VD3 supplementation on birth weight. Two meta-analyses have shown that VD3 supplementation increases mean birth weight of newborns and decreases the risk of small for gestational age fetus (SGA) [32, 33]. On the other hand, the opposite result was found by Roth et al. [34], in an RCT conducted in Bangladesh on different doses of VD3 supplementation during pregnancy and lactation. There were no effects on height and birth weight of the newborns, or SGA occurrence in any of the tested groups. However, in this study supplementation was started between 17 and 24 weeks of gestation, which might be too late to obtain a maximal positive effect of optimal VD3 level on pregnancy development.

It is controversial whether VD3 supplementation has a positive effect on birth weight. Further studies are needed in order to help explain current contradictions within the existing body of knowledge.

VD3 and gestational diabetes

VD3 plays an important role in carbohydrate metabolism; a positive correlation between VD3 concentration and tissue insulin sensitivity has been shown. VD3 deficiency diminishes insulin secretion by pancreatic β cells [35]. The insulin gene has a VDRE in a promoter region, so calcitriol can modulate insulin transcription. As previously mentioned, immune disorders lead to a general inflammatory state, which is an important element in the development of insulin resistance and diabetes type 2. Interestingly, in placentas from women with gestational diabetes, VDR expression was higher than in healthy controls. In the same study, it was found that lower concentration of VD3 was associated with higher expression of VDR in the placenta [36]. There are many studies on VD3 concentration and supplementation on gestational diabetes occurrence. A meta-analysis from 2018, consisting of 87 observational studies and 25 randomized clinical trials, which included, respectively, 55,859 and 2,445 women, showed that in a group of women with gestational diabetes, VD3 serum concentration was lower than in a healthy control group [37]. The same meta-analysis regarding the effects of VD3 supplementation on pregnancy development found that it reduces the risk of gestational diabetes [25, 30], but also that some studies included found no influence [38].

While there is strong theoretical background, the effectiveness of VD3 supplementation on gestational diabetes is debatable.

VD3 and preterm labor

VD3 deficiency can affect preterm labor in two ways. Firstly, it reduces the body's defense capability, and secondly, it increases synthesis of pro-inflammatory cytokines. Uterine activity is probably triggered by genetic factors and pro-inflammatory cytokines. When they are activated before the fetus reaches maturity, preterm labor occurs. VD3 stimulates the innate immune system, which acts immediately after coming in contact with a pathogen. In experimental studies, calcitriol induces synthesis of cathelicidin (peptide of bactericidal properties) in myeloid cells, bronchial epithelial cells and keratinocytes. The immunostimulation by 25(OH)D3 also acts in an autocrine manner. Macrophages, after encountering a pathogen, increase the expression of VDR and CYP27B1 [39]. In many studies it has been demonstrated that VD3 deficiency can increase the risk of preterm birth. Among them, a study conducted by McDonnell et al. [40], showed that women with an optimal concentration of VD3 had 60% lower risk of preterm birth occurrence. In addition, a meta-analysis from 2017 suggested a possible correlation between VD3 deficiency and preterm birth, and a probable positive effect of VD3 supplementation on this pregnancy condition [41]. In the USA, the highest percentage of preterm births, 13.3%, occurs among Afro-American women, in comparison to 9.0% in the Caucasian population. This difference is still seen after adjusting for socioeconomic factors. African-Americans also have five times greater risk of recurrent preterm birth. At the

same time, in this group, VD3 deficiency occurs more often than in different ethnic groups [42, 43].

Presented studies are in favor that VD3 supplementation reduces the risk of preterm labor, however, we could not find specific recommendations on it.

VD3 during lactation ad postpartum period

The intake of 400 IU of VD3 is recommended for born in term infants between 0–6 months of life, independently to the way of the feeding, to prevent rickets. Exclusively breastfed infants are the group with the highest risk of vitamin D deficiency because 25(OH)D3 concentration in breast milk is only about 20% of mothers' serum concentration. So, to meet newborn requirements, mothers would have to have a serum concentration of VD3 much higher than it is usually recommended. The study of Hollis et al. [44], investigated this topic and concluded that a mother's intake of 6400 IU/day safely supplies breast milk with 25(OH)VD3 to satisfy her nursing infant's requirements. Furthermore, another important issue of VD3 supplementation is prevention of pregnancy-associated osteoporosis. It is a rare syndrome affecting women during late pregnancy and the early post pregnancy period. It can cause severe loss of bone mineral density, pathological fractures in the vertebrae, hip and other bones. In the study of Eroglu et al. [45], the levels of 25(OH)D3, in postpartum women, were significantly lower in the low Bone Mineral Density group.

Proper VD3 supplementation during post-partum period is important in terms of prevention of infant rickets and mothers' osteoporosis in postpartum period.

SUMMARY

VD3 and VDR have an impact on pregnancy development in many areas. In early gestation they play a key role in the formation of the chorion, modulate the immune response, and regulate blood pressure and glucose tolerance. Contemporary lifestyle, which involves spending a long time indoors, because UVB radiation does not penetrate glass, makes VD3 skin synthesis difficult to occur in a sufficient amount. Nowadays, the Sars-CoV-2 pandemic can only increase the frequency of such a lifestyle. By proper VD3 supplementation and changes in lifestyle, it is possible to reduce the risk of many pregnancy complications. Treatment of VD3 deficiency should start at the time of pregnancy planning. The best way to treat VD3 deficiency is by dietary supplements. Because of high individual variability of skin synthesis, it is difficult to advise/prescribe a specified duration of sun exposure to treat VD3 deficiency [2]. VD3 supplementation in pregnancy seems to be a safe method to decrease VD3 deficiency, without side effects or intoxication. Many of the institutions give recommendations in this topic but they differ significantly in the advised dosage as listed in the Table 1. Most of them are consistent that a blood level of 25(OH)D3 above 30 ng/mL should be maintained [46, 47]. At the same time routine measurement of 25(OH)D3 is not advised, however, in our opinion it is worth to consider especially in a women with a higher risk of the pregnancy complications such as: RPL, PE, fetal growth disturbances, pregnancy diabetes and preterm labor. They might benefit the most of the VD3 supplementation adjusted to the serum level of 25(OH)D3.

Table 1. Reference intake of VD3 during pregnancy and lactation according to health authorities

Date	Health Authority	Dosage of VD3 supplementation in IU daily	Comments	Citation
2011	Endocrine Society	600	"in some cases at least 1500–2000 IU may be needed"	[46]
2011	The Institute of Medicine	600		[48]
2011	American College of Obstetricians and Gynaecologists	600	1000–2000 IU daily when VD3 deficiency is identified	[49]
2012	The German Nutrition Society	800		[50]
2014	Royal College of Obstetricians and Gynaecologists	400	800 IU daily, combined with calcium, recommended for women at high risk of PE 1000 IU daily recommended for women at high risk of VD3 deficiency[1]	[26]
2016	The Scientific Advisory Comittee on Nutrition	400		[51]
2020	The Polish Society of Gynecologists and Obstetrics	1500–2000	Women with a BMI > 30 kg/m^2 are advised to consider a dosage of 4000 IU daily	[47]
2020	The World Health Organization	Oral VD3 supplementation is not recommended	Women with suspected VD3 deficiency are recommended to take 200 IU daily	[52]

VD3 — vitamin D3; IU — international unit; BMI — body mass index; [1]Women with increased skin pigmentation, reduced exposure to sunlight, or those who are socially excluded or obese

Conflict of interest

The authors have no potential conflict of interests to declare.

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Dysgerminoma of the ovary in a patient with triple-X syndrome (47, XXX) and Marfanoid habitus features

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Key words: dysgerminoma; triple-X syndrome; Shprintzen-Goldberg syndrome; Marfan syndrome

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INTRODUCTION

Dysgerminoma is a malignant ovarian tumor beginning in premeiotic germ cells, with clinically aggressive behavior and good prognosis. Triple-X syndrome is characterized by an extra X chromosome due to a random error during cell division in sperm or egg formation and is not typically an inherited condition. Premature ovarian failure or ovarian abnormalities are sometimes found coincidentally. Associations between triple X syndrome and dysgerminoma have never been investigated.

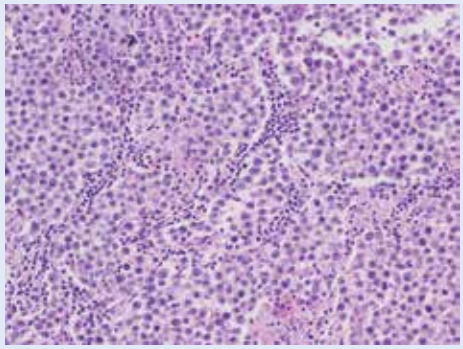

Figure 1. Dysgerminoma. Uniform tumor cells surrounded by connective tissue stroma infiltrated by lymphocytes (H&E, ×20)

CASE REPORT

A 22-year-old woman with Marfanoid habitus and an ovarian dysgerminoma (Fig.1) was admitted to the Department of Gynecology. The patient's medical history showed borderline intellectual disability and clinical suspicion of Marfan syndrome (MFS) (Fig. 2)

Surgical treatment was performed, and post-surgery, four cycles of bleomycin, etoposide, cisplatin (BEP) chemotherapy. She is alive and well with no signs of recurrence 10 years after the completion of treatment.

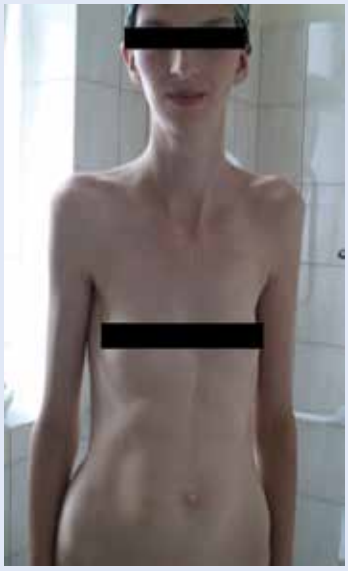

Figure 2. Clinical features of Marfanoid habitus (dolichocephaly, micrognathia, pectus carinatum, elongated extremities)

MATERIAL AND METHODS

Cytogenetic studies were performed using conventional GTG–banding of lymphocyte metaphase chromosomes at a 550-band level. DNA was isolated from the peripheral blood leukocytes of the patient and of nine anonymous healthy female volunteers (reference control DNA). Array Comparative Genomic Hybridization (aCGH) analysis was performed. Genomic imbalances identified were verified in the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation; last accessed December 2020). We searched ECARUCA [1], Decipher [2] and Medline/OMIM [3] for instances of patients with constitutional chromosomal aberrations at chromosome 4q26.

RESULTS

The patient was diagnosed with triple-X syndrome by karyotyping: 47, XXX. Further aCGH analysis confirmed the chromosome analysis and identified interstitial deletion of about 1.1Mb at chromosome 4q26.

Following the revised Ghent Marfan Syndrome Diagnostic Criteria (2010) [4], the patient having only skeletal features but no signs of other organ systems' involvement, and a negative mutation evaluation of the FBN1 gene, diagnosis excluded MFS. Differential diagnosis identified Shprintzen-Goldberg syndrome [5] as the most likely alternative diagnosis.

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DISCUSSION

Genetic studies confirmed diagnosis of the triple-X syndrome and revealed a novel interstitial deletion at chromosome 4q26. The deletion spanned 1.1Mb, comprised of one gene protein coding: translocation associated membrane protein 1 like 1 (TRAM1L1) of unknown function.

The OMIM database provided no information on this gene mutation [3], nor is such reported in the Developmental Disorders Genotype-Phenotype Database [2]. The decipher haploinsufficiency score (HI index) was estimated as 85%, and accordingly, the gene is unlikely to exhibit haploinsufficiency [2]. The adjacent genomic regions were not found to be enriched in low copy repeat sequences or segmental duplications, nor possessing significant enhancer/silencer or promotor-associated histone marks (www.genome.ucsc.edu). Therefore, it seems unlikely that the novel 4q26 deletion was responsible for the clinical features observed in the patient.

Among individuals with chromosome aberrations, a higher risk of germinal tumors has been found in cases of 45,X/46,XY mosaicism, resulting in active oncological prophylaxis in these patients. Associations between triple-X syndrome and ovarian tumors have been rarely documented. Ovarian tumors have not been clinically linked to Shprintzen-Goldberg syndrome so far. However, further research on the cooccurrence of germ cell tumors and chromosomal abnormalities is necessary to identify risk factors of these relatively rare neoplasms.

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Conflicts of interest

The authors declare no conflict of interest.

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OHVIRA syndrome in 14-year-old girl

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INTRODUCTION

Obstructed hemivagina and ipsilateral renal anomaly (OHVIRA) syndrome is an uncommon urogenital — congenital anomaly, characterized by the triad: uterine didelphys, obstructed hemivagina, unilateral renal agenesis. It is associated with abnormal development of the Mullerian and Wolffian ducts [1]. The syndrome appears at < 1/1 000 000 girls.

Patients usually present the OHVIRA when adolescent, 1–2 years after menarche. Regularly reported symptoms are dysmenorrhea and pelvic pain [2, 3]. Recommended treatment for the patients with this syndrome is a minimally invasive surgery [3].

CASE REPORT

Girl aged 14 was admitted to Chair and Department of Gynecology, Obstetrics and Gynecological Oncology in Katowice complaining about sever lower abdominal pain. During last two months pain level was increasing. The patient attained menarche at the age of 13. She experienced regular, but light menstrual cycles, and had a few episodes of a lower abdominal pain following her menses. Physical examination was unremarkable with normal secondary sexual characteristics. When the patient was six years old was diagnosed with an absent left kidney (Fig. 1).

Pelvic examination revealed a flexible, tender mass of 10 cm diameter. The pelvis ultrasonography revealed didelphys uterus, two uterine cavities and cervices. The right uterus was measured as 43 × 23 mm and were displaced to the right side, endometrium thickness — 4.5 mm. Left corpus of uterus and cervix were enlarged and filled with blood (hematometra) with the accumulation of blood in the vagina (hematocolpos), measured 80 × 60 mm (Fig. 2). Both ovaries were normal. Left kidney was absent in its anatomical location. Transverse vaginal septum was sized 5.5 mm.

The patient was treated with hemivaginal septal resection, resulting in an outflow of old menstrual blood. The incision made during the procedure should have appropriate size to prevent it from regrowing. It is extremely important to take care of the patency between the lumen of the right and left vagina for allowing the menstrual blood to outflow. The incision was marsupialized with single sutures. A foley catheter was placed into the left vagina to keep the hole created (Fig. 3). During subsequent visits, the dimensions of the left cavity and the cervix decreased due to the emptying of residual blood from these structures. The opening remained open, vaginal dilators were used by the patient with full acceptance and the pain was significantly reduced.

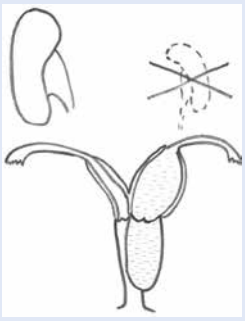

Figure 1. Schematic representation of OHVIRA syndrome [4]

Figure 2. Ultrasonography image: uterus didelphys (right uterus — 1; left uterus — 2) and hematocolpos (3)

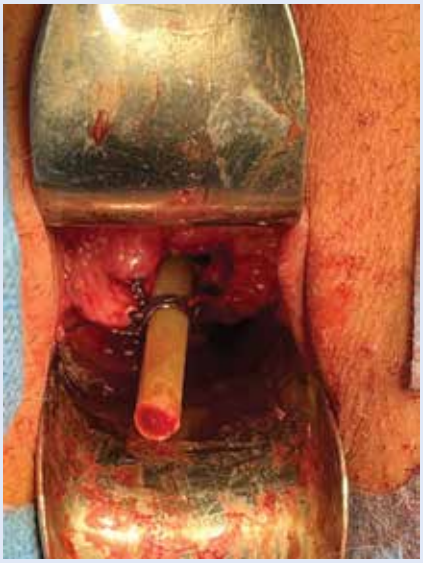

Figure 3. Vaginal operation: a foley catheter placed into the left vagina

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CONCLUSION

Pathogenesis of this syndrome is associated with development of the Mullerian and Wolffian structures. The most frequently clinical symptom reported by patients is cyclic abdominal pain which most often occurs shortly after the menarche [1]. The diagnostic methods of this syndrome are pelvic ultrasound and imaging tests; however, the golden standard is magnetic resonance imaging (MRI) [4]. The septum removal procedure should be performed with appropriate care to prevent excessive bleeding, damage to the bladder, rectum and cervix. If the patient was not treated properly it may result with retrograde tubal reflux, endometriosis and fertility problems [2]. After surgery, adolescent patients should undergo regular follow-up visits in order to prevent an adhesion formation recurrence after vaginal septum resection.

Conflicts of interest

The authors declare no conflict of interest.

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