ORIGINAL PAPERS

A. serum level of PIGF [pg/dL]

Sensitivity: 62.5
Specificity: 69.3
Criterion: ≤ 36.683

B. serum level of PAPPA [IU/dL]

Sensitivity: 58.8
Specificity: 74.8
Criterion: ≤ 1.596

VIA MEDICA

CONTENTS

REVIEW PAPERS
GYNECOLOGY

CLINICAL VIGNETTE

Laparoscopic versus vaginal cuff closure in laparoscopic hysterectomy: does it affect female sexuality?

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ABSTRACT

Objectives: To investigate the effect of vaginal cuff closure technique in laparoscopic hysterectomy on vaginal length and female sexual functions.

Material and methods: This study was conducted at a tertiary research hospital. Women who underwent laparoscopic hysterectomy were included and classified according to vaginal cuff closure technique as laparoscopic (n = 75) and vaginal route (n = 25). Vaginal lengths were measured preoperatively and at 6th month postoperatively. Golombok–Rust Inventory of Sexual Satisfaction (GRISS) was used to evaluate female sexual functions. SPSS was used for statistical analysis and the level of significance was p = 0.05.

Results: Preoperative GRISS scores and vaginal lengths were similar in two groups. The shortening of vaginal length and the worsening of GRISS scores were more prominent in vaginal cuff closure group (p = 0.002, p < 0.001). The alteration in vaginal length was positively correlated with the alteration in GRISS score in vaginal and laparoscopic route groups (r = 0.800, p < 0.001; r = 0.680, p < 0.001). The risk of female sexual dysfunction increases 69.88 fold for each 1 cm shortening of vaginal length (p = 0.039). Discriminative value of postoperative vaginal length for female sexual dysfunction in patients who underwent laparoscopic hysterectomy was investigated and a cut-off value of 7.4 cm (p < 0.001) was found.

Conclusions: Laparoscopic route instead of vaginal route in laparoscopic hysterectomy is preferable to preserve a better vaginal length, which may be an important factor for female sexual functions.

Key words: female sexual function; Golombok–Rust Inventory of Sexual Satisfaction Scale; hysterectomy; laparoscopy; vaginal cuff closure technique

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INTRODUCTION

World Health Organisation has defined sexual health as 'a state of physical, emotional, mental and social well-being in relation to sexuality; not merely the absence of disease, dysfunction or infirmity'. Sex refers to the biological characteristics that define humans as female or male. Women think their uterus as a part of their sexual identity. It is evident that the health problems associated with the reproductive system, including uterus have psychological effects on women. Benign gynecologic diseases such as myoma uteri, abnormal uterine bleeding, endometriosis, pelvic flor diseases often require uterine operations including hysterectomy. In the United States 600,000 women have hysterectomy annually, and 40% of American women are undergoing this operation until the age of 64 [1].

Hysterectomy has various complications such as bleeding, infections, thromboembolism, genitourinary, gastrointestinal tract and nerve injury, vaginal cuff dehiscence [2]. In addition to these known morbitidies, regardless of the surgical approach its effects on female sexuality and ovarian functions are controversial. Female sexual dysfunction is a multifactorial problem which is defined as a persistent or recurrent disorder of sexual desire, arousal, orgasm and pain. Social conditions such as marital problems, stress, past sexual trauma and medical conditions including diabetes, depression, neurological disorders, alcoholism and drug abuse can affect sexual functions [3]. It was emphasized that age, depression, relationship problems may be factors affecting development of sexual dysfunction after hysterectomy [4]. Dyspareunia due to shortening of vagina as

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a result of removal of cervix, vaginal dryness beginning with pelvic nerve injury and anorgasmic sexual dysfunction resulting from the breakdown of the orgasmic cycle may be underlying mechanisms of posthysterectomy sexual dysfunction [5]. In contrast, there are studies claiming that hysterectomy performed for benign gynecologic diseases may improve female sexual functions [3]. However, there is limited evidence to conclude how the surgical procedure affects sexual function.

Another challenging issue about the effects of hysterectomy on female sexuality is the cuff closure technique. There is only a few data about this issue. In a study searching the effect of horizontal and vertical vaginal cuff closure following vaginal hysterectomy for pelvic organ prolapsus it was demonstrated that there was no difference in female sexuality between two closure techniques [6]. On the other hand, another study investigating the effect of vaginal cuff closure technique after laparoscopic hysterectomy showed improvement in female sexuality through laparoscopic approach [7].

Objectıves

We aimed to investigate the effect of vaginal cuff closure technique used in laparoscopic hysterectomy on female sexuality. The secondary aim of this study was to determine a cut-off value for vaginal length to cause detoriation in female sexuality.

MATERIAL AND METHODS

A cross sectional study was conducted at a university affiliated tertiary research and training hospital in Northwestern region of Turkey among 100 women, who underwent laparoscopic hysterectomy for benign uterine pathologies between January 2016–January 2017. Women between 35–55 years old who planned to undergo laparoscopic hysterectomy for benign uterine pathologies were included in the study. Patients (n = 100) were preoperatively assessed and further classified according to the vaginal cuff closure technique as laparoscopic closure (n = 75) and vaginal closure (n = 25). The exclusion criteria of the present study were as follows; 1) Current or past psychiatric diseases, 2) Psychiatric drug use, 3) Patients with malignancy and/or endometriosis, 4) Presence of uterin prolapses at any degrees 5) History of any other gynecologic operations, 6) Presence of chronic debilitating disease, 7) Drug abuse, 8) Failure to attend the follow-up visit. All patients underwent gynecologic examination including pap-smear testing and sonographic examination. The type of surgery was determined according to medical conditions, informed consent and shared decision making. Baseline demographic characteristics including obstetric and gynecologic history such as gravida, parity, first age of coit, menarche age, education level, income level, delivery mode, age, height, weight, body mass index (BMI),

surgical and medical history were recorded. Vaginal length was measured preoperatively and at 6th month postoperatively by the surgical team. Operations were performed by the same surgical technique. Vaginal length was measured from the hymenal ring to the D point of POP-Q by speculum examination preoperatively on the day of hospitalization and postoperatively at 6th month during the routine gynecology outpatient clinic control by the same operator. Care was taken not to stretch the vagina during the measurement.

All the patients included in the present study underwent conventional laparoscopic hysterectomy. At vaginal cuff closure step of the laparoscopic hysterectomy for both vaginal and laparoscopic approaches the vaginal cuff was formed by suturing the vaginal opening antero-posteriorly in a horizontal pattern. The first bite of the suture was merged with the uterosacral ligament at one side and vaginal opening was closed through a continous running suture until the last bite of the suture which also merges with the uterosacral ligament on the contralateral side. The suture material used in either laparoscopic or vaginal closure methods was 0-Vicryl. The surgeries were done in the same hospital and by the same surgical team accepting the same surgical techniques for the conventional laparascopic hysterectomy.

Differences between the two groups in terms of sexual dysfunction were evaluated using the validated Turkish version of the Golombok–Rust Inventory of Sexual Satisfaction (GRISS) scale. Patients were asked to complete GRISS at preoperative and postoperative periods. When patients were admitted to hospital for preoperative evaluation by anesthesia care team 2 weeks before the surgery they were asked to complete the GRISS. At 6th month postoperatively, when patients admitted to gynecology outpatient clinic for routine postoperative evaluation, they were asked to complete the GRISS for one more time. The questionnaires were given to the patients by an obstetrics and gynecology assistant and they were asked to answer the questionnaires alone in a room by themselves.

GRISS has 28 questions all of which are answered over five points. It is Likert type scale and answers are as follows: always, usually, sometimes, hardly ever and never. It evaluates the different domains of sexual function which can be sorted as infrequency, non-communication, female dissatisfaction, vaginismus, female non-sensuality, female avoidence and anorgasmia. The GRISS is evaluated with a total scale score and for subdomains. High GRISS scores are indicative of more severe sexual dysfunction and disruption in relationship quality. Raw scores are converted to standard scores between 1 and 9. Scores ≥ 5 are indicative of detoriation in sexual functions. An increase in the scores for each subdomain also shows impaired sexual function [8, 9]. Differences between pre and postoperative 6th months GRISS scores were calculated and given as alteration in

GRISS scores. The alterations and preoperative GRISS scores were compared between two groups.

A post hoc power analysis was conducted using a large effect size, based upon findings of the present study. Large effect size was obtained by comparing mean alterations in vaginal length which were calculated from laparoscopic closure (1.51 ± 0.59) and vaginal closure (2.02 ± 0.68) terms for 100 participants. Using this effect size (d = 0.80) with a sample size of 100 participants, achieved power was estimated as 93% at the significance level of α = 0.05. Shapiro Wilk test was used for assessing whether the variables follow normal distribution. Continuous variables were presented as median (minimum:maximum) and mean ± standard deviation where categorical variables were reported as n (%). According to normality test independent t test and Mann Whitney U tests were used for group comparisons. Pearson chi-square and Fisher-Freeman-Halton tests were used for comparing categorical variables. Correlations between discrete and continuous variables were analyzed using Spearman correlation analysis and correlation coefficient was calculated. For postoperative vaginal length, receiver operator curve (ROC) analysis was performed for the cut-off point that could predict sexual dysfunction. Also, the sensitivity and specificity of postoperative vaginal length for predicting sexual dysfunction were calculated by ROC analysis. Area under curve (AUC) value with 95% confidence interval (CI)

were reported. To determine independent risk factors that affect sexual function, logistic regression analysis was performed. SPSS version 20 (IBM Corp. Released 2012.IBM SPSS Statistics for Windows, Armonk, NY:IBM Corp.) was used for performing statistical analysis and p ≤ 0.05 was considered statistically significant.

The study was approved by the local ethics committee and written informed consent was obtained from each participant. Also, it complies with the Declaration of Helsinki.

RESULTS

The sociodemographic findings of patients were demonstrated in Table 1. There was no difference between two groups according to age, height, weight, BMI, gravida, parity, first age of coit, menarche, delivery mode, education and income level (p > 0.05).

Preoperative vaginal length, preoperative GRISS score, alteration in GRISS score and in vaginal length were compared between two groups (Tab. 2). Although there was no difference in regard of preoperative GRISS score and vaginal length, the shortening of vaginal length was more prominent in vaginal closure group (p = 0.002) and also, the worsening in GRISS score was more apparent in vaginal group (p < 0.001).

The data about the alterations in vaginal length and GRISS score at 6th month were analyzed to show whether

Table 1. Sociodemographic characteristics of patients			
	Laparoscopic Closure (n = 75)	**Vaginal Closure (n = 25)**	**p**
Age [y]	47.41 ± 4.11	47.24 ± 4.28	0.857[a]
Height [cm]	164 (155:172)	165 (155:170)	0.051[b]
Weight [kg]	70.36 ± 10.52	73.68 ± 7.97	0.152[a]
Body mass index [kg/m²]	26.27 ± 3.58	26.99 ± 2.83	0.308[a]
Gravida [n]	3 (1:6)	3 (1:5)	0.454[b]
Parity [n]	3 (1:6)	2 (1:5)	0.455[b]
First age of coit [y]	25 (16:34)	25 (17:29)	0.722[b]
Menarche age [y]	13 (11:16)	13 (11:16)	0.240[b]
Education level — Literate (n, %) — Primary school (n, %) — Secondary school (n, %) — High school (n, %) — University (n, %)	6 (8) 18 (24) 17 (22.7) 24 (32) 10 (13.3)	3 (12) 5 (20) 6 (24) 8 (32) 3 (12)	0.976[c]
Income level — Low (n, %) — Moderate (n, %) — High (n, %)	30 (40) 32 (42.7) 13 (17.3)	8 (32) 11 (44) 6 (24)	0.688[c]
Delivery mode — Cesarean section (n, %) — Normal delivery (n, %) — Cesarean and normal delivery (n, %)	22 (29.3) 31 (41.4) 22 (29.3)	6 (24) 12 (48) 7 (28)	0.820[d]

[a] — Independent sample t test; [b] — Mann-Whitney-U Test; [c] — Fisher Freeman Halton Test; [d] — Chi-square Test

Table 2. Vaginal length and GRISS scores of patients

	Laparoscopic Closure (n = 75)	Vaginal Closure (n = 25)	p
Preoperative vaginal length [cm]	9.60 ± 0.54	9.52 ± 0.69	0.952[a]
Alterations in vaginal length [cm]	1.51 ± 0.59	2.02 ± 0.68	**0.002[a]**
Preoperative total GRISS score	5.59 ± 1.82	4.88 ± 2.15	0.076[a]
Alteration in GRISS score	1.29 ± 0.98	3.28 ± 1.49	**< 0.001[a]**

[a] — Mann Whitney U Test
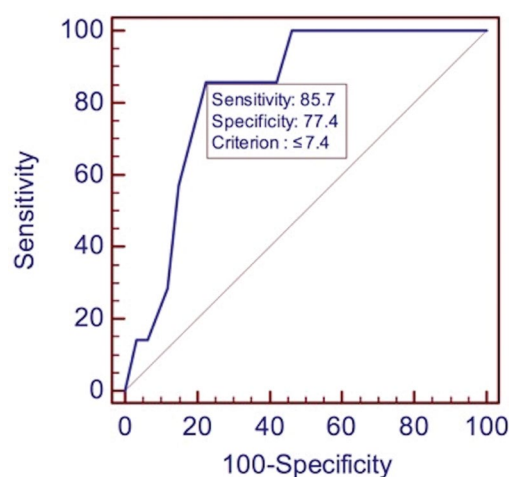
Figure 1. Receiver operating curve performed for determining the cut-off point of postoperative vaginal length that could predict sexual dysfunction

there is a correlation between the shortening of vagina and increased GRISS scores. We found that the alteration in vaginal length was positively correlated with the alteration in GRISS score for all patients (r = 0.756, p < 0.001) and also for both vaginal closure (r = 0.800, p < 0.001) and laparoscopic closure (r = 0.680, p < 0.001) groups. Furthermore, logistic regression analysis revealed that the risk of female sexual dysfunction increased 69.88 fold for each 1 cm shortening in vaginal length (p = 0.039).

The discriminative value of postoperative vaginal length for sexual dysfunction in patients who underwent laparoscopic hysterectomy was evaluated by ROC curve. AUC for postoperative vaginal length was 0.829 with a sensitivity of 85.7% and specificity of 77.4% with a cut-off value of 7.4 cm (p < 0.001) (Fig. 1).

DISCUSSION

Main findings of the present study are as follows: 1) the shortening of vaginal length and the worsening in GRISS score were more prominent in vaginal cuff closure group, 2) the shortening in vaginal length was positively correlated with the increase in GRISS scores for all patients and also for both vaginal cuff closure and laparoscopic cuff closure groups, 3) the risk of female sexual dysfunction was found to be increased 69.88 fold for each 1 cm shortening in vaginal length, 4) postoperative vaginal length with a cut-off value of 7.4 cm was supposed to be discriminative for female sexual dysfunction.

Female sexuality is a challenging issue with several confounding factors some of which are medical, psychological, physical, social, educational, religious and hormonal [10].

A majority of gynecologic surgeries have been thought to be closely related with female sexual dysfunctions. Among those, hysterectomy, which is the most frequently performed gynecological operation, has been under investigation for its effects on female sexuality for a quite long time. However, it is still controversial whether female sexual functions improve or worsen following hysterectomy [11]. Improvement in female sexual functions after hysterectomy were reported to be associated with relief of symptoms such as dysmenorrhea, dyspareunia and uterine bleeding, all of which may result in an improved quality of life including sexual functions [12]. On the other hand, neurovascular injuries due to surgical procedure, ovarian failure due to postoperative diminished blood flow, simultaneously performed bilateral salphingo-oophorectomy and decreased vaginal length following operation may provide an explanation for the worsening of sexual functions following hysterectomy [13]. Furthermore, uterus is an organ that has a psychosocial importance and so, hysterectomy may refer to loss of sexuality for most of the women [10].

It is obvious that the decisions of surgeon about the operation will have a postoperative consequence. Not only the surgical approach to hysterectomy but also the surgical technique chosen will affect surgical results. Therefore, when to prefer which surgical method should be questioned for several aspects [14].

The term laparoscopic hysterectomy defines application of laparoscopic technique for at least a part of operation [15]. It was first introduced by Reich et al in 1989 [16]. The advantages and disadvantages have been discussed since that time. However, nowadays the number of hysterectomies performed through laparoscopic approach is quite high and increasing gradually [14].

There are studies in the literature investigating the effects of laparoscopic hysterectomy on female sexuality and

comparing the results with that of abdominal and vaginal hysterectomies. The study, in which vaginal, abdominal and laparoscopic hysterectomies were compared in regard of their effects on female sexuality, revealed no differences between three groups according to their effects on orgasm, frequency and desire [10]. Sexual functions were shown to be improved in postoperative third month and second year when patients who underwent vaginal hysterectomy and total abdominal hysterectomy were compared. However, patients in total abdominal hysterectomy group experienced more pain and their self-image were poorer [17]. The impact of abdominal and laparoscopic hysterectomies on female sexuality was analyzed in a study by using Arizona Sexual Experiences Scale. It is observed that sexual functions were improved after both types of hysterectomies and total laparoscopic hysterectomy were shown to have significantly better outcomes for sexual drive and arousal [18]. A study comparing postoperative prevalence of hypoactive sexual desire disorder after different types of hysterectomy including abdominal, vaginal, laparoscopic assisted vaginal, laparoscopic supracervical and total laparoscopic hysterectomies did not display any differences between these five surgical approaches. On the other hand, female sexual function scores were reported to be better among patients in laparoscopic supracervical and total laparoscopic hysterectomy groups [19]. Probably, due to the smaller size of abdominal scar and the shorter recovery time after surgeries, laparoscopic methods were shown to be associated with positive effects on females' self-esteem and quality of life. Less invasive surgical methods of hysterectomy such as vaginal and laparoscopic routes tend to have less destruction on female sexuality [18]. From this point of view, it is possible to infer that even the different surgical techniques used during any surgical approach may have different effects on surgical outcomes [14]. For instance; total versus subtotal hysterectomy, different techniques used to support vaginal vault, to or not to perform bilateral oophorectomy during hysterectomy under elective circumstances, to or not to use several techniques in laparoscopic hysterectomies which may be helpful in avoiding several complications such as using vaginal delineators, rectal probes or illuminated ureteric stents will have different surgical outcomes [14].

How vaginal length is affected following different types of gynecologic procedures especially hysterectomies is not a widely studied topic. Pelvic reconstructive surgery and hysterectomy, regardless of type, were shown to be determinants of vaginal length with a shortening effect [20]. When vaginal length following total abdominal and vaginal hysterectomies were compared a significantly shorter vaginal length was observed after vaginal hysterectomy [21]. De La Cruz et al. compared 38 total vaginal hysterectomies and 46 robotic hysterectomies, both of which were accompanied by pelvic support surgery, with regard to vaginal length and postoperative sexual functions. Although vaginal lengths were stated to be decreased after total vaginal hysterectomy as compared to robotic hysterectomy, any differences were not observed between two groups according to the sexual functions [22].

The vaginal lengths of sexually active women were observed to be longer when compared to sexually inactive women. However, the difference between two groups was not significant. Generally, vaginal length and caliber were not accepted as determinants of sexual activity and sexual function scores were not shown to be affected by vaginal size [23]. Yet, it is important to refer that vaginal length may be an indicator of sexual functions in case of presence of a positive history for gynecologic surgery [24]. Total vaginal length was affected following total abdominal, total laparoscopic and vaginal hysterectomies. Each type of hysterectomy was shown to decrease vaginal length similarly and female sexual functions were disturbed irrespective of the surgical approach [25].

Bastu et al [7] randomized patients who underwent laparoscopic hysterectomy for the route of vaginal cuff closure where either laparoscopic or vaginal route was preferred. They reported that although female sexual functions did not differ significantly pre and three months postoperatively, vaginal lengths were significantly longer in laparoscopic route group.

In this study, the vaginal length was shown to be decreased significantly in vaginal cuff closure group. Similarly to the other studies in the literature, we evaluated the correlation between vaginal length and female sexual dysfunction and we demonstrated a positive correlation between the shortening in vaginal length and the increase in GRISS scores for all patients who underwent laparoscopic hysterectomy and also separately for vaginal cuff closure and laparoscopic cuff closure groups. In the previous studies with similar study design as ours, only postoperative female sexual functions and postoperative vaginal lengths were evaluated and the correlation analysis was performed with regard to those two parameters. However, dissimilarly, in the correlation analysis we take into account the difference between the pre and postoperative vaginal lengths and the GRISS scores, which might be more informative about the degree of vaginal shortening and the deterioration in female sexual functions, instead of taking only postoperative vaginal lengths and postoperative GRISS scores. This provide us the chance of demonstrating risk of female sexual dysfunction increased nearly 70 fold for each 1 cm shortening in vaginal length. Moreover, we found that to avoid postoperative female sexual dysfunction a vaginal length of minimum 7.4 cm was required.

There are few limitations of this study. First of all, this is a non randomized study from a single center with a small

sample size. Secondly, 6 months is a short time period for reevaluation of patients postoperatively. Thirdly, there were difficulties in measuring the vaginal length since vagina is a quite elastic organ. Moreover, pain and afraid associated with postoperative period are subjective components of sexual limitation and therefore it is difficult to differentiate their effects on sexual functions. Lastly, the surgical factors in vaginal cuff closure group and in laparoscopic cuff closure group, such as the duration of operation, blood loss, the time of passing flatus, the time of postoperative mobilization and the postoperative complications were not considered in this study.

CONCLUSIONS

In conclusion, patients should be informed about that the laparoscopic hysterectomy may cause deterioration in sexual functions to various extent. Considering the correlation of vaginal length with GRISS scores it should be better to avoid shortening of vagina as much as possible. Therefore, laparoscopic horizontal closure of the vaginal cuff instead of vaginal horizontal closure is preferable to maintain a better vaginal length which can be an important factor for female sexual functions.

Conflict of interests

The authors report no conflict of interests.

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ORIGINAL PAPER / GYNECOLOGY

Circulating sclerostin levels in relation to nutritional status, sex hormones and selected bone turnover biochemical markers levels in peri- and postmenopausal women

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ABSTRACT

Objectives: Hormonal changes during the peri- and postmenopausal age, especially decreasing estradiol levels as the result of the expired ovarian function, are an established link of the pathogenesis of postmenopausal osteoporosis. The objective of the study was to examine the association between the circulating sclerostin levels and nutritional status, sex hormones and selected bone markers turnover levels in peri- and postmenopausal women.

Material and methods: The study enrolled 84 stable-body mass women (31 perimenopausal and 54 postmenopausal). Anthropometric measurements and serum estrone, testosterone, androstenedione, DHEA-S, osteocalcin, β-CTx, 25-OH-Vitamin D and sclerostin levels were obtained.

Results: There were not any differences between body mass, BMI, body fat and waist circumference between the study groups. The serum androstenedione and DHEA-S levels were similar in both study groups. However, estrone and total testosterone levels were observed to be notably higher in the perimenopausal group, unlike in the postmenopausal group (124.1 pg/mL vs. 98.3 pg/mL, p < 0.01 and 0.3 pg/mL vs. 0.22 pg/mL, p < 0.01, respectively). Higher plasma osteocalcin and β-CTx levels were shown in the postmenopausal rather than in the perimenopausal group (19.8 ng/mL vs. 16.8 ng/mL, p < 0.001 and 0.35 ng/mL vs. 0.29 ng/mL, p < 0.05, respectively). Plasma sclerostin and 25-OH-Vitamin D levels were similar. There was not any correlation between plasma sclerostin levels and the other studied parameters. In the multivariate regression analyses, sclerostin levels were proportional to the androstenedione ones (b = 0.06; p < 0.05) but inversely related to the log10(testosterone) levels (b = -0.18; p < 0.05).

Conclusions: Circulating sclerostin levels are similar in peri- and postmenopausal women and are related to the androstenedione and testosterone levels regardless of the nutritional status.

Key words: sclerostin; bone turnover markers; sex hormones; nutritional status; menopause

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INTRODUCTION

Hormonal changes during peri- and postmenopausal age, especially decreasing estradiol levels as the effect of the expired ovarian function, are an established link of the pathogenesis of postmenopausal osteoporosis. The experimental study showed that osteogenesis decreased just 5 days after the removal of the ovary [1]. It has been suggested that estradiol inhibits the apoptosis of osteoblasts [2]. Currently, it is believed that the main signaling pathway regulating bone mass is the Wnt/β-catenin pathway [3, 4] and sex hormones may affect the activity of this pathway [5]. However, the main regulator of this pathway ac-

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tivity is sclerostin — Wnt antagonist produced by osteocytes. Sclerostin binds to LRP5 and LRP6 receptors and inhibits the activity of the Wnt/β-catenin pathway [6–9]. Higher sclerostin levels were observed in the post- rather than perimenopausal women and its levels are inversely proportional to the free estradiol index. Thus, it seems that estradiol is the factor regulating sclerostin synthesis [10]. This hypothesis confirms the observation that the administration of estradiol reduces the concentration of circulating sclerostin [11]. However, it is not known whether estradiol affects the synthesis of sclerostin directly or indirectly. Interestingly, the changes in sex hormones levels during the menstrual cycle did not affect sclerostin levels in regularly menstruating women [12]. It has also been shown that in men testosterone increased circulating sclerostin levels [11]. On the other hand, one study showed that sclerostin levels weakly correlated with bone mass density (BMD), bone turnover and parathormone (PTH) levels in postmenopausal women [13], whereas another study revealed an inverse association between bone mineral density and sclerostin in postmenopausal women. In addition, among women with osteoporosis positive association between sclerostin levels and BMI was observed. There were no correlations between sclerostin levels and circulating vitamin D, PTH, FSH, E2 and thyroid hormones [14]. However, the results assessed the relationship between circulating sclerostin levels and BMI as inconclusive. Some studies showed a positive correlation [14, 15], while others did not observe this association [16]. As a consequance, examining the association between circulating sclerostin levels and the nutritional status, sex hormones and selected bone markers turnover levels in peri- and postmenopausal women was the main objective of the study.

MATERIAL AND METHODS

The cross-sectional study involved 31 perimenopausal and 54 postmenopausal women. The inclusion criteria for perimenopausal women were irregular menstruation, hormonal confirmation of perimenopause and for postmenopausal women the time of their last menstruation, minimum 2 years. The inclusion criteria for both groups included normal thyroid function, stable body mass in the last 3 months and not using a hypocaloric diet in the last 6 months. The exclusion criteria included using any kind of a hormonal therapy, smoking and excessive drinking. Informed consent was obtained from all of the participants and the study protocol was granted the approval of the Ethical Committee of the Medical University of Silesia.

Anthropometric measurements (body mass, height and waist circumference) were carried out, and BMI was calculated in accordance with the standard formula. The participants' body composition was measured by us-

ing the bioimpedance method with the aid of Bodystat 1500 (Douglas, Isle of Man). 10 mL samples of venous blood were taken in the morning between 8.00–9.00 a.m., after an overnight period of fasting (16 h). The blood samples were accumulated following the kit manufacturer's recommendations. All the serum and plasma samples were stored frozen in -70°C.

Biochemical measurements

Total testosterone, dehydroepiandrosterone sulfate (DHEA-S) were determined by the ECLIA method using Cobas E411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with a lower limit of sensitivity 0.025 ng/mL, 0.003 μmol/L, respectively; the respective intra- and inter-assay coefficients of variations were 4.7% and 8.4% for testosterone, 2.8% and 4.7% for DHEA-S.

Estrone (BioVendor, Czech Republic) and androstendione (DRG Instruments GmbH, Marburg, Germany) were determined by using ELISA with a lower limit of sensitivity 10.0 pg/mL and 0.019 ng/mL, respectively; and the respective intra- and inter-assay coefficients of variations 7.7% and 9.1% for estrone and 9.1% and 12.1% for androstendione.

ELISA kits, all commercially available, were used to measure plasma levels of sclerostin (TECOmedical AG, Sissach, Switzerland; the mean intra- and inter-assay coefficients < 4.0% and the < 4.8%, respectively), 25-OH-Vitamin D (DRG Instruments GmbH for Hybrid XL, Marburg, Germany; the inter-assay precision < 14.2%). Osteocalcin and β-CTxwere assessed utilizing ECLIA (Roche Diagnostics GmBH, Mannheim, Germany for Cobas e 411 analyser) set up to sensitivity < 3.3% and < 4.2% respectively.

Statistical analysis

The statistical analysis was carried out utilizing the Statistica 12.0 software (TIBCO Software Inc., Palo Alto, USA). Nominal and ordinal data were expressed as percentages, while interval data were expressed as mean value ± standard deviation in the case of the normal distribution or as median with lower and upper quartile in the case of data with the skewed or non-normal distribution. The distribution of variables was evaluated by means of the Shapiro-Wilk test and quantile-quantile (Q-Q) plot, whereas the homogeneity of variances was assessed by using the Fisher test. To compare the data between the fitness and control group, the t-Student test for independent data (in the case of the normal data distribution or after logarithmic normalization — if appropriate – in the case of the skewed distribution) or the non-parametric U Mann-Whitney test (in non-normal data distribution) were used. The Pearson correlation coefficient was used as a measure of association between the analyzed variables. The multivariable stepwise backward regression analysis was carried

out for plasma sclerostin levels as an independent variable with potentially explanatory variables: postmenopausal status, body mass index BMI (model I), fat percentage (model II), waist (model III) and HOMA-IR values, serum levels of estrone, total testosterone, androstenedione, DHEA-S, 25-OH-Vitamin D, osteocalcin and β-CTx. The Cook-Weisberg test was used to test heteroskedasticity and the Remsey RESET test was used to test the linearity of regression. The variance inflation factor VIF was calculated to check multicollinearity. The goodness of fit of the acquired regression models was assessed with the adjusted determination coefficient R^2. All the tests were two-tailed. The results were regarded as statistically significant with a p-value of less than 0.05.

RESULTS

There were no differences between body mass, BMI, body fat and waist circumference between the study groups. Serum androstenedione and DHEA-S levels were similar in study groups, whereas estrone and total testosterone levels were significant higher in the peri- rather than the postmenopausal group (124.1 pg/mL vs. 98.3 pg/mL, $p < 0.01$ and 0.3 pg/mL vs. 0.22 pg/mL, $p < 0.01$, respectively). Higher plasma osteocalcin and β-CTx levels were shown in the postmenopausal rather than the perimenopausal group (19.8 ng/mL vs. 16.8 ng/mL, $p < 0.001$ and 0.35 ng/mL vs. 0.29 ng/mL, $p < 0.05$, respectively). However, plasma 25-OH-Vitamin D and sclerostin levels were similar. Table 1 presents the characteristics of the study groups.

There was a significant negative correlation between estrone levels and age, body mass and BMI ($r = -0.25$; $p < 0.01$, $r = -0.24$; $p < 0.01$, $r = -0.21$; $p < 0.01$, respectively).

The negative correlation between 25-OH-Vitamin D levels and body mass, BMI, fat mass and waist circumference and positive with androstenedione levels was found ($r = -0.24$; $p < 0.01$; $r = -0.24$, $p < 0.01$; $r = -0.25$, $p < 0.01$ and $r = -0.25$, $p < 0.01$, $r = 0.33$; $p < 0.001$, respectively). Plasma β-CTx levels correlated negatively with estrone levels ($r = -0.26$; $p < 0.01$) and plasma osteocalcin correlated positively with DHEA-S and androstenedione levels ($r = 0.29$; $p < 0.001$ and $r = 0.41$; $p < 0.0001$). No correlation between plasma sclerostin levels and the other studied parameters was detected.

Multivariate stepwise backward linear regression models for sclerostin as an independent variable, with explanatory variables: postmenopausal status, BMI values or waist circumference or fat percentage and estrone, total testosterone, androstenedione and DHEAS levels revealed that the the alterations in sclerostin levels are proportional to androstenedione levels and inversely proportional to total testosterone levels. The model with explanatory variables: vitamin D, β-CTx and osteocalcin did not show their effect on the changes in sclerostin levels (Tab. 2).

DISCUSSION

So far numerous studies assessed circulating sclerostin levels and the factors affecting them in peri- and postmenopausal women [13, 14, 17, 18]. To the best of our knowledge, the study is most likely to be the first one to assess circulating sclerostin levels and the factors influencing them in peri- and postmenopausal women. In contrast to the studies which showed higher plasma sclerostin levels in postmenopausal rather than perimenopausal women [10, 11] we did not observe any differences between perimenopau-

Table 1. Characteristics of study group			
	Perimenopausal N = 31	Postmenopausal N = 54	p
Age [years]	49.0 ± 4.0	52.2 ± 4.0	< 0.001
Body mass [kg]	76.3 ± 13.6	74.6 ± 11.5	NS
BMI [kg/m^2]	27.1 (24.0–32.5)	27.3 (24.3–30.4)	NS
Body fat [%]	37.8 ± 5.6	37.7 ± 5.8	NS
Body fat [kg]	26.5 (22.8–37.1)	28.7 (23.3–35.2)	NS
Waist circumference [cm]	88.7 ± 10.0	89.4 ± 10.2	NS
Estrone [pg/mL]	124.1 (104.3–153.3)	98.3 (74.3–118.9)	< 0.01
DHEA-S [mg/mL]	143.7 ± 77.9	141.3 ± 66.2	NS
Androstenedione [ng/mL]	2.1 ± 0.9	2.4 ± 0.9	NS
Total testosterone [pg/mL]	0.30 (0.23–0.38)	0.22 (0.13–0.29)	< 0.01
25-OH-Vitamin D [ng/mL]	28.0 (23.1–31.5)	30.0 (22.5–36.5)	NS
Osteocalcin [ng/mL]	16.84 (11.50–18.60)	19.81 (15.74–23.43)	< 0.001
Sklerostin [ng/mL]	0.63 ± 0.2	0.71 ± 0.2	NS
β-CTx [ng/mL]	0.29 (0.21–0.36)	0.35 (0.27–0.48)	< 0.05

Mean (SD) or median (lower quartile — upper quartile)

Table 2. A multivariate stepwise backward linear regression

Sclerostin [ng/mL]	b	SE(b)	p
Androstenedione [ng/mL]	0.0595	0.0263	< 0.05
$\log_{10}$ (total testosterone) [pg/mL])	-0.1786	0.0450	< 0.05
$\log_{10}$ (β-CTx[ng/mL])	-0.1859	0.0972	0.06

sal and postmenopausal women. The factor explaining the lack of differences in sclerostin levels between our study groups is the fact that women at the age of 45 and over were enrolled in our study. This hypothesis is confirmed by the observation made by Ardavi et al. [17], which made an observation of circulating sclerostin levels increasing with age, up to the age of 45. Moreover, the results of the longitudinal study revealed that sclerostin levels increased from reproductive age to menopause and from menopause to early postmenopause [18]. Additionally, Amrein et al. [15] detected a positive correlation between sclerostin levels and age in healthy subjects regardless of gender. Among other suggested factors influencing circulating sclerostin levels is the nutritional status. However, the results of recently published studies are inconclusive. Some studies showed a positive correlation between sclerostin levels and BMI [15, 19], WHR [15] and fat mass [20] as well as the percentage of visceral and gynoid fat [21]. Contrary to these studies we did not observe any associations between sclerostin levels and BMI, waist circumference, fat mass and fat percentage. It is in line with the conclusions made by Klangjareonchai et al. [16], which found a negative correlation between sclerostin levels and BMI in men, and no association in women. It should be noted that the differences in these studies may be the result of having participants of various races with distinct patterns of fat distribution. Although one study showed similar circulating sclerostin levels in Chinese-American and white women [22], the effect of race cannot be excluded. Moreover, studies performed in a large group are necessary to explain the effect of race and nutritional status on sclerostin levels. Another explanation of these differences may be the impact of gender on the fat content. Higher sclerostin levels were observed in men rather than in women [23]. Another factor influencing sclerostin levels and its association with anthropometric parameters is physical activity. Some studies showed that regular physical activity significantly reduces circulating sclerostin levels in postmenopausal women [24, 25]. However, due to the lack of objective assessment of physical activity in our study, we did not confirm its impact on the obtained results.

The circulating estradiol levels in the postmenopausal group were very low therefore we did not assess any association between sclerostin and estradiol levels. However, it should be noted that we no association between sclerostin and estrone levels was observed. Furthermore, despite significantly higher estrone levels in the peri- rather than in the postmenopausal group, sclerostin levels were similar. It is in accordance with the study that showed that bone mass positively correlates with estradiol and estrone levels in premenopausal but not postmenopausal women [26]. On the other hand, it has been observed that a 4-week-long estrogenic hormonal therapy reduced circulating sclerostin levels [27]. It should be noted that this study was performed in a very small group. Further studies are necessary to assess the effect of estrone on sclerostin levels and the role of this hormone in bone turnover. However, our study showed that changes in sclerostin levels are proportional to androstenedione levels and inversely proportional to total testosterone levels. Contrary to our results, it has been observed that in men testosterone replacement increased circulating sclerostin levels [27]. The impact of androstenedione on sclerostin levels may be explained by the results of the experimental study that showed that androstenedione could improve the proliferation and differentiation of osteoblasts in vitro [28].

In accordance with the results of the previously published study [10, 19] no association between sclerostin and vitamin D levels was observed. However, contrary to other studies [17, 22, 28, 29], we did not observe any relationships between sclerostin and osteocalcin as well as β-CTx levels. It should be noted that the results of the studies described the association between sclerostin and β-CTx as inconclusive because both a positive [28, 29] and a negative [22] correlation were found. Multivariate stepwise backward linear regression models in our study revealed that the effect of β-CTx on sclerostin levels is negative and close to significance. Further studies are necessary to explain the association between sclerostin and bone turnover markers levels in postmenopausal women.

The main limitation of the present study is the small sample size and not including women of reproductive age in the study. Other limitations are also the assessment of body composition on the basis of the bioimpedance method, not using the DXA method, which makes it impossible to assess subcutaneous and visceral fat deposits, and the lack of assessment of bone density. However, it should be noted that our study is the first one to assess the complex association between sclerostin levels and nutritional status and sex hormone levels in perimenopausal women.

CONCLUSIONS

Circulating sclerostin levels are similar in peri- and postmenopausal women and are related to androstenedione and testosterone levels regardless of the nutritional status.

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ORIGINAL PAPER / OBSTETRICS

VIA MEDICA

The adaptation of Polish version of the Readiness for Hospital Discharge Scale (RHDS) for postpartum mothers

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ABSTRACT

Objectives: The Readiness for Hospital Discharge Scale (RHDS) for postpartum women was developed and validated in the US in 2006. The aim of the research was to analyse the psychometric properties of the Polish version of the Readiness for Hospital Discharge Scale (RHDS) for subjective assessment of the bio-psycho-physical status of women after childbirth.

Material and methods: After the preparation of the Polish-language version of the questionnaire, the study was conducted among 168 postpartum women on the day they were discharged from the hospital. For the analysis of the reliability of the questionnaire, the Cronbach Alpha test was used, where the index of values above 0.7 was assumed to mean the correct reliability of the scale.

Results: Statistical analysis using the Alpha Cronbach test for the questions presented amounted to: 0.835, which indicates that the results are correctly aligned with one another.

Conclusions: The conducted analysis confirms that the Polish-language questionnaire in its current form has high reliability for the assessment of readiness to discharge in postpartum women and may be used in Polish conditions.

Key words: perinatal care; postpartum mothers; newborn; discharge

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INTRODUCTION

Childbirth is one of the greatest events in the life of every mother and is also a great challenge for a woman, especially if it is her first child. Preparation for a new social role requires many changes in their current life. The literature refers to this as "transition to motherhood" [1, 2]. This is a special period in the life of a woman and her family, requiring the dedication of both full attention and time for a baby. It is also undoubtedly an extremely stressful situation, which often raises concerns and doubts among mothers if they will manage to cope with new obligations.

The decision about discharge is made by the medical staff, taking into account both the mother's and baby's condition. In recent years, in Poland, the stay of postpartum women has been shortened to the necessary minimum, following the criteria of the National Health Fund (insurance company), which defines and recommends the length of stay depending on the clinical situation. That is why it is extremely important to prepare mothers properly before leaving the hospital, together with taking care of the newborn

and themselves during the difficult period after childbirth. Currently, there is no reliable tool in Poland for a subjective assessment of readiness to discharge of postpartum women, which is why the adaptation of the existing tool has been undertaken. The Readiness for Hospital Discharge Scale (RHDS) for postpartum mothers was developed in the USA and validated by Weiss et al. and Weiss and Piacentine [3–5].

The tool adaptation process consists of two stages: translation and evaluation of the psychometric properties of the translated scale. Its main goal is cultural adaptation and the possibility of practical scale application in Poland [6, 7].

In this paper the tool RHDS, assessing the readiness to discharge women from the hospital after the delivery, has been adapted to Polish conditions. This applies to readiness both in the physical sense (strength, energy, pain) and psychological as well as self-assessment of readiness to take care of the baby and self-care at home.

The presented study is a part of a bigger project in which adaptations were made to two other scales Quality of Dis-

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charge Teaching Scale (QDTS) and Post-Discharge Coping Difficulty Scale (PDCDS).

Objectives

The aim of the research was to analyse the psychometric properties of the Polish version of RHDS for subjective assessment of the bio-psycho-physical status of women after childbirth.

MATERIAL AND METHODS

Participants

The research was carried out among postpartum women in the Department of Gynecology and Obstetrics of Clinical Provincial Hospital no 2 in Rzeszow from June to October 2017. The inclusion criteria were the delivery method and mother's aged over 18. 168 postpartum women took part in the study, and 142 fully completed

questionnaires were analysed, which were included in the final analysis. Among the respondents, 51% were urban residents, and 49% rural residents. Most of the women had higher (52%) or secondary education (34%). The majority of them were professionally active (70%), married women (88%), 92% of their husbands/partners were employed. Over half of the women (51%) gave a vaginal birth and 49% had a Caesarean Section. For 37% of mothers, it was the first birth, and for the remaining 63% — a subsequent. Only 84% of the respondents declared exclusive breastfeeding. Almost 40% of the mothers left the hospital on the third day postpartum, 35% on the second day while 20% on the fourth day (Tab. 1).

Instruments

The study used a questionnaire to collect socio-demographic data created on the basis of the author's version,

Table 1. The characteristics of the study population			
Question	**Answer**	**Number (n = 142)**	**Percentage [%]**
Place of residence	Urban area	72	51
	Rural area	70	49
Education	Primary	1	1
	Junior high school	2	1
	Secondary	48	34
	Vocational	6	4
	Higher — undergraduate	11	8
	Higher — graduate	58	41
	Higher — other	16	11
Type of birth	Vaginal birth	73	51
	Caesarean section	69	49
Parity	First birth	53	37
	Subsequent birth	89	63
Marital state	Married	125	88
	Single	1	1
	Other	16	11
Baby feeding	Breastfeeding	119	84
	Baby formula milk	23	16
Hospitalization covered	By insurance	140	99
	On her own	2	1
Employment	Working	99	70
	Unemployed	43	30
Husband/partner's employment	Employed	131	92
	Unemployed	10	7
	I am single	1	1
Day of discharge (since the birth)	2	49	35
	3	54	38
	4	29	20
	> 4	9	7

taking into account such data as: age, place of residence, education, marital status, type of insurance, occupational situation of the postpartum woman and the baby's father. The questionnaire also included questions about the obstetric situation, *i.e.* type of delivery, parity, way of feeding the newborn, day of discharge after birth.

Readiness for Hospital Discharge Scale

RHDS was created to assess the readiness of postpartum women to leave the hospital after birth by Weiss et al. 2006 and Weiss and Placentine (2006) [4, 5]. Before the adaptation of the tool, we obtained the consent of the author of the original version to use the scale.

RHDS consists of 23 questions, where the first question concerns the subjective assessment of the patient's readiness to leave the hospital on discharge and is recorded in the dichotomic scale (answer 1 — No, answer 2 — Yes). The remaining 22 questions reflect: personal status, knowledge, self-care ability, expected support. Each question has an 11-point scale (from 0 to 10) with a description explaining the meaning of the numerical scale (*e.g.* 0 not at all — 10 completely). Scores obtained from the scale range between 0 to 220. A higher score indicated greater willingness to discharge [8].

The tool translation procedure

The scale was translated into Polish by two independent translators, and then both versions were compared and one version of the translation was created. The Polish language version has been translated back into English by another translator (back translation). The prepared version was sent to the Author of the questionnaire for the final assessment of compliance with the original version. After consultation and implementing the suggested corrections, the final Polish version of RHDS was prepared. During the preparation of the Polish version of the scale, the graphic representation of the original one was also implemented.

Ethical consideration

Prior to the tests, permission for their implementation was obtained from the management of the hospital and the Bioethics Committee at the University of Rzeszow (decision number 2/4/2017).

Pilot Study

Prior to the actual research, pilot studies were carried out on a sample of 15 women in order to check the intelligibility and readability of the tools. On the basis of the collected material, several technical corrections were made in the survey.

Data collection

The survey was conducted from June to November 2017. The questionnaires were distributed among postpartum women on the day of discharge, 3 hours before going home. Two midwife volunteers, who had been previously briefed, dealt with the questionnaire. The patients were informed about the purpose of the study, its voluntary nature and anonymity, and were asked to complete it. The completion of the questionnaires took about 5 minutes.

Methods of statistical analysis

The statistical analysis was carried out with STATISTICA 13 software. Data recorded on the nominal scale (No and Yes replies) were converted to scale 1–2. The remaining scales were treated as interval scales. For each question, the obtained results were presented with descriptive statistics using the minimum and maximum values (min and max), quartile values (Q1, Me, Q3) and mean $\bar{x}$ and standard deviation (SD). All questions from a single sheet were analysed with Pearson's correlation, for which an interpretation of the relationship between parameters was applied in accordance with Table 2.

During the verification of all analyses, a significance coefficient $\alpha = 0.05$ was used, which allowed consideration of statistically significant variables at $p < 0.05$.

The Cronbach Alpha test was used to analyse the reliability of the questionnaire. This analysis is based on the internal consistency of the responses to the questionnaire being examined. The internal consistency of the scale was measured by Cronbach Alpha. Cronbach's Alpha greater than 0.7 was adopted as satisfactory.

RESULTS

The obtained results were subjected to statistical analysis. The results obtained for the question 1 concerning the subjective assessment of readiness to discharge indicate that 96.5% of the women surveyed replied that they were ready to discharge, and only 3.5% of them gave a negative answer.

Table 2. Determination of the correlation coefficient with respect to the strength of the correlation

Correlation coefficient R	Strength of the correlation
0.0–0.3	No
0.3–0.4	Weak
0.4–0.7	Moderate
0.7–0.9	Strong
0.9–1.0	Very strong

From the remaining questions, the highest scores were noted for question 20 (emotional support after discharge), where the mean was 9.54 points. Other questions, which received equally high mean scores, were questions: 7, 18, 19, 21–23. In the case of two questions, a very low mean score was obtained. They relate to the stress (Q6 with mean score of 2.49) and the pain and discomfort experienced today (Q3 with mean score of 3.61). For the remaining questions, scores ranging from 7.66 to 8.80 were obtained. The results in terms of descriptive analysis are presented in Table 3.

Statistical analysis with the Alpha Cronbach test for the questions presented was: 0.835, which indicates that the results are correctly aligned with each other. The mean correlation between the results was 0.228. The mean result for the summed up scores of 23 questions amounted to 177.98 with a standard deviation of 19.66 points.

The current version of the questions that have the greatest negative effect on the Alpha Cronbach index are the results for the questions: 3 and 6. The biggest positive impact related to questions from 12 to 15. The results of the complete Alpha Cronbach analyses are presented in Table 4.

CONCLUSIONS

The conducted analysis confirms that the Polish-language questionnaire in its current form has a high reliability for the assessment of readiness to discharge in postpartum women and may be used in Polish conditions. Applying the scale in practice will assist in recognizing the readiness of women for self-care and care for the

Table 3. Descriptive analysis of RHDS results

Question	Mean	Standard deviation SD	Minimum Min	Lower Q_1	Median Me	Upper Q_3	Maximum Max
Q1	1.04	0.18	1	1	1	1	2
Q2	8.80	1.74	1	8	10	10	10
Q3	3.61	2.68	0	2	3	5	10
Q4	7.70	1.96	1	7	8	9	10
Q5	7.86	1.90	0	7	8	9	10
Q6	2.49	2.99	0	0	1	3	10
Q7	9.07	1.85	1	9	10	10	10
Q8	8.32	1.90	3	7	9	10	10
Q9	8.41	1.68	3	7	9	10	10
Q10	8.77	1.38	4	8	9	10	10
Q11	8.75	1.27	4	8	9	10	10
Q12	7.66	1.99	0	7	8	9	10
Q13	7.94	2.35	0	7	9	10	10
Q14	7.90	2.19	0	7	8	10	10
Q15	8.32	2.01	1	8	9	10	10
Q16	7.99	2.07	0	7	8	10	10
Q17	8.56	1.63	1	8	9	10	10
Q18	9.07	1.35	3	9	10	10	10
Q19	9.13	1.62	0	9	10	10	10
Q20	9.54	1.11	4	10	10	10	10
Q21	9.19	1.51	2	9	10	10	10
Q22	9.00	1.86	1	9	10	10	10
Q23	9.12	1.65	2	9	10	10	10

Table 4. Results of the Alpha Cronbach test for 23 questions from RHDS

Variable	Mean = 177.98 SD = 19.66 Valid N:134 Cronbach Alfa: 0.835 Mean correlation between items: 0.228	
	Square	Alpha when deleted
Q1	0.469	0.838
Q2	0.639	0.829
Q3	0.357	0.861
Q4	0.546	0.830
Q5	0.640	0.827
Q6	0.601	0.882
Q7	0.677	0.827
Q8	0.730	0.823
Q9	0.731	0.823
Q10	0.753	0.820
Q11	0.759	0.823
Q12	0.676	0.817
Q13	0.743	0.817
Q14	0.837	0.813
Q15	0.812	0.814
Q16	0.771	0.817
Q17	0.492	0.823
Q18	0.702	0.820
Q19	0.400	0.828
Q20	0.554	0.833
Q21	0.773	0.827
Q22	0.814	0.829
Q23	0.859	0.828

SD — standard deviation

newborn after discharge and will contribute to offering additional support in the bio-psycho-social dimension to patients demanding it.

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ORIGINAL PAPER / OBSTETRICS

VIA MEDICA

Serum homocysteine and vitamin B12 levels in women with gestational diabetes mellitus

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ABSTRACT

Objectives: Gestational diabetes mellitus (GDM) is described as a glucose intolerance of variable severity which begun or was firstly recognized during gravidity. Two major metabolic disorders, insulin resistance and β-cell dysfunction, currently play major role in pathogenesis of GDM. Our intention was to investigate total serum homocysteine and vitamin B12 levels in pregnant women with GDM and non-diabetic gravid women.

Material and methods: Serum homocysteine and vitamin B12 levels were prospectively measured in a total of 79 pregnant women, 60 of whom were diagnosed with GDM, and 19 of whom were healthy controls. Serum homocysteine levels were analyzed by ELISA. Vitamin B12 concentrations were determined by chemiluminescent immunoassay, and lipids were determined enzymatically.

Results: GDM and control groups did not differ in terms of the serum homocysteine levels (median 7.24 vs 7.97 umol/L, respectively, p = 0.15). Nor did we find any association between serum homocysteine levels and BMI (r = 0.06, p = 0.55, respectively). There was no correlation between serum homocysteine and fasting serum glucose (r = 0.3, p = 0.8, respectively). There was no relationship between serum homocysteine concentrations and glycosylated hemoglobin (HgbA1c) levels (r = 0.06, p = 0.67, respectively). Serum vitamin B12 concentrations did not differ between the GDM and control groups (median 286 vs 262 pg/mL, respectively, p = 0.17). We found that levels of Vitamin B12 correlated inversely with fasting serum glucose concentrations (r = -0.44, p = 0.0009). Vitamin B12 concentrations increased along with LDL (r = 0.27, p = 0.043) and HDL (r = 0.38, p = 0.004) levels, however were inversely correlated with serum triglycerides (r = -0.34, p = 0.009).

Conclusions: GDM patients with low Vitamin B12 values tend to have higher fasting serum glucose and altered lipid profiles (high triglycerides, low HDL and LDL). In women with GDM, serum homocysteine levels are not associated with HbA1c level, fasting glycemia, or BMI.

Key words: gestational diabetes mellitus; GDM; adipocytokines; serum homocysteine; Vitamin B12; pregnancy; glucose intolerance

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a frequent complaint that affects relatively 7–14% of pregnancies. GDM is specified as any degree of glucose intolerance with commencement or first diagnosis during gravidity [1, 2]. The prevalence of GDM depends to a high degree on the ethnicity of the patient and on the diagnostic criteria used [3]. Although adverse lifestyle factors (overnutrition and physical inactivity) contribute to obesity, a significant number of trials connect early-life nutrient imbalance with the development of metabolic disorders in childhood and early adulthood. Maternal obesity is characterised by the existence of an enormous amount of adipose tissue (AT) and negative effects on maternal wellbeing and the developing fetus, leading to cardiometabolic syndrome in children later in life [4].

In humans, homocysteine is formed during the methionine metabolic cycle. The re-methylation of homocysteine

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would not be possible without the enzyme methionine synthase (MS) with which vitamin B12 is a co-factor and methyltetrahydrofolate (methylTHF) a substrate. This process is indirectly controlled by the activity of the enzyme methylenetetrahydrofolate reductase (MTHFR). Disturbances in the metabolism of homocysteine caused by the deficiency of co-factors or by some genetic enzyme defect, lead to cellular accumulation of homocysteine which consequently elevates plasma homocysteine levels [5]. Compared with concentrations in nonpregnant women, serum homocysteine has been found to reduce during each trimester of gravidity (due to either a physiological answer to the pregnancy, an increase in estrogen, hemodilution from an elevated plasma volume, or enlarged demand for methionine by both women and fetus) [6]. Elevated homocysteine concentrations during pregnancy are associated with an increased incidence of spontaneous abortion, intrauterine growth restriction, placental infarction, neural tubes defects and pre-eclampsia. Some researchers showed that hyperhomocysteinemia was associated with early pregnancy losses and adverse pregnancy outcomes. [7–9]. When it comes to studies about plasma homocysteine levels and glucose tolerance in both diabetic and non-diabetic gravid women, it has also been shown that there is an association between hyperhomocysteinemia and insulin resistance.

Vitamin B12 deficiency in pregnant women is increasingly popular, and in many studies there has been shown association with higher body mass index (BMI) [10, 11], as well as with insulin resistance (IR), gestational diabetes (GDM), and type 2 diabetes (T2D) in future [12, 13].

B12 plays crucial role in the synthesis of methionine, the precursor of S-adenosyl-methionine (SAM), which is a major methyl donor for DNA methylation [13, 14].

MATERIAL AND METHODS

Based on these premises, we studied potential relationship between serum homocysteine and B12 levels in pregnant diagnosed with gestational diabetes mellitus compared with those with normal glucose tolerance. Our analysis was performed in the Department of Obstetrics and Women's Diseases of the Poznan University of Medical Sciences between August 2017 and July 2018. Research ethics approval was obtained before we begun the study and the informed consent was obtained in writing from all participating women. Subjects were qualified for the study if they did not smoke nor took any medication for at least 3 months before recruitment, did not report any vitamin deficiency or significant diseases, and had no history of cardiovascular disease or previous medical history of diabetes mellitus. Women in multiple pregnancies were debarred from the study.

The study population included 79 gravid women, 60 of whom were diagnosed with gestational diabetes and 19 of whom were healthy controls. In our department, we generaly screen our patients between 24 and 28 weeks of gestation unless they have any of the risk factors of obesity, insulin resistance, a family history of any glucose intolerance, or have given birth to a macrosomic baby. The diagnosis of our subjects' gestational diabetes mellitus was based upon the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria [15, 16]. Body mass indices (BMIs) of the study population were calculated at first visit in pregnancy.

Statistical analysis

R programming language (version 3.4.1) was used for statistical analysis. The distribution was determined with the Shapiro test. In case of normal distribution, the t-Student test (two-group comparison) or ANOVA (multigroup comparison) were applied. The sets with non-normal distribution were compared with the Mann-Whitney (two-group comparison) or the Kruskal-Wallis test (multiple groups comaprison). Linear relationships between parameters were determined by Pearson and Spearman correlation coeffcients for normal and non-normal data distribution, respectively. The *p* values below 0.05 were considered significant. R packages (ggplot2, ggpubr) were used to generate plots (geom_boxplot() function) and add labels with applied statistics and *p* values (stat_compare_means() function). Data representing individual patients were visualised as dots in the boxplots.

Blood samples

We gathered 9 mL of blood for freezing and later testing. Specimens were immediately kept at 4 degrees Celsius and processed within 4 h to avoid cell lysis. Blood fractionation was carried out centrifuge at 2500 x g for 10 min, and 3–4 mL of the blood serum's supernatant was removed and stored at -80 degrees Celsius. For homocysteine analysis, samples were determined by an enzymatic test using Cobas c501 analyzer. Vitamin B12 was analysed using electrochemiluminescent immunoassay (ECLIA) with a Cobas e601 analyser (Roche Diagnostics Poland). The inter assay coefficient of variation was 2% for plasma homocysteine, and for values above the reference range, 1.4–2%. However, the coefficient of variation for TB12 was 3.2–4.3%, and for values above the reference range, 3.8%. The reference range for vitamin B12 was 197–771 pg/mL (2.5–97.5 percentile). On the other hand, the reference range for homocysteine was 12–15 umol/L (adults 15–65 years old). Serum glucose, cholesterol, triglycerides, and HDL cholesterol were determined using a Cobas c501 auto analyser (Roche Diagnostics Poland). Using enzymatic colourimetric assay, insulin was measured by the electrochemiluminescence method with a Cobas e601 analyser (Roche Diagnostics Poland). LDL cholesterol was calculated using the Friedewald formula.

Table 1. Selected demographic and clinical data of women with gestational diabetes mellitus (GDM) and glucose tolerant pregnant women (control)

Parameter	GDM Group (n = 60)	Control Group (n = 19)
Age [years]	32.27 +/- 4.5	33.73 +/- 5.0
Weight [kg]	76.58 +/- 14.6	73.73 +/- 11.27
Height [m]	1.65 +/-0.06	1.62 +/- 0.06
BMI [kg/m2]	27.59	26.98
Gestational age [weeks]	33.26 +/- 5.54	34.88 +/- 5.66
Number of previous pregnancies:		
1st pregnancy	26 (43.33 %)	7 (36.84%)
2nd pregnancy	20 (33.33%)	8 (42.11%)
3rd pregnancy	11 (18.33%)	2 (10.53%)
4th pregnancy	3 (5.0%)	2 (10.53%)
Homocysteine [umol/L]	7.41 +/- 2.61	8.02 +/- 2.27
Vitamin B12 [pg/mL]	287.45 +/- 111.44 (215.0–321.30)*	246.30 +/- 101.69 (160.30–309.20)*
Systolic Blood Pressure- SBP [mmHg]	108.33 +/- 8.87	113.42 +/- 10.14
Diastolic Blood Pressure- DBP [mmHg]	68.25 +/- 7.74	70.78 +/- 7.68
Previous GDM	3 (5.08%)	1 (5.2%)
Gestational Age at Birth [weeks]	38.89 +/- 1.59	37.53 +/- 2.87
Birth weight [g]	3385.00 +/- 492.64	3421.42 +/- 564.18
Gender:		
Male	31.58%	50%
Female	68.42%	50%

* — median, interquartile range

Table 2. The clinical characteristics of subgroups depending on BMI intervals

BMI	< 20	20–24.9	25–29.9	30–34.9	> 35
Number of patients	2.00	14.00	17.00	19.00	6.00
Mean body mass [kg]	46.5	60.84	78.00	84.00	95.83
Homocysteine [umol/L]	6.63	7.35	7.18	7.41	7.35
B12 [pg/mL]	544.15	292.52	289.54	253.18	269.32
Mean glycemia [mg/dL]	92.5	103.5	104.38	107.21	102.16
HbA1c [mmol/L]	4.5	4.84	5.11	5.24	5.24
Fasting serum glucose [mg/dL]	70	84	92.88	92.05	91.66
Total Cholesterol [mg/dL]	299.5	254.22	256.06	219.58	209.08
HDL [mg/dL]	82.45	212.13	76.02	70.35	69.72
LDL [mg/dL]	179.65	122.3	127	95.40	94.06
TAG [mg/dL]	187.05	212.14	272.71	237.34	226.42
Delivery at term	1	8	10	11	4
Cesarean Section	0	6	7	8	4
Fetal weight at term [g]	3263	3459	3521	3341	3557

RESULTS

The age of patients in our study ranged from 22 to 43 years old. The mean age of the patients with gestational diabetes mellitus (GDM) was 32, whereas in the control (Ctrl) group it was 33 years. The clinical characteristics of the patients are shown in Table 1. There was no difference in body mass index (BMI) between the groups. The mean gestational age of diabetic patients (33 weeks) was comparable to that of the healthy controls (34 weeks). The clinical characteristics of the subgroups based on BMI intervals is presented in Table 2.

The GDM and Ctrl groups did not differ in terms of serum homocysteine levels (median 7.24 vs 7.97 umol/L, respectively, $p = 0.15$, Wilcoxon test, Figure 1A). Serum homocysteine levels did not correlate with BMI (Pearson correlation, $r = 0.06$, $p = 0.55$, Figure 1B) and nor did they differ across BMI intervals ($p = 0.95$, Kruskal-Wallis test, Figure 1C). Next, we tested whether serum homocysteine levels could reflect

Figure 1A. Serum homocysteine levels in patients with GDM and in Ctrl group

metabolic abnormalities in GDM patients. However, there was no association between serum homocysteine and fasting serum glucose (Pearson correlation, $r = 0.03$, $p = 0.8$, Figure 1D). Similarly, there was no correlation between the serum homocysteine concentration and the glycosylated
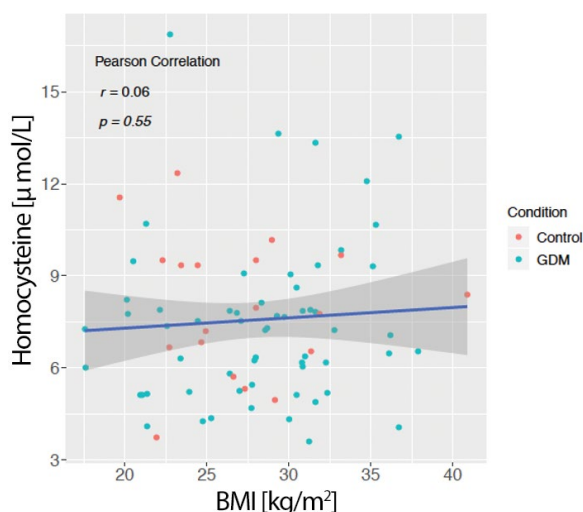
Figure 1B. Serum homocysteine levels and BMI in GDM and Ctrl group
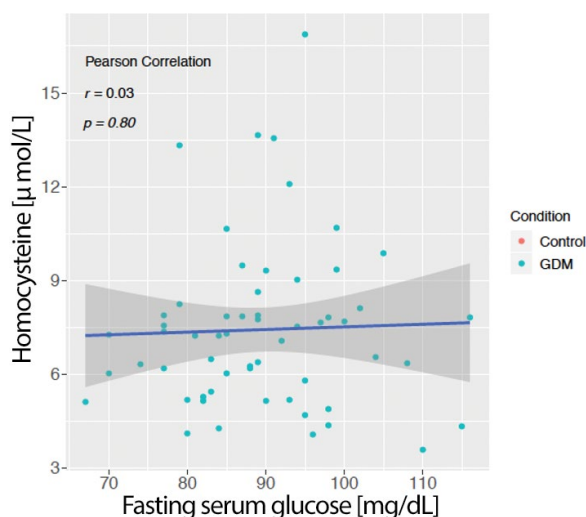
Figure 1C. Serum homocysteine levels and fasting serum glucose in GDM and Ctrl group
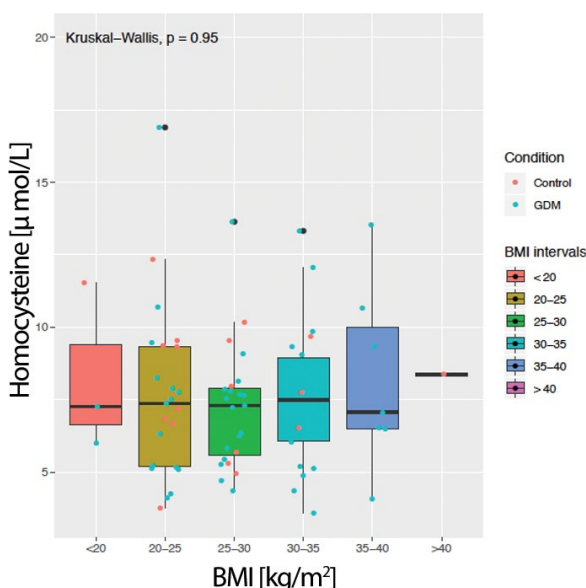
Figure 1D. Serum homocysteine and fasting serum glucose in GDM and Ctrl group

Figure 1E. Serum homocysteine concentration and the glycosylated hemoglobin (HgbA1c) in GDM and Ctrl group

hemoglobin (HgbA1c) level (Pearson correlation, r = 0.06, p = 0.67, Figure 1E).

Serum vitamin B12 did not differ between the GDM and Ctrl groups (median 286 vs 262 pg/mL, respectively, p = 0.17, Wilcoxon test, Figure 2A). Nor was serum vitamin B12 associated with BMI (Pearson correlation, r = -0.2, p = 0.08, Figure 2B), and it did not differ across BMI intervals (p = 0.74, Kruskal-Wallis test, Figure 2C). Next, we analyzed whether vitamin B12 levels were in any relationship to metabolic disturbances within the GDM group. We found that with increasing levels of Vitamin B12, fasting serum glucose concentrations were lower (Pearson correlation, r = -0.44, p = 0.0009, Figure 3A). Correspondingly, we determined that

HgbA1c increased in relation to decreasing levels of Vitamin B12 (Pearson correlation, r = -0.36, p = 0.006, Figure 3B). We demonstrated that the Vitamin B12 level was also associated with the lipid profile in patients with GDM. Vitamin B12 concentrations increased together with levels of both LDL (Pearson correlation, r = 0.27, p = 0.043, Figure 4A) and HDL (Pearson correlation, r = 0.38, p = 0.004, Figure 4B). Serum triglycerides were more likely to be elevated in GDM patients with lower vitamin B12 concentrations (Pearson correlation, r = -0.34, p = 0.009, Figure 4C).

DISCUSSION

Gestational diabetes mellitus is one of the most frequent metabolic disorders complicating pregnancy. The pathophysiology of GDM and type 2 diabetes involves abnormal
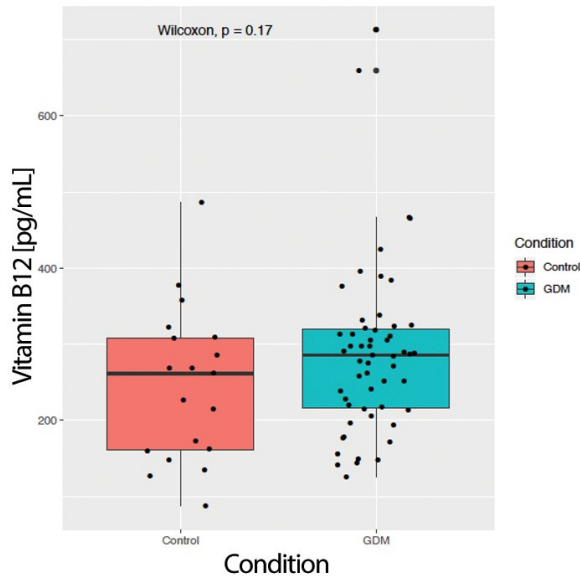
Figure 2A. Serum vitamin B12 in patients with GDM and Ctrl group

Figure 2B. Serum vitamin B12 levels and BMI in GDM and Ctrl group

Figure 2C. Serum vitamin B12 and BMI intervals in GDM and Ctrl group
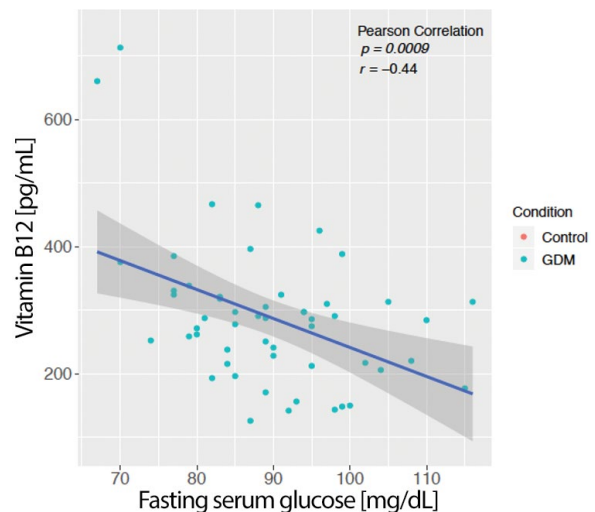
Figure 3A. Serum vitamin B12 and fasting serum glucose concentrations in GDM and Ctrl group

pancreatic insulin release and insulin resistance. The inflammatory markers, metabolic abnormalities and endothelial dysfunctions in GDM can make the pregnant woman suffering from this pathology and her developing fetus much more prone to cardiovascular diseases [17].

One of the factors responsible for this condition is the level of homocysteine. It is proved that acute and chronic exposure to homocysteine shows adverse effects on b-cell metabolism and insulin secretion [18]. Homocysteine levels are influenced by factors, such as age, gender, duration of diabetes, body mass index, nicotine addiction, kidney

failure, vitamin status and blood pressure, but also by environmental and genetic factors.

In the meta-analysis of Gong et al., serum homocysteine concentrations were higher among women with GDM than among the controls. The evidence was more consistent among women in the second trimester and for women older than 30 years of age. As a result of physiological decreases in albumin during pregnancy, as well as in association with folic acid supplementation, the serum concentrations of homocysteine decrease during pregnancy [19, 20]. Surprisingly, in our study, we did not find significant differences in those results between the GDM and control groups. The minimum value of homocysteine in our GDM group was 3.59, while the maximum was 16.87. In the control group, however, the minimum value was 3.75 and the maximum value was
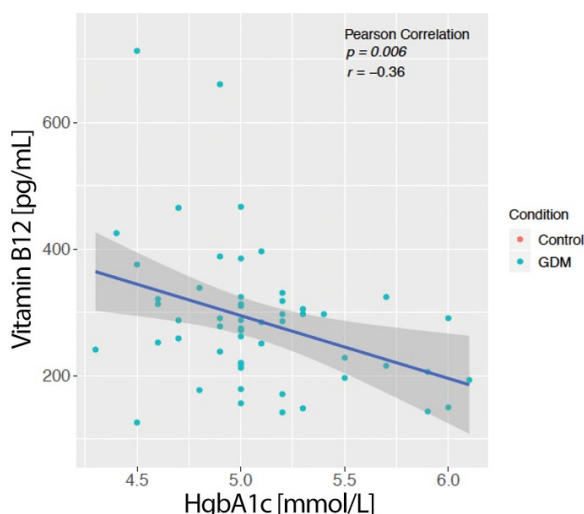
Figure 3B. Serum vitamin B12 concentration and the glycosylated hemoglobin (HgbA1c) in GDM and Ctrl group
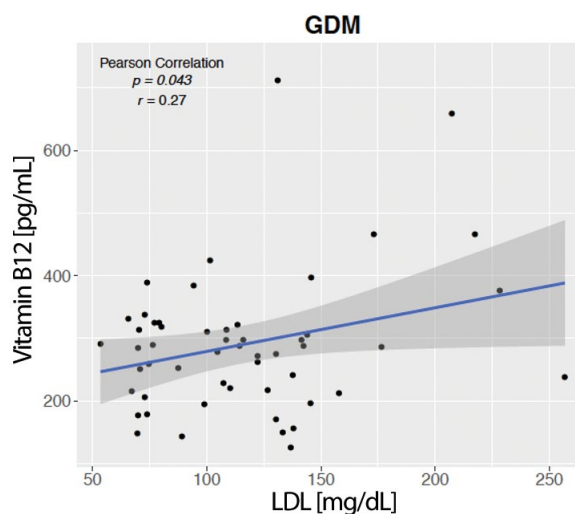
Figure 4A. Correlation between serum vitamin B12 concentration and LDL levels in GDM group
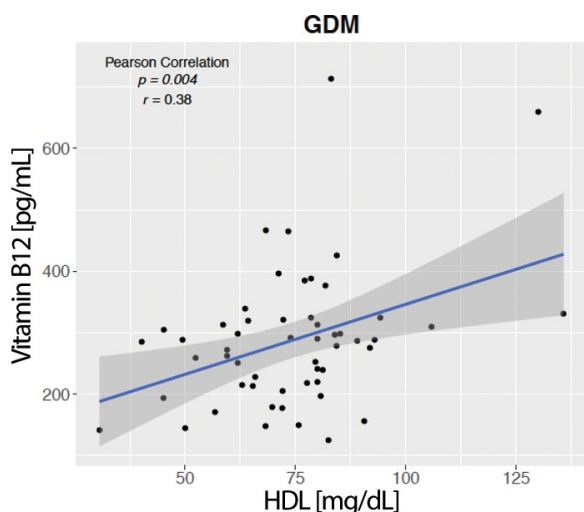
Figure 4B. Correlation between serum vitamin B12 concentration and HDL levels in GDM group
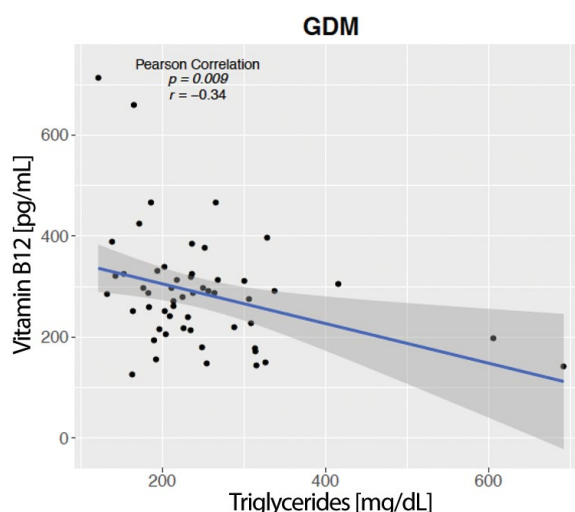
Figure 4C. Correlation between serum vitamin B12 concentration and HDL levels in GDM group

12.34. The small differences between the two groups probably result from the small population of the control group.

It has been proposed that B-vitamins may play crucial role in the pathogenesis of glucose intolerance because of their ability to regulate homocysteine synthesis [21]. Results of recent studies on concentrations of vitamin B12 and its influence on the occurence of gestational diabetes are inconsistent. In the Krishnaveni et al. [22] and Lai et al. [23] studies, it was shown that in a subpopulation of South Asian women, those with vitamin B12 deficiency were much more likely to develop GDM or type 2 diabetes mellitus (T2DM) over the course of a 5-year follow up period. Similar results of lower vitamin B12 concentrations among pregnant women diabetes in comparison with healthy controls, were pre-

sented in Seghieri's study [24]. However, there are several European studies which did not show differences in folates and vitamin B12 concentrations between women with GDM and those in the control groups [25–27, 12].

CONCLUSIONS

So far there has been conducted much research on maternal carbohydrates and fats intake and its relation to the risk of developing GDM. It has been suggested that group B vitamins may play role in the pathogenesis of glucose intolerance thank to their ability to regulate synthesis of homocysteine. In other words, vitamin B12 deficiency in pregnancy is related with a higher risk of GDM and type 2 diabetes mellitus. In women with GDM, we did not find

any correlation between serum homocysteine levels and HbA1c, fasting glycemia or BMI. Conversely, vitamin B12 values are inversely associated with glycemic status and lipid profiles. We concluded that the better the glycemic status of pregnant women with diabetes, the higher the concentration of vitamin B12. We agree that in future, large prospective cohort studies are needed to verify this finding and to evaluate the potential predictive role of these parameters for gestational diabetes mellitus.

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ORIGINAL PAPER / OBSTETRICS

Maternal factors, ultrasound and placental function parameters in early pregnancy as predictors of birth weight in low-risk populations and among patients with pre-gestational diabetes

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ABSTRACT

Objectives: The aim of our work was to assess the usefulness of maternal factors, ultrasound and placental function parameters during early pregnancy as predictors of birth weight in populations of healthy pregnant women and women suffering from pregestational diabetes.

Material and methods: A study group comprised 97 healthy women and 160 women with pregestational diabetes (PGDM, type 1), all in singleton pregnancy. Ultrasound examination was performed between weeks 11 and 14, and in weeks 20 and 30 of gestation, based on recommendations of the Polish Society of Gynecologists and Obstetricians, Ultrasonography Division. We also checked uterine artery blood flow parameters. During the first trimester consultation, all patients were surveyed and the following data were collected: age, BMI, reproductive history, comorbidities and smoking. We also collected blood samples and assessed PlGF, PAPP-A, and BhCG levels.

Results: Our study showed that newborn birth weight negatively correlated with mother's age, her diastolic blood pressure, PI of her uterine arteries and BhCG protein levels. Moreover, birth weight directly correlated with PlGF and PAPPA-A protein levels, and maternal early-pregnancy BMI.

Conclusions: LGA diagnosis in the first trimester of pregnancy allows for selection and modification of some risk factors and closer monitoring of endangered fetuses throughout the pregnancy, with emphasis on the perinatal period.

Parameters with confirmed usefulness in the prediction of birth weight in the first trimester included: maternal age, BMI, blood pressure, PAPP-A, BhCG and PlGF levels, fetal CRL and uterine artery PI.

Key words: birth weight; pre-existing diabetes; macrosomia; LGA; SGA

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INTRODUCTION

Models of modern prenatal care aim at determining the risk of pregnancy-related complications during the first trimester. They also investigate any disorders related to the fetus growth. Numerous studies carried out on a large scale allowed for development of highly sensitive screening tests for fetal growth disorders based on using data from ultrasound examinations (carried out between weeks 11–13 + 6 of pregnancy), accompanied by tests of mother blood serum.

Abnormal fetal growth, resulting in either large-for-gestational age (LGA), small-for-gestational age (SGA) or intrauterine growth restriction (IUGR), is a common fetal complication in high risk pregnancy [1, 2]. Therefore, early assessment of fetal growth and risk factors for inadequate

fetal growth now gain considerable attention in the field of maternal-fetal medicine.

Maternal hyperglycemia is the main factor responsible for excessive fetal growth. Elevated maternal glucose levels intensify glucose transport to the fetus, which results in increased fetal production of insulin and insulin-like growth factors. This consequently leads to excessive development of adipose tissue in the developing fetus. Fetal growth undergoes strong genetic regulation but it is also modified by intrauterine environment (epigenetic factors) that determines the newborn final weight [3–5]. The most important risk factors for fetal macrosomia are: maternal diabetes and obesity, older age, gestational hypertension, and pregnancy lasting over 42 weeks. Excessive weight in the fetus causes several complications during prenatal development, and perinatal and postnatal life. In the perinatal period, LGA is associated with more frequent obstructed labors, higher percentage of pelvic floor muscle and maternal anal sphincter injuries, shoulder dystocia with consequent paralysis of the shoulder plexus, hypoxia and neonatal death.

Long-term observations of children with macrosomia show that as adults they are more prone to becoming overweight or obese and more often suffer from diabetes and cardiovascular disorders. In children with birth weight exceeding 4000 g, higher frequency of insulin resistance, increased risk of metabolic syndrome, abnormal fasting blood glucose, and abnormal glucose tolerance in childhood are reported [6].

To reduce the risk of birth weight related complications, early identification of the risk group for fetal macrosomia and early elimination of potentially modifiable risk factors are important. The modern model of perinatal care called "an inverted pyramid" identifies early pregnancy as a period for perinatal risk assessment [7]. Thus, each pregnant woman is assigned to an appropriate risk group and specific interventions are commenced to reduce the identified risks. A risk analysis in early pregnancy also involves screening for abnormal fetal growth. An assessment model that draws upon data from a pregnancy history, nuchal translucency, and the levels of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP-A) in the maternal serum between weeks 11–13 of pregnancy, identifies only about 35% of women who would give birth to LGA newborns. Moreover, false positive rate is approximately 10% [8]. Further research in this field increased sensitivity and specificity of LGA screening by introducing new biomarkers (Inhibin A, selectin E, PLGF), and accounting for additional data obtained during ultrasound examination (pulsatility index PI, of the uterine artery). Despite that, majority of LGA cases remain undetected until actual delivery. Furthermore, we lack a prognostic tool that discriminates between a constitutionally large but healthy newborn and a neonate with "intrauterine obesity".

Objectives

The aim of our work was to assess the usefulness of maternal factors ultrasound and placental function parameters during early pregnancy as predictors of birth weight in populations of healthy pregnant women and women suffering from pregestational (type 1) diabetes.

MATERIAL AND METHODS

The prospective observational study included 97 healthy pregnant women (non-PGDM) and 160 pregnant women with pregestational diabetes (PGDM, type 1), all in singleton, non-malformed pregnancy, monitored on an outpatient basis during the pregnancy period at the Hospital Outpatient Clinic of the Podhalanski Specialist Hospital in Nowy Targ, Poland, or undergoing antenatal care in a tertiary referral unit of the Department of Obstetrics and Women's Diseases of the Poznan University of Medical Sciences, Poznan, Poland. All patients included in the study were informed about its purpose and scope and gave their written consent to participate.

Monitoring consisted of three follow-up visits during the pregnancy: between weeks 11 and 13 + 6, and during weeks 20 and 30. At the first visit, data were collected from each patient using a questionnaire. It requested information about the patient's age, BMI, reproductive history and co-morbidities. It particularly focused on diabetes and its type, age of the patient at onset, pregnancy-induced hypertension, pre-eclampsia in previous pregnancies, and smoking.

The study, carried out between weeks 11 and 13 + 6 of pregnancy, aimed at assessing fetal anatomy and evaluating markers of chromosomal aberrations using the recommendations of the Polish Society of Gynecologists and Obstetricians, Ultrasonography Division (crown rump length — CRL, biparietal diameter — BPD, nuchal translucency — NT, nasal bone — NB, ductus venosus — DV), and uterine artery blood flow parameters (UtA PI — pulsatility index). Blood samples collected during the visits were centrifuged, aliquoted and transported to the ISO 9000 accredited Central Laboratory of Clinical Gynecology and Obstetrics Hospital in Poznań, where PIGF and PAPP-A protein concentrations, and β-hCG, PIGF and PAPP-A serum levels were determined in an immunofluorometric assay and DELFIA Xpress analyzer. β-hCG values were assessed using monoclonal antibodies labeled with a ruthenium complex.

We defined birth weight above the 90th percentile for a gestational age and neonatal sex at delivery as a large-for-gestational age newborn (LGA). Birth weight below the 10th percentile for a gestational age at delivery and neonatal sex were defined as a small-for-gestational age newborn (SGA). Percentiles were calculated referring to the non- PGDM subgroup in the study.

Statistical analysis was carried out using SPSS for Windows 14.0.0 (SPSS Inc. Chicago, USA) and MedCalc Statisti-

cal Software, version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). Data were checked for normality and then appropriate parametric or nonparametric tests were used to check for differences between the variables studied in the PGDM and non-PGDM subgroups. Multiple regression models were built to identify predictors for the birth weight in the entire cohort, and for both researched groups. We used logistic regression and ROC analysis to identify predictors of abnormal birth weight,

defined as large-for-gestational age or small-for-gestational age. Variables were presented as mean ± standard deviation or median. P < 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the characteristics of the study groups. Of the entire cohort, 9.7% of participants had pregnancy-related hypertensive disorders, 9.7% reported smoking during pregnancy, 30.7% were overweight or

Table 1. Characteristics of the study groups							
Variables	Patient group	Mean	Standard deviation (SD)	Median	Min	Max	Mann-Whitney Test
Age [years]	PGDM	29.8	4.7	29.3	19.4	44.5	p = 0.030
	non-PGDM	28.5	5.3	27.0	19.0	41.0	
Height [cm]	PGDM	166.0	6.3	165.0	153.0	186.0	p = 0.569
	non-PGDM	165.2	6.0	165.0	150.0	176.0	
Weight [kg]	PGDM	65.3	14.6	62.0	47.0	124.0	p = 0.227
	non-PGDM	66.2	12.4	62.9	44.6	99.0	
BMI	PGDM	23.7	5.1	22.6	16.7	47.8	p = 0.161
	non-PGDM	24.3	4.7	23.0	17.6	39.0	
Systolic BR	PGDM	112.3	14.6	111.9	80.0	150.8	p = 0.003
	non-PGDM	107.6	12.8	105.0	80.0	145.0	
Diastolic BP	PGDM	70.6	9.9	70.0	50.0	97.0	p = 0.006
	non-PGDM	67.3	9.7	65.0	50.0	90.0	
Mean BP	PGDM	84.5	11.0	83.3	60.0	112.9	p = 0.005
	non-PGDM	80.7	9.9	79.2	60.0	106.7	
Right UtA PI (I trimester)	PGDM	1.55	0.63	1.44	0.46	3.57	p = 0.426
	non-PGDM	1.60	0.58	1.49	0.57	3.49	
Left UtA PI (I trimester)	PGDM	1.53	0.60	1.52	0.45	3.43	p = 0.455
	non-PGDM	1.55	0.47	1.51	0.53	2.82	
Low UtAPI	PGDM	1.29	0.49	1.23	0.45	2.89	p = 0.225
	non-PGDM	1.20	0.40	1.17	0.40	2.64	
High UtAPI	PGDM	1.79	0.62	1.77	0.56	3.57	p = 0.001
	non-PGDM	2.10	0.67	1.98	0.90	3.98	
Mean UtAPI	PGDM	1.54	0.51	1.52	0.55	2.99	p = 0.469
	non-PGDM	1.59	0.44	1.52	0.85	2.88	
CRL [mm]	PGDM	65.1	8.3	64.0	48.0	83.0	p = 0.186
	non-PGDM	63.4	10.9	64.5	45.0	86.0	
NT [mm]	PGDM	1.38	0.33	1.40	0.70	2.50	p < 0.001
	non-PGDM	2.49	8.40	1.70	1.00	83.00	
β-hCG U/l	PGDM	53.0	56.0	42.7	8.3	522.2	p = 0.033
	non-PGDM	41.5	31.2	33.1	6.3	232.8	
PAPP-A U/L	PGDM	3.07	1.98	2.77	0.22	9.43	p = 0.689
	non-PGDM	3.26	3.94	2.44	0.67	36.45	
PLGF [pg/L]	PGDM	43.5	15.0	39.9	1.7	100.0	p < 0.001
	non-PGDM	34.0	14.5	30.4	13.6	90.8	
Birth weight [g]	PGDM	3388	596	3415	980	4660	p = 0.191
	non-PGDM	3314	512	3350	1660	4600	

obese (compared with 13.2% of our subgroup). In the PGDM subgroup, 10.1% of participants had vascular complications (retinopathy, and/or nephropathy).

There were no statistically significant differences between patients with pre-gestational diabetes and nondiabetic participants regarding height, body weight, and BMI, PI in the right and left uterine artery, the lowest and average PI value of the uterine artery, crown rump length (CRL), PAPP-A levels or child birth weight.

Data concerning correlations between the fetal and maternal parameters collected during early pregnancy and neonatal body weight are summarized in Table 2.

We found that in the non-PGDM subgroup birth weight significantly correlated only with maternal BMI, whereas among the diabetic patients significant correlations were confirmed for maternal age, CRL, vascular flow and concentration of placental proteins.

In order to investigate the influence of selected early pregnancy parameters on birth weight in the entire cohort, stepwise regression was built. The best-fit model for the entire cohort is presented in Table 3.

After investigating for early pregnancy maternal and fetal parameters, we found that PAPPA concentrations, early pregnancy maternal body weight and hypertensive status remained statistically significant predictors of birth weight across the entire study group.

In a separate analysis, we looked for predictors of abnormal fetal growth, defined as either LGA or SGA. In our study group, we had 39 cases of LGA out of 226 mother-infant pairs (17.2%, no data available for 31 patients). After investigating for fetal and maternal parameters, we confirmed that only mean UtAPI remained a statistically significant predictor of LGA (Tab. 4). However, we also identified several independent predictors of LGA in our cohort that included PlGF, NT, and CRL (Fig. 1 A–D).

Table 2. Correlations between birth weight and fetal/maternal characteristics

Pairs of correlated variables	STUDY GROUP		PGDM		non-PGDM	
	R	p	R	p	R	p
Birth weight [g] & Age	-0.129	0.049	-0.165	0.044	-0.100	0.356
Birth weight [g] & BMI	0.076	0.247	0.014	0.861	0.224	0.039
Birth weight [g] & systolic BP	0.021	0.747	-0.040	0.626	0.067	0.543
Birth weight [g] & diastolic BP	-0.028	0.674	-0.076	0.358	0.037	0.738
Birth weight [g] & Mean BP	-0.002	0.978	-0.055	0.508	0.047	0.666
Birth weight [g] & right UtAPI	-0.065	0.327	-0.109	0.194	0.032	0.770
Birth weight [g] & left UtAPI	-0.168	0.014	-0.177	0.033	-0.131	0.282
Birth weight [g] & low UtAPI	-0.087	0.189	-0.131	0.116	-0.010	0.925
Birth weight [g] & high UtAPI	-0.131	0.048	-0.167	0.044	-0.022	0.839
Birth weight [g] & mean UtAPI	-0.128	0.052	-0.161	0.054	-0.062	0.570
Birth weight [g] & CRL [mm]	0.085	0.194	0.164	0.046	-0.053	0.631
Birth weight [g] & NT [mm]	0.006	0.930	0.049	0.554	0.042	0.704
Birth weight [g] & B-hCG IU/L	-0.043	0.516	-0.080	0.336	0.009	0.935
Birth weight [g] & PAPP-A U/L	0.119	0.072	0.258	0.002	-0.163	0.140
Birth weight [g] & PLGF [pg/L]	0.187	0.006	0.284	0.001	0.042	0.734

p — level of significance; R — Spearman's rank correlation coefficient

Table 3. Predictors of birth weight in the entire cohort — multiple regression

Summary of dependent variable regression: Birth weight [g]
$R = 0.341$ $R^2 = 0.116$ correct; $R^2 = 0.102$; $F (5.185) = 3.9103$; $p < 0.0001$; standard estimation error: 560.279

Regression model	Standardized coefficients	Non-standardized coefficients		t (185)	p
	beta	B	SE		
Intercept		2257.217	250.085	9.027	p < 0.0001
PAPPA serum level	0.274	79.907	21.711	3.680	p < 0.0001
Maternal body weight	0.309	13.397	3.341	4.010	p < 0.0001
Hypertensive disorders of pregnancy YES	-0.149	-287.703	140.234	-2.052	p = 0.042

In the entire cohort we had 24 cases of SGA (10.6%, no data available for 31 patients). None of the analyzed variables significantly predicted birth weight below the 10th percentile.

In a separate analysis of the subgroups, we identified several predictors of birth weight in PGDM subgroup that remained statistically significant after adjustment: the moth-er's age, gestational age at examination, glycemic levels below a pathological limit, and duration of diabetes. Data from the multiple regression model are presented in Table 5.

To identify predictive factors for excessive fetal growth in diabetic pregnancy, we built a model of logistic regression with LGA as a dependent variable. After adjustments for fetal and maternal confounders, mean UtAPI and PAPPA serum level remained as statistically significant predictors of LGA in this subgroup (Tab. 6). However, we also identified CRL and PLGF as independent LGA predictors (Fig. 2 A–B).

In PGDM subgroup, we had 17 cases of SGA. After adjusting for confounders, maternal PLGF serum level was found to be a statistically significant predictor of low birth weight but with a minimal actual impact on this outcome (Tab. 7). We also noted that PAPPA serum levels independently predicted SGA in this cohort (Fig. 3 A–B).

In a separate analysis of non-PGDM subgroup, none of the parameters contributed significantly to the risk of LGA.

DISCUSSION

Early detection of pregnant women with high risk of fetal growth disorders allows for closer monitoring of these patients, possible modification of risk factors and informed decisions concerning the mode of delivery. Recent research in this field abounds in algorithms based on data obtained during the first trimester screening, supplemented with
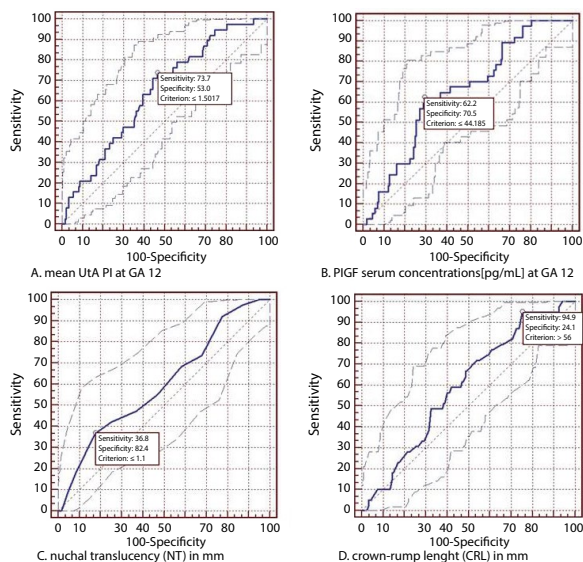
Figure 1. Independent predictors of LGA in the whole cohort

Table 4. Predictors of LGA in the entire cohort — logistic regression

Logistic regression FSTEP model	Regression coefficient B	p	Odds ratio (OR)	95% confidence interval for OR	
Intercept	0.64	p = 0.329	1.90		
mean UtAPI	-1.496	p = 0.001	0.22	0.09	0.56

Table 5. Predictors of birth weight in PGDM patients — multiple regression

Summary of dependent variable regression: Birth weight [g]
R = 0.454; R2 = 0.206 Correct; R2 = 0.182; F (6.133) = 6.2581; p < 0.00001; standard estimation error: 539.602

Regression model	Standardized coefficients	Non-standardized coefficients		t (185)	p
	beta	B	SE		
Intercept		860.804	1220.459	0.705	p = 0.482
PAPP-A U/L	0.353	103.3065	23.036	4.487	p < 0.0001
β-hCG IU/L	-0.197	-2.015	0.791	-2.546	p = 0.012
Maternal height [cm]	0.196	18.402	7.251	2,.538	p = 0.012
Diastolic BP at the examination	-0.182	-10.801	4.702	-2.297	p = 0.023

Table 6. Predictors of LGA in PGDM patients — logistic regression

Logistic regression FSTEP model	Regression coefficient B	p	Odds ratio (OR)	95% confidence interval for OR	
Intercept	-0.379	p = 0.643	0.684		
PAPP-A U/L	0.205	p = 0.043	1.23	1.01	1.50
Mean UtAPI	-1.162	p = 0.018	0.313	0.12	0.82

Table 7. Predictors of SGA in PGDM patients — logistic regression

Logistic regression FSTEP model	Regression coefficient B	p	Odds ratio (OR)	95% confidence interval for OR	
Intercept	0.147	p = 0.880	0.95		
PLGF [pg/L]	-0.065	p = 0.030	1.16	0.901	0.995
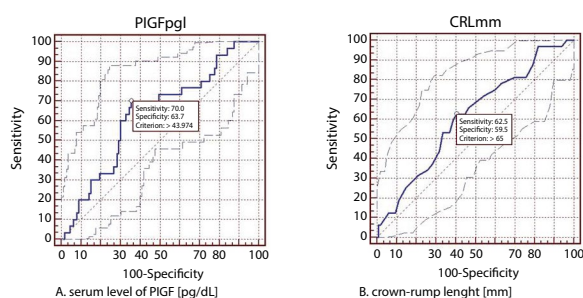
Figure 2. Independent predictors of LGA in PGDM patients
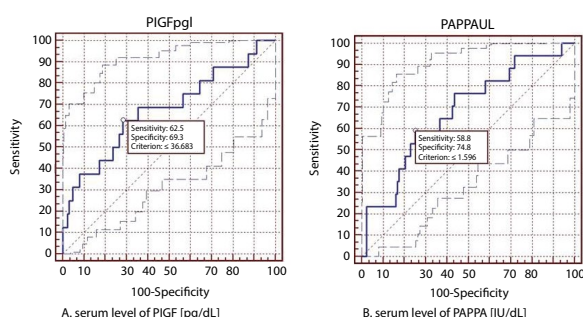
Figure 3. Predictors of SGA in PGDM patients

additional data from ultrasound examination and on plasma protein concentrations.

In 2013, Papastefanou et al. performed prenatal examinations in 702 first trimester patients. They created a diagnostic model in which significant, independent SGA predictors included patient height, multiparity, smoking, assisted reproduction, CRL, NT and PAPP-A and β-hCG levels. They also found that the weight and height of the pregnant women, cigarette smoking, and CRL and NT levels were significant, independent predictors of LGA. Sensitivity of both models was relatively low — 48% for LGA and 55% for SGA [9]. The importance of the body mass index in LGA prediction was confirmed in another prospective screening study, based on examination of 41.577 pregnancies. Regression analysis showed a significant contribution from maternal BMI, in addition to maternal characteristics and obstetric history, in the prediction of subsequent delivery of small and large for gestational age neonates. The risk of LGA increased exponentially with increasing maternal BMI [10].

In 2016, Frick et al. published an observational study of a large cohort of pregnant women who were having fetal growth monitored throughout their pregnancies. The

likelihood of developing LGA grew with increasing weight and height of the mothers, and decreased in tobacco-smoking and nulliparous women. Higher LGA risk was also found in patients with pre-gestational diabetes type 1, but dropped in patients with chronic hypertension. In multiparous women, LGA risk increased if LGA newborn was delivered before, and decreased in patients with previous gestational diabetes and short interval between consecutive pregnancies. The screening study was solely based on medical history data taken from 76.300, 54.999, 25.727 and 6.181 singleton pregnancies at 11–13, 19–24, 30–34, and 35–37 weeks' gestation, respectively. Screening by maternal factors at 11–13 weeks predicted 32%, 44% and 60% of LGA > 95th at false-positive rates (FPRs) of 5%, 10% and 20%, respectively. With the addition of fetal biometry, the detection rates improved to 37%, 51% and 68% at 19–24 weeks, 50%, 65% and 81% at 30–34 weeks and 60%, 73% and 85% at 35–37 weeks at FPRs of 5%, 10% and 20%, respectively. The addition of biomarkers did not improve the detection rates achieved when screening by a combination of maternal history and fetal biometry [11].

An LGA prediction algorithm similar to that mentioned above was used in an Italian study of 72 pregnant women between weeks 11–14 of pregnancy. The LGA prediction used historical data and PAPP-A concentration levels. Maternal growth, age, smoking, assisted reproduction and PAPP-A levels were found to be important, independent predictors of LGA [12]. In another study, Gonzalez et al. conducted a prenatal examination of 2097 pregnant women, including a Doppler study of the uterine artery in the first trimester and an assessment of fetal growth and the uterine artery Doppler in the second trimester. The study algorithm based on maternal history, PAPP-A protein concentrations, and β-hCG, NT, and PI values in the uterine arteries, allowed for identification of 30.2% of LGA cases, with a false positive rate of 10%. When data from the second trimester were added, the sensitivity increased to 56.2% with FPR of 20% [13]. Another biomarker that turned out useful in the prediction of macrosomic neonates was adiponectin. Examination of 350 cases showed that in the macrosomic group the median serum adiponectin was significantly lower than in the non-macrosomic controls. A detection rate of macrosomia, based on maternal characteristics and obstetric history was 34.6% with false positive rate of

10%. Inclusion of adiponectin to this algorithm increased the detection rate to 38.2% [14].

Boucoiran at al. used only the parameters from the first trimester screening for aneuploidies to predict the birth weight. They examined 4110 patients in a singleton pregnancy. NT was significantly higher in LGA group as compared with the unaffected group but biomarkers (PAPPA and BhCG) were at the same levels in both groups. After controlling for gestational age, maternal weight, smoking status, ethnicity, and fetal sex, first-trimester markers contributed to the prediction of birth weight in a multiple linear model but did not significantly improve the prediction of LGA as compared with maternal characteristics alone [15].

There are few studies referring to growth disorders in the fetuses of mothers with pre-gestational diabetes, which used plasma protein concentrations and data from prenatal ultrasound examination between weeks 11–14 of pregnancy in their algorithm. The most frequently assessed predictors of growth disorders in such fetuses were fasting glycemia and gyrated hemoglobin concentrations. Some information about the risk of LGA and fetal macrosomy in pregnancies complicated by a metabolic syndrome and obesity can be found in the first trimester screening carried out by Migda et al. in 123 Caucasian patients with the metabolic syndrome. In that study, BMI above 25.5 was found an important risk factor for excessive fetal weight. Mother blood glucose and concentrations of adiponectin and soluble E-selectin were predictive of LGA and fetal macrosomia. The mother weight of or exceeding 67 kg in the first trimester showed high sensitivity and specificity in detecting LGA and macrosomia [16]. However, that study investigated a completely different population than ours.

Based on our cohort, we can conclude that placentation and placental function during early pregnancy strongly affect fetal growth, particularly in PGDM complicated pregnancy. Importantly, early pregnancy vascular function seems to be even more crucial for fetal growth than maternal pre-pregnancy glycemic control. The latter needs to be achieved if a reduced risk of fetal malformation or miscarriage is aimed at, while uteroplacental capacity ensures adequate transfer of oxygen and nutrients.

Our observations confirm that genetic potential (seen as CRL), uteroplacental vascular capacity (measured as UtAPI), and placental function (measured as serum levels of specific placental proteins) shape the growth trajectory for fetuses. Those predictors, supported by data commonly available from the patient's history (age, BMI, and blood pressure) can be used in the algorithm for the first trimester. In our study, birth weight of the newborn negatively correlated with the age of the mother, her diastolic blood pressure, PI of the uterine artery and BhCG protein levels. PlGF and PAPPA-A protein levels and maternal early-pregnancy BMI

correlated positively with birth weight of the newborn. Importantly, using an LGA prediction algorithm in the first trimester of pregnancies complicated by PGDM, we are able to estimate the baseline risk of fetal growth disorders in the diabetic population before the maternal glycemia affected the fetal growth trajectory.

CONCLUSIONS

1. LGA diagnosis in the first trimester of pregnancy allows for the selection and modification of some risk factors and closer monitoring of endangered fetuses throughout the pregnancy, with emphasis on the perinatal period.
2. Parameters with confirmed usefulness in the prediction of the birth weight in the first trimester include maternal age, BMI, blood pressure, PAPP-A, BhCG and PlGF values; CRL measurement and uterine artery PI values.

Authors' contributions

1. Anna Gąsiorowska — designed the experiment, collected the material, drafted the manuscript and researched the literature.
2. Agnieszka Zawiejska — analyzed and interpreted the results, critically revised the manuscript.
3. Piotr Dydowicz — collected the material.
4. Ewa Wender-Ożegowska — critically revised the manuscript.
5. Grzegorz Poprawski — collected the material.
6. Kinga Toboła-Wróbel — analyzed and interpreted the data.
7. Katarzyna Ziółkowska — collected the material and carried out biochemical tests.
8. Marek Pietryga — designed the experiment, proofread, verified and finally approved the manuscript.

Conflict of interests

The authors declare no conflicts of interest. No financial remuneration was received relative to the technologies and concepts presented in the manuscript.

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ORIGINAL PAPER / OBSTETRICS

Secondary cervical cancer prevention in routine prenatal care — coverage, results and lessons for the future

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ABSTRACT

Objectives: Cervical cancer is the fourth most common type of cancer among women worldwide and one of the most common malignancies diagnosed in gravidas. Therefore, routine antenatal Pap smear is such an important examination. The aim of the study was to assess the prevalence of Pap smear performance during prenatal care and to determine possible factors affecting it.

Material and methods: A self-composed questionnaire was distributed among 638 women managed in a tertiary obstetric referral center. 33 questions regarded cervical cancer prevention and risk factors.

Results: 96.9% of respondents had undergone Pap smear and 80.6% had it performed during pregnancy. For 11.5% women Pap smear in pregnancy was the first one in their life. The most common reasons for lack of Pap smear performance were: no subjective need to perform it (40.9%), no doctor's recommendation (28.6%) and lack of gynecological care (16.3%). Among professionally active women the percentage of those who had not undergone Pap smear during pregnancy was statistically higher (28.5%) than among those who were on sick leave (13.5%) (p = 0.0003). Also, younger women were at risk of less frequent participation in cervical cancer screening

Conclusions: Although performance of Pap smear among surveyed patients was relatively high, there was a significant group of women who had undergone their first test during pregnancy, which makes secondary cervical cancer prevention in prenatal care a useful prophylactic strategy. Special attention should be given to younger and professionally active women.

Key words: cervical cancer; pap smear; prenatal care; pregnancy; secondary prevention

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INTRODUCTION

According to GLOBOCAN cervical cancer is the fourth most common type of cancer among women worldwide, with the incidence of 569,847 cases and the cause of 311,365 deaths a year [1]. In Poland, in 2018 the number of new cases was 3,220 with an incidence age-standardized rate of 9.4 per 100,000 and the number of deaths was 1,947 with a mortality age-standardized rate of 4.9 per 100,000 [1]. Nevertheless, according to GLOBOCAN, the age-adjusted cervical cancer incidence in 2018 was lower in Poland than in Europe as a whole (9.4 vs 11.2) and the number of new cases decreased by almost 2.3 times during the end of the first decade of the 21st century, which was possibly due to the introduction of a population based cervical cancer screening program initiated in 2006 by the Polish National Health Fund [1, 2]. The program is dedicated to women covered by health insurance between 25 and 59 years of age and offers performance of Pap smear once in three years — in case of negative results. Whereas patients classified as high-risk groups — HIV or HPV positive or on immunosuppressive therapy - can participate in examinations every 12 months [2]. Over the years participation in the cytology screening program in Poland increased from 12.7% in 2006 to 42.11% in 2015 [3]. This suggests that the universal, free access to cytology examinations may result in increased population coverage.

Another factor, which contributes to incidence reduction, is the vaccination against human papillomavirus (HPV)

which is recommended in Poland, but is not among the publicly funded healthcare benefits [4]. Only two EU member states, including Poland, are not financing HPV vaccinations in selected age groups (10–15) [5]. However, various local governments and autonomies as well as charity organizations and schools conduct free, preventive HPV vaccination actions. The Polish National Vaccination Program recommends HPV vaccination prior to sexual initiation, however it does not indicate the specific age when such vaccination should be performed [4].

Additionally, in order to improve cervical cancer screening effectiveness, according to the Ordinance of the Polish Minister of Health, Pap smear should be performed in every pregnant woman till the end of the 10th gestational week as a part of standard prenatal care, unless she had undergone it within the last six months [6]. For most women, pregnancy is a period of increased medical supervision, which makes it a good opportunity for secondary cervical cancer prevention.

Objectives

The aim of the study was to assess the performance of Pap smear in pregnant women and to determine possible factors affecting it.

MATERIAL AND METHODS

A cross-sectional study was performed by means of a self-composed questionnaire (*see* Annex no. 1) distributed among 638 patients from different regions of Poland who were managed in the 3rd trimester or within the first month after birth in a tertiary referral obstetric center. The entire group of patients who agreed to take part in the study was surveyed between December 2017 and February 2018. The survey was composed of 33 questions regarding obstetric history, performance, frequency and results of Pap smear examinations, reasons for not performing it (if applicable), gynecological

care, vaccination against HPV, use of hormonal contraception, family history of cervical cancer, and performance of further diagnostic procedures: colposcopy or histopathological examination. Informed consent was obtained from all individual participants included in the study. Statistical analysis was performed with the use of Statistica 13.3. Since the differences between groups were based on the categorical variables, they were tested with the use of chi-squared test. P-values < 0.05 were considered statistically significant.

RESULTS

The average age of surveyed women was 25.8 ± 4.89 years (17–45 years). The study group was diverse in terms of place of residence, level of education, professional activity and marital status, which is presented in Table 1.

More than half of respondents were primigravid (54.4%; n = 347), 29.9% (n = 191) were in their second pregnancy and for 15.7% (n = 100) of women it was the third or subsequent pregnancy.

All surveyed women were under gynecological supervision during pregnancy but as many as 10.2% (n = 65) of them had never visited a gynecologist before pregnancy. Out of those who were attending appointments regularly before pregnancy, 34.1% (n = 206) did it once in 6 months or more often, 41.9% (n = 253) once a year, 12.7% (n = 77) once in 2 or 3 years and 6% (n = 36) less often.

The analysis of Pap smear performance among surveyed women revealed that the vast majority of them (96.9%; n = 618) had undergone Pap smear examination, yet fewer (80.6%; n = 497) did it during pregnancy. This includes a small group of women (4.9%; n = 23) who performed the test as a preparation for planned pregnancy. Among women who underwent Pap smear during pregnancy those who had it in the first trimester (72.2%; n = 324) dominated, whereas 23.6% (n = 106) had it in the second and 4.2% (n = 19) in the third trimester. Among multiparas the per-

Table 1. Group characteristics: number of women (%)				
Level of highest achieved education				
Primary	**Vocational**	**Secondary**	**Higher**	
19 (3%)	36 (6%)	238 (37%)	345 (54%)	
Place of residence				
Village	**City < 10,000 inhabitants**	**City 10,000–100,000 inhabitants**	**City > 100,000 inhabitants**	
128 (20%)	55 (9%)	149 (23%)	306 (48%)	
Professional activity				
Unemployed	**Professionally active**	**Sick leave during pregnancy**	**Maternity leave**	**Other**
99 (16%)	137 (21%)	283 (44%)	75 (12%)	44 (7%)
Marital status				
Married		**Unmarried**		
457 (72%)		181 (28%)		

centage of Pap smear performance in previous pregnancies was 79.9% (n = 226).

The analysis of Pap smear results performed during pregnancy revealed that in 67.8% (n = 328) of cases they were normal, in 23.6% (n = 114) the test detected inflammation and 2.3% (n = 11) of women had an abnormal test result such as: atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL) (Fig. 1).

The analysis of questions regarding frequency of Pap smear performance showed that almost half of the women (47.4%; n = 293) performed the test regularly every year, 22.7% (n = 140) every 2 years, 7.8% (n = 47) every 3 years and 7.9% (n = 49) less often. For 11.5% (n = 71) of women Pap smear in pregnancy was the first in their lifetime and 1% (n = 6) underwent the test mainly in pregnancies. The average age during the first Pap smear was 20.6 ± 3.4 years (12–35 years).

Similarly, among women who were over 25 years old and were qualified for the population-based cervical cancer screening program, almost half (48.1%; n = 232) performed the test once a year, 24.5% (n = 118) every 2 years, 9.3% (n = 45) every 3 years and 8.5% (n = 41) less often. For 7.5% (n = 36) of women Pap smear in pregnancy was the first in their lifetime.

The most common reasons for not performing Pap smear were: no subjective need to perform it (40.9%; n = 83), no doctor's recommendation (28.6%; n = 58) and lack of gynecological care (16.3%; n = 33). No doctor's recommendation was reported by 26% (n = 39) women who were

over 25 years old and were covered by a population-based screening program. Other listed reasons were: feeling of shame (5.4%; n = 11), cost of the test (3%; n = 6), fear of pain (2.5%; n = 5) and concern about the test result (2%; n = 4).

As for the reasons which affected the Pap smear performance, it was observed that among professionally active women the percentage of those who did not perform Pap smear during pregnancy was statistically higher (n = 41; 28.5%) than among those who were on sick leave (n = 37; 13.5%) (p = 0.0003). Another statistically significant factor was women's age (Tab. 2). Analysis of the following age groups: under 25 years (< 25), between 25 and 35 years (≤ 25 and < 35) and over 35 years (> 35) revealed that the youngest women much more often had never performed Pap smear (9.1% vs. 1.2% in other age groups; p = 0.0001) and that the test during pregnancy was more often the first one in their life (25.7% vs. 7.2% in other age groups; p = 0.0001). The most important fact is that they much more frequently did not perform Pap smear in their current pregnancy (27.1% vs. 16.7% in other age groups; p = 0.02). No significant association between place of residence, level of education or marital status and Pap smear performance was observed.

8.8% of women (n = 56) admitted to having a positive family history of cervical cancer. Yet the majority of them (67.9%; n = 38) did not indicate any impact of this fact on the frequency of Pap smear performance. 50.9% (n = 324) of women declared their use of hormonal contraception (HC) in the past, 85.4% (n = 274) of them reported no association between HC use and regular Pap smear control.

The study also showed that vaccination against HPV was not widespread among respondents (6.1%; n = 39). Those who had been vaccinated were asked whether they had undergone it before or after sexual initiation, but no marked predominance of either option was observed (38.5% vs. 46.2%, respectively).

A history of further diagnostic procedures due to suspicion of cervical abnormalities was reported by 10.1% (n = 64) of surveyed women, revealing one case of LSIL and four cases of HSIL in histology examinations.

DISCUSSION

Despite the development of a National Population-Based Cervical Cancer Screening Program in 2006 by the Polish Ministry of Health, the National Health Fund and
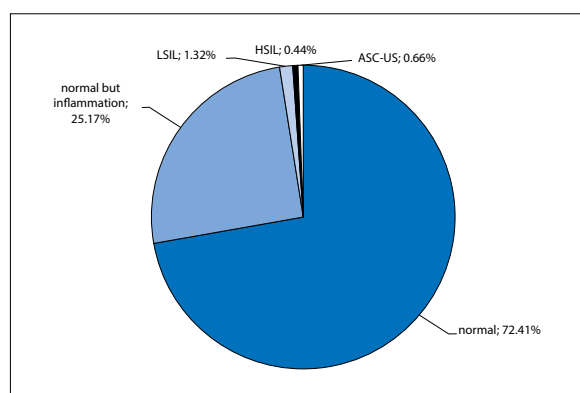
Figure 1. Results of performed Pap smears in current pregnancy

Table 2. Pap smear performance in different age groups: number of patients (%)				
	< 25 years n = 150	25–34 years n = 404	≥ 35 years n = 70	P
Had never performed Pap smear	14 (9.1%)	4 (1.2%)	1 (1.4%)	0.0001
Pap smear in pregnancy was the first in a lifetime	39 (25.7%)	31 (7.6%)	4 (5.6%)	0.0001
Did not perform Pap smear in current pregnancy	41 (27.1%)	69 (17%)	12 (17.1%)	0.02

the Polish Gynecological Society, following World Health Organization and International Agency for Research on Cancer (WHO/IARC) guidelines, percentage of women performing the test was still unsatisfactorily low according to the Supreme Audit Office of Poland performed in the Lubelskie Province [7]. In order to increase cervical cancer screening coverage, since 2012 every pregnant woman should undergo Pap smear during her initial prenatal visit as ordinated by the Polish Ministry of Health [8]. However, it is stated that cervical samples collected during pregnancy are more difficult to interpret because of hormonal changes in the epithelial cells [9]. Similarly, WHO provides the information that cervical specimens taken during pregnancy can give misleading results [10]. Nevertheless, results of Pap smear performed in pregnant women are not less accurate than in non-pregnant patients and it is recommended to perform the test if the woman is likely to give up gynecological care after delivery and when she is in the target age group [11, 12]. This includes women over 20 years old or sexually active for more than 3 years [13]. On the contrary, several publications reported that pregnancy did not modify or affect HPV infection, nor cervical cytomorphology [14, 15]. It is also proved that the intraepithelial lesions in gravidas are cytometrically identical to those in nonpregnat women [13]. Therefore, a specimen collected during pregnancy is adequate for evaluation but only if the pathologist is notified of patient's pregnancy status [9, 16]. It may be even more difficult to interpret the colposcopic appearance of the cervix, even in the first trimester. Nevertheless, it should be always performed in women with an abnormal Pap test result [13].

Cervical cancer is one of the most common malignancies diagnosed in gravidas, complicating 1 in 2200 pregnancies [13, 17]. Therefore, routine antenatal Pap smear is such an important examination. Abnormal cervical cytology is observed in about 5% of gravidas [17–19]. In our study the percentage was slightly lower than the average — 2.3%. Pregnancy is a special period and sometimes the only one when women pay more attention to their health condition so especially during this time physicians should motivate their patients to conduct screening examinations. In the Norwegian study Nygård et al. [20] indicated that screening during pregnancy increases the coverage of the cervical cancer prevention program. They reported that 69% of pregnant women had the Pap smear performed during one year of follow-up since the beginning of pregnancy and that the majority of tests were performed before the delivery [21]. Our study showed that the percentage of Pap smear performance during pregnancy in Poland was even higher (80.6%). However, more important is the fact that for 11.5% of women cervical cytology performed during pregnancy was the first in their life and it does not apply only to women who were under the age of 25, not routinely covered by screening. As many as 16.7% of women over 25 years of age had never had the test performed before pregnancy, while according to the screening guidelines — should have had [22]. Similarly, a French study conducted by Brun-Micaleff et al. suggested that Pap smear combined with HPV infection testing may be an effective method of covering young women with poor adherence to cervical cancer screening. It is estimated that in France 40% of women do not perform regular cytology examinations. By enrolling in the study women with poor adherence the researchers detected high risk HPV-infections in 20.2% gravidas and cervical intraepithelial neoplasia grade 1 or 2 in 2% of the tested population [23].

It may be the subject of controversy whether it is wrong that women under the age of 25 much more often do not perform Pap tests. It is believed that the majority of dysplastic cervical lesions, which are common in this age group have a tendency to regress spontaneously [21]. Therefore, covering this age group with screening may lead to overdiagnosis and subsequently — overtreatment of precancerous lesions [24]. However, Nygård et al. [20] showed that women who perform Pap smear frequently have a tendency to follow this pattern, whereas those who do it seldomly or never, have no tendency to participate in screening in the near future. This is why teaching young women a regular Pap smear scheme may lead to increased screening coverage when they are older. This approach is also in line with the study conducted by Brun-Micaleff et al. Although they detected that 20% of the young pregnant women were positive for HPV infection, they were aware that most of them developed transient infections. Nevertheless, they stated that a positive result may prompt women to repeat Pap smears in the following years at regular intervals [23].

In a Polish study regarding awareness of cervical cancer prevention performed by Ulman-Włodarz et al. [25] the most common reasons for avoiding Pap smear performance were: fear of pain (40.9%), no symptoms of the disease (18%) and carelessness (15%). These causes are quite different in our results, where no subjective need to perform the test was the most frequently reported reason (40.9%) and fear of pain was quoted only by 2.5% of respondents. Nevertheless, the alarming fact is that in both studies a significant number of patients — 28.6% in our study and 11.0% in Ulman-Włodarz et al. [25] study — reported no doctor's recommendation to perform the test. Monteiro et al. [26] showed that the problem does not only concern the Polish society. In their study from Brazil the majority of gravidas who did not undergo cytological examination did not receive an offer from healthcare professional to do so. They emphasized that it was crucial to perform the Pap smear during antenatal consultations as women less frequently schedule an appointment exclusively in order to undergo Pap smear but they usually participate in prenatal consultations, which

makes pregnancy a good opportunity for screening. Similar recommendations are given by the authors of the Thai research [18]. In their study 31% of gravidas had no previous Pap smear screening. Therefore, the proposition of the examination is clinically relevant because these women may not attend regular gynecological appointments in future. In addition, in a Swedish study by Eaker et al. [27] respondents stated that receiving an invitation or offer from a gynecologist motivated them to perform the test. Thus, it is crucial to put emphasis on the greater frequency of these proposals. In addition, the most commonly reported reason for avoiding Pap smear performance, which was 'no subjective need to perform the test', can be interpreted as no disease symptoms. This misunderstanding that Pap smear should be performed only in the presence of gynecological symptoms is quite common among patients all over the world. Augusto et al. [28] in their Brazilian study proved that the absence of symptomatic episodes of sexually transmitted disease was one of the most common reasons for no participation in the screening program. Also, research by Khaengkhor et al. [18] revealed that for 53% of women Pap smear performance seemed necessary only in case of symptoms, such as vaginal bleeding or leucorrhea.

Authors of different studies tried to identify factors affecting the Pap smear performance in order to define under-screened groups requiring special attention. Spaczyński et al. [29] reported that the place of residence and level of education have an impact on cervical cancer screening. According to their study, living in the village and low level of education contribute to less frequent secondary prevention. On the contrary, our study did not show any influence of the place of residence, level of education or marital status on cervical cancer screening — both during pregnancy and in general. The only association we observed was the fact that women who were professionally active during pregnancy less often underwent Pap smear in comparison to women who were on sick leave (28.5% vs. 13.5%; $p = 0.0003$). This problem does not only affect our population — in the Brazilian study Augusto et al. [28] revealed that for 30.4% of women no participation in the screening program was caused by time-consuming job responsibilities and childcare. It may indicate that numerous duties affect prenatal testing and that this group of women requires more attention.

Ulman-Włodarz et al. [25] mentioned that almost 31% of patients who had an incidence of cervical cancer in their family were more motivated to perform Pap smear. It is consistent with the results obtained in our study (32.1%). Other forms of cervical cancer prophylaxis such as vaccination against HPV for primary prevention is not widespread in Poland — Ulman-Włodarz et al. [25] reported that only 4% of respondents were vaccinated, whereas in our study it was declared by 6.1% of surveyed women.

CONCLUSIONS

In conclusion, although the prevalence of Pap smear performance in analyzed population was relatively high, there was a substantial group of women who performed their first Pap smear as a part of prenatal care. Therefore, secondary cervical cancer prevention should remain an important element of prenatal consultations, combined with educating women about the necessity of regular control in future and more frequent doctors' proposals to perform it. Special attention should be given to younger and professionally active women as they are at risk of less frequent participation in cervical cancer screening in comparison to older women and those who resign from work during pregnancy.

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12 c.	The evaluation of the last cytology was made with the use of scale:
	Papanicolau Scale: result as group I, II, III, IV or V.
	Bethesda scale: normal result, ASCUS, LSIL, HSIL, AGUS, ASC-H, cancer cells
	I do not know
13.	If you answered "Yes" to question number 8 and were pregnant before: Did you perform cytology during your previous pregnancy(ies)?
	Yes
	No
14.	If you answered "Yes" to question number 8: When was the last time you have had undergone Pap smear (before pregnancy)?
	< 1 year
	1–2 years ago
	2–3 years ago
	3 years
	I do not remember
	Never
15.	If you answered "Yes" to question number 8: What were the results of these cytology examinations?
	Always correct
	Mostly correct
	Usually incorrect — exactly how? ....
	Always incorrect — exactly how? ....
	I do not remember
16.	If you answered "Yes" to question number 8: Did the abnormal cytology result affected the frequency of your Pap smear performance?
	Yes
	No
17.	If you have not undergone Pap smear before, why was that?
	No subjective need to perform it
	Concern about the test result
	No doctor's recommendation
	Lack of gynecological care
	Fear of pain
	Feeling of shame
	Cost of the test
	Other — what? ....
18.	Have you ever been to a gynecologist before your pregnancy?
	Yes
	No
19.	If you answered "Yes" to question number 18: How often did you go to the gynecologist for a check-up before pregnancy?
	Once every 6 months or more often
	Once a year
	Once every 2–3 years
	Less often than every three years
	Never

20.	Have you been vaccinated against HPV (human papilloma virus causing cervical cancer)?
	Yes
	No
20 a.	If you answered "Yes" to question number 20, has it been before your sexual initiation?
	Yes
	No.
21.	Has anyone in your family had cervical cancer?
	Yes — who? ....
	No
	I do not know
21 a.	If you answered "Yes" to question number 21, has it affected the frequency of your Pap smear performance?
	Yes
	No
22.	Did you use hormonal contraception before getting pregnant?
	Yes
	No
22 a.	If you answered "Yes" to question number 22, what kind of contraception did you use?
	Oral contraception
	Intrauterine device
	Vaginal ring
	Subcutaneous implant
	Injections
	Contraceptive patch
	Other - what? ....
22 b.	Have you performed Pap smear more often while using hormonal contraception?
	Yes
	No
23.	Have you ever had a colposcopy (endoscopic examination of the cervix, assessing the cervix by a doctor using an optical device - colposcope)?
	Yes
	No
	I do not know
23 a.	If you answered "Yes" to question number 23, do you remember the result of the colposcopy?
	Correct result
	Incorrect result: ....
24.	Have you had a histopathological examination of the cervical tissue?
	Yes
	No
	I do not know
24 a.	If you answered "Yes" to question number 23, do you remember the result of the histopathological examination?
	Correct result
	Incorrect result: ....

ORIGINAL PAPER / OBSTETRICS

Therapeutic hypothermia in asphyxiated newborns: selective head cooling vs. whole body cooling — comparison of short term outcomes

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ABSTRACT

Objectives: Therapeutic hypothermia TH became broadly used in the management of the asphyxiated newborns. Although two cooling methods are used, so far the superiority of none of them has been established. The purpose of the study is to compare two cooling methods: selective head cooling (SHC) and whole body cooling (WBC)

Material and methods: We conducted a prospective observational study in newborns with HIE. The patients received one of methods: SHC or WBC. The eligibility criteria were similar to previous studies. Stability of cardio-respiratory parameters and short term outcomes were analyzed.

Results: 78 neonates with hypoxic-ischemic encephalopathy due to perinatal asphyxia were involved in this study. The SHC group consisted of 51 newborns, the WBC group consisted of 27 patients. Both study groups had similar baseline characteristics and condition at birth. There were no significant differences in hospital course, neurological status and adverse effects associated with cooling procedure between groups. Analyzing the rate of thrombocytopenia and the number of transfusions of blood components no statistically significant differences were found between the groups.

Conclusions: Results of our study indicate that two compared methods of TH despite varied target core temperature ranges do not differ significantly according to clinical course and risk of adverse events. Further observations are conducted and we look forward to the results of the long neurodevelopmental care.

Key words: asphyxia; hypoxic–ischemic encephalopathy; newborn; selective head cooling; whole-body cooling

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INTRODUCTION

Despite the advances in perinatal care, asphyxia of neonates remains a serious condition leading to significant mortality and morbidity. According to WHO about 2.6 million of deaths occur annually in the neonatal period. Perinatal asphyxia represents the third most common cause of neonatal death (23%) after preterm birth (28%) and severe infections (26%). It means that all over the world, almost 600 000 newborns die every year and at least the same number develop severe consequences such as epilepsy, cerebral palsy and developmental delay due to acute perinatal sentinel events [1].

In the 90s of the last century, it was confirmed that the decline in brain temperature by 2°C to 5°C provided neuroprotection [2–4]. Encouraging results of the first applications of therapeutic hypothermia in human neonates enabled to construct prospective randomized trials, initially with a control (not cooled) group of asphyxiated newborns. Research results, since the pioneering studies [5, 6] through the multicenter trials, were convincing and led to immediate acceptance of medical devices for TH by the FDA [7–12]. The outcomes of RCT studies confirmed the lower mortality rates and improvement of neurodevelopmental outcomes in cooled neonates. Current guidelines for neonatal resuscitation (since 2010) recommend the use of therapeutic hypothermia as a standard treatment in newborns with moderate or severe hypoxic-ischemic encephalopathy.

The Olympic Cool-Cap providing selective head cooling with mild systemic hypothermia was the first method of cooling approved by FDA in 2006 to be used in newborns with HIE.

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Subsequently, in 2010 Tecotherm Neo device — Inspiration was introduced into the European market and at the same time (2010 and 2014) Arctic Sun TMS and Blanketrol AE; received the acceptance of the FDA. Apart from the Cool-Cap all the other devices mentioned above were dedicated to the second method of hypothermia: whole body cooling (WBC). The indications for hypothermia include: an acute hypoxia incident, Apgar score ≤ 5 in 10 min, resuscitation for 10 min after birth or severe acidosis (pH < 7 or BE > 16 mmol/L) (in umbilical cord blood or in the first 60 min after birth), neurological symptoms of moderate or severe hypoxic-ischemic encephalopathy and pathological aEEG recording [13]. Because of a very short therapeutic window after perinatal sentinel events the treatment with TH should be started in the first 6 hours of life and continued for 72 hours. The inclusion criteria also contain: the maturity above 36 weeks of pregnancy and birth body weight > 1800g. Although whole body cooling is now more popular and more commonly exploited, both methods are used and considered as appropriate and equivalent [14]. So far we have only a few studies comparing the two aforementioned methods of therapeutic hypothermia of newborns [15]. The superiority of either, SBC or WBC, has not been established.

MATERIAL AND METHODS

The prospective observational study was carried out in three centers. Two NICUs at level III perinatal centers and one Pediatric Intensive Care Unit participated in this study. The inclusion criteria were consistent with earlier randomized control trials: post menstrual age ≥ 36 weeks, Apgar ≤ 5 at 10 minutes of life and umbilical cord blood pH < 7.1 and/or base excess (BE) ≥ 16 mmol/L, respiratory support until 10th minute of life. The exclusion criteria involved: major congenital malformations, life-threatening abnormalities and extremely poor prognosis (i.e. Apgar score 0 for longer than 10 minutes of life). Subsequently, all the considered infants were neurologically examined according to Sarnat classification [16]. Newborns with clinical symptoms of moderate or severe HIE were further assessed with integrated EEG by means of infant electroencephalography Monitor Olympic CFM™ 6000, Natus Medical, USA. Moderately or severely abnormal aEEG traces were the entry criteria for therapeutic hypothermia. The selection of the cooling method, either WBC or SHC, depended on the cooling equipment available at the time in the care unit. The study protocol received the ethics approval of the bioethical board — PMMH — Research Institute Ethical Committee. Informed consent was obtained from all the legally designated representatives of the study neonates.

Therapeutic hypothermia protocol

Therapeutic hypothermia was initiated in the first 6 hours of life and continued for 72 hours. The cooling period was followed by a rewarming phase and the rewarming speed was set at 0.5°C per hour. Throughout the course of the cooling and rewarming process, vital signs (heart rate, arterial blood pressure, rectal temperature) and parameters of respiratory support (FiO_2, MAP, level of respiratory support) were monitored and recorded every hour for the first 6 hours, then every three hours, and again every hour during the rewarming. A total of 36 measurements were taken. The vital signs were monitored using Philips IntelliVue MP70 Neonatal Monitors or Drager Infinity® Delta Monitors.

The use of medicines like: vasopressors, anticonvulsants, steroids, volume expanders and inhaled nitric oxide were recorded. Parameters of coagulation were followed closely and the number of blood components transfusions (platelets, fresh frozen plasma, red blood cells) during the first 7 days of life were noted. Additionally, clinical data such as the number of seizure episodes, the incidence of IVH detected by cranial USG, the rate of PPHN confirmed by ECHO exam and feeding difficulties were analyzed. The assessment of the neurological status of the studied infants was carried out daily by trained personnel using the Thompson Encephalopathy Score [17].

Statistical analysis

In order to compare the percent accuracy between groups *Chi-square* or *Fisher* exact *test* were applied. One-way or two-way analysis of variance (ANOVA) was used for continuous data to investigate the differences between groups and changes observed over time. Fishers LSD (least significant difference) post hoc comparison tests were used to identify any significant differences between mean values. Differences in the means of the ranks of the values between groups were analyzed with the Student's t-test or Mann-Whitney U test. Statistical analysis was carried out using STATISTICA (StatSoft, Tulsa, OK, USA, version 6.0 PL). Statistical significance was assumed at p-value < 0.05.

RESULTS

Seventy nine neonates were included in this study. The SHC group consisted of 51 newborns, the WBC group was composed of 27 patients. Table 1 shows demographic and clinical data of the study population.

Groups had similar baseline characteristics and condition at birth. pH, BE values in umbilical cord blood, rectal temperature at admission and neurological assessment on the initiation of the cooling procedure were also similar between the groups. The majority of the analysed patients: 21 out of 27 (77.8%) of patients in WBC and 42 out of 51 (82.4 %) patients in the SHC were outborn so neonatal interfacility transport was used. Emergency C-section procedure rates did not differ between groups: 74.1% vs. 69.8% . Therapeutic hypothermia initiation time equaled

Table 1. Demographic data and clinical characteristics of the study subjects			
	SHC 51	WBC 27	p-value
Postmenstrual age [weeks]; mean (± SD)	38.53 (± 1.7)	38.96 (± 1.9)	0.71
Female gender, number (%)	23 (43.4)	19 (70.3)	0.33
Birth body weight [g]; mean (±SD)	3213.6 (576.2)	3276.9 (625.7)	0.31
Cesarean delivery (%)	37 (69.8)	20 (74.1)	0.69
Apgar score: @ 5 minutes; @10 minutes,	2.84 (± 1.71) 3.54 (± 1.70)	2.11 (± 1.89) 2.92 (± 1.87)	0.08 0.15
Umbilical blood pH[a] (± SD)	6.89 (0.151)	6.87 (0.184)	0.62
Outborns n (%)	42 (82.4)	21 (77.8)	0.63
Base excess (BE)*	-19.92 (± 4.8)	-19.78 (± 4.8)	0.92
Rectal temperature at admission [^{0}C]	33.8 (± 1.81)	33.7 (± 1.63)	0.86
HIES (Thompson scale) at < 6 hrs of age 7th day of life	10.36 1.87	11.06 2.66	0.49 0.75
Neonatal seizures prior to cooling (n; %)	26 (51.0)	7 (26.9)	0.04
TH initiation time [minutes of life]	317 (± 167)	357.0 (± 122)	0.08

The difference p-value of < 0.05 was considered statistically significant.
[a]the worst values: umbilical cord blood or any blood sampling during the first hour after birth
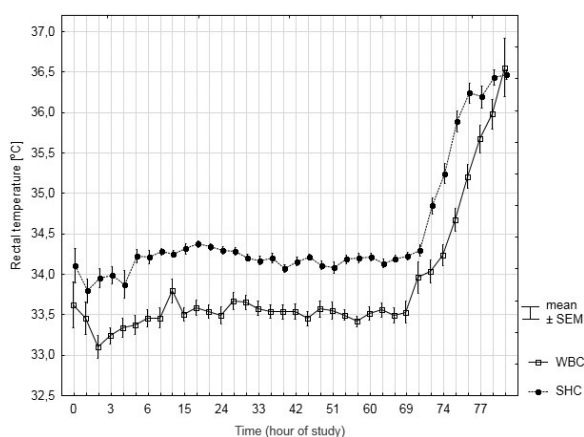
Figure 1. Time sequence of changes in rectal temperature. The average rectal temperature during the maintenance and re-warming phase (WBC vs. SHC)

Figure 2. Mean arterial blood pressure during the whole TH procedure in the WBC and SHC groups [mmHg]

(mean, ± SD) 357 (± 122) and 317 (± 167) minutes in the WBC and SHC respectively (p-value 0.08).

The rectal temperature evidently varied between the groups: during the maintenance phase of hypothermia the average temperature in the WBC group was 33.5°C whereas in the SHC it amounted to 34.2°C (p-value 0.000). During the rewarming phase the differences gradually decreased reaching normothermia (Fig. 1).

In the WBC group the mean HR values equaled 116.4 (SD ± 21.1) bpm, whereas in the SHC group 114.9

(± 20.1) bpm; (p-value 0.58). A similar observation concerned measurements of the mean arterial blood pressure which amounted to (mean ± SD) 51.1 (± 10.2) mmHg in the WBC group and to 49.4 (± 9.9) in the SHC group, however, a tendency to lower MABP was found in the SHC, (p-value 0.099). Nonetheless, the difference between the groups was not statistically significant. Mean arterial pressure (MAP) in the 36 points of measurement in WBC and SHC groups are shown in Figure 2. Time 0 in these and subsequent figures designates the initiation of the cooling procedure.

Table 2. Hospital Course and Status at Discharge. Analysis of drug and blood components administration in the first week of life			
	SHC 51	**WBC 27**	**p-value**
Respiratory support No [%] mean [days] (± SD)[a]	4.8 (± 3.6)	6.0 (± 6.6)	0.37
Noninvasive support [days] mean (± SD)[aa]	0.7 (± 1.3)	1.5 (± 3.6)	0.26
Oxygen therapy [days] mean (± SD)	5.6 (± 7.6)	4.5 (± 3.8)	0.47
Diagnosis of PPHN Administration of inhaled NO (INO)	6 (11.3) 2 (3.8)	5 (18.5) 4 (14.8)	0.38 0.08
Thrombocytopenia 100 000/microliter (µL) Number; (%)	23 45.1	12 45.1	0.93
RBC transfusion (No; No of patients) No/patient	10/6 0.2	7/5 0.28	0.46
FFP transfusion (No; No of patients) No/all patients	20/12 0.39	24/13 0.89	0.36
PLT transfusion No/patient	5/5 0.1	4/3 0.16	0.30
Catecholamine use: none 1 ≥ 2 Mean No of days/all patients	18 (35.3%) 19 (31.4%) 17 (33.3%) 3.14	12 (44.4%) 1 (3.7%) 14 (51.8%) 4.04	0.08 0.19
Volume replacement therapy (No; No of patients) No/patient	5/5 0.2	13/9 0.25	0.20
Anticonvulsant administration (No of patients) No of doses/all patients	40 141/2.8	24 80/2.9	0.94
Antibiotic therapy [days] mean (±SD)	14.5 (± 7.6)	13.1 (± 6.8)	0.43
Full enteral feeding Oral feedings (sucking) [days] mean (± SD)	14.5 (± 5.9) 16.7 (± 10.2)	14.7 (± 5.1) 14.3 (± 5.4)	0.92 0.37
Age at discharge/DOL mean (± SD)	27.26 (± 13.0)	21.91(± 11.3)	0.09
Death during hospital stay n (%)	3 (6.0)	2(8.3)	0.71

The p-value of < 0.05 was considered statistically significant
[a]any type of mechanical ventilation (SIMV, PC, HFOV)
[aa]any type of noninvasive respiratory support (noninvasive ventilation, nasal CPAP)

The analysis of the frequency of inotropes use did not reveal significant changes. Additionally, the mean number of days of inotropes administration calculated on the number of people in the group was not statistically significant (Tab. 2) .

When analyzing the course of hospitalization and the rate of complications, no differences were found. The respiratory support and oxygen supplementation time were not significantly different. However, the rate of neonates who required any respiratory support (MV or noninvasive support) between 36–80 hour of cooling was significantly higher in the WBC vs. SHC group, 83.6% vs. 72 % respectively; (p-value 0.047). During the whole therapeutic hypothermia procedure the fraction of inspired oxygen (p-value 0.99) and mean airway pressure (p-value 0.68) did not differ between the groups.

The rate of PPHN decreased and the need for inhaled nitric oxide (INO) was reduced three-fold in the SHC group but the rate of its incidence was very low. The average platelets count in all controlled tests was similar in both groups. In the WBC group a statistically significant decrease in mean PLTs values was observed on the 2nd, 3rd and 4th days compared to baseline values in the first 24 hours. The reduction in the following days reached 38% of the initial value. A smaller reduction in average platelets values was observed in the SHC group, by only 21%. The incidence of
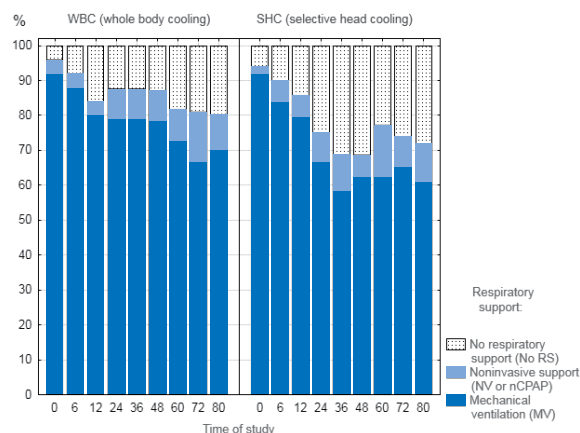
Figure 3. The mode of respiratory support in the WBC and SHC groups during the whole TH procedure. Any type of applied mechanical ventilation (SIMV, PC, HFOV) and noninvasive respiratory support (noninvasive ventilation, nasal CPAP) were analyzed jointly

thrombocytopenia 100×10^9/L found in each of the groups did not differ statistically significantly; (p-value 0.93). Also, the number of transfusions of different blood components *(fresh frozen plasma, erythrocytes and platelets)* did not differ significantly (Tab. 2).

Results of neonatal neurological status assessment performed daily according to Thompson scale are presented in Figure 3. On the following days of the treatment, to the fifth day of study (after the hypothermia was completed), the score was comparable in both groups. The rate of neurological status improvement expressed as a percentage decrease in Thompson scores was slightly better in SHC i.e. by 83.1% vs. 74.3% in WBC group in comparison with initial values.

DISCUSSION

Currently, therapeutic hypothermia is a standard treatment of hypoxic-ischemic encephalopathy in neonates [18]. The SHC was the first method of therapeutic hypothermia utilized in the neonatal period. The only device is a system utilizing a special cooling cap to provide selective brain cooling while maintaining core temperature at safe levels through the use of a radiant warmer. The Cool-Cap works by maintaining a steady flow of cool water at a selected temperature through a plastic cap covering the infant's head. The temperature is manually controlled. In this way the newborn's brain is the coldest part of the body. The rectal temperature is maintained at the level of 34.0–35.0°C.

When using the second method, whole body cooling (WHC), the temperature distribution is reversed. In such case, hypothermia is delivered in *newborn* infants using different types of *mattresses* or *wraps* around the body. The newborn's brain cools backward through transfer of low temperature from the rest of the body. The temperature of the neonatal brain is probably higher compared to the tem-

perature in the rectum. Therefore, the target rectal temperature in the WBC method is slightly lower and maintained at the level of 33.5 ± 0.5°C. Nowadays, the whole body cooling method seems more popular and more often used. However, both methods are used as before and considered equivalent in terms of effectiveness. Because the target ranges of deep body temperature are different, many users pose a question of the side effects as well as the effectiveness of both methods. In the animal model (piglet), which has a much smaller head compared with the human newborn infant, using SHC it was possible to cool the basal ganglia to the temperature lower by 5°C than rectal temperature [18], however, in adults, a considerable temperature gradient between the surface of the head and deep brain structures, where the temperature is similar to rectal temperature (Trec), was observed. Therefore, deep brain temperature remains almost equal to core temperature even when extreme cold is applied to the surface of the head [19]. It seems possible that the temperature gradient between head surface and deep brain structure could be substantial and may have an influence on regional brain temperature. Theoretically, it is possible that fluctuations in Trec and, in consequence, brain temperature may adversely affect the neuroprotective effect of TH. There is evidence that brain temperature is an important factor in regulating BBB permeability, alterations in brain water homeostasis and subsequent structural abnormalities of brain cells [20]. So far, we have only a few studies comparing the two methods of therapeutic hypothermia employed in neonatal period [15].

As expected, in our studied group differences in Trec were observed. The mean Trec in WBC group was 33.5°C and was the same as noticed in TOBY trial [21]. In the SHC group mean Trec was only 34.2°C, which means that the differences were smaller than expected and amounted only to 0.66°C. It may express the tendency of the medical team to maintain a target temperature in the lower ranges in manually controlled devices. The stability of vital parameters: mean target Trec, ABP, and HR at hourly intervals were previously assessed by Hoque et al. [22]. They compared four groups of newborns: 1 group treated with SHC, and 3 groups receiving different variants of WBC. The authors did not find differences in mean ABP or mean HR between groups during the maintenance phase of cooling. They noticed greater variation in Trec in the SHC group during rewarming phase. No other clinical outcomes were analyzed.

Earlier studies have shown the effect of hypothermia on the frequency of inotropic agents used [7]. Meta-analysis of previous RCT (TH vs. control group) assessed cardiovascular effect and revealed increased risk of hypotension treated with inotropes in hypothermia group RR 1.17 (95% CI 1.00, 1.38), RD 0.08 (0.00, 0.17) [23]. In our research, despite the obvious depth temperature measurements (Trec) dif-

ferences, we did not notice differences in the mean HR as well as mean ABP between groups. The rate and duration of inotropes administration were also similar. This observation is consistent with reports by other experts [24]. Although the SHC group was cooled using the standardized Cool Cap device, the low-tech method (cooling the baby's environment using a room air conditioner) was used in the WBC group. No differences in the following clinical parameters: low blood pressure, bradycardia, thrombocytopenia, coagulopathy, renal failure, necessity of respiratory support and discontinuation of mechanical ventilation or PPHN were identified during the cooling and rewarming procedure. Likewise, a non-randomized trial conducted at University of Michigan (WBC-28, SHC-31) confirmed that important components of multi-organ system dysfunction and hypotension treated with inotropes for > 24 h, commonly required in newborns with HIE, were similar for both methods of TH [25, 26].

The requirement for invasive mechanical ventilation, ability to perform extubation during the period of cooling and risk of PPHN were analyzed in two studies [25, 27]. No differences were found between groups in regard to the need of mechanical ventilation during the cooling or ability to be extubated during the cooling. In both analyses the rate of mechanical support while the cooling was being carried out was above 90%. In addition, the possibility of extubation and newborns breathing on their own during the cooling procedure was confirmed (WBC 42.8% vs. SHC 37.9%). The highest values of peak inspiratory pressure (PIP), positive end-expiratory pressure (PEEP), FiO_2 (fraction of inspired oxygen), and $PaCO_2$ (partial pressure of carbon dioxide) were investigated during the cooling procedure. No differences were found in the above mentioned respiratory settings between WBC and SHC groups [28]. Our findings are consistent with previous studies. Initially mechanical support was required by 92% of neonates in both groups, but at 72 hours of cooling artificial ventilation was used only in 65.2% SHC vs. 66.6% WBC. As was the case in the literature quoted earlier, we did not observe significant differences between analyzed groups. (Fig. 4, Tab. 2). In all three studies, the PPHN risk was higher in the WBC group but it did not reach statistical significance.

Thrombocytopenia is one of the most frequently reported side-effects of therapeutic hypothermia and its incidence is significantly different and varies from 3–65%. Differences are probably related to definitions of thrombocytopenia and differences in timing of platelet count assessment [28]. Meta-analysis of four trials [5, 6, 19] showed statistically significantly increased thrombocytopaenia in the hypothermic groups (RR: 1.55, 95% CI: 1.14, 2.11, NNH: 8, 95% CI: 5, 50) [29]. The next analysis (8 RCT) confirmed a risk of thrombocytopenia in the hypothermia group RR
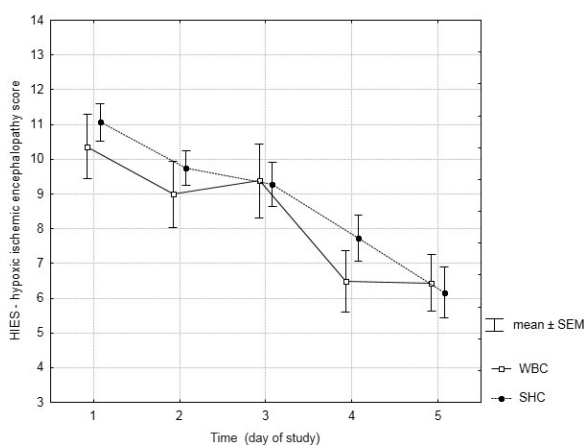
Figure 4. HIES — hypoxic ischemic encephalopathy score during the 5th first days of life

1.21 (95% CI 1.05–1.40) [23, 30]. The observed difference suggests an additional effect of hypothermia on platelet count in infants with perinatal asphyxia. A slight statistical increase in the risk of thrombocytopenia was detected in the group of infants treated with SHC [23]. In our study the frequency of thrombocytopenia was 32.7% (SHC) vs. 37% (WBC) and no statistical differences were observed. The aforementioned research conducted by Atici did not notice the differences in the risk of thrombocytopenia either [24]. Moreover, other researchers reported no differences in organ failure parameters including haematological, coagulogical and cardiovascular ones [31].

The same team of researchers conducted a small randomized study (17 in SHC vs. 12 in WBC group) and recorded the death rate at 41 vs. 33% and survivors without disability of 18 vs. 33% in the SHC and WBC respectively [32]. However, the differences did not reach the level of statistical significance, which may be ascribed to a limited study group. Finally, the same authors analyzed certain neural and inflammatory biomarkers. The serum interleukin (IL)-1β, IL-6, neuron-specific enolase (NSE), brain-specific creatine kinase (CK-BB), tumor necrosis factor-alpha (TNF-α), protein S100 levels, the urine S100B level and the urine lactate/creatinine (L/C) ratio were evaluated 6 and 72 hrs after birth. They did not observe significant differences between groups at assessed time points, neither did they find such differences when the changes between 6 and 72 h were determined in percentages [33].

Consistently with previous studies, also in our analysis no method has been proven superior. Although our research included the largest number of newborn infants investigated so far, we did not observe additional clinical benefit of WBC technique. Despite the lack of clinical advantages, the simplicity of usage of WBC (due to servo-control) should be emphasized.

The limitation of this study was a lack of randomization and a smaller sample of WBC group.

CONCLUSIONS

Both methods of therapeutic hypothermia are comparable. We did not find significant differences in short term outcomes and the risk of adverse effects between SHC vs. WBC in newborns with HIE. Our results require confirmation in the follow-up observation. The tendency to higher rate of PPHN in WBC group should be confirmed in subsequent studies

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ORIGINAL PAPER / OBSTETRICS

Exposure to dolutegravir in pregnant women living with HIV in Central and Eastern Europe and neighboring countries — data from the ECEE Network Group

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ABSTRACT

Objectives: The purpose of this study was to investigate dolutegravir (DTG) use among women and exposure to DTG during pregnancy in real world in Central and Eastern Europe and neighboring countries.

Material and methods: Centres from 20 countries included in the Euroguidelines in Central and Eastern Europe (ECEE) Network and Finland were asked to complete an on-line questionnaire.

Results: Seven centres from Czech Republic, Finland, Greece, Poland, Slovakia, and Turkey provided detailed information. DTG exposure was reported in 415 women, of which 26 were during pregnancy. Of those, 22 were on DTG at the time of conception and 4 had started DTG during pregnancy. Few women had conventional risk factors. The data on folic acid usage was unknown for eight women; 14 were using and four were not using folic acid. Four pregnancies were ongoing at the time of the study and of those with an outcome, 77.3% resulted with term, 13.6% preterm delivery, 4.5% spontaneous and 4.5% medical abortion.

Conclusions: The DTG signal report indicates the importance of safety research for drug use in pregnancy and highlights the urgent need for systematic surveillance of pregnancy outcomes and neonatal surveillance. Countries with low- or moderate HIV prevalence should be included in studies reviewing pregnancy outcomes and in any surveillance system to ensure the accuracy of drug safety revision.

Key words: HIV infection; dolutegravir; pregnancy; Central and Eastern Europe

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INTRODUCTION

Integrase strand transfer inhibitors (InSTIs) have become the mainstay of antiretroviral treatment (ART) recently owing to their high virologic activity, durability, minimized drug-drug interactions, low adverse event profile, and tolerability [1]. They are recommended as first-line antiretroviral drugs to be included in the treatment regimens of ART-naive patients in major guidelines [2, 3] and becoming more widely accessible across national guidelines in the Sentral and Eastern European (CEE) region [4]. Dolutegravir (DTG) has been studied extensively among adults and children living with HIV and has replaced efavirenz (EFV) in the latest updated World Health Organization (WHO) guidelines [3]. However, the interim analysis of the Tsepamo study comparing DTG with EFV revealed a higher rate of neural tube defects (NTDs) in infants born to mothers using DTG at conception compared

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to those using EFV or non-DTG-containing regimens [5]. This unexpected finding resulted in safety warnings for the use of DTG in pregnant women and women of childbearing age, which restricted its use significantly [6]. It is estimated that restricting DTG use may compromise the overall benefit and access to modern combination ART for women living with HIV [7]. In order to balance the risks and benefits at the population level it is necessary to assess what the size of the population at risk is i.e. the number of women of childbearing age and the number of women conceiving on the drug.

Objectives

The aim of this study was therefore to investigate DTG use among women and exposure to DTG during pregnancy in real world in Central and Eastern Europe and neighboring countries where epidemiological data are few and HIV prevalence is low- or moderate-level.

MATERIAL AND METHODS

Centres from 20 countries included in the Euroguidelines in Central and Eastern Europe (ECEE) Network along with Finland were approached in June 2018 and were asked to complete an on-line questionnaire about DTG availability, the scale of its use among women, and exposure to DTG during pregnancy (Tab. 1). DTG was available in twelve centres, and eight centres reported use of DTG in pregnant women. Seven centres from six countries (Czech Republic, Finland, Greece, Poland, Slovakia, and Turkey) provided detailed information on the use of DTG during pregnancy, and data on other risk factors. Follow-up was censored at 31st July, 2018.

RESULTS

A total of 415 women were exposed to DTG in seven centres and 26 were exposed during pregnancy. Of those, 22 were on DTG at the time of conception and 4 had started DTG during pregnancy. In only one patient who conceived a month before the warning for DTG use in pregnancy was released, the ART regimen was switched to darunavir/ritonavir + raltegravir (RAL). The remaining patients stayed on DTG.

Few women had conventional risk factors. Only one woman continued to use psychoactive substances and alcohol during pregnancy. Two women stopped psychoactive drugs and one alcohol after they became pregnant. Four were smokers at the time of conception; two of those stopped after being pregnant.

The status of acute or reactive TORCH diseases was available for 23 women and for all it was negative. Hepatitis C antibodies were negative for 23 women, two had positive antibodies with confirmed HCV RNA positivity, and one had positive antibodies but an unknown RNA status.

Other concomitant illnesses included three hypothyreosis, one autoimmune hepatitis, one insulin-dependent diabe-

tes, and one systemic lupus erythematosus. One woman had gestational diabetes stage A and one Rh incompatibility.

The data on folic acid usage was unknown for eight women, 14 were using and four were not using folic acid.

The median number of prior pregnancies was one [interquartile range (IQR): 0–3]. Six had a prior caesarian section (CS), six were reported to have an induced abortion, and four a spontaneous abortion when HIV positive.

Four pregnancies were ongoing at the time of the study. Of the 22 pregnancies with an outcome, there were 17 (77.3%) term deliveries, three (13.6%) preterm deliveries, one (4.5%) spontaneous and one (4.5%) medical abortion.

DISCUSSION

This study is the first to report on DTG use among women and during pregnancy in ECEE Network countries showing that a substantial number of women were exposed to DTG in the region. The introduction of InSTIs to the market in Central and Eastern Europe, especially in low and lower-middle income countries was delayed. In our study only 12 out of 20 centres had access to the drug, all of which are high-income or upper-middle income countries. There seems to be no improvement over time, as similarly in a study from 2015 including 24 countries from the Eastern and Central Europe, DTG was available in half, RAL in 70%, and elvitegravir fixed-dose combination only in 20% and again the majority (75%) was high or upper-middle income countries [8]. The relatively high number of women exposed to DTG in our study, is an indicator that DTG has become a preferable antiretroviral drug in the region.

Initially a few small cohorts from Europe and North America and a larger series from Botswana (the Tsepamo study) reported no evidence of increased birth defects in infants born to mothers who conceived while using DTG [9–13]. However, in April 2018, an unplanned interim analysis requested by the WHO of the Tsepamo-cohort revealed an unexpectedly higher rate of NTDs among infants born to mothers who conceived on DTG compared to those using non-DTG regimens (0.94% and 0.12%, respectively) [5].

This unexpected finding resulted with a warning message from the European Medicines Agency (EMA) and the WHO for designing ART regimens for women of reproductive age and pregnant women based on drugs with reliable efficacy and safety data such as an efavirenz–based regimen. [6, 14]. Similarly, all major guidelines issued new recommendations regarding DTG use in women of childbearing age and in pregnant women [2].

In African countries the warnings on DTG resulted in almost immediate banning of the drug use among women of reproductive age [15]. Shutting off access to a superior regimen without extensive discussions with the relevant party was considered unacceptable and was widely criticized by

Table 1. Questionnaire used for data collection		
Question	**Response choice**	**Open ended question**
Number of women on DTG in your centre:	Number	n/a
Number of women who got pregnant while being on DTG:	Number	n/a
Number of women who started DTG during pregnancy:	Number	n/a
Date of starting DTG:	Date/Time	
If DTG was switched provide date of stopping DTG:	Date/Time	
If DTG was switched provide name of new ARVs:	Text	Yes
NNRTI used in this pregnancy:	Text	Yes
Concurrent use of other medication (not ARV):	Yes (please specify)/no/unknown	Yes
Concurrent use of folic acid:	Yes (please specify)/no/unknown	Yes
Any health problems during pregnancy:	Yes (please specify)/no/unknown	Yes
HCV status:	HCV antibodies negative	No
	HCV antibodies positive	
	HCV antibodies positive confirmed with HCV RNA	
	HCV antibodies positive but HCV RNA not performed	
Date of pregnancy start:	Date/Time	n/a
Number of pregnancies:	Number	n/a
Outcome of past pregnancies (please describe):	Text	Yes
Outcome of pregnancy exposed to DTG:	Term delivery	Yes
	Preterm delivery (< 37 gestation week)	
	Abortion	
	Other (please describe)	
Newborn outcome:	Text	Yes
Smoking:	Yes and continued in pregnancy	No
	Yes, but stopped at the begining of pregnancy	
	No	
Alcohol:	Yes and continued in pregnancy	No
	Yes, but stopped at the begining of pregnancy	
	No	
Psychoactive substance use:	Yes and continued in pregnancy	No
	Yes, but stopped at the begining of pregnancy	
	No	
TORCH diseases:	Yes (please specify)/no/unknown	Yes
Date of delivery:	Date/Time	n/a
If not yet delivered - date of expected delivery:	Date/Time	n/a

ARV — antiretroviral; DTG — dolutegravir; HCV — hepatitis C virus; NNRTI — non-nucleoside reverse transcriptase inhibitor; RNA — ribonucleic acid; TORCH — toxoplasma-other-rubella-cytomegalovirus-herpes

women's organizations in Africa [15]. A modelling study to compare various scenarios of ART policy in a hypothetical southern African population showed that the benefits of DTG should not be sacrificed for the potential risk of NTDs in a woman who has not conceived yet [7]. Although the HIV epidemic in ECEE Network is concerning in terms of women, the implications of such an approach has not been discussed for this region. Most of the Central and Eastern European region has experienced a low- or moderate-level epidemic and thus has not received enough attention for

many years [4, 16]. However, in many of those countries the epidemic is rapidly rising and mother-to-child transmission is still of concern [17]. DTG-based therapy proved to be effective in achieving rapid viral suppression in pregnant women, even in late presenters and those with highly resistant virus, and in preventing mother-to-child transmission. This makes it an attractive choice in many settings including pregnancy [12, 18–21]. DTG is becoming a preferred antiretroviral drug in the region and reliable data are required urgently for such settings.

In the general population NTDs were responsible for the highest disability and mortality rate among all birth defects. Although the etiology of NTDs is not clear yet several factors such as genetics, nutrition, and environmental factors were suggested. Two randomized controlled studies and several observational studies showed that NTDs were prevented by 50% if women consumed a folic acid-containing supplement before and during early pregnancy [22, 23]. An important finding in our study was that almost half of the women who conceived on DTG were either not using folic acid or had no available data on its use, which seems like a significant gap in prevention of NTDs. In addition to folic acid depletion, obesity, poor control of diabetes mellitus, and use of some anti-epileptic drugs were also associated with a higher risk of spina bifida or anencephaly in the infant [24–28]. In our study concomitant illnesses reported among pregnant women exposed to DTG were three hypothyreosis, one autoimmune hepatitis, one insulin-dependent diabetes, and one systemic lupus erythematosus. One woman had gestational diabetes stage A and one Rh incompatibility. We would like therefore to highlight the importance of complete assessment of medical history in women exposed to antiviral drugs in pregnancy. Neglecting to collect and analyze these data may result in overestimating the toxicity related to antiretroviral drugs and undermining women's rights to best possible standard of care [29]. The latest results of the Tsepamo study from Botswana suggest that although lower than the previous rate (0.67%), the increased risk still remains. On the other hand, these findings were not yet replicated either by case series, observational cohorts or joined pregnancy surveillance reports [12, 30–33]. Therefore, the current scene is that the controversy has not resolved yet.

The DTG story indicates the importance of safety research for drug use in pregnancy. Moreover, it highlights the urgent need for systematic surveillance of pregnancy outcomes and neonatal surveillance. Mechanisms ensuring such reporting with necessary quality, *e.g.* excluding double reporting, are necessary to ascertain prolonged benefit from ART irrespective of gender [34]. Another subject of discussion was that in order to refute an NTD signal a large number of preconception exposures would be required. As estimated by Schomaker et al [35] if one new defect would be reported we would need 2000 exposures to see a lower confidence interval to overlap non-DTG prevalence. Therefore countries with low- or moderate HIV prevalence should be included in studies reviewing pregnancy outcomes as well as in any surviellance system in order to ascertain the completeness of drug safety revision.

Conflict of interests

On behalf of all authors, the corresponding author states that there is no conflict of interests.

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ORIGINAL PAPER / OBSTETRICS

Neonatal survival and kidney function after prenatal interventions for obstructive uropathies

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ABSTRACT

Objectives: Prenatal interventions in LUTO (lower urinary tract obstruction) usually are still question of a debate between gynaecologist and paediatric nephrologist. We aimed the study to assess the early survival rate and renal outcome in LUTO foetuses.

Material and methods: The study was a prospective data analysis of 39 foetuses from singleton pregnancies. All pregnant women with LUTO in the foetus were qualified for VAS based on a local practice. The mean time of first urine analysis ranged between 13–30 weeks of pregnancy. Primary end-point analysis included live birth, 28d-survival, pulmonary and renal function assessment in neonatal period.

Results: From initial number of 39, six patients miscarried before the procedure was performed. Overall, 33 VAS were performed at the mean 21 week of pregnancy (range 14–30 weeks). 25/39 foetuses survived until delivery. Three neonates died in first 3 days of life. In the first month 3 children required peritoneal dialysis, but at 28 day all children were dialysis-free. Overall survival rate at 28 day was 56%. Renal function preservation of the initial group (39) turned out to be low — 18% (7/39).

Conclusions: Our study showed average survival curves and complications. LUTO in the foetus had mostly unfavourable outcome in the neonatal period. The prenatal intervention did not increase it significantly and did not guarantee the preservation of normal kidney function.

Key words: obstructive uropathy; posterior urethral valves; vesico-amniotic shunting; kidney function; prenatal; neonate

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INTRODUCTION

Proper diagnosis and prenatal treatment of foetal lower urinary tract obstruction (LUTO) still constitutes a great challenge for obstetricians and neonatologists despite the significant progress in last 30 years [1, 2]. Clinically significant LUTO is detected in 1:10–20 000 pregnancies [3] . Male foetuses with posterior urethral valves (PUV) prevail, but female foetuses may also develop LUTO due to urethral stenosis or atresia [3, 4].

According to the latest studies success rates measured by 2-y survival ranges from 21–72%, depending on the study intervention [5, 6]. It might be assumed that the long-term renal function depends mostly on the number of nephrons, dysplastic and cystic changes that had occurred before urine

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production start. This may be related to multiple genetic, epigenetic and environmental factors of early pregnancy, before any intervention is possible [7].

Prenatal interventions in LUTO usually comprise vesi-co-amniotic shunting (VAS) or laser valve ablation, which in selected cases are accompanied by amnioinfusions. These procedures may prevent pulmonary hypoplasia and progression of irreversible renal damage [3]. There was at least one attempt to prove the effectiveness of VAS in randomised controlled trial, but it did not show enough strength to draw firm conclusions [8]. However, some observational data and metaanalyses suggested that VAS may improve perinatal survival, but without significant influence of renal survival [9].

Because of the lack of randomised control trials and controversies about the interventions, there is still a place for observational trials in this field. Thus, the aim of our study was to assess the neonatal outcome in an unselected cohort of prenatally detected LUTO qualified for VAS.

MATERIAL AND METHODS

The study was a prospective observational trial (y.:2016–2018) led in the tertiary multidisciplinary reference centre on neonatal outcome in the cohort of foetuses diagnosed prenatally with LUTO and qualified for prenatal intervention. The study team comprised of 10 physicians (obstetricians, neonatologists, urologists and nephrologists), who were trained in the protocol.

The study was accepted by the Local Ethics Committee of Polish Mother's Memorial Hospital Research Institute (No:1/2016) and conducted according to the Declaration of Helsinki. All the pregnant women, and later parents and guardians of the neonates gave informed consents for participation in the study.

Inclusion criteria of the study were:
1. obstructive uropathy by ultrasound with megabladder detected in at least 2 separate examinations with or without hydronephrosis, with normal or reduced amniotic fluid volume

2. singleton pregnancy
3. patient consent for prenatal intervention

Foetuses with multiple genetic abnormalities and/or abnormal karyotype were excluded from further evaluation.

Study group characteristics

All the patients diagnosed with LUTO between January 2016 and December 2017 in whom the LUTO was detected by repeated ultrasound were offered a prenatal intervention of VAS and a prenatal and postnatal follow-up in the reference centre. The decision of prenatal intervention was based on clinical experience of an experienced obstetric team and the parents (local criteria for intervention: enlarged bladder, reduced amniotic fluid, hydronephrosis — uni or bilateral). The urinary analysis was done but did not constitute a contraindication for the intervention. Parenchymal kidney changes were noted but did not influence the qualification. Finally, 39 patients entered the study. Clinical characteristics of patients is presented in Table 1.

Preoperatively, a detailed ultrasound examination (Voluson E8, GE Healthcare) was carried out to confirm the diagnosis of obstructive uropathy and exclude any other major defects. Obstructive uropathy was diagnosed by the presence of enlarged bladder (megabladder) with or without "keyhole" sign. A diagnostic "keyhole" sign is seen in PUV, indicating continuity between distended bladder and the dilated posterior urethra proximal to the valves. Changes in renal parenchyma — increased echogenicity or structure and cyst formation were also described (Tab. 2). Oligohydramnios was defined by single deepest pocket of less than 2 cm.

Vesico-amniotic shunting procedure

Ultrasound scanning was used to obtain a transverse section of the enlarged bladder and define the appropriate site of entry on the maternal abdomen which was infiltrated with local anesthetic (10 mL of 1% lignocaine) down to the myometrium. Under continuous ultrasound guidance, the

Table 1. Prenatal characteristics of the study group. Continuous data presented as median value and min–max range)				
	All foetuses N = 39	Survivors at 28d. n = 22	Non–Surivors n = 17	Statistical difference
Mother age [y]	29 (18–38)	29 (18–38)	29 (19–37)	> 0.05
Pregnancy	1.5 (1–5)	2 (1–5)	1.5 (1–3)	> 0.05
Week of diagnosis of LUTO	18 (12–30)	18 (13–30)	17 (12–21)	> 0.05
AFI [cm]	6 (0–21)	8 (0–21)	3 (0–21)	0.04
Week of intervention – shunting	21 (14–30)	22 (14–30)	20 (16–23)	0.06
Number of shunts/patient	1.5 (1–5)	2 (1–5)	1.5 (1–3)*	> 0.05
Need for amnioinfusions	18/39	12/22	6/17	NA

* only 11 were shunted; AFI — amniotic fluid index; LUTO — lower urinary tract obstruction

Table 2. Initial description of ultrasound images in the study group

Megabladder only	Megabladder and hydronephrosis	Only bilateral hydronephrosis	Renal parenchymal changes — echogenicity	Renal cysts present	Renal cysts or changes in echogenicity	Oligohydramnios
19/39 51%	14/39 36%	5/39 13%	22/39 56%	25/39 64%	29/39 74%	30/39 85%

Table 3. Biochemical predictive factors in the fetal urine analysis (median and min–max range)

	All subjects	Survivors at 28 d	Non–survivors	Statistical difference
sodium [mmol/L]	104 (50–146)	91 (50–146)	116 (50–127)	0.17
chloride [mmol/L]	84 (5–145)	80 (5–146)	95 (66–114)	0.17
B2–microglobuline	7.1 (0.4–24.7)	6.7 (0.4–17.0)	10.7 (2.2–24.7)	0.14
Osmolarity [mOsm/kg H_2O]	223 (96–289)	187 (96–289)	241 (164–264)	0.08

shunt (diameter 2 mm, length 12 cm; Rocket KCH Fetal Bladder Catheter, Washington, United Kingdom) was inserted.

In the foetal urine selected markers of prognosis were assessed: sodium (normal < 100 mmol/l), chloride (normal < 90 mmol/L), β2microglobulin (SO-314.501 LIAISON beta 2- microglobulin; normal < 4.0 mg/L) concentration and urine osmolarity (normal < 200 mOsm/kg/H_2O). The values of these predictive indices in the study group are given in Table 3. They were analysed according to the criteria cited in the last review by Smith-Harrison [3]. Pregnancy duration and delivery procedures were analysed according to the outcome (demise, termination, still birth, live birth).

Neonatal assessment

After delivery the neonate was assessed with basic anthropometry and Apgar score. On the 4th day clinical and biochemical variables were analysed: survival renal function (eGFR and absolute creatinine [10]), albuminuria (albumin/creatinine ratio: ACR) and urine output, pulmonary function (ventilation dependency, clinical and radiological signs of pulmonary hypoplasia) and central nervous system (CNS) injury. Within first 28 days LUTO was confirmed/excluded by micturating cystogram/cystoscopy. Urologic interventions also were assessed. Clinical data were gathered with regard to the urine output, ventilation support and vasopressors need. Blood pressure was measured with oscillometric devices to exclude hypertension.

Urinary tract was assessed by a radiologist experienced in paediatric ultrasound. The Samsung RS80 device with convex/microconvex and linear probes were used.

Kidney injury was measured by serum creatinine (ELISA) compared with reference values of Rudd et al. [10] eGFR calculation was performed according to the Schwartz formula (with k value of 0.33 for neonates) and presence of albuminuria (albumin/creatinine ratio — ACR) [11]. Albumin excretion was compared to the data obtained in preterm

babies from the study of Gubhaju et al [12]. Children were tested for presence of acidosis. The study had 2 primary endpoints: survival and kidney injury symptoms at 28th day of life.

Statistical methods

Data analysis was performed using Statistica package version 12 (StatSoft Inc., USA) with medical add-in. All parameters were tested for normal distribution using Shapiro-Wilk W test and were presented as means with standard deviation. Comparisons of differences in characteristics between two groups (survivors and non-survivors) were performed using the Student's t-test and Mann-Whitney U test depending on the distribution of data. The differences between proportions were evaluated by chi-squared test.

The assessment of the validity of prenatal markers for 28-day survival was performed with discriminative analysis and a probabilistic predictive Naïve Bayes model. A Wilks lambda test was used to test for significant differences between the groups on the individual predictor variables. A significance level of $p < 0.05$ was considered significant. A receiver operating characteristic (ROC) curve with sensitivity and precision were applied to examine the correctness of the predictive model. The Kaplan-Meyer estimator was used for estimation the survival function.

RESULTS

Amongst the study group all pregnant women accepted the prenatal intervention. However, six patients miscarried before the procedure was performed. Overall, 33 VAS were performed at the mean 21 week of pregnancy (range 14–30 weeks). No pregnancy lost was noted as a complication of prenatal interventions. In 6 cases there was a dislodgement/blockage of the shunt and procedure was repeated. Mean number of interventions was 1.5/foetus (range 1–5) — detailed data are presented in Figure 1. In 18 cases prior
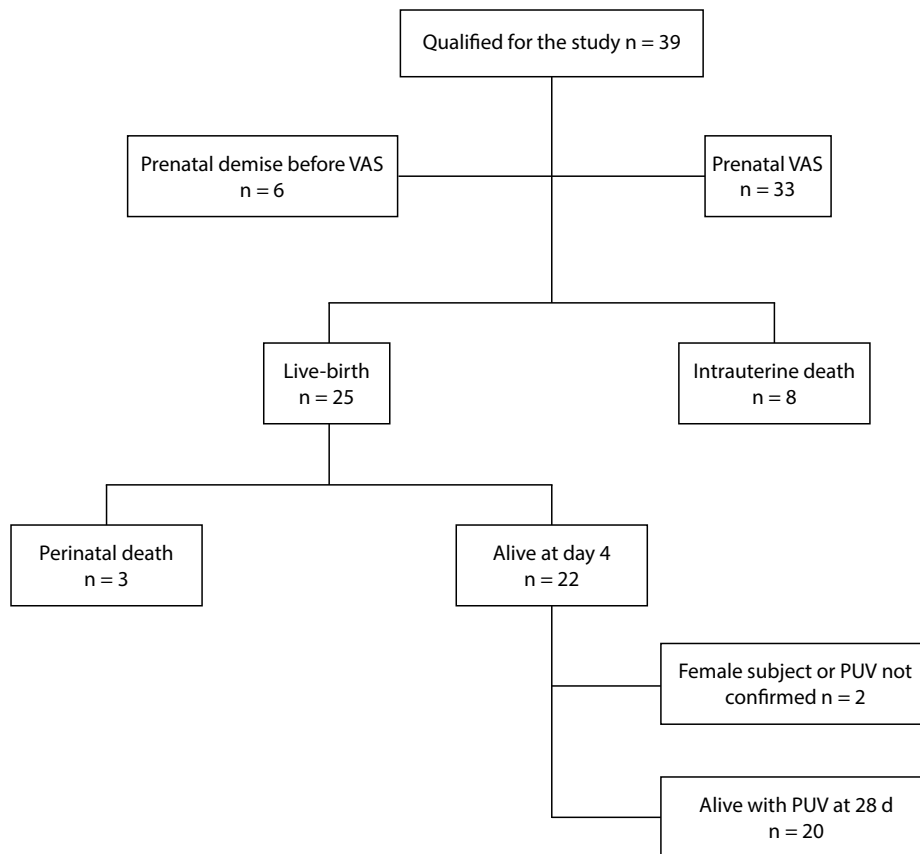
Figure 1. Outcome flowchart in the study group

to shunt insertion, amnioinfusion was performed due to technical difficulties (anhydramnios). Clinical characteristics of pregnancies are presented in Table 1.

20 patients were confirmed by postnatal evaluation to have PUV. In 10 foetuses diagnosis was confirmed by pathomorphology evaluation. There were also 1 urethral atresia (UA, in female) and in one no significant malformation of urinary tract was detected. There were 8 intrauterine deaths at mean age of 26 hbd (range 18–29). The deaths were noted with functioning shunts in a mean time of 3 weeks after shunting. 25 children were born at the mean age of 34 weeks (range 24–39 hbd) with mean Apgar score of 8 (range 1–10).

Anthropometrical, clinical and biochemical data of the live-born children with comparison of non-survivors and survivors at 28 day are presented in Table 4. We observed that those neonates who died after birth had lower birth age, body mass, head circumference and Apgar score.

Radiological and clinical investigations confirmed LUTO in 24/25 newborns (PUV-23, UA-1). 6 children had urinary tract diversions in neonatal period and 16 cystoscopy with electroresection of the PUV within 28 days. No patient had hypertension. 21/25 required intensive care with cardiac support with vasoactive amines and ventilation support (for the mean 2.4 days — range 1–20). Mean ICU stay was

36 days (1–150 d.). Radiological features of lung hypoplasia were detected in 6/25 neonates. We noted 3 deaths within first 3 days of life because of cardio-pulmonary insufficiency with lung hypoplasia. At 4th day 18/22 required mechanical ventilation but at 28th day all children were free of mechanical support. In 2/22 clinical features of pulmonary hypoplasia were present at this time. No child amongst those who survived required oxygen supplementation at the end of neonatal period.

Renal function was compromised in 13/22 of subjects who survived with mean eGFR on 4th day of 16.2 (4–47) mL/min/1.73 sqm. Serum creatinine was elevated also in 13/22 when compared to the reference from 7th day published by Rudd. [10]. 12 out of 22 neonates had significant acidosis and 11/22 proteinuria (mean: 102; r: 25–339 mg/dL). ACR was significantly elevated in 86% of cases (Tab. 4).

Three out of 25 (12%) children were treated with peritoneal dialysis at 4th day (for 3 to 19 days) – and all survived, while no one required it at the 28th day because of renal function improvement.

Overall survival rate at 28th day was 56% among whole LUTO group of patients. In PUV patients it was lower 20/37 — 54%, but in those who underwent VAS — 20/30 — 66%.

Table 4. Clinical and biochemical characteristics of neonates from the study group (median and min–max range)

	All subjects	Survivors at 28d	Non-survivors (demise/ stillbirth/death)	Statistical difference
OU confirmed	38/39	21/22	10/17*	NA
Gender (M:F)	38/1	21/1	17/0	NA
Birth age [weeks]	33 (24–39)	35 (29–39)	30 (24–32)	0.001
Apgar score at 5 min.	8 (1–10)	9 (4–10)	6 (1–6)	0.001
Weight [g]	2.4 (0.5–4.4)	2.6 (1.5–4.4)	1.8 (0.5–1.9)	0.005
Lenght [cm]	47 (28–57)	49 (41–57)	41 (28–42)	0.002
Head circumferrence	31 (15–38)	32 (27–38)	30 (15–32)	0.002
Ventilation support after birth [%]	21/25	18/22	3/3	NA
Oxygen dependency at birth [%]	24/25	21/22	3/3	NA
Pulmonary hypoplasia at 28 d [%]	NA	2/22	NA	NA
ACR at 4 d [mg/mg]	NA	7.7 ** (2.6–30)	NA	NA
Serum creatinine at 4d [mg/dL]	NA	1.7 (0.5–1.74)	NA	NA
eGFR at 4 d. [mL/min/1.73 BSA]	NA	10 (4–37)	NA	NA
ACR at 28 d [mg/mg]	NA	4.7*** (0.05–13.5)	NA	NA
Serum creatinine at 28d [mg/dL]	NA	0.95 (0.25–4.7)	NA	NA
eGFR at 28d. [mL/min/1.73 BSA]	NA	20.6 (5–79)	NA	NA
Urine output [mL/kg/h] at 4 d	NA	3.4 (1.0–7.5)	NA	NA
Dialysis need [%]	3/25	3/22	0/3	NA
Dialysis lenght [d]	3–19	NA	NA	NA
ICU stay [d]	NA	19 (1–150)	NA	NA
Acidosis at 28day	NA	9/22	NA	NA
HCO3 [mmol/l]	NA	10 (17–25)	NA	NA

* — only 10 were analysed by pathomorphology; ** — maximum excretion for preterm 0.21 mg/mg (22 mg/mmol) by Gubhaju et al. [12]; *** — maximum excretion for preterm 0.11 mg/mg (15 mg/mmol) Gubhaju et al. [12]; eGFR — estimated glomerular filtration rate; NA — not applicable; OU — obstructive uropathy

Kidney injury markers were also tested at the end of neonatal period. 12/22 (54%) neonates had impaired eGFR (13/33 elevated creatinine) with eGFR of 24 (range 5–19) mL/min/1.73 m^{2}BSA, 18 (82%) had increased albumin excretion (mean 5.4 range 0.05–13.5 mg/mg). When the combined renal survival (free of any injury) of the initial group (39) was analysed, it turned out to be significantly low — 18% (7/39). When only who survived neonatal period were qualified the percentage rose to 32% (7/22).

When we compared the data of foetuses (children) who survived with the other only several significant differences were detected. Normal foetal urine osmolarity was detected in 10/22 foetuses who survived and in only 1/17 who did not (p = 0.03). We also found that in the former group AFI was higher (8 *vs.* 4, p = 0.04). Borderline significance was achieved with the age of the first VAS (21 *vs.* 19 hbd, p = 0.056).

We aimed at determining any prenatal marker of 28-day survival in the study group. Clinical markers of the pregnancy and VAS intervention, sodium, chloride, β2-microglobulin urine concentration, urine osmolarity, amniotic fluid index,

presence of dysplastic changes in renal parenchyma were initially included. Using common statistical tests and discriminant analysis, we only found that the survival rate was higher in those who were shunted. To validate the discriminative quality of shunting for prediction of survival we used a probabilistic predictive Naïve Bayes model. The assessed sensitivity the model was of 0.718 and precision of 0.812 with a low value of AUC equalled 0.528. The combination AFI, shunting and normal urine osmolarity assessed at the moment of diagnosis were characterised similarly: sensitivity of 0.744, precision of 0.743 with AUC 0.850. No other single predictor or combination of predictors were recorded as prognostic.

In the Kaplan Meyer analysis at 28th day we observed that foetuses with at least 2 (out of 3 standard) abnormalities in the urine analysis differed with regard to outcome (Fig. 2).

DISCUSSION

We aimed our study to assess short-term outcome in LUTO. The patients remained under care of the same team
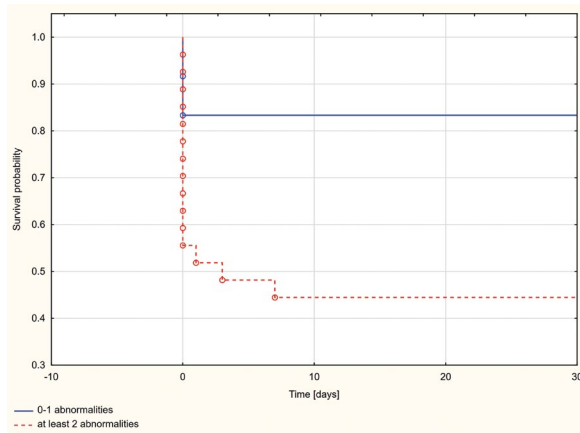
Figure 2. Kaplan-Meyer survival curves for all subjects and adjusted to the presence of negative prognostic factors in fetal urine analysis

until the end of pregnancy and at least up to 28th day after delivery.

Prenatal diagnosis was confirmed in 87.6% cases. We found that overall survival rate in LUTO qualified for shunting at 28d was 56%, however when only shunted foetuses were included it rose to 66%. When the renal function preservation was analysed, we showed that significant number of children had compromised eGFR at 4th day from delivery with need for temporary dialysis in 3/22. Proteinuria in this group of patients was also a frequent marker of kidney injury. Only 18% of initially qualified subject survived until neonatal period with no kidney injury (eGFR decrease or significant proteinuria). VAS preserved renal function in 7/22 children who survived (32%). The overall 28d survival rate was similar to those reported in other single and multicentre trials.

The analysis conducted by Morris et al. done on basis of PLUTO randomised controlled trial and prospective observational cohort study reported 28 day survival rate as 50% and 40% respectively [6]. What was surprising, this survival rate was significantly lower than in the conservatively managed patients. Survival to 28 days after delivery was higher in the conservative-management group, at 69% (24/35), compared to 40% (4/10) in the VAS group (p = 0.02). Compared to the conservative-management group of the trial, a higher proportion of women in this registry opting for conservative management had a normal amniotic fluid volume at diagnosis (p = 0.05) and a diagnosis of LUTO ≥ 24 weeks' gestation (p = 0.003). On multivariable logistic regression analysis, these variables showed a significant association with perinatal survival (p < 0.001) [6].

Ruano group assessed the 2-y outcome after foetal intervention in 50 patients with LUTO. They found PUV in 31 (62%) foetuses, urethral atresia (UA) in 14 (28%) foetuses, and urethral stenosis (US) in 5 (10%) foetuses. There were no survivors in the UA group. Eleven (22.9%) infants died during

the neonatal period because of prematurity, lung hypoplasia or renal failure [13]. Short term survival rate in all abnormalities was 36% (18/50) which is comparable to our data.

Ethun et al. [14] analysed outcome of 14 patients after prenatal intervention of LUTO and showed similar results to our observation with regard to success and complications. Jeong et al. [15] showed among the 32 foetuses examined that: 5 died because of termination of pregnancy, and 2 died in utero. Three neonatal deaths occurred, resulting in an overall perinatal survival rate of 68.8% (22 of 32). Fontanella et al. [16] analysed the data of foetuses at high-risk of isolated LUTO and managed conservatively. The survival rate was 42% and was similar to our observation. Martinez et al. performed 20 fetal PUV ablations at the median gestational age of 18.1 weeks (range 15.0–25.6). Overall, there were 9 (45%) terminations of pregnancy and 11 women (55%) delivered a liveborn baby at a mean gestational age of 37.3 (29.1–40.2) weeks. No infants who survived developed pulmonary hypoplasia and all were alive at 15–110 months [17]. Johnson et al. [18] in the cohort study on LUTO in USA reported even higher percentage of perinatal survival (97%), but surprisingly dialysis was required in 32% of patients.

Ruano et al. analysed retrospectively a cohort of 111 foetuses with LUTO. They showed that the probability of survival was significantly higher with foetal cystoscopy and VAS when compared to no intervention [adjusted relative risk (ARR) 1.86 (95% CI, 1.01–3.42; p = 0.048) and ARR, 1.73 (95% CI, 1.01–3.08; p = 0.04) respectively]. Unfortunately, there was no statistically significant chance for maintaining normal kidney function in short and long-term observation. However, when they analysed only PUV patients, the situation turned out to be more optimistic. Foetal cystoscopy was effective in improving both the 6-month survival rate and renal function [ARR 4.10 (95% CI, 1.75–9.62; p < 0.01)] and 2.66 [(95% CI, 1.25–5.70; p = 0.01) respectively] while VAS was associated only with an improvement in the 6-month survival rate [ARR 3.76 (95% CI, 1.42–9.97; p < 0.01)] with no effect on renal function (ARR 1.03 [95% CI, 0.49–2.17, p = 0.93]) [5].

We found that shunting with VAS was successful in prevention of renal injury in the neonatal period only in 18% of patients from the initially qualified group. From those who survived 32% were free of any injury. In the study of Martinez et al. [17] on 20 PUV ablations, 8 (40% of all foetuses, 72.7% of newborns) had normal renal function and 3 (27.3%) had CKD awaiting renal transplantation. They assessed the kidney injury by eGFR decrease with no data on proteinuria. Similarly, Ruano et al. [13] reported that among the infants with PUV 17/30 (56.7%) survived and 13/17 (76.5%) had normal renal function at 1 year of life; 15/28 (53.6%) survived and 11/15 (73.3%) had normal renal function at 2 years.

Our study showed that classical prognostic factors did not differ between the survivors and non survivors group.

Only a combination of predictors (shunting, urine osmolarity and AFI) showed little value in this prediction. In the Kaplan-Meier curve analysis foetuses with at least 2 increased classical biochemical parameters (osmolarity, sodium and chloride concentration) had lower survival rate. These results are concordant with other studies, where only combined prenatal factors were potentially qualified as significant in multivariate analysis [5, 14, 18, 19].

We are aware of the weaknesses of the study. The number of patients included was relatively small, but that was a single centre analysis. Only multicentre studies presented significantly higher number of patients. Furthermore, there was no comparator (conservative treatment) arm in the study group, nor the randomisation was possible. However, this weakness may turn into some strength. Because of specific treatment policy of the centre and profile of the country our cohort was complete and all of the pregnant women with LUTO diagnosis referred in second trimester were proposed an intervention with no option of termination of pregnancy based on patient decision. The number of patients at the first sight would look low, but when it is compared to single arms of PLUTO studies and other papers it could be qualified as one of the largest prospective cohort of VAS ever published [6]. One can postulate that the location of neonatal and urology-nephrology centre in once place increases the value of observation and quality of data. The data gathered in the neonatal period concerning comorbidities and development should enrich the value of observation. The broad range of VAS intervention (up to 30 week) also decreases the precision and value of observation but similar approach was also presented by other authors.

CONCLUSIONS

Our study led to clinically important conclusions that pregnancies with LUTO fulfilling criteria for prenatal interventions had significant percentage of unfavourable outcome. The full prevention of the kidney injury in live-born subject was difficult to achieve. However, the need of early neonatal dialysis was relatively low.

Conflict of Interests

The authors declare that they have no conflict of interests.

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VIA MEDICA

Metrorrhagia iuvenilis and Premenstrual Syndrome as frequent problems of adolescent gynecology with aspects of diet therapy

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ABSTRACT

Painful menstruation, premenstrual syndrome and metrorrhagia iuvenilis are one of the most common problems related to the sexual cycle in adolescent girls.

Metrorrhagia iuvenilis is acyclic bleeding that occurs in adolescents and lasts from over 10 days even up to 3 months. These bleeds are very abundant and have a tendency to relapse. They cause anemia, and severe cases can be life-threatening.

Premenstrual Syndrome (PMS) is a cluster of somatic, emotional and behavioural symptoms occurring in the luteal phase of the menstrual cycle. The aetiology of PMS remains unknown. According to strict diagnostic criteria, an estimated 2.5–5% of girls and women are affected by PMS. However, some researchers maintain that the symptoms of PMS may be prevalent in as many as 40–80% of girls and women.

This article it has been discussed premenstrual syndrome and metrorrhagia iuvenilis and aspects related to dietotherapy were included.

Key words: adolescent gynaecology; iuvenile metrorrhagia; premenstrual syndrome; diet therapy

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INTRODUCTION

Premenstrual syndrome and metrorrhagia iuvenilis are frequent problems in adolescent gynecology. In the prevention and treatment of both disease entities, it is very important to include diet therapy as a minimally invasive method, which is very important in developmental age.

Metrorrhagia iuvenilis

Metrorrhagia iuvenilis (heavy menstrual bleeds) is acyclic bleeding that occurs in adolescents and lasts over 10 days, even up to 3 months. These bleeds are very abundant and have a tendency to relapse. They cause anemia, and severe cases can be life-threatening [1–6].

In 2009, at the FIGO congress, the so-called criteria were set. normal menstruation: the frequency of menstruation (from 24 to 38 days), the regularity of menstruation over a period of more than 12 months, the duration of bleeding (from 4.5 to 8.0 days), the volume of blood loss (from 5 mL to 80 mL).

During normal menstruation 90% of wasted blood is in the first three days, and the bleeding in the first two days is the most abundant. Bleeding that does not meet the above conditions is treated as abnormal uterine bleeding (AUB) [7].

Heavy menstrual bleeding (HMB) (above 80 mL) of a cyclic nature concerns women most often in the premeonopausal age. The clinical definition set by The National Institute for Health and Care Excellence (NICE) in 2007 is more practical. Defines HMB as excessive loss of menstrual blood, interfering with and interfering with the physical, emotional, social and material quality of a woman's life, which can also be combined with other symptoms. Prevalence according to different authors, it is in the range of 4–9% .

Heavy and prolonged menstrual bleeding (HPMB) is abundant and prolonged menstrual bleeding above 80 mL and lasting more than 8 days, while intermenstrual bleeding (IMB) this is intermenstrual bleeding. Postmenopausal bleeding (PMB) defines postmenopausal bleeding. It is

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possible to use the term amenorrhea (absent menstrual bleeding [AMB]) in the absence of menstrual bleeding over a period of more than 90 days.

The causes of abnormal bleeding from the genital tract (eg. from the vagina) can be divided into two groups: structural and non-structural. The structural reasons include: changes in the cervix (eg erosions of the vaginal part of the cervix, cervical cancer, polyps of the cervix), changes in the endometrium (endometrial hyperplasia, uterine polyps, fibroids, adenomyosis, stem cancer). Non-structural causes include: ovulation disorders/lack of ovulation (eg progesterone deficiency, estrogen deficiency, inhibin deficiency, PCOS, hyperprolactinemia), thyroid disease (hypothyroidism), adrenal diseases (Cushing's disease), haematological diseases (eg coagulopathy, thrombocytopenia), diseases of other organs (eg ovarian, adrenal or pituitary tumors, cirrhosis of the liver, some kidney diseases). Other causes include: obesity, injuries, foreign body, stress, medicines used (e.g., antiepileptic, antidepressant, anticoagulants, NSAIDs, hormones).

In 2009, the FIGO committee recommended the introduction of a new classification of abnormal bleeding from the uterus — The PALM-COEIN Classification System. The name is an acronym from the first letters of causes that can cause bleeding from the uterine cavity:

P — polyp;

A — adenomyosis;

L — fibroids of the uterus;

M — endometrial cancer and proliferation;

C — coagulopathies;

O — ovulation disorders;

E — other causes of disorders;

I — iatrogenic (IUD insert, steroid hormones, anticoagulants, antidepressants);

N — not yet classified [7].

Juvenile bleeding usually occurs shortly after menarche, when the cycles are anovulatory. Juvenile bleeding is functional uterine bleeding and rarely disappears spontaneously. It is assumed that irregular bleeding from the genital tracts in pre-pubescent girls up to 2–3 years from menarche may occur as a physiological phenomenon. Situations with profuse bleeding and secondary anemia are dangerous and require treatment [8–15]. When the cycles are anovulatory, bleeding is not cyclical, and there are no typical premenstrual symptoms associated with premenstrual syndrome, such as fluid retention, breast tenderness, abdominal distension. Juvenile bleedings usually have a sudden onset without any symptoms which predict them [1, 3, 6, 10, 15].

As a result of the lack of ovulation, there is a situation in which uncyclic (often unexpected) exfoliation of the expanded and thickened mucous membrane of the uterine cavity occurs, which stems from the relative hyperestrogen-ism (deficiency of progesterone, because ovulation does not occur). This results in bleeding. Acyclic bleeding may also be due to insufficient exfoliation of the endometrium (i.e. endometrium). First, there is a slow endometrial hyperplasia for up to 3 months, and then slow functional exfoliation of the endometrium takes place. The exfoliation of the endometrium to its basal layer can last up to a month. Estrogens stimulate the endometrium for growth constantly. In the etiology of profuse bleeding is involved the abnormal process in the endometrium, which controls the supply of arachidonic acid for the production of prostaglandins too. In women and girls with profuse bleeding, endometrium occurs on a much larger scale than in women with normal menstruation, and causes the formation of prostaglandins series 2, which affect the functions of the blood vessels and affects the sensation of pain [5, 11, 15].

In the case of abrupt juvenile hemorrhage, early diagnosis is important to avoid complications that threaten health and life [2, 4, 9, 15]. A gynecologist decides whether the patient's condition is acute and requires immediate hospitalization, or whether it will be possible to monitor it in as an outpatient. During a medical interview, information about family diseases is important. This includes hematological diseases, information about how the patient's development progressed, other diseases, the history of menarche, the course of cycles, and the period during which abnormal bleedings started and their duration. It is important to establish whether the patient takes drugs or dietary supplements, and if so, what kind. Of particularly importance is information that excludes or confirms hematological problems, whether the patient is suffering from hemorrhagic diathesis or whether there are bleeds from other places [8, 10, 15].

Excessive menstrual bleeding in girls and women with bleeding disorders occurs with a frequency of 10 to 100%, depending on the particular disorder. In turn, disturbances of thrombosis are also more frequent among girls and women with excessive menstrual bleeding. Research already carried out indicates that coagulation disorders occur in 5–32% of girls and women with heavy menstruation. On the basis of the number of tampons and sanitary towels used, the amount of lost blood can be estimated, as the use of more than 10 sanitary towels or tampons within 24 hours can be a sign of bleeding over 30 mL. During the test, the basic parameters (blood pressure, heart rate, conjunctival assessment, and petechiae evaluation) are measured. In a gynecological examination, it is important to exclude possible pregnancy in juveniles and its consequences of this (an imminent abortion or an ectopic pregnancy). In view of the bleeding and the fact that it is usually their first contact with the gynecologist, girls can be reluctant to undergo an examination with the use of a speculum. In gynecology

of the developmental age, a long narrow speculum is used if necessary, which makes it possible to assess the vagina and cervix. It is worth noting that bleeding can be the result of sexual violence. Moreover, the cause of bleeding may also be injuries experienced while practicing sports. During the examination, the gynecologist must rule out the tearing of the vaginal walls, polyps, foreign bodies, and must check whether the bleeding comes from the uterus. A two-handed study in girls who have not had a sexual relationship yet should be performed via the anus. This may be particularly unpleasant and some authors recommend withdrawing from this practice. An ultrasound examination is performed via the rectum or ultrasound examination is carried out through the abdominal walls, and in the case of patients who have had a sexual relationship, an ultrasound examination is performed. Laboratory tests are recommended: complete blood count, coagulation system, FSH, LH, estradiol, prolactin, thyroid hormones and TSH [1, 5, 9, 10, 15]. On the basis of these, the doctor is able to assess the condition of the patient (exclude anemia, thrombocytopenia, plasma defects) and the hormonal causes of acyclic bleeding. The results of the tests allow decisions to be made as to how to proceed. Bleedings in the case of anovulatory cycles do not always involve excessive blood loss: there are no changes in peripheral blood morphology, and girls may not have additional symptoms at that time. However, patients are always advised to lead a healthy lifestyle, a diet rich in iron ions, and sometimes its supplementation. Advice from a gynecologist, in the absence of patient's knowledge, also has an educational significance [4, 9, 13–15].

As mentioned above, it is very important to exclude hematological causes of excessive menstrual bleeding and juvenile bleeding.

VON WILLEBRAND'S DISEASE

Among women with abundant menstruation, the incidence of VWD is 5–20%, and among teenagers with juvenile bleeding it may be as high as 36% [4]. In girls and women, Von Willebrand's disease (vWD) is often not recognized due to the aforementioned lack of pro-health education.

Von Willebrand's disease is the most common hereditary coagulation problem. It is due to deficiency, dysfunction or lack of von Willebrand factor, which is necessary for platelet adhesion in vascular sites, and for the protection of factor VIII with pre-proteolysis in the bloodstream. Von Willebrand's disease appears through bleeding within the skin and mucous membranes, including uterine bleeding. The incidence of vWD in the general population is 0.6–1.3%, depending on the prevalence of people in a given region with bleeding symptoms, family history and laboratory abnormalities [1–5].

HAEMOPHILIA

Haemophilia is the most common severe hereditary disorder of coagulation, but as a disease conjugated to the X chromosome, it occurs almost exclusively in men and boys, while women are carriers of it.

In women who are carriers of haemophilia, clinical signs of hemorrhagic diathesis may or may not occur, and the symptomology of bleeding varies widely, from asymptomatic to severe bleeding [1–5].

THROMBOCYTOPENIA CONDITIONED IMMUNOLOGICALLY

Primary immune-mediated thrombocytopenia (immune thrombocytopenia, ITP) is an acquired immune disease characterized by isolated thrombocytopenia, defined as the number of platelets in peripheral blood below 100x109/l in the absence of an obvious initiating or primary cause. ITP occurs with the frequency of about 1 per 10000 patients, but more often among women between their 30s and 60s [10, 11]. Depending on the intensity of thrombocytopenia, there is a higher risk of abundant periods and other abnormal birth canal bleedings.

The treatment of juvenile metrorrhagia (heavy menstrual bleeds) depends on their etiology, the patient's contraceptive needs, her individual ability to comply with medical recommendations, tolerability of side effects, costs and medical interventions. In the case of girls with adolescent bleeding, estrogen-progesterone therapy, clotting drugs, and iron preparations are individually selected. Sometimes these girls require hospital treatment. Oral progestogens may also be effective, but the choice of therapy is specific to each patient, depending on her age, puberty, ultrasound and laboratory tests. The management of patients with and without coagulation disorders is similar [8–12,15].

Increased iron loss, caused by e.g. heavy menstruation, also requires proper dietary treatment because it threatens with anemia and anemia occurring in children and adolescents may results in a wide range of serious health consequences such as impaired mental development and physical growth and reduces school performance and work capacity [16, 17]. Adolescent females are particularly vulnerable to iron deficiency because of both high iron losses during menstruation and insufficient dietary iron intake. Based on a study carried out for a representative national Polish sample of adolescents, girls were characterized by a significantly lower intake of all forms of iron (total iron, heme iron, non-heme iron, anmal iron, plant iron) compared to male adolescents [18]. The results of Hamułka et al. [19] and Mizgier et al. [20] studies confirm that female individuals commonly have too low iron intakes, which in Poland is confirmed both for pregnant young women and those who are not pregnant.

Dietary restrictions and a vegetarian diet may also lead to limiting the intake of meat and meat products, and ultimately lead to lower iron intake [21].

Iron is present in food products in two forms, as heme iron, which is found in meat, fish and other animal products, and as non-heme iron, which is found in plant as well as animal products. Heme iron is highly bioavailable (25–30% of this form is absorbed), although it represents a minor part of dietary iron. The absorption of non-heme iron is only about 1–10% The best sources of the both forms of iron in the diet are: offal, dry legume seeds (peas, beans, lentils, chickpeas), poultry, fish, eggs, cereal products (oatmeal, whole grain bread, whole grain pasta), and some vegetables (beets, chard, peas) [20, 22, 23].

Premenstrual Syndrome (PMS)

Premenstrual Syndrome (PMS) is a cluster of somatic, emotional and behavioral symptoms occurring in the luteal phase of the menstrual cycle. Many studies have been conducted, but the etiology of PMS remains unknown. PMS symptoms substantially affect the quality of a woman's life. According to strict diagnostic criteria, an estimated 2.5–5% of girls and women are affected by PMS. However, some researchers maintain that the symptoms of PMS may be prevalent in as many as 40–80% of girls and women. Due to this considerable discrepancy, it is necessary to make the definition of PMS more precise. Patients with PMS experience mood disturbances together with physical and emotional symptoms, which recur in the luteal phase and disappear in the follicular phase of the menstrual cycle [24, 25].

In the last decade, the methods of PMS treatment have undergone significant changes. Although the pathophysiology of the syndrome has not been fully elucidated, effective treatment is possible for most patients. In women with premenstrual syndrome, body composition should be assessed and the appropriate diet and physical activity should be taken into consideration.

The results of the studies conducted confirm the theory of fluid retention in the body as associated with the occurrence of PMS. The theory of fluid retention in the body, which suggests that estrogens induce an increased synthesis of angiotensinogen in the liver, which leads to increased production of aldosterone, which directly affects the retention of sodium and potassium loss. It was also found that in PMS, there is a slight increase in prolactin concentration in the second phase of the sexual cycle, perhaps due to the impaired synthesis or secretion of dopamine and serotonin in the central nervous system. It has been proven that dopamine exerts direct neurotransmission on the kidneys, and serotonin is responsible for excessive sensitivity. The feeling of weight in the lower abdomen and pain in the sacral region are a consequence of retention in the uterus and venous stasis in the pelvis. Swelling of the central nervous system is the cause of headaches, dizziness, nausea, mental tension, insomnia, anxiety, depression, and increased appetite. At such times, women very often have a craving for something sweet. An increase in body weight of 2 to 4 kg usually disappears in the first days of menstruation [24–27].

The results of Mizgier et al.'s study seem to confirm the fluid retention theory, since statistically significant differences were observed in the two study groups for total body water (TBW) in kilograms. Higher values were reported for PMS women ($p < 0.005$). This statistical significance was confirmed for both the whole study population and for women with BMI ≥ 25 kg/m2. Although the pathophysiology behind PMS has not been fully explained, it is recommended to assess the state of nutrition and body composition which, as the current study shows, are related to premenstrual syndrome prevalence. Since they are modifiable factors, PMS treatment should include a balanced diet and physical activity, which will help to modify not only body mass but also fat mass. Further studies on PMS women should also offer recommendations for lifestyle interventions [24].

The results of the study suggest the importance of proper eating habits and physical activity on the course of menstrual cycles [28]. Physical exertion, e.g. aerobics, but also relaxation or relaxation exercises may be beneficial.

It should be added that conventional methods of treatment of cyclic pain and discomfort over a time period of approximately one mnth, although numerous and diverse, have disadvantages, including side effects, disruptions of women's functioning. Many of these women also turn to herbal medicine. Clinical evidence supports the efficacy of Vitex agnus-castus, but other drugs commonly used by western herbalists to treat menstrual symptoms are not supported by clinical trials. This raises concerns about the effectiveness and safety of these herbs. Treatment options for women must be extended and individualized when current conventional strategies fail, which requires appropriate clinical trials of potentially useful herbal medicines [29].

Some women with PMS want to be treated with acupuncture or acupressure. Limited available evidence suggests that acupuncture and acupressure may improve both the physical and mental symptoms of premenstrual syndrome. There was insufficient evidence to determine whether there is a difference between groups in the frequency of adverse events. There is also no evidence comparing acupuncture or acupressure with currently recommended therapies for PMS, such as selective serotonin reuptake inhibitors (SSRIs). Further research is necessary, using approved measures for PMS, appropriate blindness and appropriate comparative groups reflecting the best practice currently [30].

The basic elements of the diagnosis are: the need to prospectively determine the time of appearance of

symptoms and the occurrence of at least 30% increase in the severity of symptoms in the luteal phase compared to the follicular phase. An additional criterion is the exclusion of mental illness and the condition that women did not use oral contraception and have regular monthly cycles [26–27].

Any woman who notices the severity of symptoms should consult a doctor. It is necessary to exclude other diseases, both internal, neurological and gynecological, which may give similar ailments. Any ailment that contributes to the deterioration of the comfort of life, although it does not cause negative medical effects, should be treated. A typical premenstrual syndrome should be distinguished from monthly complaints, the primary cause of which is not stress, anxiety, depression, emotional disorders, family or professional problems. The study should exclude women with dysmenorrhea, in which local ailments occurring only at the beginning of bleeding, and persistent during its duration, accompanied by abnormalities of the sexual cycle, prevail.

Internal and gynecological examinations are necessary. These aim to identify possible organic changes that may be responsible for ailments and at the same time may have serious health consequences. Similar discomfort is often felt by women after gynecological operations, after childbirth or abortions, and those suffering from other organic diseases of the reproductive organs. In premenstrual syndrome, the doctor may not find any palpable lesions in the genital organ during a pelvic examination. Sometimes the determination of hormonal activity of the ovaries is indicated and helpful in further proceedings. The simplest method is to measure the basic temperature of the body daily, immediately after waking up throughout the menstrual cycle. The doctor may also order the a cytohormonic smear to be taken from the vagina, a cervical mucus crystallization test or, in justified cases, determination of the level of sex hormones (ovarian and pituitary) in the blood serum. People with milder symptoms of premenstrual syndrome may try nonpharmacological methods.

If possible, it is good to avoid stress, and more difficult tasks should be planned for the first half of the cycle. Inhalation of essential oils may be helpful, and it is recommended to reduce the consumption of sodium and caffeine. Writing a diary and noting the changes in the mood, and the ways of reacting and physical ailments can help to understand changes in the body and to control behavior. Understanding and acceptance on the part of relatives is of course very important, and a woman has the right to expect various forms of help and tolerance during this period. With a higher severity of premenstrual symptoms, especially in full-blown PMDD, non-pharmacological methods usually fail and should not be used as the sole method of treatment for more than 3 months. After this period, pharmacotherapy should be considered.

As first-line drugs with proven efficacy, antidepressants from the SSRI (serotoninergic) group are currently used. It is interesting that studies indicate similar effectiveness of these drugs used both continuously and intermittently only in the second phase of the cycle. Recently, the latter strategy has been preferred. These drugs may cause resolution or outstanding relief of most of the symptoms of PMDD. Other pharmaceuticals are less effective, act on some symptoms or are associated with a higher risk of use. Some efficacy of synthetic analogues of hypothalamic gonadotropin-releasing hormone has been demonstrated, but they are not routinely recommended. These drugs radically change the body's hormonal status, for example, they can overcome ovulation, can have other important side effects, and are also expensive. There are several drugs that are useful in some cases to alleviate individual syndromes of the syndrome. Alprazolam relieves psychical tension, but should be avoided due to the possibility of substitution. Only spironolactone is recommended for diuretics, and can reduce body swelling, while non-steroidal anti-inflammatory drugs, especially for pro-zene and mefenamic acid, can be beneficial especially when pain is an important symptom of the syndrome.

Breast engorgement and tenderness may be ameliorated, through vitamin E administered only in the second half of the cycle, and gammalinolenic acid (e.g. in evening primrose oil). Some studies have demonstrated the benefit of taking calcium carbonate at 1.200 mg per day during three monthly cycles. There is no evidence for the effectiveness of supplementing magnesium, manganese and other micronutrients. The validity of the use of contraceptives was also not confirmed. Several randomized clinical trials were performed in which vitex agnus-castus (monk's pepper) was used to alleviate the symptoms of PMS syndrome. The results indicate a significant improvement in the symptoms of PMS syndrome in 42% to 77% of women taking the drug for 3 months. No side effects were noted. The randomized study also compared the effectiveness of Mastodynon® with pyridoxine. A better therapeutic effect was demonstrated in patients treated with Mastodynon®: in 80% of patients a significant improvement in symptom perception was obtained compared to 21% in the group treated with vitamin B6. The effectiveness of vitex agnus-castus extract in alleviating emotional disorders in the most severe form of PMS, pre-menstrual dysphoric disorder (PMDD) has also been confirmed. Vitex agnus-castus showed efficacy comparable with fluoxetine, one of the most frequently used in PMDD selective serotonin reuptake inhibitors (SSRIs) [27]. Mastodynon's ingredients have been selected so as to have the greatest possible extent to alleviate the various symptoms observed in PMS [26].

It was also shown that aromatherapy with Citrus aurantium flower improved the symptoms of premenstrual tension [31].

The methods of treatment also include the administration of:

— gestagens in the second half of the cycle (17–26 days), e.g. didrogesterone (10 mg 1 once 1 tablet) or progesterone (50 mg 1 once 1 tablet),

— contraceptives that stop ovulation when contraception is indicated,

— prolactin inhibitors, when the dominant symptom is mastodynia with abdominal discomfort and edema tendency, e.g. bromocriptine (½–1 tablet), from day 14–28 of the cycle,

— diuretics as a supplement to therapy or exclusive treatment, when the dominant symptom is swelling and weight gain, e.g. hydrochlorothiazide 12.5–25 mg per day,

— a set of vitamins A, B (especially B6), D and E.

Risk factors for the occurrence of PMS symptoms may be vitamin and mineral deficiencies. To synthesize neurotransmitters potentially involved in the pathogenesis of this syndrome, B vitamins and folic acid are needed, among others [32]. Increased amounts of thiamine (vitamin B1), riboflavin (vitamin B2) and pyridoxine (vitamin B6) have a beneficial effect on the alleviation of PMS symptoms. Bertone-Johnson et al. [33] emphasize the importance of vitamin D, which at a dose of ≥ 2.5 µg reduces the risk of premenstrual syndrome. Siuda and Rabe-Jabłońska [34] suggest calcium substitution from ovulation to menstruation, at a dose of 1200 mg/day, with the simultaneous use of tryptophan at a dose of 6 g/day.

In view of the quite diverse positions in the literature regarding the supplementation and dosage of vitamins and microelements, it is safest to use a balanced diet. In this way, overdose is avoided and the effect is reversed to that of a therapeutic one. Selecting the correct, balanced diet is a task for the dietitian. Based on the nutritional interview, he or she has the opportunity to recognize the mistakes made and determine the need and scope of diet modification, which will contribute to reducing the perceived PMS symptoms [32].

SUMMARY

Adolescent girls often complain of heavy menstrual bleeding, and premenstrual symptoms are also common. Differential diagnosis is extensive and requires vigilance, especially in the area of coagulation disorders. In establishing the diagnosis, the gynecologist's cooperation with the hematologist may be helpful, which may prove invaluable in achieving success.

With reference to the occurrence of above mentioned problems, there is a need for nutritional education among adolescents. Dietary intervention is the most appropriate way to improve knowledge status in young women and can act as an alternative to conventional treatment. Unfortunately, there are no educational campaigns in Poland related to diet therapy possible in prementrual syndrome or iron deficiency. Such education should be targeted at broadening knowledge by identifying and promoting various sources of well-absorbed iron. Such a strategy will be beneficial for an individual's health during adolescence and for their future health.

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VM **VIA MEDICA**

Pregnancy in a 29-year-old woman in cerebral death

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Patient aged 29 in 21 + 4th weeks of first pregnancy admitted to the Intensive Care Department after sudden cardiac arrest, after effective 15 min. resuscitation, even undertaken at home by the husband. In an interview with the husband: the patient reported a severe headache, vomited, then lost consciousness.

In the ward there was retained the amine infusion, anti-swelling treatment in accordance with the recommendations of the neurosurgeon, anticoagulant therapy due to the high thromboembolic risk. Disorder of water, electrolyte and acid-base balance was compensated. Due to an interview indicating the neurosurgical cause of SCA, the CT scan was performed with radiological protection of the fetus. It was found: in the topography of the cerebellar vermis and ventricle IV, a large hematoma measuring 29 x 20 x 21 mm, blood visible in the foremost reservoir, over the tent of the cerebellum, in the crevices of the cerebellum, around the medulla (Fig.1).

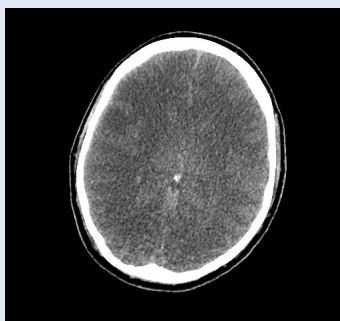**Figure 1.** A hematoma in the topography of the cerebellar vermis and ventricle IV

Figure 2. Deletion of cortico-subcortical diversity

Therefore, ventricular drainage was applied and endovascular embolization was performed on arteriovenous malformation of the area of the quadratic plaque, which was the source of bleeding. Due to the significant damage to the CSN, after the consultation and conversation with the patient's relatives, it was decided to maintain the vital functions of the patient to prolong the pregnancy until the fetus' maturity gave the chance of survival.

FHR was controlled daily, hormones were supplemented due to disruption of the hypothalamic pituitary axis, and microbiological tests were performed. After 24 weeks steroid therapy, pentoxifylline infusion and DHA replacement were included. In CT scan, deletion of cortico-subcortical diversity was observed (Fig.2). Clinically, the patient with dilated, areactic pupils, in a state indicating the death of the brain.

The condition of the levonor infusion gradually deteriorated, the circulatory instability appeared, and the spinal reflexes were observed indicating the progressive degradation of the nervous system. In view of the deteriorating condition of the patient, a decision was made on a planned cesarean section. A steroid-like dose and a magnesium infusion were administered in a neuroprotective regimen. Caesarean section was performed in planned mode at 27th week of gestation, a newborn born with a weight of 1250 g Apgar 5/6/7/7 was born and

Figure 3. The intracerebral arteries without the flow

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given to the neonatal team. The next day, angio-CT was performed by angiography of the intracerebral arteries without showing the flow (Fig.3). After a series of clinical trials, the commission for the adjudication of brain death determined the death of the patient.

The death of the pregnant woman's brain is an extremely complex situation, requiring not only a well-thought-out plan of medical treatment but also requiring prudent decision-making in ethical and legal situation. Brain death in pregnant women is a very rare phenomenon, from which the birth of a viable child takes place in less than half of the patients. From this year to 2017, 37 such cases were registered in the world.