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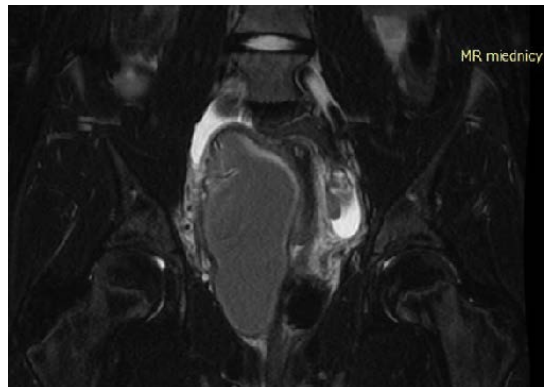

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Prognostic value of tissue plasminogen activator (tPA) in patients with epithelial ovarian cancer undergoing chemotherapy

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ABSTRACT

Objectives: Tissue plasminogen activator (tPA) is a key enzyme for fibrin degradation and the proteolytic defense against formation of the thrombotic endothelial deposits. tPA is involved in carcinogenesis but its exact role in tumor biology is not very well understood and a prognostic value of tPA remains ambiguous in different cancers. The aim of the study was to assess the prognostic value of plasma tPA in patients with epithelial ovarian cancer (EOC) in the course of the first line chemotherapy.

Material and methods: the study covered 60 patients with EOC who underwent the 1st line chemotherapy. Plasma tPA was assessed at onset, after 3 and 6 cycles of chemotherapy. The groups were stratified according to tPA level at onset of chemotherapy (low tPA group < 6.5 mg/L, N = 37 and high tPA group > 6.5 mg/L, N = 23). Survival analysis was repeated for the cut-off of tPA level at 6.5 mg/L and 5.1 mg/L after 3 and 6 cycles.

Results: Only subjects with tPA > 6.5 mg/L at onset of chemotherapy had a significantly lower probability of a 5-year survival (34.8% vs. 72.7%, P < 0.006) and lower chance for disease free survival within 5 years (39.3% vs. 72.7%, P < 0.014). tPA < 6.5 mg/L plasma level evaluated at onset of chemotherapy was an independent marker of better overall survival (RR = 0.44, 95%CI = 0.19–0.98) but not disease-free survival.

Conclusions: Plasma tPA may serve as a marker of survival if assessed at onset of the first line chemotherapy in patients with ovarian cancer.

Key words: tissue plasminogen activator; tPA; epithelial ovarian cancer; chemotherapy; overall survival; disease free survival

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INTRODUCTION

Hemostatic markers are predictors for both thrombosis and hemorrhage. Malignancy enhances the process of fibrin formation and degradation. Local blood clotting is a process strictly associated with cancer — both platelet aggregates and fibrin depositions are found in tumors during histopathological investigations. It is known that hypercoagulability promotes thromboembolic events in cancer with frequency much higher than in benign conditions [1]. Cancer associated thrombosis (CAT) can be linked to oncogenic lesions that account for the onset and progression of malignant disease [2]. Abnormal results of hemeostatic laboratory tests change with cancer progression, which confirms the impact of tumor on blood clotting [3]. As the connection between fibrinolysis and metastasis is established, different fibrinolytic biomarkers are investigated with the aim of foreseeing a prognosis. Two types of plasminogen activators are found in plasminogen activator system — tissue type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA). They both can turn inactive plasminogen to active plasmin. tPA is present in both normal and malignant tissues and is mainly involved in the conversion of plasminogen to plasmin during blood clot dissolution, while uPA, which is mainly associated with malignancy,

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plays a role in proteolysis during cell migration and tissue remodeling [4]. Cell migration is increased by promotion of tPA and uPA mediated proteolytic cleavage of plasminogen to generate plasmin. That subsequently affects breakdown of basement membrane to facilitate cell migration [5, 6].

tPA is considered a key enzyme in fibrin degradation and is crucial for the proteolytic defense against formation of the thrombotic endothelial deposits. However, tPA is also produced by epithelial cells, fibroblasts, and macrophages. The role of tPA in tumor biology is not well understood. In breast cancer contradictory results have been reported: high tumor tissue content of tPA was observed to be associated with good prognosis, whereas high content of the tPA:PAI-1 complex was associated with shorter disease-free survival (DFS) and overall survival (OS) [7]. In ovarian cancer large amounts of fibrin are deposited within and around ovarian tumors secondary to the release of tissue factor from tumor cells and macrophages. Fibrin may enhance tumor cell adhesion to endothelial and mesothelial surfaces. The fibrin deposition leads to the activation of plasminogen mediated by tPA which results in degradation of matrix by plasmin [8]. As an evidence of the above process, fibrin degradation products are detectable in high concentrations in peripheral blood and ascitic fluid [9]. Experimental data on mechanism of action of tPA encouraged scientist to evaluate its role as a prognostic factor in EOC. There are some studies which showed that a high expression of tPA in tumor tissue is a negative predictor of survival [10, 11]. However, very little is known about possible predictive meaning of plasma tPA especially at various stages of EOC treatment [12].

Objectives

The aim of our study was to investigate the prognostic significance of plasma tPA in patients with epithelial ovarian cancer who underwent the first line chemotherapy.

MATERIALS AND METHODS

Ethics statement

The study was conducted under the approval of the local Bioethics Committee at Medical University of Warsaw. All subjects have provided written informed consent.

Patients and samples

The study was carried out between 2011 and 2018. 60 patients with a diagnosis of epithelial ovarian cancer (EOC), who had previously undergone surgery, were enrolled into the study at the onset of the first line adjuvant chemotherapy. Chemotherapy regimen included 6 cycles of intravenous paclitaxel and carboplatin. Blood samples were taken from the patients at the onset of chemotherapy, and again after 3 and 6 cycles of chemotherapy. tPA, fibrinogen, D-dimer and antithrombin III (AT III) were assessed in blood plasma. Besides prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR) test was performed. The IMUBIND® tPA ELISA (Sekisui Diagnostics, LLC, USA) test was used for the quantitative measurement of tPA in plasma.

Response to chemotherapy was assessed according to RECIST 1.1 criteria [13]. OS was defined as time from the start of treatment until death from any cause and DFS was defined as time from the start of treatment until disease progression or death (according to Food and Drug Administration guidance) [14]. The clinicopathological data were obtained from medical records.

Two groups of patients were identified according to tPA level at the onset of chemotherapy: tPA < 6.5 mg/L group and tPA > 6.5 mg/L group. The choice of the tPA cut-off level was based on analysis of tPA level distribution. The mean follow-up time from the initial treatment which included surgery was 57.0 ± 22.9 months (2.6 to 87.6 months).

Statistical analysis

The results were presented as mean ± standard deviation (SD). Significant statistical differences between groups were assessed applying the chi-square test, exact Fisher test or Student's *t* test. The Kaplan–Meier method was employed to plot survival curves, and differences in survival were compared using the log-rank test. The Cox regression model was used to ascertain the value of independent prognosis for patients with epithelial ovarian cancer. $P < 0.05$ was considered statistically significant.

RESULTS

The low tPA (< 6.5 mg/L) group consisted of 37 patients and the high tPA group (> 6.5 ng/mL) was represented by 23 subjects. The clinicopathological characteristic of the groups is presented in Table 1.

No differences between the groups were observed regarding patient age, tumor stage and grade, and histological type of malignancy. Only the rate of overweight/obese patients (BMI > 25 kg/m2/) was significantly higher in the high tPA group ($P = 0.006$). The results of the first-line chemotherapy are presented in Table 2.

No differences in response to chemotherapy were observed in the low and high tPA group regardless of the way the response was stratified (complete remission — partial remission — stabilization — progression or any response vs. no response).

We evaluated tPA plasma level in both groups after 3 and 6 cycles of chemotherapy and found that tPA concentration was significantly higher in the high tPA group throughout the whole 1st line of chemotherapy. PT and INR were other coagulation parameters significantly elevated from the on-

Table 1. Clinicopathological characteristic of the low and high tPA group. Values are mean +/-SD or present a number [%] of cases in groups. Student's t test or exact Fisher test were applied, respectively

	Low tPA group (< 6.5 mg/L) N = 37	High tPA group (> 6.5 mg/L) N = 23	P
Age [years]	53.4 ± 10.6	59.1 ± 12.5	0.06
BMI =< 25 [kg/m^2]	11 (29.7%)	16 (69.6%)	0.006
Histological type n [%] • Serous • Endometrioid • Clear cell • Mucinous	 15 (40.6%) 12 (32.4%) 8 (21.6%) 2 (5.4%)	 14 (73.7%) 1 (5.2%) 4 (21.1%) 0 (0%)	0.06
Stage n (%) I II III IV	 12 (32.4%) 4 (10.8%) 21 (56.8%) 0 (0%)	 6 (26.1%) 1 (4.3%) 15 (65.2%) 1 (4.4%)	0.44
Grade n (%) 1 2 3	 9 (24.3%) 17 (46.0 %) 11 (29.7%)	 6 (26.1%) 4 (17.4%) 13 (56.5%)	0.06
Early cancer (FIGO I-II) Advanced cancer (FIGO III-IV)	16 (43.2%) 21 (56.8%)	7 (30.4%) 16 (69.6%)	0.47

Table 2. Correlation between tPA serum level and the clinical results of the first line chemotherapy. Values are mean +/- SD or present a number [%] of cases in groups. Student's t test or exact Fisher test were applied, respectively

	Low tPA group (< 6.5 mg/L) N = 37	High tPA group (> 6.5 mg/L) N = 23	P
Response to treatment • Complete remission • Partial remission • Stabilization • Progression	 33 (89.2%) 2 (5.4%) 0 (0%) 2 (5.4%)	 17 (73.9% 1 (4.4%) 1 (4.4%) 4 (17.3%)	0.25
Any response vs. progression	35 (0.94.6%) 2 (0.05.4%)	19 (82.6%) 4 (17.4%)	0.29

set till the end of chemotherapy when compared between the low and high tPA group (Tab. 3).

Survival analyses

We plotted Kaplan-Meier curves (Fig. 1) to analyze OS and DFS according to tPA level at different stages of chemotherapy.

Subjects with tPA > 6.5 mg/L at the onset of chemotherapy had a significantly lower probability of a 5-year survival (34.8% vs. 72.7%, P < 0.006) and chance for disease free survival within 5 years was significantly lower in this group (39.3% vs. 72.7%, P < 0.014), (Fig. 1). We

Table 3. The results of coagulation tests in the low and high tPA group at the onset, after 3 and 6 cycles of chemotherapy. Values present mean +/- SD. Student's t test

	Low tPA group (< 6.5 mg/L) N = 37	High tPA group (> 6.5 mg/L) N = 37	P
At onset of chemotherapy			
PT [s] APTT [s] D-dimer [mg/L] Fibrinogen [g/L]	11 .32 ± 0 .75 28 .26 ± 2 .93 2 .08 ± 4 .66 3 .52 ± 0 .76	11 .93 ± 1 .24 28 .67 ± 4 .04 2 .80 ± 2 .28 3 .64 ± 0 .84	0 .02 0 .65 0 .49 0 .56
After 3 cycles of chemotherapy			
tPA [mg/L] PT [s] APTT [s] D-dimer [mg/L] Fibrinogen [g/L]	3.57 ± 1.08 10.94 ± 0.63 27.92 ± 2.97 0.67 ± 0.47 3.51 ± 0.59	9.83 ± 7.75 11.44 ± 0.85 28.01 ± 3.31 1.27 ± 1.45 3.66 ± 0.73	0.0007 0.03 0.92 0.06 0.47
After 6 cycles of chemotherapy			
tPA [mg/L] PT [s] APTT [s] D-dimer [mg/L] Fibrinogen [g/L]	4.13 ± 1.64 11.01 ± 0.70 27.30 ± 2.80 1.49 ± 3.73 3.22 ± 0.64	7.45 ± 4.35 11.48 ± 0.86 27.88 ± 3.21 2.17 ± 3.61 3.46 ± 0.62	0.002 0.06 0.52 0.56 0.24

repeated the analysis after 3 and 6 cycles of chemotherapy for the cut-off of tPA level at 6.5 mg/L and 5.1 mg/L (the level of 5.1 mg/L corresponded the best with the results presented in Table 3). However, we found no significant differences in DFS and OS in all those groups after 3 and 6 cycles of chemotherapy (Fig. 2 and 3).

Univariate analysis showed that a high tPA plasma level at the onset of chemotherapy, advanced tumor stage, tumor grade 2 or 3 and residual disease were associated with worse OS and DFS. BMI > 25 kg/m^2 was only related to shorter DFS (Tab. 4).

Multivariate analysis revealed that tPA plasma level at onset of chemotherapy was an independent marker of OS but not DFS (Tab. 5). Tumor stage and grade were also independent predictors of OS.

DISCUSSION

The pathogenesis of hemostatic disorders in cancer is complex and reflects an interaction of coagulation and fibrinolytic system, vascular endothelium, leukocytes, and platelets. Circulating biomarkers of hemostasis have been extensively studied to predict cancer outcomes along with predicting the thrombotic risk. Plasminogen activators released from cancer cells lead to degradation of basement membrane proteins and extracellular matrix and facilitate cancer cell invasion into surrounding tissues and blood stream. It has been shown that tPA plays a major role in invasion in case of different types of highly aggressive tumors such as melanoma, glioblastoma, pancreatic, breast and endometrial cancer [15–19]. Majority of authors investigated

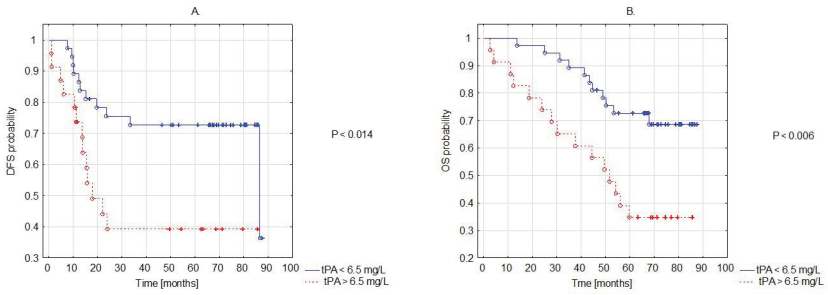

Figure 1. Kaplan-Meier analysis of disease-free survival (DFS) and overall survival (OS) according to tPA level at onset of chemotherapy. **A.** — DFS, 6.5 mg/L < tPA < 6.5 mg/L; **B.** — OS, 6.5 mg/L < tPA < 6.5 mg/L, Log rank test

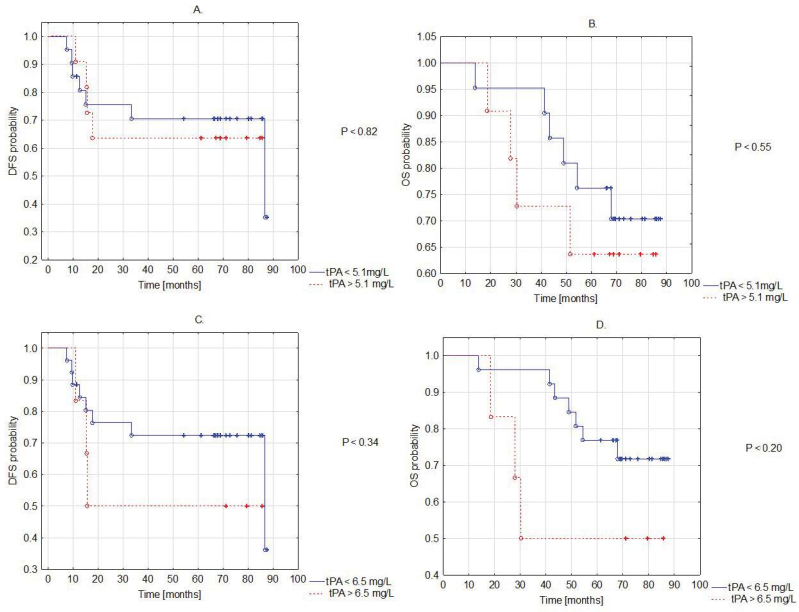

Figure 2. Kaplan-Meier analysis of disease-free survival (DFS) and overall survival (OS) according to tPA level after 3 cycles of chemotherapy. **A.** DFS, 5.1 mg/L < tPA <5.1 mg/L; **B.** — OS, 5.1 mg/L < tPA < 5.1 mg/L; **C.** — DFS, 6.5 mg/L < tPA < 6.5 mg/L; **D.** — OS, 6.5 mg/L < tPA < 6.5 mg/L; Log rank test

tissue tPA levels and obtained data came from the removed tumor at the time of surgery [15–17, 19]. In our opinion this approach has got a weakness: tumor state in vivo is much more complex than in cell culture. Neoplastic and nonneoplastic cells manifest migratory and invasive properties, linked to the cancer cell-induced process of tissue reconstruction. Expression of the plasminogen activation system differs between various tumors originating from the same tissue or from the same organ, depending on the extent of their histological differentiation [13]. In neoplastic cells the degradation of the extracellular matrix proteins is facilitated by excessive expression of t-PA. In many forms of carcinoma increased expression of fibrinolytic biomarkers is associated with significantly shorter survival. In acute nonlymphocytic leukemias, poor outcome correlates with high t-PA levels [14]. In the study by Chernicky et al. on breast cancer, low expression of tissue tPA correlated with better prognosis and longer DFS [15].

We decided to investigate the prognostic significance of plasma tPA in patients with epithelial ovarian cancer who underwent the first line chemotherapy. We found only one study which investigated prognostic value of plasma levels of selected fibrinolytic parameters in ovarian cancer [12]. Ho et al. showed that blood tPA did not differ in patients with malignant tumors, benign gynecological diseases and healthy controls. The expression of tissue tPA was significantly lower inside the tumor compared with adjacent ovarian tissue but there was no significant correlation between plasma and tissue concentration of tPA. In our study plasma concentration of tPA < 6.5 mg/L

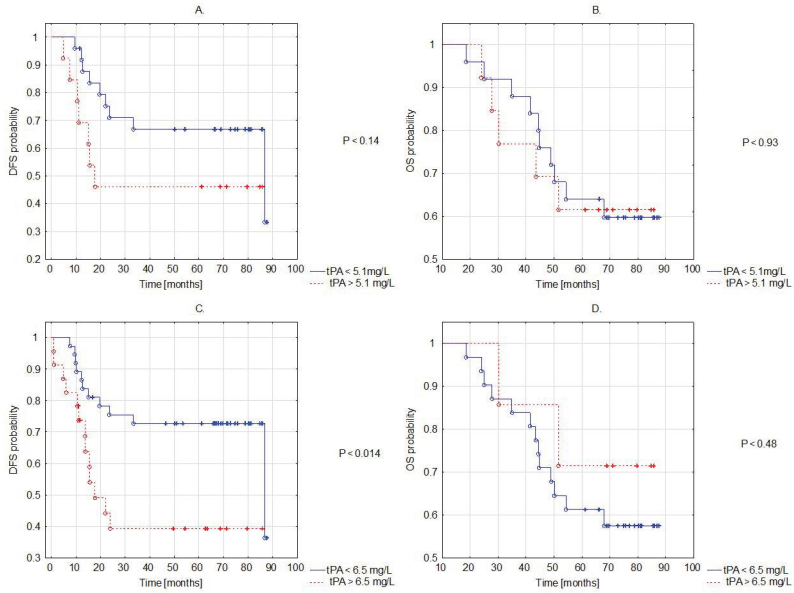

Figure 3. Kaplan-Meier analysis of disease-free survival (DFS) and overall survival (OS) according to tPA level after 6 cycles of chemotherapy. **A.** — DFS, 5.1 mg/L < tPA < 5.1 mg/L; **B.** — OS, 5.1 mg/L < tPA <5.1 mg/L; **C.** — DFS, 6.5 mg/L < tPA < 6.5mg/L; **D.** — OS, 6.5 mg/L < tPA < 6.5 mg/L; Log rank test

Table 4. Univariate analysis of factors associated with OS and DFS. The Cox regression model. RR presents relative risk of death or relapse/progression, respectively

Variable	OS			DFS		
	RR	95% CI	P	RR	95% CI	P
tPA < 6.5 mg/L vs. > 6.5 mg/L	0.34	0.15–0.73	0.006	0.35	0.15–0.80	0.01
FIGO I-II vs. III–IV	0.09	0.02–0.36	0.0009	0.12	0.03–0.50	0.005
Grade 1 vs. 2–3	0.29	0.13–0.65	0.003	0.43	0.19–0.98	0.04
Residual disease	0.25	0.11–0.54	0.0005	0.20	0.09–0.47	0.0002
BMI < 25 kg/m2 vs. ≥ 25 kg/m2	0.55	0.25–1.20	0.13	0.36	0.15–0.84	0.02
Serous vs. non-serous tumor	0.61	0.25–1.46	0.27	0.70	0.29–1.71	0.43
Age ≤ 60 years vs. > 60 years	0.38	0.50–2.15	0.93	0.94	0.40–2.20	0.88

Table 5. Multivariate Cox regression analysis of factors associated with OS and DFS. RR presents relative risk of death or relapse/progression, respectively

Variable	OS			DFS		
	RR	95% CI	P	RR	95% CI	P
Low vs. high tPA	0.44	0.19–0.98	0.044	0.59	0.25-1.40	0.23
FIGO 1–2 vs. 3–4	0.12	0.03–0.55	0.006	0.26	0.05-1.27	0.09
Grade 1 vs. 2–3	0.33	0.15–0.76	0.009	0.57	0.24-1.34	0.20
Residual disease	0.52	0.23–1.18	0.117	0.41	0.16-1.02	0.06
BMI < 25 vs. ≥ 25				0.61	0.25–1.48	0.28

at the onset of chemotherapy was associated with longer OS and DFS. Our decision to determine plasma tPA just before the beginning of chemotherapy was based on the fact that this is the time when discussion with a patient about prognosis takes place, adjuvant therapy is outlined and the need for reliable prognostic factors is indisputable. For the same reason we decided to repeat our analysis after 3 and 6 cycles of chemotherapy. We chose two cut-off levels

of plasma tPA: 6.5 mg/L and 5.1 mg/L. There were no significant differences neither in OS nor in DFS in all analyzed settings. These results have proven the clinical prognostic usefulness of plasma tPA determination only at the onset of the first line chemotherapy, not later. There is no data in the literature that could be compared with our findings. The above-mentioned Ho et al. didn't give any information on the time of collecting plasma samples, whether it was in any way connected with surgery or chemotherapy [12]. We also performed Cox regression analysis which proved that plasma tPA level of 6.5 mg/L at the onset of chemotherapy was a marker of short OS and DFS in univariate analysis and an independent marker of poor OS in multivariate analysis. This result makes tPA a good candidate for a clinical biomarker of EOC prognosis.

It is important to underline that our low and high plasma tPA groups were homogenic in terms of patients age, tumor stage, grade and histological type of malignancy as well as response to chemotherapy. Only the rate of overweight and obese patients was significantly higher in the high plasma tPA group. The impact of BMI on hemostatic markers has been widely discussed, although data concerning tPA is conflicting [20–22]. Smith et al. found that plasma tPA concentration positively correlated with triglyceride levels and BMI and negatively with HDL cholesterol level whereas Morgan et al. didn't confirm that relationship [23, 24]. Our univariate analysis revealed that BMI > 25 kg/m^2 didn't increase the risk of death but was associated with shorter DFS. In multivariate analysis BMI was not an independent factor of OS and DFS.

We also specifically looked at the clotting global tests as their availability in clinical settings is widely known [11, 25–27]. Mean PT was statistically longer in the high tPA group but didn't exceed the upper reference value at the onset and after 3 and 6 cycles of chemotherapy. Our findings were consistent with the study by Tas et al. where prolonged PT was associated with poorer OS (P = 0.03) and progression free survival (PFS) (P = 0.04) [28].

Independent prognostic value of the tumor stage, tumor grade and residual disease associated with poor OS in our study is consistent with many other studies regarding EOC [7, 17, 29]. Lack of significance in DFS comparison may reflect the limitation of our study design which is the sample size.

CONCLUSIONS

Plasma tPA is an independent marker of survival if assessed at the onset of the first line chemotherapy in patients with ovarian cancer.

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The expression of Platelet-Derived Growth Factor Receptors (PDGFRs) and their correlation with overall survival of patients with ovarian cancer

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ABSTRACT

Objectives: The main aim of the study was to investigate the expression of Platelet-Derived Growth Factor Receptors alpha (PDGFR-alpha) and beta (PDGFR-beta) in malignant and benign ovarian tumors. We performed an analysis of the correlation of PDGFRs expression and stage of the disease, tumor grade and histopathological type of epithelial ovarian cancer (EOC). Additionally, we evaluated patient prognosis according to PDGFR expression.

Material and methods: Our study group was composed of 52 samples of EOCs, 35 samples of benign ovarian tumors (BOTs), and 21 samples of unchanged ovaries (UOs). The samples were collected from patients who had been operated on in the Division of Gynecological Surgery of the Poznan University of Medical Sciences.

Results: PDGFR-alpha was found to be expressed more frequently in cancer cells of EOCs, when compared with tumor cells of BOTs and epithelium of UOs. On the other hand, PDGFR-alpha receptors were present less frequently in the stroma of EOCs, when compared with the stroma of BOTs and UOs. Comparing the studied groups, there were no statistically significant differences in the expression of PDGFR-beta. The expression of both PDGFRs was not related to the FIGO stage, grade or histopathological type of EOCs. The expression of the PDGFR-beta receptor in cancer cells was associated with an improved overall survival among patients with EOCs. Patient prognosis was not affected by either PDGFR-alpha expression or by PDGFR-beta tumor stroma expression.

Conclusions: The expression of PDGFR-alpha is significantly different when comparing EOCs, BOTs and UOs. However, the prognosis of EOC only seems to be affected by PDGFR-beta expression in cancer cells.

Key words: platelet-derived growth factor; platelet-derived growth factor receptor; ovarian malignancies, epithelial ovarian cancer

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecological malignancies and the fifth leading cause of cancer-related death among women in the Western World [1]. Although complete remission after primary treatment is achieved in approximately half of patients, the majority will relapse, and the disease then becomes fatal [2, 3]. The poor prognosis of ovarian cancer patients has motivated the development of new anti-cancer therapies. Recently, antiangiogenic treatments have been introduced, and several trials have reported encouraging results in the management of patients with ovarian cancer. Two 3rd phase clinical trials evaluated the addition of anti-VEGF monoclonal antibody (bevacizumab) to the primary chemotherapy in patients with ovarian cancer [4]. However the results were far from what was expected — only the ICON7 study indicated a pro-

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longed overall survival rate in the group of patients with high-risk, non-optimally debulked ovarian cancer [5].There are multiple theories trying to explain lack of the efficacy of an anti-VEGF blockade, and one of them postulates the role of other than VEGF proangiogenic factors, which may stimulate the development of new blood vessels [6]. Thus, it is believed, that a combined inhibition of various proangiogenic pathways may exert more pronounced clinical benefits [7].

One group of growth factors which contributes to angiogenesis are the platelet-derived growth factors (PDGFs). The PDGFs family includes five growth factors: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. These growth factors are homo- or hetero-dimers, each composed of two of the following polypeptides: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. The PDGFs are ligands for receptors: PDGFR-alpha (for PDGF- AA, AB, BB, CC) and PDGFR-beta (for PDGF-BB, DD and less for AB). PDGFs play an important role in many physiological and pathological processes — like wound healing, bone development, erythropoiesis, atherosclerosis, and fibrosis. They act as typical growth factors, thus, they also play a role in cancerogenesis [8]. PDGF-PDGFR pathway activation is observed in multiple steps essential for cancer development, including uncontrolled proliferation, evading growth suppressors, resisting cell death, infiltration, metastasis, and immune system evasion [9–12]. Matei et al. [13], have shown that autocrine activation of PDGFR-alpha stimulates proliferation of ovarian cancer cells. Lassus et al. [14] reported that the expression of PDGFR-alpha in serous ovarian cancer cells correlates with a high mitotic index. Furthermore, inhibition of PDGFR signaling leads to tumor cell apoptosis, and a decrease in microvascular density and of the tumor cell proliferation rate [15]. Matsuo et al. [16], showed that blocking PDGFR-alpha activity with monoclonal antibodies increases the sensitivity of carcinoma cells to docetaxel. PDGFs are also suspected to facilitate angiogenesis during cancer development. PDGF-AA, PDGF-BB, and PDGF-AB are expressed within endothelial cells (ECs). Of these three growth factors, probably PDGF-BB has the most important impact on angiogenesis, as it directly stimulates ECs proliferation, migration, and tube formation, and inhibits ECs apoptosis [17]. Additionally, PDGF-B action through PDGFR-beta is responsible for pericyte vessel coverage, and thus, for vessel maturation [18, 19]. On the other hand, it was shown that significant vascular abnormalities develop in PDGFR-alpha knockout mice [20]. PDGFs can also indirectly stimulate angiogenesis by increasing expression of VEGF [21]. Furthermore, recent studies indicate PDGFs are involved not only in vascular development, but they also have a prominent role in lymphaniogenesis [22]. Taken together, these data support the important role of PDGFs in ovarian cancer development and angiogenesis.

Recently, protein kinase inhibitors, targeting among others the PDGF-PDGFRs axis, were introduced for clinical trial in ovarian cancer [4]. However, targeted therapy requires the appropriate selection of patients who may benefit from this novel therapy. Thus, the main aim of our study was to investigate the expression of PDGFR-alpha and PDGFR-beta in epithelial ovarian cancer. We have compared the expression of both receptors with their presence in BOTs and UOs. Additionally, we performed an analysis of PDGFRs expression and its correlation with selected clinicopathological features of the disease. Finally, because data on the impact of PDGFRs expression on EOC patient prognosis is sparse, we evaluated the expression of PDGFRs in terms of the overall survival (OS) rates of patients with EOC.

MATERIAL AND METHODS

The study group was composed of 52 samples of epithelial ovarian cancer (EOCs). All samples were collected from patients operated on in the Division of Gynecological Surgery, at the Poznan University of Medical Sciences, during primary surgery. The control group included 35 benign ovarian tumors (BOTs) and 21 samples of unchanged ovaries (UOs) obtained for non-oncological reasons. The tumors and ovarian samples were fixed in 10% formalin for immunohistochemical study. The expression of PDGFR-alpha and PDGFR-beta was assessed by means of immunohistochemistry using the ImmunoMax technique [23]. The following antibodies were used for the immunohistochemical evaluation: PDGF Receptor alpha – monoclonal mouse anti-human antibody; dilution 1:1000; clone MM0004-8A89, Novus Biologicals®, catalogue number: NB110-60969; and PDGF Receptor beta – monoclonal mouse anti-human antibody; dilution 1:500, clone MM0005-5C37, Novus Biologicals®, catalogue number: NB110-60970. Both, the tumor cells/ovarian epithelium and tumor/ovarian stroma were evaluated for the presence of the PDGFR-alpha and -beta. We observed only cytoplasmic PDGFRs staining pat tern, both in the tumors and in the UOs. The expression of PDGFRs was evaluated using subjective assessment of PDGFRs immunoreactivity. The PDGFRs expression was assessed as negative, when less than 5% of cells presented immunoreactivity for PDGFR. Representative images are presented in the Figure 1.

Forty patients were available for follow-up. In this group, the patients' survival rates were analyzed in relation to PDGFRs expression. The median patient follow-up was 1238 days (range 28–4550). Information on patients who died was retrieved from the database of the regional office of the National Health System of Poland.

The statistical analysis was performed using MedCalc (11.4.2.0) and GraphPad (3.06) software. The distribution of the variables in the study groups was verified using the Shapiro-Wilk test. Parametric or non-parametric tests were

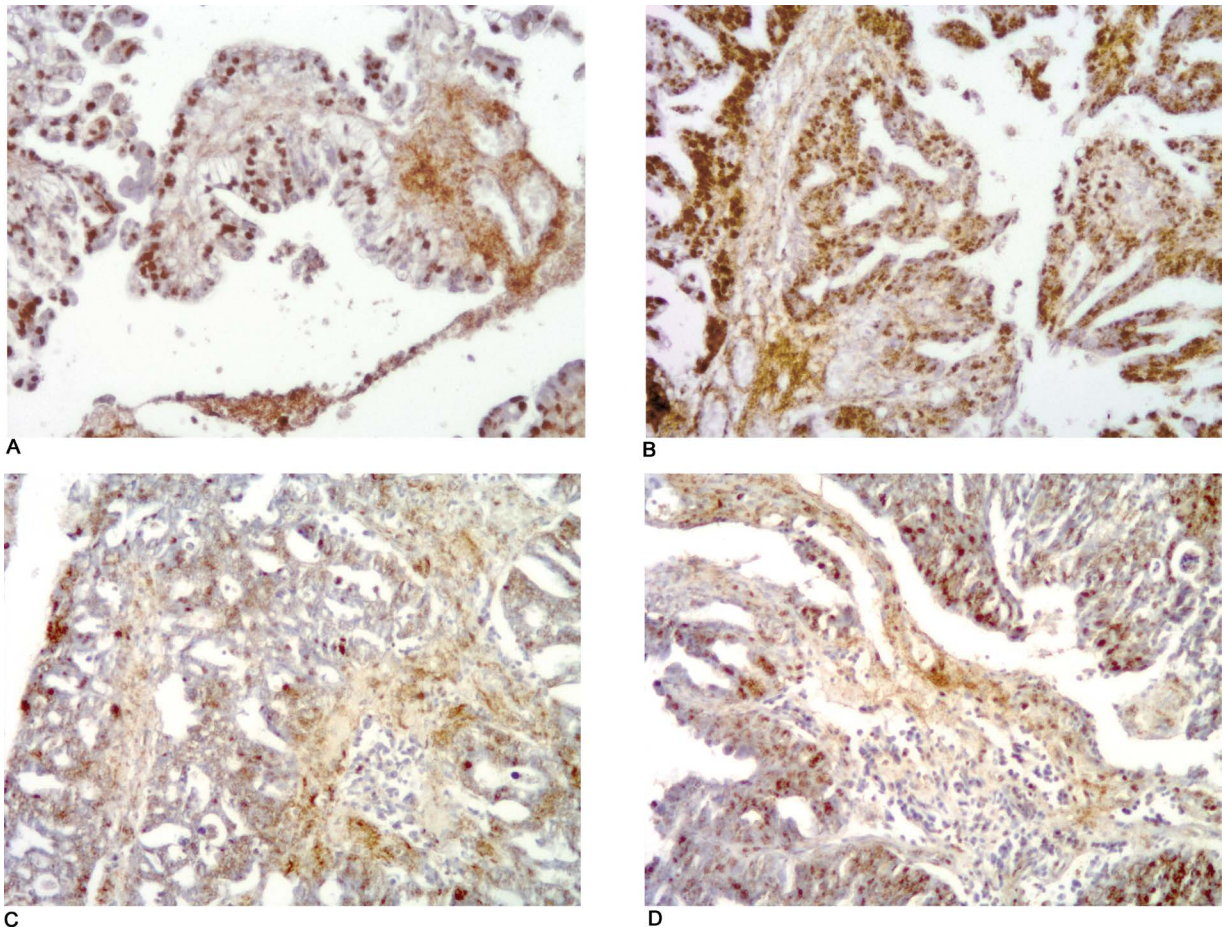

Figure 1. Representative pictures of PDGFRs expression in cancer cells and tumor stroma. **A.** Ovarian serous adenocarcinoma with strong PDGFR-alpha immunoreactivity in cancer cell cytoplasms, and moderate PDGFR-alpha immunoreactivity in the tumor stroma. **B.** The same tumor as on the picture A, showing strong PDGFR-beta immunoreactivity in cancer cell cytoplasm, and moderate PDGFR-beta immunoreactivity in the tumor stroma. **C.** Ovarian serous adenocarcinoma with weak PDGFR-alpha immunoreactivity in cancer cell cytoplasms, and weak PDGFR-alpha immunoreactivity in the tumor stroma. **D.** The same tumor as on the picture C, showing moderate PDGFR-beta immunoreactivity in cancer cell cytoplasm, and weak PDGFR-beta immunoreactivity in the tumor stroma. Magnification 200x for A–D

used for evaluation according to the data distribution. The differences in patient age and BMI were determined using the Kruskal-Wallis Test with post-hoc Dunn's Multiple Comparisons Test. The differences between the expressions of PDGFRs between studied groups were analyzed using the Fisher exact test. In the case of malignant ovarian tumors, the Fisher exact test was also used for the analysis of the differences in PDGFRs expression according to the tumor grade and the FIGO stage of the disease. We have used Chi square test for the analysis of PDGFR expression according to the histopathological type of the disease. Survival analysis was conducted using Kaplan-Meier survival curves and the differences in patient survival were determined using log-rank test.

Our research plan was approved by the University of Medical Science Poznan's Bioethical Committee (Number: 181/07).

RESULTS

The median age of patients in the group with EOCs was 53 years (range: 34–85), whereas in the group with BOTs it was 43 years (14–80). The median age in the UOs group was 50 years (44–67). The difference between the patients' ages was statistically significant ($P = 0.002$), whereas the posthoc tests only showed statistically significant differences between the group with malignant ovarian tumors and that with benign ovarian tumors ($P < 0.01$). The median body mass index (BMI) in the group with EOCs was 25.7 (range: 17.9–49.7), while in the group with BOTs and in the UOs group, the median BMI was 24.8 (18.5–37.3) and 25.2 (17.8–29.4) respectively. This latter difference was not statistically significant ($P = 0.199$).

The histopathological diagnoses of the tumors are summarized in Table 1. Among the EOCs there were nine tumors at stage I, 10 at stage II, 24 at stage III and 9 at (FIGO) stage IV.

Table 1. Histopathological diagnoses of ovarian tumors in the study group	
Malignant ovarian tumors group (n = 52)	
Serous adenocarcinoma	25
Mucinous adenocarcinoma	8
Endometrioid adenocarcinoma	4
Clear — cell adenocarcinoma	4
Undifferentiated carcinoma	11
Benign ovarian tumors group (n = 35)	
Serous cystadenoma	10
Mucinous cystadenoma	5
Endometrioma	7
Adult teratoma	10
Fibrothecoma	3

Grading of malignant tumors was as follows: G1 — 11 tumors, G2 — 10 tumors and G3 — 31 tumors.

The expression of the PDGF-alpha receptor was analyzed in 52 samples of EOCs, in 34 BOTs and in 18 samples of UOs, while the expression of the PDGF-beta receptor was analyzed in 52, 35 and 18 samples respectively. PDGFR-alpha expression was found in neoplastic cells in 33% of the malignant ovarian tumors and in 20% of the benign ovarian tumors. There was no PDGFR-alpha expression in the epithelium of the UOs. PDGFR-alpha expression between each of the groups studied was found to differ, and the difference was statistically significant (P = 0.008). Similarly, there were statistically significant differences in PDGFR-alpha expression in the analyzed groups (P = 0.005). PDGFR-alpha was found in the stroma of 83% of the UOs, 40% of the EOCs and in 58% of the BOTs. There were no significant differences in the expression of PDGFR-beta between the studied groups, both in either of the neoplastic cells/epithelium (P = 0.07) or the stroma (P = 0.29). The PDGF receptors expression results are shown in Table 2.

PDGFR-alpha expression differed in neither the tumor cells (P = 0.76) nor in the stroma (P = 0.55) between early and advanced malignant ovarian tumors (Tab. 3). Similarly, PDGFR-beta expression did not differ between the analyzed subgroups (tumor cells — P = 0.09; stroma — P = 0.76). There were no differences in PDGFRs expression between the EOCs of G1 and G2/3. These results are shown in Table 4.There were no differences in the expression of PDGF receptors between different histological types of ovarian cancer. Those results are summarized in Table 5.

Patients with PDGFR-beta expression in cancer cells of EOC (10 patients) had significantly higher median OS compared with patients without PDGFR-beta expression (30 patients) in cancer cells (3506 days, range 526–3966 vs 891 days, 28–4550, respectively, P = 0.04). There was no significant difference regarding patient survival in relation to PDGFR-beta expression in the stroma of EOC. Patients with PDGFR-beta expression in the tumor stroma (21 patients) had a median survival of 1247 days (range

Table 2. Expression of alpha and beta receptors for PDGF in epithelial ovarian cancer, benign ovarian tumors,and unchanged ovaries

	Malignant ovarian tumors	Benign ovarian tumors	Unchanged ovaries	P value
PDGFR-alpha neoplastic cells/epithelial cells	33% (17/52)	20% (7/34)	0 (0/18)	P = 0.008
PDGFR-alpha stroma neoplasm/normal ovary	40% (21/52)	58% (20/34)	83% (15/18)	P = 0.005
PDGFR-beta neoplastic cells/epithelial cells	23% (12/52)	14% (5/35)	0 (0/18)	P = 0.07
PDGFR-beta stroma neoplasm/normal ovary	63% (33/52)	50% (17/35)	67% (12/18)	P = 0.29

Table 3. Expression of PDGF receptors according to FIGO stage

	FIGO I and II	FIGO III and IV	P value
PDGFR-alpha carcinoma cells (+)	37% (7/19)	30% (10/33)	P = 0.76
PDGFR-alpha stroma (+)	47% (9/19)	36% (12/33)	P = 0.55
PDGFR-beta carcinoma cells (+)	37% (7/19)	15% (5/33)	P = 0.09
PDGFR-beta stroma (+)	68% (13/19)	60% (20/33)	P = 0.76

Table 4. Expression of PDGF receptors according to tumor grade

	G1	G2/3	P value
PDGFR-alpha carcinoma cells (+)	36% (4/11)	32% (13/41)	P = 1.0
PDGFR-alpha stroma (+)	54% (6/11)	37% (15/41)	P = 0.31
PDGFR-beta carcinoma cells (+)	27% (3/11)	22% (9/41)	P = 0.68
PDGFR-beta stroma (+)	64% (7/11)	63% (26/41)	P = 1.0

Table 5. Expression of PDGF receptors between different histopathological types of ovarian cancer

	Serous adenocarcinoma	Mucinous adenocarcinoma	Endometrioid adenocarcinoma	Clear — cell adenocarcinoma	Undifferentiated carcinoma	P value
PDGFR-alpha carcinoma cells (+)	36% (9/25)	37.5% (3/8)	0% (0/4)	25% (1/4)	35.7% (4/11)	P = 0.67
PDGFR-alpha stroma (+)	40% (10/25)	50% (4/8)	25% (1/4)	0% (0/4)	54.5% (6/11)	P = 0.36
PDGFR-beta carcinoma cells (+)	24% (6/25)	37.5% (3/8)	25% (1/4)	0% (0/4)	21.4% (2/11)	P = 0.68
PDGFR-beta stroma (+)	64% (16/25)	75% (6/8)	25% (1/4)	50% (2/4)	54.5% (6/11)	P = 0.52

28–4550), when compared with 1229 days (range 106–3402) for the patients without PDGFR-beta expression in the tumor stroma (P = 0.84). Patient prognosis was unaffected by PDGFR-alpha expression. Patients with PDGFR-alpha expression (10 patients) in cancer cells had a median survival of 2314 days (range 526–4550), while patients with no PDGFR-alpha expression (30 patients) in cancer cells had a median OS of 1012 (28–3966 days; P = 0.19). Patients with PDGFR-alpha expression in tumor stroma (14 patients) had an insignificantly higher median OS compared with patients without PDGFR-alpha expression (26 patients) in tumor stroma (1924 days, range 364–4550 vs 913 days, 28–3966, respectively, P = 0.40). Figure 2 presents the survival curves corresponding to the elements referred to here.

DISCUSSION

In our study we observed significant differences in PDGFR-alpha expression between EOCs, BOTs and UOs. The differences were found both in the cancer cells and in the tumor stroma. On the other hand, there were no differences in the expression of PDGFR-beta between the groups analyzed. Henriksen et al. assessed the expression of alpha and beta receptors for PDGF in ovarian cancer, benign ovarian tumors and normal ovaries [24]. They demonstrated PDGFR-alpha expression in 16 of the 45 malignant ovarian tumors, while they found no expression in the BOTs and the UOs. Expression in the stroma occurred in 17 of the 45 malignant tumors, 9 of the 20 benign tumors and in all of the normal ovaires. These results are very similar to ours, except for the expression of PDGFR-alpha in the benign tumors, which occurred in 20% of the tumors in our study. Additionally, we have found PDGF-alpha expression in the stroma of 83% of the UOs [24]. Madsen et al. [25] observed PDGFR-alpha expression in cancer cells in 43% of EOCs, and in 32% of their stroma. A study by Wilczynski et al. [26] revealed similar results, namely, PDGFR-alpha expression in 58% ovarian cancers and no expression in the epithelium of normal ovaries. We may conclude that the findings of these reports are compatible. In summary, about 30–58% of EOC express PDGFR-alpha in cancer cells. PDGFR-alpha expression is less frequently presented in benign ovarian tumors. Three reports showed no expression of PDGFR-alpha in normal ovarian surface epithelium. In the case of PDGFR-alpha expression in the stroma, PDGFR-alpha immunoreactivity in the stroma is found in about one-third of EOCs, one-half of BOTs, and in most UOs.

The main difference between our study and the study by Henriksen et al. is the expression of PDGFR-beta in neoplastic cells. Henriksen et al. did not observe PDGFR-beta expression in either ovarian cancer or benign ovarian tumors cells, nor in normal ovarian epithelial cells. However, our study showed PDGFR-beta expression in 20% of ovarian cancers and 14% of benign ovarian tumors. The presence of PDGFR-beta expression in ovarian cancer cells

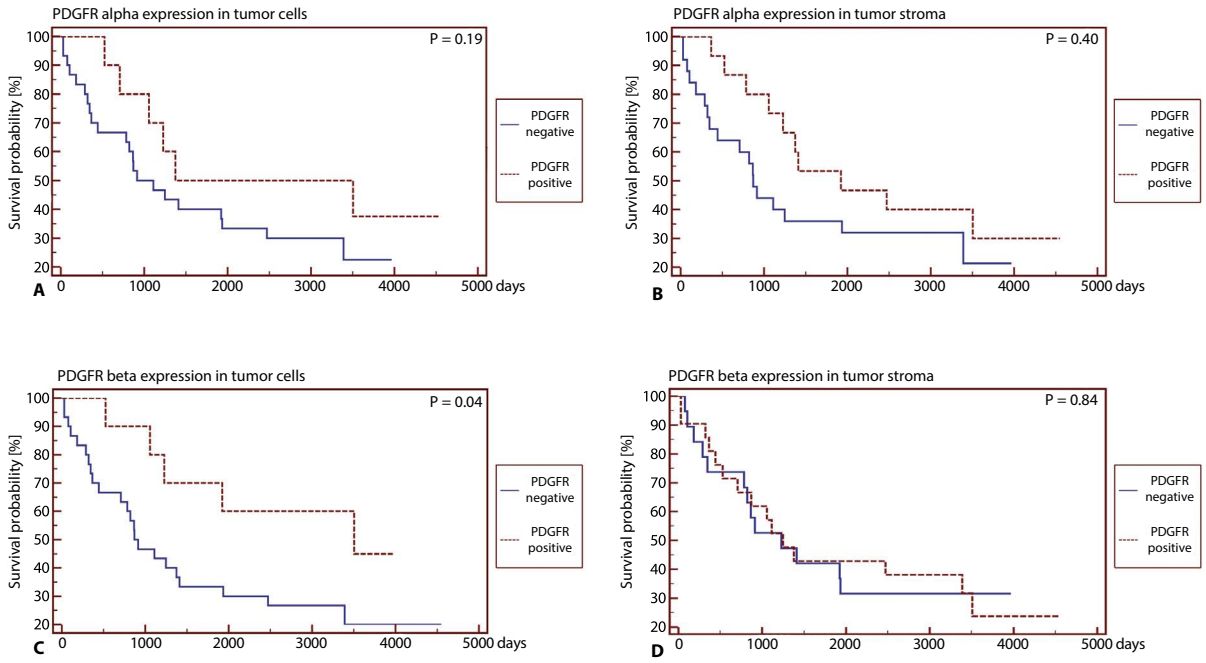

Figure 2. Survival analyses according to PDGFR expression. **A)** Patients with PDGFR-alpha expression (10 patients) in cancer cells of EOC had a median overall survival (OS) of 2314 days (range 526–4550), vs 1012 days (28–3966) for the patients with no PDGFR-alpha expression (30 patients) in cancer cells (P = 0.19). **B)** Patients with PDGFR-alpha expression in tumor stroma (14 patients) had a median n median OS of 1924 days (range 364–4550) vs 913 days (28–3966) for the patients without PDGFR-alpha expression (26 patients) in tumor stroma (P = 0.40). **C)** Patients with PDGFR-beta expression (10 patients) in cancer cells of EOC had a median OS of 3506 days (range 526–3966) vs 891 days (28–4550) for the patients without PDGFR-beta expression (30 patients) in cancer cells (P = 0.04). **D)** Patients with PDGFR-beta expression in the tumor stroma (21 patients) had a median survival of 1247 days (range 28–4550) vs 1229 days (range 106–3402) for the patients without PDGFR-beta expression in the tumor stroma (P = 0.84)

was also confirmed by other studies [24]. Apte et al. [15, 17] reported both PDGFR-alpha and PDGFR-beta expressions in ovarian cancer cells from HeyA8 and SKOV3ip1 cell lines. Madsen et al. and Wilczynski et al. [25, 26] reported PDGFR-beta expression in 41% and 29% of ovarian cancers respectively.

Madsen et al. [25] showed PDGFR-beta expression in the stroma of 44% of ovarian cancers. Henriksen et al. [24] reported PDGFR-beta expression in the stroma of 20 of the 21 unchanged ovaries studied, 21 of the 23 benign tumors, and 29 of the 45 ovarian cancers. The results of the two studies cited here corresponded with our observations.

Henriksen et al. showed more frequent expression of PDGFR-alpha in serous ovarian cancer than in mucinous and endometrioid. In our study there were no statistically significant differences in the expression of PDGFR-alpha between the various histopathological types of malignant ovarian tumors. Madsen et al. also did not find a correlation between PDGFRs expression and the histopathological type of the tumor. In the Henriksen et al. [24] study previously cited, the authors showed no differences in the expression of PDGF receptors between the different grades and stages of malignant tumors; and this finding also corresponds with our results. Similarly, no relationships were noted between PDGFRs expression and tumor stage and grade in the study by Madsen et al. [25].

In our study we found the expression of PDGFR-beta receptor in cancer cells to be associated with improved overall survival of EOCs patients. Similar results were obtained by Dabrow et al. [27], who reported a two times higher median relapse-free survival in patients with PDGFR-beta expression in cancer cells. These results are in contrast to those of the study by Avril et al. [28], which found high expression of PDGFR-beta to be associated with shortened survival rates and with platinum-resistance. However, both we and Dabrow et al. have based our study on immunohistochemistry techniques (assessment of PDGFR-beta immunoreactivity), while Avril et al. used reverse phase protein arrays to evaluate protein expression [27, 28]. Thus, the differences between the two sets of results may be explained by the studies' different methods of assessment.

We found no differences in EOC patient survival relating to the expression of PDGFR-beta in tumor stroma. Similarly, in the study by Madsen et al. [25], PDGFR-beta expression in tumor stroma did not affect patient survival. PDGFR-beta is expressed by a variety of non-cancerous cells infiltrating the tumor stroma, mainly Cancer-Associated Fibroblasts (CAFs) and pericytes. CAFs contribute to tumor stroma re-

modeling, creating a tumor-friendly microenvironment. Multiple studies confirm the role of tumor stroma and CAFs in ovarian cancer development and progression [29, 30]. Additionally, the number of CAFs seems to correlate with patients' poor prognosis, since EOC patients with stroma-rich tumors have a worse prognosis than patients with tumors characterized by poorly developed stroma [31]. On the other hand, recent studies have shown that pericyte coverage of vessels, by preventing cell migration and hematogenous metastasis, correlates with better patient prognoses [32]. In our paper we did not differentiate PDGFR-beta expressing stroma cells. We presume that further studies evaluating the source of PDGFR-beta expression in tumor stroma may contribute to a better understanding of the prognostic role of PDGFR-beta expression in the stroma of EOCs.

In our study, patient prognoses were not affected by PDGFR-alpha expression. Similar results were obtained by Madsen et al. [25]. However, in the study by Henriksen et al., patients with tumors expressing PDGFR-alpha in cancer cells had significantly shortened overall survival when compared with those with PDGFR-alpha negative tumors. The association was also significant, when the evaluation was limited to twenty-three stage III EOC patients [24]. Similar results were obtained by Matsuo et al. [16], where the authors found that increased PDGFR-alpha expression is associated with poorer overall survival when compared with low, or no, PDGFR-alpha expression. Our study did not confirm the above observations. The discrepancy between these three studies may be explained by the fact that each of the studies was based on small populations of patients. Additionally, there is significant variability in the specificity of various sets of anti-PDGFR-alpha antibodies. Thus, the different antibodies used in each of the studies may have influenced the differences between the studies' results [33].

Several trials have been conducted to evaluate the clinical utility of multipotential kinase inhibitors in the management of EOC. These drugs inhibit numerous signaling pathways involved in cancer development, including those of PDGFR-alpha and -beta. However, most of these drugs yield weak clinical responses, with no impact on patient prognosis [34]. The most promising results are related to the use of cediranib. Cediranib is a tyrosine kinase inhibitor that inhibits not only PDGFR-beta, but also all three members of the VEGFR family and c-KIT. A recent trial by Ledermann et al. [35] has shown significant prolongation of progression-free survival with cediranib when given during chemotherapy and then continued as maintenance therapy in women suffering from platinum-sensitive EOC. However, considering that PDGFRs are expressed in less than half of EOC patients, and their impact on patient prognosis is not clear, it seems reasonable to investigate PDGFR when conducting trials of PDGFR-inhibitors.

CONCLUSIONS

The expression of PDGFR-alpha, in contrast to PDGFR-beta, is significantly different between EOCs, BOTs and UOs. The expression of both PDGFRs is not affected by the clinical stage of the diseases, the tumor grade and the histopathological type of EOC. The prognostic role of PDGFR-alpha expression in EOC needs further evaluation. However, the prognosis of EOC seems to be affected by PDGFR-beta expression in cancer cells.

Conflict of interests

All authors have approved the final article and declare no conflicts of interest in relation to the current work.

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A study of physical activity levels of pregnant women using the Polish version of Pregnancy Physical Activity Questionnaire (PPAQ-PL)

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ABSTRACT

Objectives: The aim of the present study was to determine the optimal level of physical activity during pregnancy and discuss whether and to what extent biological, social and demographic variables affect the level of total physical activity in studied women.

Material and methods: The respondents were 267 pregnant women from Poland aged 28.16 ± 4.67 years. The majority of women under study had a higher and a secondary education and lived in villages near Poznań, i.e. a large urban agglomeration in Poland. Most of the women were in the first or second pregnancies, at the mean gestational age of 24th week. The study used the Polish version of PPAQ questionnaire to determine the weekly energy expenditure (MET hour/week -1) (Krzepota, Sadowska 2017). The respondents self-assessed their physical activity levels by filling in a questionnaire consisting of 33 items grouped into the following activity categories: household/caregiving (15 items), occupational (5 items), sports/exercises (7–9 items), transportation (3 items), and inactivity (3 items).

Results: Pregnant women prefer physical exercises of low and moderate intensity. The test results indicated a significant impact of variables such as age, trimester of pregnancy, and number (sequence) of pregnancies on the women's physical activity.

Conclusions: The results of the present study confirm that actions propagating active lifestyles among pregnant women are necessary. It also appears that the recommendations of the Polish Society of Gynecologists and Obstetricians regarding the physical activity of pregnant and postnatal women require adjustments and improvements.

Key words: physical activity; pregnancy; pregnancy outcomes; excessive weight gain

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INTRODUCTION

The results of epidemiological studies from a number of countries have confirmed beneficial effects of physical activity during pregnancy on the mother's and child's health [1, 2]. Regular physical exercises were shown to have a positive impact on cardiovascular endurance, lower the risk of excessive body weight gain [3], relieve spinal pains, and reduce depressive symptoms during pregnancy [4] and after childbirth as well as contribute to regaining proper body weight after childbirth. Increased physical activity during pregnancy is also associated with a lower risk of caesarean delivery [5], respiratory diseases, and macrosomia in newborns. Physical activity greatly influences the development of cognitive abilities in unborn children, preventing SI (Sensory Integration) disorders. Furthermore, physical exercises during pregnancy have a positive effect on the woman's blood pressure, and cholesterol and glucose levels. They also improve sleep quality. However, recommendations regarding the types, intensity and duration of physical activity during pregnancy may invoke certain controversies.

Research teams from different countries have attempted to estimate the optimal volume of physical activity for pregnant women using different methods and techniques. Krzepota and Sadowska [6] noted that the determination of the type, duration and intensity of physical activity during pregnancy is key to the health condition of pregnant women. Insufficient physical activity will not produce desired physiological effects, while excessive exercise unadjusted for age, health status, and physical capabilities, can be even harmful.

Evenson et al. [7] in their review *Guidelines for Physical Activity during Pregnancy: Comparisons From Around the World* indicated the fundamental differences in the assessment

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of physical activity levels of pregnant women. Their comparison of national sets of recommendations from eleven countries revealed that the differences mainly concerned contraindications to physical exercise, general training exercise guidelines, including the form and intensity of physical activity, both recommended and not recommended. In fact, the number of studies in this area appear to be rather insignificant.

The recommendations of the Polish Society of Gynecologists and Obstetricians [8] regarding physical activity during physiological pregnancy focus on the outcome of exercises of excessive intensity and volume. Hazards to the fetus may include disorders of maternal-placental circulation, hyperthermia, dehydration, limited maternal-fetal exchange, and growth disorders. On the other hand, exercising pregnant mothers may be susceptible to traumas, fatigue, fainting, and loosening of the joints (especially of the spine). These recommendations do not encourage physicians to propagate physical exercises among pregnant women.

Another problem is the lack of uniform and standardized research tools. In Poland, despite numerous publications examining the levels of physical activity of pregnant women, there have been very few studies using standardized measurement tools, e.g. Wojtyła [9], Krzepota [6], Suliga [10]. Those authors made use of the Pregnancy Physical Activity Questionnaire (PPAQ) Chasan-Taber [11]. PPAQ has been transculturally adapted for Vietnamese [12], Japanese [13,14], French [15], Turkish [16] and Polish [6, 10] populations. It can serve as a uniform criterion for measurement of physical activity of pregnant women that can be used for a comparative analysis considering cultural differences.

Objectives

The aim of the present study was to determine the optimal level of physical activity during pregnancy and discuss whether and to what extent biological, social and demographic variables affect the level of total physical activity in studied women.

MATERIAL AND METHODS

The respondents were pregnant women from Poland aged 28.16 ± 4.67 years, the majority of whom were in the age range of 25 to 35 years. The majority of women under study had a higher and a secondary education (52% and 33%, respectively) and lived in villages near Poznań, i.e. a large urban agglomeration in Poland. Most of the women were in the first or second pregnancies, at the mean gestational age of 24th week (Tab. 1).

The study used the Polish version of PPAQ questionnaire to determine the weekly energy expenditure (MET hour/week -1) [6]. The respondents self-assessed their physical activity levels by filling in a questionnaire consisting of 33 items grouped into the following activity categories: household/caregiving (15 items), occupational (5 items), sports/exercises (7–9 items), transportation (3 items), and inactivity (3 items). The declared duration of performance of particular tasks was assigned fixed numbers of minutes (0; 0.12; 0.50; 1.0; 2.0; 3.0) and then multiplied by the number of days of performance of tasks per week [6]. The obtained values were then multiplied by intensity (MET) in accordance with the guidelines in "Compendium of Physical Activities: an update of activity codes and MET intensities" [16]. The following activity intensity ranges were used: sedentary < 1.5 METs; light 1.5 – < 3.0 METs; moderate ≥ 3.0 – ≤ 6.0 METs; and vigorous > 6.0 METs.

Etical approval

The study has been approved by Poznan Medical University Ethical Committee.

Table 1. Characteristics of pregnant women under study

Variable		Total	1st trimester	2nd trimester	3rd trimester
Number of pregnant women [n/%]		267	48/18	113/42	106/40
Age [years]		28.16	28.17	27.29	29.08
Education [%]	higher	51.82	29.03	43.86	65.71
	secondary	42.73	64.52	38.64	28.57
	vocation	5.45	6.45	4.55	5.71
Place of residence [%]	city	29.09	25.81	53.13	21.88
	country	70.91	29.49	34.62	35.90
Week of pregnancy (in months)		24.22	9.96	21.58	33.04
Sequence of pregnancies [%]	first	50.00	19.28	50.60	30.12
	second	42.17	12.86	42.86	44.29
	≥ third	7.83	36.36	36.36	28.47

RESULTS

By using the Kruskal-Wallis test by ranks and the Mann-Whitney U test (Tab. 2) the authors determined first whether and to what extent socio-demographic and biological variables affected the women's total physical activity. The test results indicated a significant impact of variables such as age, trimester of pregnancy, and number (sequence) of pregnancies on the women's physical activity.

Next the women's physical activities were compared with regard to type and intensity of exercise. The highest percentage of women reported undertaking light and moderate physical activity (Tab. 3). No woman in the third trimester of pregnancy reported vigorous physical activity (> 6.0 MET) or activities associated with occupation or transportation. The respondents revealed the highest physical activity levels in the household category and the lowest in the transportation category. The mean sports activity of studied women amounted to 12.92 9 MET h/week and constituted 6% of total physical activity. Furthermore, the intensity of undertaken activities in each trimester of pregnancy decreased; however, significant differences were only found in total physical activity ($p \leq 0.0463$) and light physical activity ($p \leq 0.0247$). At the same time, lower occupational and household activity levels in the consecutive pregnancy trimesters were found, with statistically significant differences only in the former activity category ($p \leq 0.000$).

The analysis of relationships between the sequence of pregnancies and the level of physical activity produced interesting results (Tab. 4). It was revealed that the sequence of pregnancies affected significantly the women's total physical activity as well as their sedentary, light, moderate, household activities and inactivity (passive recreation). Women in their second pregnancy displayed the highest levels of total, light, moderate, occupational and household physical activity. *Ad hoc* comparisons showed that out of the five studied activity categories four were at significant levels in women in their second pregnancy. Furthermore, women in their third and subsequent pregnancies featured a significantly lower level of sedentary activity, and spent less time on passive recreation (inactivity) compared to women in their first and second pregnancies.

A factor significantly affecting the pregnant women's total physical activity was their age. A thorough analysis (ad hoc comparison) showed that age significantly differentiated only the total physical activity levels ($H = 8.13$; $p = 0.0171$) and occupational activity levels ($H = 8.73$; $p = 0.0127$) of studied pregnant women. Despite the significant correlation (coefficient of correlation $r = 0.14$; $p = 0.0184$) women's age only insignificantly (coefficient of determination $R^2 = 0.4$; $p \leq 0.01$) contributed to the variability of total physical activity levels.

Table 2. Kruskal-Wallis test and Mann-Whitney U test results for levels of total physical activity with regard to socio-biological variables

variable	Kruskal-Wallis test Mann-Whitney U test	*p* value
Age	8.13	**0.0171**
Pregnancy trimester	6.15	**0.0463**
Sequence of pregnancies	24.04	**0.0000**
Education	3.02	0.2150
Place of residence	0.35	0.7248

Values in bold are statistically significant

DISCUSSION

There have been very few Polish studies utilizing standardized research tools for assessing the level of physical activity of pregnant women. There is no research considering the frequency, duration, and type of physical activity

Table 3. Physical activity of studied women in relation to pregnancy trimester

Types of physical activity			Total	1st trimester	2nd trimester	3rd trimester	Kruskal-Wallis test	p value
Total physical activity			213.17	**265.69**	**202.43**	186.28	6.15	**0.0463**
Physical activity	by intensity	sedentary	40.18	40.26	38.43	44.10	1.39	0.4990
		light	89.49	**112.76**	**84.99**	**79.78**	7.40	**0.0247**
		moderate	68.38	99.10	62.81	40.13	1.93	0.3814
		vigorous	1.65	1.48	1.39	0	3.71	0.1561
	by type	household	112.00	123.24	114.91	88.81	0.21	0.9016
		occupational	29.83	**78.57**	**17.20**	**0**	25.33	**0.0000**
		sports	12.92	9.24	12.85	9.375	5.11	0.0779
		transportation	3.61	1.26	2.958	0	5.34	0.0729
		inactivity	54.81	53.39	54.51	53.55	0.43	0.8067

Values in bold are statistically significant

Table 4. Physical activity with regard to sequence of pregnancies

Physical activity			First pregnancy	Second pregnancy	Third and subsequent pregnancies	Kruskal-Wallis test	P value
Total physical activity			**186.93**	**263.95**	209.66	24.04	**0.0000**
Physical activity	by intensity	sedentary	**42.41**	39.06	**30.64**	7.23	**0.0270**
		light	**74.44**	**113.86**	**102.13**	36.66	**0.0000**
		moderate	**53.22**	**97.66**	66.48	27.39	**0.0000**
		vigorous	1.58	1.98	0.96	3.59	0.1658
	by type	household	**78.93**	**163.64**	**145.53**	73.89	**0.0000**
		occupational	29.69	35.13	14.30	1.54	0.4627
		sports	14.49	11.86	7.07	4.58	0.1012
		transportation	4.82	1.89	1.85	3.09	0.2130
		inactivity	**59.00**	51.43	**40.91**	14.27	**0.0000**

Values in bold are statistically significant

focusing on how these variables change during pregnancy. Very few publications have focused on relations between biological and social factors and the level of physical activity of pregnant women. The present study is an attempt to fill this void.

Research results show that pregnant women are less physically active than non-pregnant women, and that pregnancy leads to a decrease in physical activity [17]. Moreover, women have a tendency to reduce the volume, duration, and intensity of physical exercise during pregnancy [18, 19]. The level of physical activity of pregnant women is usually assessed as low [20].

In our study low (light) physical activity (< 600 METs) was characteristic of 91.2 % of studied pregnant women, moderate physical activity (600–1500 METs) of 8.8 %, and no woman under study displayed a vigorous level of physical activity. Thus in comparison with results of other Polish authors, the level of total physical activity of pregnant women in the present study (213.17 MET) was slightly lower than in Krzepota and Sadowska [6] — 246.41 MET, but higher than in Wojtyła [9] — 190.83 MET. The studied women featured a higher contribution of moderate-intensity exercise (3.0–6.0 MET), and lower contribution of sedentary physical activity (< 1.5 METs), compared with women studied by other Polish authors. The women from Poznań spent less time watching TV or video movies, reading or making job unrelated telephone calls than women studied by Wojtyła [9], Krzepota [6] and Suliga [10].

Experts indicate that pregnant women tend to replace moderate-intensity exercises with low-intensity sedentary exercises [21]. A review of Polish literature revealed that lower levels of education were associated with a lower interest of Polish society in sports and recreational forms of leisure [22]. Similar results were produced in the 2009 Eurobarometer [23] survey conducted among 26 788 citizens of 27 EU member states. The survey revealed a strong correlation between education and frequency of undertaking active leisure. 64% of persons who completed their education at the age of 15 were shown to have never undertaken regular physical activity. The respective percentages were 39% of those who finished their education at the age of 16–19 years, and 24% of those who completed their education at the age of 20 years and above. The Eurobarometer survey authors postulated that a higher education level was associated with a better standard of living, and that better educated EU citizens associated physical fitness with better quality of life. In the present study most women had a higher and secondary education, which could have significantly affected the level of their total physical activity that was higher than the level of physical activity of pregnant women in other studies using the same assessment tool (Tab. 5). It can be suggested that research designs should consider biological and socio-demographic factors that can significantly affect the level of physical activity.

The present study also revealed a disturbing tendency of decreasing physical activity levels in the later trimesters of pregnancy. Significant differences were found in total physical activity level in the first and second trimesters, and in low-intensity activity level in all trimesters. Although the respondents were not asked to provide reasons for cessation of exercising, the observed decrease in physical activity in the second and the third trimesters of pregnancy can be related to women's mood changes and fetal growth which leads to gaining weight and discomforts such as back pain, fatigue, and sleeplessness [24, 25]. As indicated by other authors decreased physical activity can also result from the lack of exercising habits related to the development of the awareness of physical activity. Experts propose that

Table 5. Comparison of study results by different authors

Activity	Authors' own research (2018) Polish N = 267	Wojtyła (2012) Polish N = 2852	Suliga (2017) Polish N = 164	Chandonnet (2012) French N = 49	Ota (2008) Vietnamese N = 60
Total activity	213.17 ± 167.42	190.83	no data	180.00	137.97
Sedentary	40.18 ± 27.98	59.50	60.70	60.00	28.00
Light	89.49 ± 36.36	83.65	44.10	73.00	119.70
Moderate	68.38 ± 129.33	31.75	20.40	34.00	3.10
Vigorous	1.65 ± 92.66	0	0.80	0	0
Household/caregiving	78.93 ± 95.96	142.54	72.70	74.00	11.30
Occupational	29.69 ± 30.24	0	37.00	0	0
Sports/exercises	14.49 ± 122.16	20.13	6.80	11.00	0.60
Transportation	4.82 ± 29	0	9.50	14.00	2.80

women who led a physically active lifestyle before becoming pregnant, should not abandon their active lifestyle habits but only modify them. The intensity and type of exercises should be adjusted for pregnancy trimester and woman's general disposition. During physiological pregnancy with no complications, medical professionals see no contraindications to undertaking physical activity during pregnancy. Healthy women should begin or continue moderate-intensity aerobic activity during pregnancy, accumulating at least 150 minutes per week. [3, 26, 27]. The most beneficial forms of physical activity for pregnant women are walking, gymnastics, yoga, and swimming.

Experts also point to the fact that a decrease in physical activity during pregnancy can be noted in the performance of physical exercises as well as activities of daily living such as household chores, caregiving, transportation, and occupational activities. Research shows that undertaking physical activity during pregnancy has a significant influence on women's self-assessment of the course of the pregnancy and on their life satisfaction [28]. Non-employed women and pregnant women on sick leave experience health problems much more often. Professionally active women cope with emotional problems more effectively, feel stronger, have a higher self-esteem, and display greater physical endurance. They also recover faster and are more resourceful in their daily living (Nowakowska-Głąb, Maniecka-Bryła 2012). On the other hand, very intensive and intensive professional activities negatively affect intrauterine fetal development and contribute to a decrease in newborns' birthweight [29]. In the present study the highest levels of occupational activity were found in women in the first trimester of pregnancy. In the second trimester the level of occupational activity was significantly lower, and in the third trimester no women undertook any professional activities.

The above results appear to be very disturbing. The pregnant women under study prefer low- and moderate-intensity exercises, while their level of total physical activity is slightly higher than in respondents from other studies. These results could have been affected by the women's higher education level than in women studied by other authors. On the other hand, the study groups evaluated by other researchers were not that numerous (except for Wojtyła et al.). It was rather difficult to find comparable studies in Polish literature The available research data on the physical activity of pregnant women are spotty and inconsistent, and often based on various authors' own questionnaires. This makes any solid comparative analysis of results rather difficult. There is a pressing need to develop and implement the educational component of maternity care, increase pregnant women's motivation to take up physical exercises, and modify and adjust relevant recommendations of the Polish Society of Gynecologists and Obstetricians.

CONCLUSIONS

1. Pregnant women prefer physical exercises of low and moderate intensity. The research results show that only biological variables, i.e. age, trimester, (number) sequence of pregnancies, significantly affect pregnant women's level of total physical activity.
2. The general awareness of benefits of physical activity is not enough to encourage women to take up physical exercises during pregnancy. The results of the present study confirm that actions propagating active lifestyles among pregnant women are necessary.
3. The results of the present multi-characteristic analysis can be used to define the approaches for maternity care and healthcare professionals and institutions to raise the awareness of benefits of physical activity for the course of pregnancy and fetal development.
4. A prospective thorough analysis of physical activity of pregnant women will require designing an extra questionnaire that would include items related to respond-

ents' pre-conception health behaviors (e.g. physical activity, sport career); socio-demographic data (occupation, education, marital status, number of children); and pre-conception body height and body weight.

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The relationship between body mass index, body composition and premenstrual syndrome prevalence in girls

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ABSTRACT

Objectives: Premenstrual Syndrome (PMS) is a cluster of physical and emotional symptoms occurring in the luteal phase of the menstrual cycle. The study aim was to determine the relationship between PMS, and state of nutrition expressed as Body Mass Index (BMI) and body composition in 18-year-old females.

Material and methods: The study was conducted on 476 women divided into two groups i.e. those suffering from PMS (n = 233) and those without symptoms (n = 243). The women were examined during their luteal phase using bioelectrical impedance analysis to determine their body composition. Height and weight were measured using digital medical scales with an electronic height rod. BMI was calculated thus: BMI = body mass (weight) (kg)/height (m^2). The subjects were divided into two sub-groups: BMI < 25 kg/m^2 and BMI ≥ 25 kg/m^2. Statistical analysis was carried out using STATISTICA 10 PL software and the Mann-Whitney test.

Results: The women with normal BMI suffered from PMS twice as often as the women with BMI ≥ 25 kg/m^2 (68.2% vs. 31.8%). There were significantly higher values for Fat Mass (FM) (%) ($p < 0.01$) and FM (kg) ($p < 0.001$) in women without PMS, and significantly higher values for Fat-Free Mass (FFM) (%) ($p < 0.001$) and Total Body Water (TBW) (%) ($p < 0.001$) in women with PMS. Higher values were reported for FFM (kg) and TBW (kg) ($p < 0.05$) in girls with PMS and BMI ≥ 25.

Conclusions: These results show PMS is more frequent in patients with BMI < 25, and less frequent in patients with higher FM (kg) and FM (%). Moreover, significant frequency of PMS was observed in patients with higher FFM and TBW. Such statistical significance was not confirmed in girls with a BMI < 25.

Key words: premenstrual syndrome; gynaecology; body composition

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INTRODUCTION

Premenstrual Syndrome (PMS) is a cluster of somatic, emotional and behavioural symptoms occurring in the luteal phase of the menstrual cycle. Many studies have been conducted but the aetiology of PMS remains unknown [1–3]. PMS symptoms substantially affect the quality of a woman's life [2, 3].

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. No other psychiatric or health conditions that might cause similar symptoms are observed. The symptoms, with irritability being a major one, occur to a degree that they impair some aspects of a women's life, in particular, their sexuality. Such

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symptoms usually last from 10 to 14 days every month. The basic diagnostic criteria for PMS include:

1. Prospectively establishing the timing of the symptoms, confirmed by prospective daily ratings for at least two menstrual cycles, with changes documented in a diary (at least one affective or physical symptom during the last five days before menses and remission of the symptoms within the first four days of the menses).
2. A 30% increase in the intensity of symptoms in the luteal phase, as compared with the follicular phase.

It is made sure that the subjects do not have histories of psychiatric disorders or hormonal medication such as oral contraceptive pills or irregular menstrual cycles. The most common PMS symptoms include physical symptoms: breast tenderness or breast pain (mastalgia), accompanied by breast swelling and tenderness of the nipples (70%), abdominal pains, bloating, abdominal swelling and sometimes constipation (50%), headaches and migraines (30%), swelling and weight gain (45%), cramps and back pains, dizziness, digestive disturbances, nausea, vomiting, diarrhoea, swelling of extremities (both hands and feet), joint or muscle pain, palpitations, frequent micturition, increased thirst, decreased alcohol tolerance, and skin lesions. Symptoms of chronic diseases are frequently aggravated e.g. allergic symptoms, including hay fever, bronchial asthma and allergic dermatitis, chronic digestive disorders and nervous system diseases (e.g. epilepsy). Other psychologic and behavioural PMS symptoms include: sugar and salt cravings, tension or anxiety, irritability, crying and tearfulness, fatigue, depressed mood, insomnia or hypersomnia, forgetfulness or confusion, panic attacks, aggression and depression [4–9].

The prevalence of PMS is related to the ovarian cycle and the influence of oestrogen and progesterone on centrally acting neurotransmitters, serotonin and GABA (γ-*aminobutyric acid*). Arguing for this hypothesis is that PMS is not prevalent before puberty, during pregnancy and after menopause. Oestrogen and progesterone influence serotonin receptors. The evidence suggests that treatment with selective serotonin reuptake inhibitors (SSRI PMS) alleviates PMS symptoms. Allopregnanolone, a metabolite of progesterone, modulates GABA and symptomatic women seem to have lower levels of allopregnanolone. Some studies find that PMS may be determined by genetic factors; however, no specific genotype has been identified yet. Childhood emotional experiences are linked with PMS prevalence in adulthood. In addition, the occurrence of PMS is influenced by lifestyle (physical activity, diet), personality disorders and addiction to psychoactive substances, as well as high body mass index (BMI) [10–21].

Research has been going on for many years on the changes in body composition that are related to age, malnutrition or overnutrition. However, to our knowledge, no previous studies have examined whether body composition may be a premenstrual syndrome indicator in young women.

In this study, bioelectrical impedance analysis (BIA) was used to assess body composition of the patients [1]. BIA is a non-invasive diagnostic method using the ability of tissues to impede electric current and providing accurate analysis of body composition. BIA approach is also used in total body water and fat content measurements [1, 4].

Techniques for assessing body composition emerged in the first half of the 20th century. Initially, an indirect way of this assessment relied on the analysis of body fluids. The discovery of deuterium enabled accurate assessment of total body water, and the use of other isotopes of hydrogen helped to develop assessment techniques of totally replaceable electrolytes – sodium and potassium. The early studies by Barnett and other investigators focused on the relationship between bioelectrical impedance measurements and total body water. In the 1970's, Nyboer et al. started their pioneer research on impedance plethysmography, in which they highlighted the relationship between the changes in body impedance and the changes in pulsatile blood, pulse and breathing. Simultaneously, hydrodensitometry was being investigated as a method of estimating body composition. At the time, most of the body mass measurement methods relied on the distinction between the two main components i.e. fat and non-fatty matter. Hydrodensitometry apparatus became standard equipment for measuring body composition. A distinction was made between fat mass (FM) and fat-free mass (FFM). Fat mass components include triglycerides and lipids but no water or potassium. The composition of fat-free mass include 72–74% of water and 60–66 mmol/kg of potassium. This 2-compartment model became a basis for the modern research on body composition. In the years that followed, a 4-compartment model was developed which divided the body into measured water, protein, bones, mineral mass (ash mass) and fat. As in the 2-compartment model, total body potassium (TBK) measurements and total body water (TBW) measurements were used to estimate body components. The division based on TBK and TBW measurements led to the development of the 4-compartment model. The 4-compartment model includes: FM, BCM (Body Cell Mass), ECW (extracellular water; in girls about 45% of TBW) and other fat-free extracellular solids (FFECS). Later on, intracellular water (ICW), which constitutes approximately 55% of total body water, was distinguished from ECW. Bioelectrical impedance (BIA) was first used in the mid-1980s. Today many BIA devices are in use, with different electrode configurations and different frequencies [1, 4].

The objective of the present study was to investigate the relationship between state of nutrition, expressed as BMI, body composition in 18-year-old girls, and premenstrual syndrome prevalence.

MATERIALS AND METHODS

The study was conducted among the patients of Gynaecology and Perinatology Medical Centre in Gynaecology and Obstetrics Hospital of Poznan University of Medical Sciences. The sample of 476 Caucasian women aged 18 was divided into two groups: experimental one with PMS (n = 233) and controls without PMS (n = 243).

Diagnosis of PMS in this study was made according to the diagnostic criteria suggested by Royal College of Obstetricians and Gynaecologists (RCOG) [16].

Testing for PMS included a symptom diary in which the patients prospectively recorded the symptoms within two full monthly cycles.

PMS was diagnosed if the patient reported at least one of the following symptoms including depression, irritability, anxiety, confusion or social withdrawal and one of the somatic symptoms (breast tenderness, abdominal bloating, headache, swelling of extremities) during the 5 days before menses in the two prior menstrual cycles. The patients were diagnosed by a practitioner during the medical examination.

Exclusion criteria were based on the exclusion of disease entities that could be similar to PMS, i.e. mental disorders such as depression, bipolar disorder, anxiety and personality disorders, as well as drug dependence, endometriosis, thyroid function disorders, allergies, and autoimmune diseases [17].

Body composition was assessed with Tanita MC 780 device which uses bioelectrical impedance analysis (BIA method) [4]. In this model, the accuracy of measurement for the individual components, including adipose tissue, is 100 grams. The measurements of fat mass FM, fat-free mass FFM and total body water TBW have been expressed as percentage (%) and kilograms (kg). All the patients had their height and body mass measured. Weight was measured to the nearest 0.1 kg using a SECA 899 digital medical scales. Height was measured to the nearest mm with a SECA 217 stadiometer attached to the scales. The patients were asked to remove their shoes and outer garments.

The measurements were taken according to the standards for anthropometric assessment (The World Health Organisation, 1987). BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2).

The participants were grouped according to their BMI: < 25 kg/m^2 and BMI ≥ 25 kg/m^2.

Statistical analysis was carried out using STATISTICA 10 PL software. Due to non-normal distribution and inability to ensure constant homogeneity of variances, Mann-Whitney test was used to assess parametric differences in PMS and non-PMS women. Statistical significance was set at $P < 0.5$.

Before the study began, all the patients had been informed about the procedures, aims, and scope of the study. Informed consent was obtained from all the participants. The study was approved by the Bioethics Committee of Karol Marcinkowski Poznan University of Medical Sciences (Resolution No 553/18).

Table 1. Characteristics of participants according to PMS prevalence and BMI

BMI in kg/m2	prevalence of PMS			
	yes n = 233		no n = 243	
	n	[%]	n	[%]
< 25	159	68.2	127	52.3
≥ 25	74	31.8	116	47.7

Source: authors' own research

RESULTS

The study was conducted among 476 women aged 18 who were divided two groups: women with PMS (n = 233) and those without PMS (n = 243). Then, two sub-groups were identified: women with BMI < 25 kg/m^2 and women with BMI ≥ 25 (Tab. 1). It was observed that among the women with normal body mass (BMI < 25 kg/m$^{2)}$), twice as many had PMS, compared to those with a BMI ≥ 25 (68.2 vs. 31.8).

Statistically significant differences between PMS and non-PMS groups are given in Table 2.

The parameters for analysis included: fat mass (FM), fat-free mass (FFM) and total body water (TBW), expressed as percentage and kilograms.

There were statistically significant differences between the PMS and non-PMS groups for FM, expressed as percentage and kilograms, with significantly higher values for women without PMS ($p = 0.001$ and $p = 0.0001$ respectively). Higher values for FFM (%) ($p = 0.0001$) and TBW (%) ($p = 0.0001$) were reported for women with PMS.

As shown in Table 3, there was no statistically significant difference in the examined parameters between trials in women with PMS and the control group with normal body mass i.e. BMI < 25.

Body composition of the two groups in patients with BMI ≥ 25 was compared. Statistically significant differences were observed. In women with PMS, higher values were recorded both for FFM (%) ($p = 0.02$) and TBW (kg) ($p = 0.02$) (Tab. 4).

DISCUSSION

In the present study we have assessed the state of nutrition and body composition in 18-year-old girls, with the use of BMI and BIA respectively. The objective of the study was to determine the relationship between BMI, fat mass, fat-free mass and water with PMS prevalence.

Our study reported that there was a statistically significant relationship between PMS and BMI. PMS is significantly

Table 2. Descriptive statistics for body composition parameters analysis in PMS and non-PMS women

Parameter	PMS prevalence	n	Mean	SD	Median	Min.	Max.	Mann-Whitney test	
								Z	p
FM [kg]	yes	233	20.2	10.1	17.3	2.1	61.5	-3.29	0.001*
	no	243	23.0	10.8	21.2	2.1	61.7		
FM [%]	yes	233	29.2	8.2	28.3	5.7	50.1	-3.96	0.0001*
	no	243	32.0	8.8	32.1	5.0	53.7		
FFM [kg]	yes	233	45.7	6.6	44.3	28.7	70.5	-0.35	ns
	no	243	45.4	5.5	45.2	30.8	65.9		
FFM [%]	yes	233	70.8	8.2	71.7	50.0	94.4	3.96	0.0001*
	no	243	68.0	8.8	67.9	46.3	95.0		
TBW [kg]	yes	233	33.5	4.8	32.4	21.0	51.6	-0.35	ns
	no	243	33.2	4.0	33.1	22.6	48.2		
TBW [%]	yes	233	51.8	6.0	52.4	36.6	69.0	3.95	0.0001*
	no	243	49.8	6.5	49.6	33.8	69.5		

*statistically significant, p < 0.05; ns — not statistically significant; SD — standard deviation
Source: authors' own research

Table 3. Descriptive statistics for body composition parameters analysis in PMS and non-PMS women with BMI < 25 kg/m2

Parameter	Prevalence of PMS	n	Mean	SD	Median	Min.	Max.	Mann-Whitney test*	
								Z	p
FM [kg]	yes	159	14.92	4.49	14.70	2.10	28.10	-0.49	ns
	no	127	15.26	5.32	15.20	2.10	27.80		
FM [%]	yes	159	25.18	5.52	25.50	5.70	36.20	-1.07	ns
	no	127	25.70	6.41	26.70	5.00	38.60		
FFM [kg]	yes	159	43.48	5.26	42.70	28.70	70.50	0.67	ns
	no	127	42.63	3.85	42.40	30.80	54.30		
FFM [%]	yes	159	74.85	5.56	74.58	63.74	94.39	1.08	ns
	no	127	74.31	6.40	73.33	61.45	94.99		
TBW [kg]	yes	159	31.83	3.85	31.30	21.00	51.60	0.66	ns
	no	127	31.21	2.82	31.00	22.60	39.80		
TBW [%]	yes	159	54.79	4.04	54.58	46.71	68.98	1.07	ns
	no	127	54.41	4.69	53.71	45.02	69.45		

* statistically significant, p < 0.05; ns — not statistically significant; SD — standard deviation
Source: authors' own research.

more prevalent in girls with normal BMI (Tab. 1) and with significantly lower FM, higher FFM and higher TBW.

Fat mass average in non-PMS women was approximately 3 kg higher than in PMS women. Also, non-PMS women had statistically lower fat-free mass and lower total body water (Tab. 2).

PMS women with BMI ≥ 25 kg/m^2 had statistically higher fat-free mass (FFM, kg) and water (TBW, %). No such statistical significance was found in women with BMI < 25.

The present findings are not consistent with the findings from several previous studies which concluded that PMS is more prevalent in obese women with BMI > 30 [14] and BMI > 27.5 kg [15].

These previous studies have relied on the theory that in premenstrual period, glucose-induced metabolism of food deviates from the brain to the reproductive system and its surroundings including an increase in the level of blood in uterus vessels, which provides menstruation. Subsequently, this deviation leads to changes in brain control adjustment processes, which in turn cause impulsive symptoms characteristic of PMS. However, this hypothesis is not fully understood, because it has been proven that increased fat deposits compensate for a lack of metabolism required by the body and simply enhances glucose consumption. Since the premenstrual phase is characterized by a decrease in hormones in the bloodstream, no

Table 4. Descriptive statistics for body composition parameters analysis in PMS and non-PMS women with BMI ≥ 25 kg/m^2

Parameter	PMS prevalence	n	Mean	SD	Median	Min.	Max.	Mann-Whitney test*	
								Z	p
FM [kg]	yes	74	31.52	9.38	28.90	16.90	61.50	-0.52	ns
	no	116	31.54	8.64	30.60	15.20	61.70		
FM [%]	yes	74	37.85	5.85	36.95	19.30	50.10	-1.63	ns
	no	116	38.87	5.24	39.00	18.70	53.70		
FFM [kg]	yes	74	50.58	6.62	49.75	38.50	70.50	2.33	0.02*
	no	116	48.45	5.34	47.15	39.60	65.90		
FFM [%]	yes	74	62.16	5.86	63.11	49.95	80.76	1.63	ns
	no	116	61.13	5.24	60.97	46.27	81.26		
TBW [kg]	yes	74	37.03	4.85	36.40	28.20	51.60	2.34	0.02*
	no	116	35.47	3.90	34.55	29.00	48.20		
TBW [%]	yes	74	45.50	4.29	46.21	36.56	59.11	1.62	ns
	no	116	44.75	3.84	44.64	33.84	59.43		

*statistically significant, $p < 0.05$; ns — not statistically significant; SD — standard deviation
Source: authors' own research

significant increase in the function of the ovary and the uterus at the beginning of and during the next cycle is observed, and there is no clear reason for increased metabolic costs which are different during the premenstrual phase [16, 18]. This theory partly explains the results of our research.

Moreover, it has to be noted that the cited authors [14, 15] previously conducted their studies on younger and older women aged 18–44. Our study group only included 18-hear-olds.

It is likely that obesity and being overweight will play a role in the etiology of PMS, although the relationship between PMS and anthropometric indicators in ambiguous. In some studies, BMI is directly or indirectly related to the body's hormonal balance mechanism [14, 19, 20, 22].

Most investigators relate PMS to the falling levels of progesterone during the luteal phase of the menstrual cycle, which leads to the lower oestrogen/progesterone ratio. One of the etiologic theories is related to fluid retention. The theory suggests that oestrogen induces increased hepatic angiotensinogen synthesis, which results in higher aldosterone levels. This in turn, leads to sodium retention and potassium-depleting effect. It has also been found that in PMS, there is little increase in prolactin levels in the luteal phase, which may be the result of abnormalities in the synthesis and the release of dopamine and serotonin. It has been proven that dopamine has a direct renal diuretic effect, whereas serotonin makes kidneys more sensitive [1, 2].

The feeling of pressure in the lower abdomen and low back pains result from fluid retention in the uterus and the venous stasis in the lesser pelvis. The swelling of central nervous system cells is the cause of headaches, dizziness, nausea, mental tension, insomnia, anxiety, tendency for depression and increased appetite. Weight gain by 2–4 kg resolves within the first days of menstruation [2].

The results of our 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 form women with BMI ≥ 25 kg/m^2.

The pathophysiology of premenstrual syndrome is still unclear, and its causes may be of multifactorial nature.

Previous studies not very comprehensive assessed body composition in relation to PMS, it is impossible then to establish comparative and evolving nature of this relationship. Therefore, it is recommended that further studies should be conducted to confirm correlations between the prevalence of PMS and body composition expressed as fat mass, fat free mass and total body water content. In order to develop and understanding of their importance for future treatment of women with PMS.

A limited number of previous studies have evaluated the relationship between body mass index body composition and premenstrual syndrome and to our knowledge, this is the first study that has investigated the relationship between PMS prevalence, state of nutrition and body composition in developmental age girls. Further study is recommended to determine the optimal BMI and fat mass values, which will help minimize PMS prevalence.

CONCLUSIONS

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.

It is also vital to ensure that PMS-related psychosexual disorders are diagnosed, and proper treatment is administered.

The strengths of our study include sample size (476 women) and its homogeneity (all the patients were Caucasian women aged 18). A limitation of the study is that we did not analyze lifestyle factors such as diet, physical activity and the use of stimulants, all of which may be potentially linked to PMS prevalence.

Conflict of interests

The authors declare no conflict of interests.

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Is polycystic ovarian syndrome and insulin resistance associated with abnormal uterine bleeding in adolescents?

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ABSTRACT

Objectives: The aim of the study was to determine if adolescents with juvenile bleeding had polycystic ovarian syndrome (PCOS) and insulin resistance.

Material and methods: The study was conducted in a group of 43 females aged 12–18 years, diagnosed with juvenile menorrhagia, and 37 healthy female adolescents aged 12–18 years. The study was conducted during the early follicular phase of the menstrual cycle. Menstrual cycle disturbances, acne and hirsutism were recorded. Ultrasound scan determining the condition of the ovaries was conducted. Laboratory tests of the glucose level, cholesterol, LDL and HDL cholesterol and triglycerides fraction, DHEAS, FSH, LH, insulin, SHGB, total testosterone, androstenedione, and free testosterone have been established.

Results: The occurrence of regular menstrual cycles (30.23%, p = 0.006) was significantly lower in the juvenile bleeding group. Also, secondary amenorrhea was significantly more likely to be recognized in this group of females (p = 0.03). The concentration of FSH was considerably lower (p = 0.0002) in the group of adolescents with AUB.

Conclusions: Adolescents with abnormal uterine bleeding (AUB) are often diagnosed with secondary amenorrhea, and PCOS. The group with a diagnosis of juvenile bleeding was also diagnosed with higher rates of insulin resistance.

Key words: abnormal uterine bleeding; polycystic ovarian syndrome; hyperandrogenism; insulin resistance

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INTRODUCTION

Abnormal uterine bleeding (AUB), or juvenile bleeding, is defined as heavy bleeding with blood clots, lasting more than 7 days and often leads to anemia. Menstrual blood loss exceeds 80 mL [1–5].

It has been observed that abnormal menstrual bleeding occurs in approximately 20–30% of adolescent females, and one fifth experience features relating to AUB during their first period. It is estimated that 80% of adolescent bleeding occurs one to two years after menarche [3, 4].

Etiopathogenesis of juvenile bleeding has not yet been fully established. It is believed that heavy menstrual bleeding in adolescence has no organic etiology, but is due to a lack of ovulation and luteal insufficiency. In these cases, menstrual cycles are not yet regular, there are no premenstrual symptoms and bleeding may start without any signals indicating the approach of menstruation [4–7].

It has also been suggested that juvenile bleeding may be associated with hormonal disorders such as polycystic ovarian syndrome (PCOS). It was observed that among some young women with PCOS, incidents of heavy menstrual bleeding had previously occurred [8].

Currently, there are only few results from large epidemiological studies which would affect the risk factors

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of the polycystic ovarian syndrome. In studies conducted on small groups of women, a significantly higher incidence of PCOS was observed among women with low birth weight, premature start of puberty and the onset of menarche over 15 years of age. A risk factor for PCOS is obesity and there are also observations being carried out on the genetics of this syndrome [9–12].

Heavy menstrual bleeding occurs frequently and can significantly impair the quality of life of adolescent females. Thorough analysis and research can help in revealing the causes of abnormal bleeding and prevent intensification of co-endocrine disorders such as acne, hirsutism, PCOS, metabolic syndrome and insulin resistance in future life [12–16].

Objectives

The aim of this research project was to assess the presence of polycystic ovarian syndrome and insulin resistance in females diagnosed with juvenile bleeding.

MATERIALS AND METHODS

Study population

The study included 43 females aged between 12 and 18 years old, who were diagnosed with abnormal uterine bleeding (AUB), and 37 healthy females of the same age range, who constituted the control group. All respondents gave written consent to carry out all the procedures included in the study protocol (in case of underage patient, consent was taken both from the participant and their parent or legal guardian). The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (No. of consent KNW /0022/KBI/74/12).

Methods

All participants of the study had a gynecological assessment, which established menstrual cycles (length, regularity of menstrual cycle, presence of secondary amenorrhea and the length of menstrual bleeding). According to the American Society of Obstetricians and Gynecologists (ACOG), normal menstrual cycles in adolescent females last 21–45 days, and the duration of menstrual bleeding ≤ 7 days [14]. Heavy menstrual bleeding is defined as being > 80 mL [2, 4, 17]. Irregular menstrual cycles, are when the length of the cycle is shorter than 21 days (*polymenorrhoea*) or longer than 45 days (*oligomenorrhoea*) [17]. Secondary amenorrhea was defined as lack of menstrual bleeding for more than 6 months in those with a history of normal menstruation [18].

Clinical features of hyperandrogenism were based on the occurrence of features such as hirsutism, acne (diagnosed based on a subjective 10-step scale), and androgenetic alopecia. Hirsutism was assessed using the Ferriman and Gallwey scale and was diagnosed if the score was 8 or more points [7, 18]. In addition, between the 3rd and 5th day of the menstrual cycle a transabdominal ultrasound scan was conducted to assess the structure and volume of the ovaries. The diagnosis of PCOS was based on the ESHRE/ASRM criteria. Assuming that the adolescents satisfied all three criteria, and had been assessed for at least two years after menarche [19–22]. All participants also had anthropometric measurements (weight, height, waist and hips).

The survey excluded adolescents who also had significant additional systemic illnesses (e.g. coagulation disorders, hyperthyroidism, hypothyroidism, cardiovascular disease, diabetes, peptic ulcer, autoimmune diseases, other endocrine disorders, epilepsy). The study did not include females who were taking hormonal or contraceptive pills, non-steroidal anti-inflammatory drugs or have been on a restrictive diet in the last six months.

The examined adolescents in the early follicular phase of the menstrual cycle (between the 3rd and 5th day of the menstrual cycle), between 8:00 am and 9:00 am fasting, 16 hours after their last meal, had 15 mL of venous blood for biochemical markers taken.

Measurements of serum levels of glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were made by colorimetric method (COBAS e 411 Roche), while immunoenzymatic assays (ELISA) were used for determination of serum levels of DHEA-S, FSH, LH, insulin, SHGB, total testosterone, androstenedione and free testosterone.

Insulin resistance was assessed by an indirect method based on the results obtained after calculating the index value HOMA-IR = insulin concentration in the serum of fasting (μIU/mL) × fasting plasma glucose levels (mmol/L) /22.5 [18].

The free androgen index (FAI) was calculated in accordance with the standard formula (FAI = [total testosterone/SHBG] × 100%).

Statistical analysis

The statistical tests were conducted using computer software Excel 2007 and STATISTICA 10. The results of the survey were considered statistically significant when p values ≤ 0.05.

Data were analyzed using the Mann-Whitney U test, Fisher's exact test and chi-squared test with Yates correction.

RESULTS

General characteristics of the study groups

The group of participants with abnormal uterine bleeding (AUB) and the control group were homogeneous in terms of age, height and age at menarche. The statistical

significance for body weight, BMI, waist circumference and hip circumference, for the mean, standard deviation, and median in both groups has been proved. (p-values were respectively: p = 0.03; p = 0.03; p = 0.05; p = 0.04; p = 0.006) (Tab. 1).

Analysis of the menstrual cycle and the androgens features in both bleeding and control groups

The occurrence of regular menstrual cycles (30.23%, p = 0.006) was significantly lower in the juvenile bleeding group. Also, secondary amenorrhea was significantly more likely to be recognized in this group of females (p = 0.03) (Tab. 1).

The maximum duration of menstrual bleeding in the study group averaged at 18.1 ± 15.5 days, and in the control group 6.3 ± 0.6 days (p < 0.000001). In contrast, the shortest menstrual bleeding in the group of females with abnormal bleeding profile averaged 8.6 ± 5.0 days, respectively, and 4.9 ± 0.7 in the control group (p < 0.000001) (Tab. 1).

Hirsutism has been significantly more frequently observed in the group of bleeding in comparison to the control group (51.16% vs. 18.92%, P = 0.003). The length of the abnormal hair growth did not differ much between the two groups (17.8 ± 6.4 months vs 20.6 ± 9.1 months). There were also significant differences between hair loss, acne, and sometimes from what occurred these ailments. Although in a group of juvenile bleeding acne was relatively more frequent (86.05% vs 78.38%), but these differences were not statistically significant (p = 0.27). Significant differences in the severity scale of acne between the two groups of females was observed (p = 0.04). In the group of females with abnormal menstrual bleeding seborrhea is also much more frequent (60.47% vs 16.22%, p = 0.00005) (Tab. 2).

Pelvic ultrasound scan examination

Pelvic ultrasound scan in the examined females was performed between the 3rd and 5th days of the menstrual cycle. In the juvenile bleeding group, the mean volume of the right ovary was 6.5 ± 2.8 cm^3, while in the control group 5.1 ± 2.4 cm3 (p = 0.03). The average volume of left ovary was 6.2 ± 2.7 cm^3 of the test group and 4.7 ± 2.8 cm^3 for the control group (p = 0.01). (Tab. 3).

Hormones and metabolic parameters in blood serum

FSH levels in serum were significantly lower in the group of bleeding adolescents than in the control group, while the LH concentrations in blood serum showed no difference between the two groups. The ratio of LH / FSH in the group of females with juvenile bleeding was 1.34 ± 0.95 and 0.92 ± 0.39 respectively for the control group. These differences were not statistically

Table 1. General characteristics and course of the menstrual cycles in both study and control group (mean ± SD, median, 5th percentile, 95th percentile)

	Study group	Control group	p
Age [years]	15.3 ± 1.6 [16.0] (13.0; 18.0)	15.6 ± 1.6 [16.0] (13.0; 18.0)	NS (p = 0.50)[a]
Height [cm]	163.2 ± 6.1 [163] (151; 172)	163.6 ± 5.0 [164] (155; 172)	NS (p = 0.84)[a]
Weight [kg]	56.8 ± 10.4 [55.0] (45.1; 75.3)	61.7 ± 10.4 [57.8] (50.0; 81.0)	p = 0.03[a]
BMI	21.4 ± 3.9 [20.4] (16.5; 28.8)	22.9 ± 3.5 [22.0] (19.0; 30.7)	p = 0.03[a]
Waist circumference [cm]	70.7 ± 7.8 [68.5] (61.1; 84.9)	74.5 ± 8.6 [72.0] (65.5; 93.0)	p = 0.04[a]
Hip circumference [cm]	93.3 ± 8.1 [92.0] (84.0; 107.7)	98.5 ± 7.5 [97.5] (84.5; 110.1)	p = 0.006[a]
Menarche [age]	12.6 ± 1.2 [12] (11.0; 15.0)	13.0 ± 1.0 [13] (11.8; 14.2)	NS (p = 0.06)[a]
The longest bleeding in the last 6 months [days]	18.1 ± 15.5 [11] (8; 55)	6.3 ± 0.6 [6] (6; 7)	p < 0.000001[a]
The shortest bleeding in the last 6 months [days]	8.6 ± 5.0 [7] (4; 15)	4.9 ± 0.7 [5] (4; 6)	p < 0.000001[a]
Irregular cycles occurrence %	69.77	40.54	p = 0.006[b]
Secondary amenorrhea %	30.23	10.81	p = 0.03[b]

Statistical tests:
[a] — Mann-Whitney U test
[b] — Chi-squared test with Yates correction test

Table 2. Analysis of the androgenization features occurrence and the size of the ovaries in both study and control group (mean ± SD, median, 5th percentile, 95th percentile)

	Study group	Control group	p
Hirsutism occurrence	22 (51.16%)	7 (18.92%)	p = 0.003[a]
Length of hirsutism occurrence [months]	17.8 ± 6.4 [18] (8.2; 24.0)	20.6 ± 9.1 [24] (12.0; 32.4)	NS (p = 0.53)[b]
Ferriman-Gallwey score [points]	10.3 ± 2.5 [9] (8; 15)	10.1 ± 1.6 [10] (8; 12)	NS (p = 0.75)[b]
Hair loss	7 (16.28%)	2 (5.41%)	NS (p = 0.12)[a]
Acne	37 (86.05%)	29 (78.38%)	NS (p = 0.27)[a]
Acne occurrence [months]	37.8 ± 16.6 [36] (17; 53]	32.0 ± 12.5 [30] (18; 49)	NS (p = 0.17)[b]
The severity scale of acne [points]	4.5 ± 2.3 [4] (1; 8)	3.3 ± 1.8 [3] (1; 7)	p = 0.04[b]
Seborrhoea	26 (60.47%)	6 (16.22%)	p = 0.00005[a]

Statistical tests:
[a] — Fisher's Exact Test
[b] — Mann-Whitney U test

Table 3. The results of analysis of ultrasound scan in both study and the control group (mean ± SD, median, 5th percentile, 95th percentile)

	Study group	Control group	p
The size of the right ovary [cm^3]	6.5 ± 2.8 [5.9] (2.6; 11.9)	5.1 ± 2.4 [4.3] (2.8; 9.0)	p = 0.03[a]
The size of the left ovary [cm^3]	6.2 ± 2.7 [6.6] (2.6; 11.3)	4.7 ± 2.8 [3.5] (1.8; 9.1)	p = 0.01[a]
Endometrium length [cm]	0.73 ± 0.22 [0.75] (0.42; 1.00]	0.54 ± 0.17 [0.55] (0.29; 0.80]	p = 0.003[a]
Length of uterus [cm]	3.63 ± 0.62 [3.60] (2.72; 4.51)	3.28 ± 0.58 [3.37] (2.50; 4.12)	NS (p = 0.06)[a]
Length of cervix [cm]	2.97 ± 0.57 [2.80] (2.29; 3.97)	2.84 ± 0.46 [2.80] (2.20; 3.55)	NS (p = 0.44)[a]
Ratio of uterus and cervix length	1.24 ± 0.21 [1.24] (0.93; 1.65)	1.16 ± 0.09 [1.12] (1.06; 1.33)	NS (p = 0.10)[a]

Statistical tests:
[a] — Mann-Whitney U test

significant (p = 0.15). There were no statistically significant differences in concentrations of DHEA-S, free testosterone, total testosterone, androstenedione, and SHBG. The value of the FAI also did not differ statistically significant between the two groups (Tab. 4).

The group of females with juvenile bleeding and the control group did not differ in levels of: total cholesterol, triglycerides and HDL cholesterol fractions in blood serum. Insulin concentrations in serum did not differ significantly, although in the test group showed a generally higher concentration (10.02 ± 5.02 uU/mL) than controls (7.62 ± 4.11 uU/mL). LDL lipoprotein cholesterol in serum was much lower in the treatment group (78.6 ± 19.8 mg/dL) than in the control group (86.0 ± 22.3 mg/dL). Serum glucose levels were significantly higher in the test group (4.66 ± 0.29 nmol/L) than in the control group (4.09 ± 0.80 nmol/L). Importantly it was found that the value of HOMA-IR was statistically higher in the group of females with juvenile bleeding (2.10 ± 1.04) than in the control group (1.49 ± 0.79) (Tab. 4).

In the group of females with abnormal uterine bleeding, insulin resistance by HOMA-IR ≥ 2.5 was diagnosed significantly more often than in healthy females (26.19% versus 5.26%), (Fig. 1).

PCOS occurrence in bleeding and control groups

PCOS occurred more often when bleeding was of at least moderate intensity (33.33% of this group). However in the group with mild bleeding, PCOS occurrence was 9.52% and in the control group, occurrence was only 5.41% (Fig. 2).

Table 4. The concentration of hormones in the blood serum, and metabolic parameters in both test and control group (mean ± SD, median 5th percentile, 95th percentile)

	Study group	Control group	p
FSH [mIU/mL]	5.09 ± 1.51 [4.89] (2.14; 7.46)	7.49 ± 3.03 [7.15] (3.16; 11.91)	p = 0.0002[a]
LH [mIU/mL]	6.60 ± 4.84 [5.10] (2.46; 18.53)	6.77 ± 3.85 [6.19] (1.37; 11.82)	NS (p = 0.59)[a]
LH/FSH	1.34 ± 0.95 [1.01] (0.47; 3.21)	0.92 ± 0.39 [0.95] (0.34; 1.61)	NS (p = 0.15)[a]
DHEA-S [µg/dL]	252.6 ± 96.2 [244.1] (107.5; 419.4)	212.6 ± 87.7 [198.1] (81.4 346.7)	NS (p = 0.13)[a]
Free testosterone [pg/mL]	3.69 ± 6.72 [2.02] (0.64; 11.45)	2.17 ± 1.52 [1.97] (0.64; 3.40)	NS (p = 0.53)[a]
Total testosterone [ng/mL]	0.33 ± 0.17 [0.29] (0.12; 0.62)	0.26 ± 0.1 [0.28] (0.09; 0.42)	NS (p = 0.19)[a]
Androstenedione [ng/mL]	2.56 ± 1.46 [2.24] (1.11; 5.23)	2.23 ± 0.77 [2.31] (1.24; 2.86)	NS (p = 0.60)[a]
SHGB [nmol/L]	59.78 ± 31.55 [55.07] (23.61; 108.4)	53.03 ± 14.69 [51.35] (32.08; 76.82)	NS (p = 0.64)[a]
FAI	0.81 ± 0.77 [0.59] (0.14; 2.27)	0.53 ± 0.27 [0.48] (0.21; 0.91)	NS (p = 0.44)[a]
Total cholesterol [mg/dL]	139.4 ± 25.1 [136] (97; 183)	146.5 ± 26.0 [146] (96; 191)	NS (p = 0.18)[a]
Triglycerides [mg/dL]	83.2 ± 30.3 [82] (38; 136)	83.2 ± 44.5 [67] (41; 190)	NS (p = 0.26)[a]
HDL [mg/dL]	51.9 ± 12.8 [51] (35; 72)	51.0 ± 14.9 [48] (31; 86)	NS (p = 0.48)[a]
LDL [mg/dL]	78.6 ± 19.8 [95] (56; 126)	86.0 ± 22.3 [86] (55; 124)	p = 0.001[a]
Glucose (nmol/L)	4.66 ± 0.29 [4.60] (4.30; 5.28)	4.09 ± 0.80 [4.24] (3.35; 4.74)	p < 0.000001[a]
Insulin [µU/mL]	10.02 ± 5.02 [8.86] (3.92; 19.46)	7.62 ± 4.11 [6.76] (3.49; 11.75)	NS (p = 0.53)[a]
HOMA-IR	2.10 ± 1.04 [1.86] (0.89; 4.32)	1.49 ± 0.79 [1.27] (0.72; 2.33)	p = 0.01[a]

Statistical tests: [a] – Mann-Whitney U test

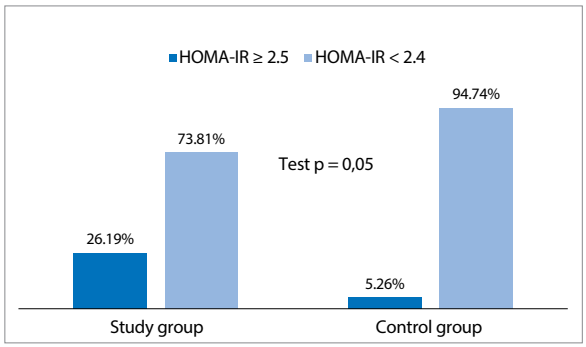

Figure 1. Insulin resistance measured by HOMA-IR in the study and control group. Statistical tests: Fisher's Exact Test

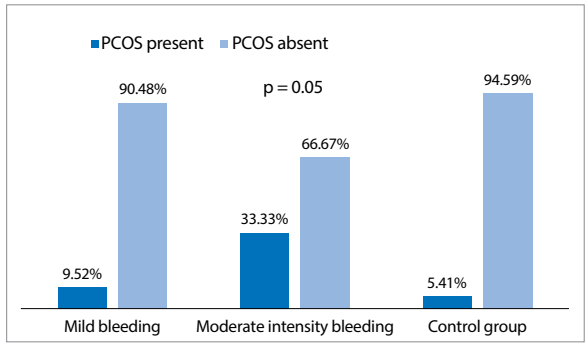

Figure 2. Frequency of PCOS in both test and control groups. Statistical tests: Mann-Whitney U test

DISCUSSION

The average duration of menstrual bleeding within the group of females with heavy menstrual bleeding was 18.1 ± 15.5 days, and the range was high, (8 to 55 days, between the 5th and 95th percentiles). This demonstrated a complex clinical picture of juvenile bleeding.

The limited existing research in young females with heavy and prolonged menstrual bleeding does not allow comparison of the results of this study with other studies accurately.

For instance, one study conducted in a group of young Turkish females with heavy menstrual bleeding also assessed the duration. This included 31 females who were diagnosed with abnormal uterine bleeding (AUB) and anemia, with an average age of 15.3 years (12–19 years). Their results were similar to those in this study; demonstrating a large discrepancy in the length of menstrual bleeding (4–90 days). The average length of menstrual bleeding was 22.6 ± 20.0 days. The limitation of these results is, unfortunately, a small study group [23].

Our study showed AUB was significantly more frequent in the group of females with juvenile bleeding in comparison the control group ($p = 0.006$). AUB was diagnosed in 69.77% of females with juvenile bleeding, whereas 59.46% of females in the control group had a regular menstrual period. This study also highlighted the presence of secondary amenorrhea among the surveyed females, being diagnosed more frequently in the group of females with AUB ($p = 0.03$) [24].

Similar observations were obtained by a study of Brazilian females aged between 12–19 years old. It is worth noting that only females who were at least 2 years after menarche qualified for this particular study. Out of all the participants, more than half (61%) had irregular menstrual cycles and 69% were diagnosed with PCOS. Secondary amenorrhea was identified in 13.56% of all females who have taken part in this study [25].

A study by Shah et al. [26] also showed a high frequency of irregular menstrual cycles among young females (50.9%), and 29.4% of teenagers had secondary amenorrhea (absence of menstrual bleeding for > 3 months).

Our study also found that more than half of females diagnosed with juvenile bleeding (51.16%) also had hirsutism. An increased incidence of acne was also observed (86.05%), and the scale of severity was significantly higher compared to the control group ($p = 0.04$). Females with juvenile bleeding experienced seborrhea significantly more often compared to the control group ($p = 0.00005$). These results indicate a higher occurrence of features of androgenization in the group experiencing juvenile bleeding. This group may be more prone to developing PCOS in adolescence or adulthood (in adolescence some females may not present the complete clinical picture of the disease).

Hyperandrogenism (or androgen excess) in young females has been the subject of several studies. The example of this is a prospective cohort study conducted in Finland with a large population of females, confirmed that hyperandrogenism in adolescence was associated with a higher incidence of PCOS in adulthood. West et al., conducted a study on a group of 2.448 females aged between 15 and 16 years old. They assessed the occurrence of PCOS, hirsutism, acne and pregnancy problems in a group of females after 10 years of follow-up. The study found that females with irregular menstrual cycles at the age of 16 also experienced significantly more skin problems, such as acne and hirsutism, at age 26, compared to females with regular menses ($p = 0.049$). The study also found that adult women with PCOS had significantly higher serum testosterone levels ($p = 0.011$) and higher FAI ($p = 0.013$) in adolescence compared to the group with regular menses. Similarly, women with hirsutism aged 16 presented significantly lower concentrations of SHBG in the blood serum ($p = 0.003$) and higher FAI ($p = 0.001$). Also, women with a history of acne also had significantly higher levels of testosterone ($P = 0.041$), higher FAI ($p < 0.001$) and significantly lower levels of SHBG ($p = 0.003$) compared to women without a history of acne [27].

On the other hand, a study by Pinola et al. [28] on a large group of Finnish females aged 15–16 years confirmed significantly higher levels of testosterone ($p = 0.0010$), lower levels of SHBG ($p = 0.042$) and higher values FAI ($p = 0.002$) in the group of females with irregular menstrual cycles compared to those with regular cycles. These studies show that menstrual disorders in adolescence are a possible indicator of hyperandrogenism, and may be an early risk factor for polycystic ovarian syndrome in adulthood [23].

Analyzing the biochemical parameters between the group diagnosed with juvenile bleeding and the control groups demonstrated that the concentration of FSH was significantly lower ($p = 0.0002$) in the juvenile bleeding group compared to controls. No significant differences in concentrations of DHEA-S, free testosterone, total, androstenedione, SHBG and FAI values between the two groups of females were found. The glucose concentration ($p < 0.000001$) and the fraction of LDL cholesterol ($p = 0.001$) in blood serum differed significantly between the study group and control group. This study also found that the value of HOMA-IR was significantly higher ($p = 0.01$) in the juvenile bleeding group than in controls.

It has been hypothesized that young adolescents who experience juvenile bleeding are at risk of PCOS. This study is supported by the current research available. PCOS was more often diagnosed in the group with abnormal menstrual bleeding. There was also a correlation between the severity of bleeding and the incidence of PCOS syndrome. PCOS was more often diagnosed in the group of moderate and severe bleeding (33.3%). In the group with mild bleeding PCOS was diagnosed in 9.24% of the females, while in the control group it was diagnosed in 5.41% of females ($p = 0.05$). These studies differ slightly from the

results of the Basaran et al. on the Turkish population of females with heavy menstrual bleeding. This study involved 36 young females (mean age 13.7 ± 1.4 years) diagnosed with juvenile bleeding. Only one patient was diagnosed with PCOS (2.8%). However, 25% of the study group of females were diagnosed with coagulation disorders [23]. It would appear that the differences between our studies and those conducted in Turkey are due to the different etiologies of heavy menstrual bleeding. Unfortunately, in the study by Basaran et al., there is no information about the duration of menstrual cycles, relation of LH/FSH and diagnostic criteria used for diagnosis of PCOS. It was therefore difficult to determine whether abnormal menstrual bleeding was connected to the lack of ovulation, and to what extent [23].

Vrbikova et al. [29], studied the prevalence of metabolic syndrome among Czech young females with PCOS and demonstrated that juvenile bleeding occurred in 6.97% of teens with PCOS. This American study also confirmed the presence of heavy menstrual bleeding in adolescent females with PCOS (3.9%) [26].

Literature available about the presence of PCOS in groups of females with the juvenile bleeding is limited. Few studies show the existence of PCOS in this group, but do not define a direct relation between juvenile bleeding and PCOS [23, 26, 29].

There were some limitations in this research which may affect its interpretation. The first limitation was the relatively small number of cases. This makes it more difficult to generalize findings to the entire group of females with juvenile bleeding, and the results should be treated as preliminary. Another limitation of the study was that the assessment of menstrual cycles and the duration of menstrual bleeding were retrospective in nature and therefore their assessment may have been less accurate.

Despite these limitations of the study, its novel findings should be highlighted, and the results should be considered as preliminary to extend the research on a larger population of females. To assess the long-term consequences of juvenile bleeding in adolescence, a prospective cohort study assessing the health of the same study group when they are fully mature should be also carried out.

CONCLUSIONS

Juvenile bleeding may be a risk factor of PCOS. The authors recommend performing screening studies in females with a high degree of risk for the development of polycystic ovarian syndrome, such as irregular menstrual cycles, the occurrence of excessive male type hair and acne. Such measures may reduce the risk of developing metabolic disorders and consequently improve the long-term health of young women [20, 30–32].

Author's Contributions

E.U. — concept and design, data analysis, interpretation of data collection, compilation and preparation of the manuscript. L.H. — final approval of the manuscript. M.O-G. — data analysis and interpretation, final approval of the manuscript. V.S.-P. - final approval of the manuscript.
A.S-F. — analysis and data interpretation. A.D-C. — concept and design, data analysis and interpretation, the introduction of important intellectual content of the manuscript.

Conflict of interests

The authors declare no conflict of interest. The authors report no financial, personal, political, intellectual or religious conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Non-invasive prenatal testing for detection of trisomy 13, 18, 21 and sex chromosome aneuploidies in 8594 cases

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ABSTRACT

Objectives: Cell-free fetal DNA has been widely used in prenatal genetic testing during recent years. We explored the feasibility of non-invasive prenatal testing (NIPT) for analysis of common fetal aneuploidies among pregnancies in northwest China.

Material and methods: A total of 8594 maternal blood samples were collected from October 2014 to December 2017 in the Department of Obstetrics and Gynecology at the First Affiliated Hospital of the Air Force Medical University. Cases with positive screening results by NIPT detection were validated using karyotype analysis.

Results: Of 8594 clinical pregnancies, 88 had positive NIPT results and 78 of 88 (88.6%) positive NIPT results were shown to be false-positive by amniotic fluid puncture and chromosome karyotyping analysis. There were 44 cases (49.44%) with trisomy 21, 18, and 13 syndromes (30 cases of trisomy 21, 9 cases of trisomy 18, and 5 cases of trisomy 13). There were 44 cases (50.56%) with sex chromosome abnormalities, including 11 cases with Turner syndrome (45, X), 17 cases with Triple X syndrome (47, XXX), 2 cases with Klinefelter syndrome (47, XXY), and 14 cases with 47, XYY syndrome (47, XYY).

Conclusions: The accuracy, specificity, high efficiency, and acceptance of NIPT can effectively avoid birth defects and improve the quality of the birth population. We should deepen mining and analysis of the clinical data and explore ways to use NIPT. It is recommended that the NIPT guidelines be extended to low-risk patients to further explore the impact of a significant increase in screening.

Key words: non-invasive prenatal testing; fetal aneuploidy; karyotype analysis

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INTRODUCTION

In 1997, cell-free fetal DNA fragments were discovered in maternal blood, and in 2011 non-invasive prenatal testing (NIPT) analyzed cell-free fetal DNA via massive parallel sequencing. The testing was introduced into clinical practice and became more widely available [1], Clinical trial data and reports based on actual clinical experience have demonstrated efficacy in screening for the most common autosomies that occur at birth (trisomies 21, 18, and 13), and sex chromosome aneuploidies [2–5]. Some studies have reported a detection and false-positive rates of 99.2% and 0.09% for trisomy 21, 96.3% and 0.13% for trisomy 18, and 91.0% and 0.13% for trisomy13 [6]**.** Other studies have reported trisomy 21 detection rates of 98.6% to > 99% [7–9], and the trisomy 18 detection rate is > 97.2% [8]. According to a meta-analysis conducted by Taylor-Phillip et al. [10], the pooled sensitivity was 99.3% for trisomy 21, 97.4% for trisomy 18, and 97.4% for trisomy13. Positive predictive values are significantly lower, particularly in low-risk populations [11]. Therefore, NIPT is an accurate screening test that offers the opportunity to improve the detection of aneuploidies, while reducing the use of invasive diagnostic procedures.

In our hospital we have used NIPT since 2014 and > 8594 pregnant women have undergone NIPT. We mined and analyzed the clinical data and showed that NIPT is feasible for prenatal screening of common chromosomal abnormalities. Herein we explored the use of NIPT from the perspective of evidence-based medicine.

MATERIAL AND METHODS

Non-invasive prenatal testing

A total of 8594 pregnant women who underwent NIPT and prenatal diagnosis at the First Affiliated Hospital of the Air Force Medical University from October 2014 to Decem-

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ber 2017 were enrolled in this study. The inclusion criteria were as follows: (i) singleton pregnancy; (ii) 18–50 years of age; and (iii) gestational age 13–27 weeks. All patients had a risk indication, which included advanced maternal age (≥ 35 years), a sonographic abnormality, a history of a prior aneuploidy, and abnormal traditional aneuploidy screening. Informed consent was obtained from the patients prior to enrollment in the study. Standard procedures were followed, including plasma separation, isolation of cell-free DNA, library construction (end repair, joint connection, and gap repair), an accurate quantitative library, sequencing detection, and bioinformatics analysis. An Illumina NextSeq CN500 or Ion Torrent Sequencing System (BioelectronSeq 4000) was used for sequencing detection. Sequencing data were analyzed using a proprietary algorithm. The binary hypothesis Z-score of specific chromosomes in each sample was determined; the normal range for chromosomes was $3 < z < 3$. Briefly, samples with a Z-score ≥ 3.0 for these chromosomes was classified as positive, whereas a Z-score < 3.0 was classified as negative for the indicated trisomy.

Invasive procedure and karyotyping

Pregnant women with positive screening results on NIPT consented to undergo an invasive prenatal diagnosis procedure, which is the gold standard for diagnosing chromosome aneuploidies. Karyotyping was performed on cell cultures from trophoblastic or fetal cells. Routine fixation, production and dyeing treatment, microscopic examination, and analysis of the karyotype (G-banding) of amniotic fluid were performed. The resolution of metaphase G-banding was 320 bands.

Follow-up

Prenatal and postnatal telephone follow-up were conducted to determine whether or not there were false-negative results.

Statistical analyses

All statistical analyses were performed using SPSS 19.0 software. Data are presented as the mean ± SD. Analysis of variance was used to compare the differences between different groups. A P-value < 0.05 was considered statistically significant.

RESULTS

Efficiency of NIPT for T21/T18/T13

Among 8594 prenatal women who received effective NIPT results, 44 had positive results for T21/T18/T13, including 30 cases with T21, 9 cases with T18, and 5 cases with T13. After informed consent, the gravidas accepted prenatal diagnosis by amniotic fluid cell analysis. Table 1 shows the prenatal diagnosis results. The detection rate, specificity, and positive predictive value (PPV) was 97.37%, 99.92%, and 82.22%, respectively. After follow-up, we identified 1 false-negative result and 7 false-positive results; the false-positive rate was 0.08%.

Efficiency of NIPT for sex chromosome aneuploidy (SCA)

The NIPT results indicated that 44 cases had fetal sex chromosome abnormalities, including 2 cases with Klinefelter syndrome (47, XXY), 11 cases with Turner syndrome (45, X), 14 cases with 47 XYY syndrome, and 17 cases with XXX syndrome (47, XXX). After informed consent, 33 gravidas accepted prenatal diagnosis via amniocentesis. As shown in Table 2, 18 cases were confirmed to be true-positive results

Table 1. 44 cases of NIPT-positive results for T21/T18/T13

NIPT result	NIPT positive result	True positive	False positive	False negative	Detection rate [%]	Specificity [%]	Positive predictive value [%]
T21	30	30	0	1	96.77%	100%	96.77%
T18	9	6	3	0	100%	100%	66.67%
T13	5	1	4	0	100%	100%	20.00%
Total	44	37	7	1	97.37%	99.92%	82.22%

Table 2. 44 cases of NIPT-positive SCA detection results

NIPT-positive SCA	Positive NIPT cases [n]	Karyotype validated	True positive	False positive	Positive predictive value [%]	Without karyotype validated
47, XXY	2	2	2	0	100.00%	0
45, X	11	9	4	5	44.44%	2
47, XYY	14	10	5	5	50.00%	4
47, XXX	17	12	7	5	58.33%	5
Total	44	33	18	15	54.55%	11

and 15 cases were confirmed to be false-positive results. The PPV of NIPT for fetal SCAs was 54.55%. Table 3 shows the PPV of NIPT for different types of SCAs. The PPV for Turner syndrome was the lowest (44.44%).

Distribution of the indications

Among 8594 prenatal women who received NIPT results, we diagnosed a total of 88 cases with abnormal NIPT results. Different detection pointers detect different positive NIPT numbers. The positive distribution of each indication through NIPT was different (Tab. 3).

DISCUSSION

Aneuploidies, which are chromosomal abnormalities characterized by anomalous of chromosomes than the 23 pairs normally present in humans,concluding Down syndrome (trisomy 21 or T21), Edward syndrome (trisomy 18 or T18), Patau syndrome (trisomy 13 or T13), Turner syndrome (45, X), Klinefelter syndrome (47, XXY), Triple X syndrome (47, XXX) and 47, XYY syndrome (47, XYY). These chromosomal anomalies are contributed to the morbidity or death of both childhood and adulthood. [12] During recent years, NIPT has been increasingly used in the detection of common chromosome aneuploidies for T21, T18, and T13 in fetuses during prenatal screening in many countries [13]. NIPT is helpful for the early detection of birth defects to reduce the incidence of birth defects. In addition, it can reduce the occurance of spontaneous miscarriages due to invasive prenatal testing such as amniocentesis and expand the prenatal testing options as well as reduce the need for invasive testing [14–17].

According to our clinical data from 8594 gravidas, we confirmed that the detection rate, specificity, and PPV were 97.37%, 99.92%, and 82.22%, respectively, which were in accordance with other studies [16–19]. Moreover, the specificity of the prior serologic screening test for T21 was 29.13% compared to 100% for NIPT; the PPV was 96.77%. Compared with conventional methods, the specificity and PPV of NIPT in screening for T21 were greater. For T18, the specificity was 98.20% for conventional screening [20] compared to 100% for NIPT; the PPV was 66.77%. The PPV for T13 was 20.00%; the result was not satisfactory, suggesting that large samples are still needed to focus on the performance of NIPT for T13 screening.

NIPT is used in prenatal screening for fetal SCAs; however, positive SCA results are not a direct indication to induce labor. Our results were positive for SCAs in 44 cases; there were 18 true-positive cases. The overall true-positive rate for SCA detection by NIPT was 54.55 %. Among the different types of SCAs, the PPV for Turner syndrome was the lowest (44.44%). Prediction of other SCAs was relatively accurate; the PPVs of pregnancies with 47, XXX, 47, XXY, and 47, XYY karyotypes predicted by NIPT were 58.33%, 100%, and 50.00%, respectively. Cheung [21] reported that the false-positive rate of monosomy X karyotype detected by NIPT was 62%, which is much higher compared to the false-rates for 47, XXX, 47, XXY, and 47, XYY karyotypes. Therefore, our findings demonstrated that NIPT was a more accurate predictor of triple X and Klinefelter syndrome compared with fetal Turner syndrome. In addition, patients with SCAs are usually mildly symptomatic during the neonatal period without any physical or intellectual disabilities. The traditional method of follow-up could not achieve accurate results of SCAs disease. Therefore, it is necessary to improve the detection rate of sex chromosomes by NIPT and reduce the false-positive rate.

Our results suggest that NIPT is suitable for high- and intermediate-risk prenatal screening. It has been reported that among 56 Down syndrome fetuses, 14% were associated with intermediate-risk mothers [22]. In our study, NIPT for intermediate-risk samples detected 10 positive cases, among which 7 were true positive (70.00%). NIPT for low-risk samples detected 12 positive cases, among which 7 were true positive (58.33%).

The current experimental studies involving clinical effectiveness have focused on high-risk populations, such as advanced maternal age, and screening to reduce unnecessary interventional prenatal diagnosis, which have not been fully validated in low-risk populations. Based on our data, the experimental failure rate was not significantly increased in low-risk groups and the false-positive rate

Table 3. The positive distribution of each indication for 88 cases with NIPT positive results

Indication	Cases [%]	Positive NIPT cases [%]	True positive [%]
High-risk prenatal screening	2614 (30.42%)	32(35.95%)	20 (60.61%)
Intermediate-risk prenatal screening	2111 (24.56%)	10 (11.24%)	7 (70.00%)
Low-risk prenatal screening	1513 (17.61%)	12 (13.48%)	7 (58.33%)
Ultrasound structural abnormality	572 (15.65%)	9 (10.11%)	6 (66.67)
Advanced maternal age (≥ 35)	973 (11.32%)	19 (1.95%)	11 (57.89%)
Abnormal pregnancy	590 (6.87%)	5 (21.35%)	3 (60.00%)
Assisted reproduction conception	221 (2.57%)	2 (2.25%)	1 (50.00%)

was similar to the high-risk group. It is our opinion that if guidelines expand NIPT recommendations to include low-risk patients, the implications of significantly increased screening should be further explored.

The development and application of NIPT in southern and southeastern China has advanced further than in western and northern China; there are clearly regional differences in the use of NIPT [23]. Our study was conducted mainly in northwestern China. We found that there were fewer pregnant women undergoing NIPT, with the main reason being cost. The costs for NIPT screening are currently higher than for other screening protocols [24]. If the technology can reduce the cost of testing, more pregnant women will undergo NIPT and will gradually replace serologic screening.

The accuracy, specificity, high efficiency, and acceptance of NIPT has great advantages, which can effectively avoid the birth defects and improve the quality of the birth population compared with the classical invasion testing. But the disadvantage of NIPT is obvious. The false positives could occur because of NIPT [17]. On this account, we must use invasive testing to confirm positive results before any irreversible procedure is performed. It can avoid the harm of patients' interests from detection errors [12].

CONCLUSIONS

We should deepen mining and analysis of the clinical data and explore ways to use NIPT. It is recommended that the NIPT guidelines be extended to low-risk patients to further explore the significance of a significant increase in screening and we can consider NIPT as an important supplementary diagnosis to conventional invasion testing for detection of trisomy 13, 18, 21 and sex chromosome aneuploidies.

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Use of alternative methods in the treatment of anemia in pregnant women – prospective observational study

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ABSTRACT

Objectives: Anemia in pregnant women is a common condition, diagnosed when the concentration of hemoglobin falls below 11 g/dL. Taking into consideration the accounts of nephrologists about good results of treatment of secondary anemia using erythropoietin in patients with renal failure, we tried to use EPO to cure anemia in pregnant women.

The aim of the study was to evaluate the results of EPO treatment on pregnant women diagnosed with iron deficiency anemia, as well as possible side effects.

Material and methods: The study consisted of 25 patients:

Group I — treated with iron supplement administered parenterally — Ferrum Lek every two days intramuscularly.

Group II — treated with recombinant human erythropoietin — 1000 j intravenously every three days, with oral iron supplements.

Results: After a week of treatment the positive response was higher in the second group (92.3% in II, vs 33.3% in I, $p < 0.005$).

The average increase of hemoglobin and RBC was significantly higher in II group.

An increase in hemoglobin did not correlate with the age of women ($r = 0.07$) or with the duration of pregnancy ($r = 0.08$). However, a negative correlation was found between basic hemoglobin level and its increase after treatment ($r = 0.602$).

Conclusions: EPO administered with the oral dose of iron in pregnant women with anemia caused by iron deficiency shows higher effectiveness than the use of iron preparations parenterally.

The usage of EPO during pregnancy is not related to any dangerous side effects for the mother or fetus.

Key words: pregnancy; anemia; erythropoetin

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INTRODUCTION

Anemia in pregnant women is a commonly diagnosed condition, especially in the 2nd and 3rd trimesters. It is estimated that in Poland, 30–41% of women suffer from it, in the United States, up to 25%, and in Europe, less than 10%. It is most commonly found in women from environments with low social and economic status [1]. According to the WHO (World Health Organization), 11 g% (6.82 mmol/L) of hemoglobin in the blood is considered to be the lower limit of the normal, independent of the advancement of pregnancy.

A small degree of anemia is a natural physiological phenomenon during pregnancy. It is caused by the increased volume of plasma in proportion to the increase in the volume of the morphotic blood elements (anemia due to "dilution"). Pathological anemia is diagnosed when the concentration of hemoglobin falls below 11 g/dL (according to the WHO). We distinguish three types of anemia: mild (10.9–10 g/dL), moderate (9.9–7 g/dL) and severe (< 7 g/dL) [2]. The most common cause of anemia in pregnant women is iron deficiency, less commonly folic acid or vitamin B12 deficiency [1]. It is also often diagnosed in multiple pregnancies, in those infected with the HIV (human immunodeficiency virus), and in women suffering from chronic renal failure.

Anemia caused by iron deficiency constitutes approximately 80% of all cases of anemia [1].

The consequences of anemia during pregnancy include, among other things, the following: placental function

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disorder and related complications (intrauterine growth restriction, premature birth, intrauterine fetal demise), abnormal uterine activity during birth or post-partum atony related to the metabolic process disorders of the uterine muscle. There is also an increased risk of perinatal infections that result from changes in the immune system. Severe circulatory decompensation and DIC (disseminated intravascular coagulation) may also occur due to the lack of erythrocyte reserve in case of increased bleeding during birth. Children born to anemic mothers are often premature, and even if they are born on predicted due dates, they usually have a lower birth weight, adaptation process disorders and deficiency anemia [3].

Due to the most commonly occurring anemia pathogenesis during pregnancy, first-line treatment is the iron supplementation in the form of an oral preparation or parenterally in case of suspected problems with iron assimilation from the gastrointestinal tract [3].

Endogenic EPO (*erythropoietin*) is a glycoprotein with a 34 kDa mass composed of 166 amino acids. Its principal source are Leydig cells in kidneys (80–90%) and Ito cells in the liver (10–20%). Its small amounts are also produced in the liver, lungs, testicles, uterus, spleen, lymphocytes, megakaryocytes of the brain, and cornea. During fetal life, its primary source is the fetus' liver and placenta [3]. EPO coding gene is located on chromosome 7, whereas the factor regulating its production is the concentration of oxygen in the blood flowing through the kidneys.

The target spot of the erythropoietin activity is the hematopoietic system located in the bone marrow, and its principal function is to regulate erythrocyte production. EPO has an effect on the target cells through a receptor (EPO-R) that belongs to the first family of cytokine receptors. The combination of EPO and EPO-R activates tyrosine kinase JAK-2, which leads to the activation of genes Bcl-2 and Bcl-XL through the cytoplasmic transcription factor STAT5. Those genes stop apoptosis of precursor cells of the erythrocytic system and stimulate their maturation to the stadium of the mature erythrocyte [4, 5]. An increased number of mature erythrocytes appears as a result of that. Hypoxia causes an increased production of EPO and an increased number of red blood cells, which, in the reverse mechanism, causes the decrease of synthesis of this cytokine and the decrease of its concentration in blood. Taking into consideration the accounts of nephrologists about very good results of treatment of secondary anemia using erythropoietin in patients with renal failure, research concerning the use of that substance in pregnant women has been conducted since the end of the 20th century [6].

The production of human recombinant erythropoietin (rHuEpo) through genetic engineering has allowed for its practical use in the treatment of anemia. There have also been publications regarding its use in pregnant women. Not all the aspects of EPO's activity are known though, and some mentions of the potential threats related to its application found in literature require research. It should be remembered that there is a limited number of academic papers considering this topic and as a result the population of this study is also limited.

Aim of the study

The objective of the conducted research was the assessment of treatment results in pregnant women with diagnosed anemia caused by iron deficiency with the use of EPO, as well as possible side effects. Literature provides data regarding the use of EPO together with Fe preparations in the treatment of postpartum anemia.

We have to remember that biological EPO activity reaches beyond hematological changes. Many studies have demonstrated that cytokine has autocrine and paracrine effect on other tissues showing neuroprotective, anti-inflammatory, and antioxidant activity; it stimulates nitric oxide and intensifies angiogenesis [4, 5].

Currently, apart from the interest of the effect of EPO on hematopoiesis, more and more attention in being brought to its potential effect on the fetus and the role it plays in the development of pregnancy. It was demonstrated that the concentration of cytokine in blood of pregnant women changes and reaches maximum values around the middle of pregnancy, which is probably related to the physiological blood hemodilution. Due to the size of the particle, EPO does not penetrate the placenta, which was also proved in the conditions of experimental perfusion of a fragment of placenta in an in vitro test [7, 8].

Fetus produces EPO primarily in the liver. Its third source during pregnancy is the placenta. Due to the existing separate co-parameters for the mother and the fetus and the fact that what stimulates its production is hypoxia, the concentration of EPO is treated by many authors as a sensitive marker of fetal asphyxia [4, 9].

Literature points out the increase in the EPO concentration in the plasma of the mother during preeclampsia, and, according to Hershkovitz et al., the reason for that is the diminished flow through placenta, its hypoxia and related to it increased production of EPO by the trophoblast's cells [10]. Although mother's EPO has no effect on the fetus, its potential effect on the placenta is unknown, particularly since Resh et al. found in an in vitro study a vasoconstrictive effect of EPO on the umbilical cells, especially the umbilical vein. However, other scientists concentrate on the development of an early pregnancy and stress the positive of EPO on the angiogenesis of the trophoblast's cells. Considerable differences have been found in the concentration of cytokine in plasm in

pregnant women and the expression of EPO-R receptors on the trophoblast in cases of regular and pathological pregnancies [9, 11].

MATERIAL AND METHODS

The study group included 25 patients with gestational age between 18 and 35 weeks, treated for treatment-resistant anemia with the use of oral iron preparations. The study was conducted with pregnant women from the Pregnant Pathology Department, Medical University of Lodz, in 2006–2012. Every patient was informed about possible ways of treatment and gave informed consent to iron and EPO therapy. The size of the group depended on the number of patients with the above mentioned diagnosis hospitalized in the ward during that period of time. The patients were divided into two groups:

Group I — patients treated with iron preparations administered parenterally (Ferrum Lek 1 amp every other day, intramuscular). Women with the initial level of hemoglobin equal or over 9.2 g%, but not higher than 10.9 g%, were placed in this group.

Group II — patients treated with human recombinant erythropoietin (rHuEpo) of 1000 u. intravenously, every three days (initial hemoglobin level of HB < 9.2 g%). In this group, iron supplementation with oral preparations was also used.

The reason for that group selection was the profile of the patient hospitalized in the pathological pregnancy ward. Light and moderate anemias are the most common types of disorders found in pregnant women. It seemed reasonable to use these two groups of patients to carry out the tests.

In both groups, patients received orally 5 mg of folic acid daily.

All important information, as a standard medical interview, had been collected from the patients before the implementation of the treatment. No patient had symptoms of eating disorders or used a restrictive or vegetarian diet. None of them were under treatment for hypertension or chronic infections, either. Before the examination, each patient had a blood test, iron concentration in plasma test, and a total and latent iron binding capacity tests, in order to corroborate the diagnosis of deficiency anemia. A follow-up test of the erythrocytic system was performed again in the eighth day of the treatment.

Afterwards, the differences in the values of particular parameters in relation to the test results from before the treatment were assessed. Those differences were marked as Д. Treatment effectiveness was compared taking into account the concentration of hemoglobin and the numerical value of erythrocytes. The general condition of the pregnant women and the well-being of the fetuses were supervised with the use of cardiotocographic data and fetal motor activity.

Statistical analysis

The statistical analysis was performed with the use of a computer program named Statistica 12. In order to assess the normality of distribution, Shapiro-Wilk test was used, whereas equality of variances was tested using Brown – Forsythe test. The comparisons between the groups were conducted using t-Student, Mann – Whitney U, and chi2 tests. $P < 0.05$ was adopted as the level of statistical significance

RESULTS

Results obtained in the compared groups can be observed in the table below.

The evaluated factors were: pregnant women's age, body weight, weight gain during pregnancy (Tab. 1).

The parameters of the erythrocytic system, platelets, and leucocytes were assessed, and the results can be found in Table 2.

In accordance with the assumed method of the patients' placement in groups, the average hemoglobin concentration was lower in Group II in comparison to Group I ($p = 0.0011$). However, when taking into account the average number of erythrocytes in patients of both groups before treatment, no significant statistical difference was observed ($p = 0.1628$).

Table 3 presents the obtained differences of the formerly assessed parameters 7 days into the treatment, illustrated as Д.

One week into the treatment, 33.3% of the tested patients from Group I and 92.3% of those from Group II ($p < 0.005$) responded to the treatment positively.

Table 1. Groups characteristics

	Group 1 — Ferrum N = 12		Group 2 — EPO N = 13		P
	Average	Standard deviation	Average	Standard deviation	
Gestational age [w]	28.6	6.47	29.5	4.6	0.8939
Body weight before pregnancy [kg]	66.7	4.83	66.2	10.94	0.7330
Weight gain [kg]	9.7	4.22	9.46	3.59	0.8878
Pregnant women's age	30.1	5.53	28.8	6.63	0.1425

Table 2. Parameter values in peripheral blood

	Group 1 — Ferrum N = 12		Group 2 — EPO N- = 13		P
	Average	Standard deviation	Average	Standard deviation	
WBC [thous.]	9.73	3.16	10.05	2.39	0.7927
Erythrocytes [mln]	3.58	0.65	3.15	0.41	0.1628
Hb [g/dL]	9.83	1.15	8.52	0.62	0.0011
PLT [thous.]	233.1	55.05	269.2	97.14	0.3143

Table 3. Differences of evaluated parameters

	Group 1 — Ferrum N = 12		Group 2 — EPO N- = 13		P
	Average	Standard deviation	Average	Standard deviation	
Д WBC [thous.]	1.45	1.94	0.61	2.68	0.3671
Д Erytrocyty [mln]	0.2	0.52	0.22	0.29	0.0235
Д Hb [g/dL]	0.2	0.61	0.69	0.59	0.0436
Д PLT [thous.]	26.73	40.27	3.76	95.66	0.7098

Comparing the results obtained after the treatment, it was demonstrated that the patients from Group II, who were treated with EPO, had a significantly higher average hemoglobin and erythrocytes growth than the pregnant women in Group I, who were treated exclusively with an iron preparation.

Besides, it was found that the growth of hemoglobin concentration after the treatment was not related to the age of the woman (Pearson coefficient value r = 0.07) or the gestational period (r = 0.08). A negative correlation was noticed though between the initial hemoglobin concentration before the treatment and its growth after the research was concluded (r = 0.602).

Only one patient from Group II demonstrated a short-term temporary increase of blood pressure to the value of 150/94 mm Hg. No pathological reactions in the pregnant women's bodies to the administered erythropoietin or any threat to the fetus in any pregnant women were observed.

The research draws attention to the important and often forgotten pregnancy pathology which is anemia. The presented study has numerous limitations, caused mainly by the number of patients participating in it, but it shows promising data for research in the future. It is worth mentioning that future studies should include patients with severe anemia.

DISCUSSION

Due to the high prevalence and the consequences it has for both the mother and the fetus, anemia caused by iron deficiency constitutes a significant problem in modern perinatology. The traditional treatment method involves leveling the quantity of iron in the body through its supplementation administered orally. In case of suspected problems with iron assimilation in the gastrointestinal tract, it should be administered parenterally.

The normalization of existing iron deficiency from before pregnancy requires time and it also depends on a patient's acceptance and her cooperation with medications and diet. If the treatment results are ineffective, a blood transfusion can be an alternative, which, however, can cause numerous undesirable post-transfusion complications, both immediate and remote. Moreover, for a large group of women, treatment with blood derivatives is unacceptable for religious reasons. This is why the use of EPO with pregnant women has become a promising method of therapy in the treatment of anemia.

The results of the performed study were assessed one week into the treatment. The positive effect was reached in 92.3% of women treated with iron + EPO, but only in 33.3% of those who received only iron. Positive results were also obtained by Breymann et al. [12], who stress that treatment with both iron only and iron plus EPO are effective; however, pregnant women who were given the combination of both reached the desired level of hemoglobin, i.e. 11 g/dL, sooner. Similar good results are presented by Krafft et al. [13], who simultaneously point out the need to search for the causes why some patients did not show any improvement after being treated with the same iron preparations, despite being diagnosed with deficiency anemia. Many authors report positive effects of using EPO in the treatment of anemia in pregnant women. Sifakis et al. [14] report a quick positive reaction to the treatment in 73% of the pregnant

women, observing an increase of the Hb (haemoglobin) concentration by 3 g/dL in the first two weeks.

From among the patients treated with EPO, one had an excessive increase of blood pressure, which could have been an example of undesirable side effects.

As noted by Fisher, the administration of EPO may carry the risk of adverse side effects, especially hypertension and prothrombotic action [11]. Patomechanism of this disorder consists in the retention of Ca ions in the intercellular space and decrease of the reactivity to the vasodilatory effect of nitric oxide. According to Fisher, EPO may stimulate increased production of endothelin and changes in the production of prostacyclin and may foster the production of renin and angiotensin [15]. In existing literature, however, there are no data on serious complications in mothers during the EPO treatment. In our study, we have observed only once an increase of blood pressure in the pregnant women- one patient demonstrated a short-term temporary increase of blood pressure to the value of 150/94 mm Hg. Single reports found in literature concern cases of some significant increase of blood pressure after the administration of EPO; however, they occurred to women with prior chronic renal disease [11].

Literature provides data regarding the use of EPO together with Fe preparations in the treatment of postpartum anemia. Wagstrom et al. compared the results of treatment of pregnant women given iron only and those given iron + EPO. They did not observe any significant differences and both methods proved effective [11]. On the other hand, Meyer found in pregnant women treated with EPO less severe symptoms of baby blues in comparison to the patients treated exclusively with Fe [16].

The research draws attention to important and often forgotten pregnancy pathology, which is anemia. Presented study has numerous limitations, caused mainly by the number of patients participating, but shows promising data for research in the future. It is worth mentioning that future studies should include patients with severe anemia.

Erythropoietin is a cytokine not entirely explored in the context of its application in pregnant women. Its basic use as a substance assisting erythropoiesis in the treatment of patients with anemia is rather well explored and often practised with a good result. On the basis of the available literature and our own research, it should be regarded as a valuable form of therapy. Positive effects of EPO as a medication normalizing hemoglobin and erythrocyte levels concern a great majority of the treated patients and so far no severe negative effects have been found. Further research regarding the significance of erythropoietin should be conducted as it seems that there are considerably higher possibilities of the application of this cytokine in therapy than currently used.

CONCLUSIONS

Erythropoietin administered together with the oral dose of iron in pregnant women with anemia caused by iron deficiency shows higher effectiveness than the therapy with the use of iron preparations administered parenterally.

The treatment with the use of erythropoietin during pregnancy is not related to any dangerous side effects for the mother or the fetus.

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Randomized comparison of two methods of the epidural space identification during regional labour analgesia

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ABSTRACT

Objectives: Conventional loss of resistance (LOR) technique for identifying the epidural space (EDS) predominantly depends on experience of the anaesthetist. A technique using automated syringe for EDS identification was invented as an alternative to the traditional method. The aim of the study was to compare the efficacy and risk for complications between automatic LOR syringe — Epimatic® (Vygon, Ecouen, France) and conventional LOR — Perifix® (B.Braun Melsungen AG, Melsungen, Germany) techniques for EDS identification.

Material and methods: A total of 170 patients were enrolled into the study and 153 cases were analysed. Number of attempts, time to EDS identification, ease of EDS identification, complication rate and patient procedure-related discomfort were evaluated and compared.

Results: No statistically significant differences were found in the number of needle insertion attempts (1.3 in both groups), time to EDS identification (31 sec. vs. 27 sec.), efficacy of epidural analgesia (100% in both groups), or complication rate between both groups.

Conclusions: The automatic and the conventional LOR techniques are comparable in terms of efficacy and safety for the epidural space identification.

Key words: epidural analgesia; epidural space; loss-of-resistance technique; automatic identification; labour analgesia

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INTRODUCTION

Lumbosacral epidural analgesia (EDA) remains the most effective way of alleviating labour pain [1], as it increases patient comfort and quality of patient-personnel cooperation during labour. The procedure involves 3 crucial steps: (i) identification of vertebrae level, (ii) selection of desired puncture site and angle, (iii) needle insertion into epidural space (EDS) between ligamentum flavum (LF) and dura mater covering a spinal cord [2].

Identification of EDS, which has only several millimetres, is the key element and the necessary prerequisite of effective EDA [2]. The fact that there is negative pressure within the epidural space is clinically important [3, 4]. Negative pressure can be magnified by increased flexion and reduced by decreased flexion of the spine [5]. Several techniques detecting a change in the resistance or pressure have been proposed for EDS identification: a dual technique [6], balloon technique [4], drip infusion technique [4], acoustic signalization [7, 8] Despite the claimed advantages, none of them is widely used in clinical practice [8].

The current gold standard is the loss of resistance (LOR) technique described in 1921 by Pages [6]. However, regardless of saline or air usage, it is associated with a significant failure rate of up to 32% and may be time-consuming [9–11]. It remains a particular change among pregnant women due to impaired palpability of anatomical landmarks and flawed identification of the entry to EDS — as ligament complex (supraspinous ligament, interspinous ligament, ligamentum flavum) become softer and more inhomogeneous, which may mimic the feeling of lost resistance. It is happening

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Figure 1. Epimatic® syringe

because of increased relaxin and estrogen levels, which change the ligament structure and the fact that ligaments are bearing greater burden due to 15–20 kg weight, gain [12]. Moreover, epidural pressure during pregnancy is elevated secondary to increased oedema, elevated vena cava inferior and intreperitoneal pressure and enlarged venous plexus [13]. However, the blind technique may lead to accidental dural puncture with a following post-procedural headache as well as accidental plexus venous puncture [5, 6].

Thus, the search for a simple, objective, effective, safe and reliable method of EDS localization has been underway for many years, with the automatic Epimatic® syringe (Vygon) as an example of a tool for EDS identification (Fig. 1). Epimatic® is a low-resistance syringe where continuous positive pressure on the plunger is achieved by an elastic strip. When the Tuohy needle enters the epidural space, LOR is visible by the sudden movement of the syringe plunger, which removes operator subjectivity and variability [14]. The other advantage of the spring-loaded syringe is that both hands can be used for needle's advance and stabilization [15].

Objectives

The aim of the study was to compare the effectiveness and complications of EDS identification with the use of conventional LOR technique and the automatic syringe system during EDA.

MATERIAL AND METHODS

This manuscript adheres to the applicable EQUATOR guidelines [16]. The study was a prospective, single-blinded, randomized study conducted in one centre between January and September 2015. Prior to the study onset, a randomization list was prepared based on the coin toss by Primary Investigator. Patients were blind and randomized according to the list to undergo standard EDA (control group) or Epimatic® syringe EDA (study group).

Inclusion criteria were (i) patient's choice of EDA during labour and (ii) informed consent to participate in the study. Exclusion criteria were following clinical contraindications for EDS: (i) skin lesion at the puncture area, (ii) coagulopathy, (iii) lack of informed consent to participate in the study.

All the procedures were following standard preparation for the neuraxial block with life sign's monitoring. EDA was performed in sitting or lateral position at the preference of the one out of four study anaesthetist, each with at least 10 years of clinical experience. For epidural space identification during LOR technique, all of them preferred air to be used.

In both groups, total aseptic technique was used, the appropriate intervertebral space was identified, and lidocaine (*Lignocainum hydrochloricum* WZF 1% 2 mL, Polfa Warszawa SA) was administered as local analgesia for the skin and the subcutaneous tissue, a Tuohy needle was inserted and attached to the automatic or conventional syringe. The needle was positioned and pushed towards the ED space until LOR was felt (conventional method) or plunger movement was observed (automatic method), thus confirming entry into the epidural space.

In the control group, EDA was performed using the *Perifix*® kit (B.Braun Melsungen AG, Melsungen, Germany) — including a Tuohy Perican® 18G epidural needle (diameter: 1.3 mm, length: 80 mm) and a Perifix® LOR syringe with continuous pressure applied by the anesthetist using his/her thumb. The syringe was filled with air. In the study group, a Tuohy Perican® 18G epidural needle (diameter: 1.3 mm, length: 80 mm) and an Epimatic® syringe (Vygon, Ecouen, France), filled with air, were used.

After EDA procedure and delivery, each patient was followed-up for 24 hours.

The principal aim of our study was to establish the efficacy of Epimatic® syringe and to estimate rates of complications in each group. Therefore, the number of randomized patients (170) was not selected to power any hypothesis test, but to provide the most accurate estimation of population rates giving the time and resources available. The following criteria were used for comparative assessment:

- patient's demographic and anthropometric data: age, height, BMI, primiparous/multiparous status;
- procedural data: positioning, LOR depth, number of puncture attempts until EDA, time to EDA, time to LOR feeling;
- operator's views: ease of catheterization;
- patient's view: level of discomfort during the procedure, procedural efficacy scored in Numerical Rating Scale (NRS), satisfaction measured by willingness to undergo the same procedure in the future;
- post-procedural complications: regional pain, skin irritation/redness, accidental dural puncture (ADP), accidental catheterization of epidural venous plexus.

Local Ethics Committee approved of the study (no. KB/226/2014). Each participant signed informed consent.

STATISTICAL ANALYSIS

A total of 170 patients without contraindications to neuraxial analgesia, who received EDA during labour, were included in the study (Fig. 2).

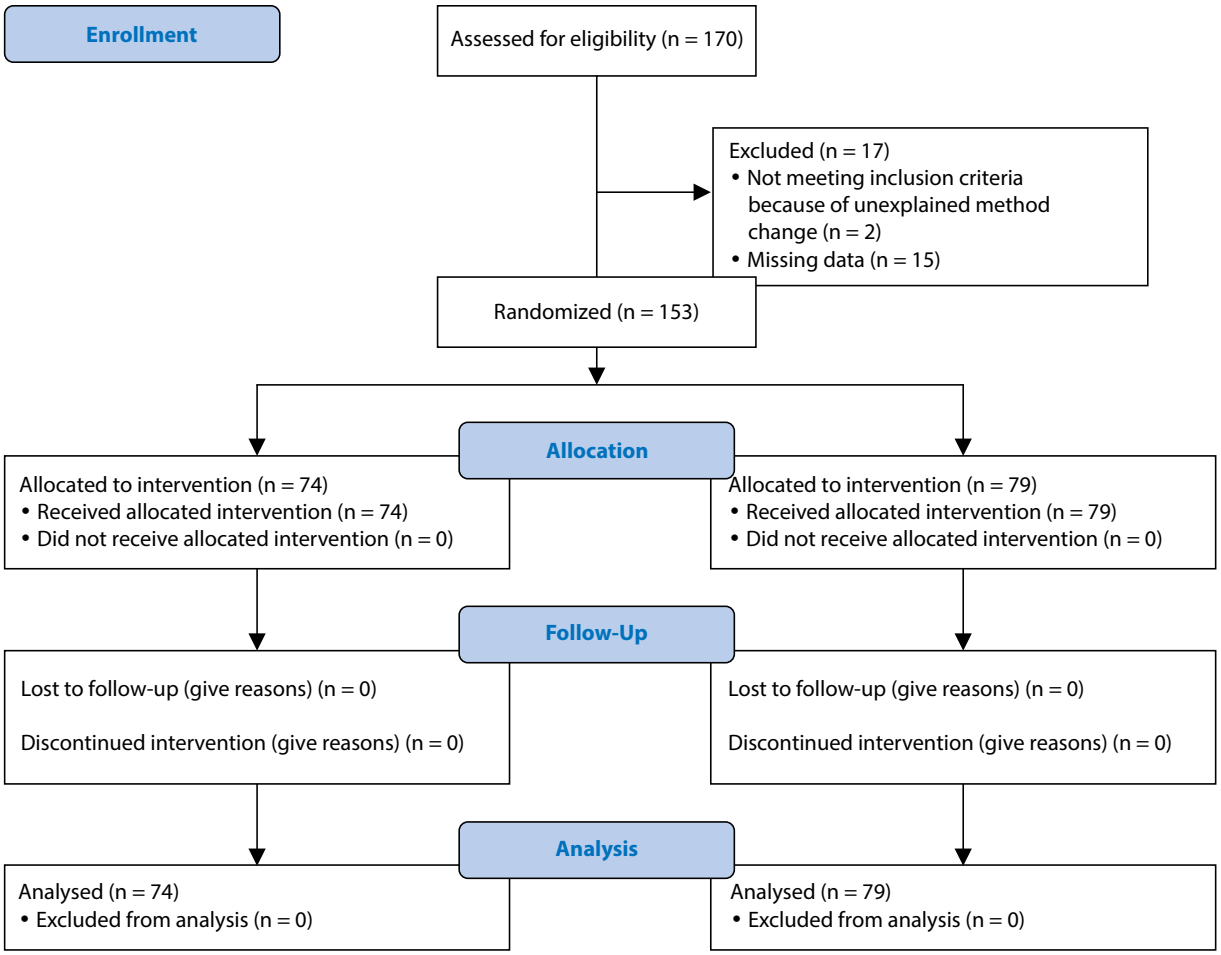

Figure 2. CONSORT 2010 Flow diagram of the progress through the phases of a parallel, ranodomised trial of two groups (enrolment, intervention allocation, follow-up, data analysis)

In case of continuous dependent variables, two-sample one-sided t-Student test was used to compare the differences in means between two independent groups (sample size of > 30 for both groups allows to use the central limit theorem). Fisher's test was used to verify equality of variances of the investigated variables. Additionally, Chi^2 Pearson's test and Fisher's exact test were used to compare distributions of categorical dependent variable between the independent groups [17]. In case of multiple regression analysis, depending on the specificity of the analyze variable, one of the following three models was used: linear regression (continuous distribution variables), logit regression (dichotomous outcome variables), or Poisson regression (set of natural numbers distribution variable) [18]. The p-value of < 0.05 was considered as statistically significant.

RESULTS

A total of 170 patients were randomized for the study and 153 women were evaluated. Their baseline characteristics are presented in the Table 1.

Table 1. Patient demographic and anthropometric data (presented as mean and standard deviation, SD or percentage, %)

Parameter	Perifix® (n — 74)	Epimatic® (n — 79)	p
Age [years]	30.95 (4.56)	30.51 (4.42)	0.27
Height [cm]	167.18 (4.43)	165.71 (6.70)	0.06
BMI	28.11 (3.80)	27.26 (3.74)	0.08
Primiparous	44 (59%)	58 (73%)	0.067*
Multiparous	30 (41%)	21 (27%)	0.086**

*Chi^2 Pearson's test; **Fischer's exact test

Data on maternal position during epidural analgesia are presented in Table 2. No statistically significant differences between the modes of identification were found.

Data on the procedure-related parameters are presented in Table 3. No statistically significant differences between the two methods were found.

No statistically significant differences in tactile sensation of resistance loss felt by the anesthetist performing the pro-

Table 2. Maternal position during epidural analgesia

Parameter		Perifix® (n - 74)	Epimatic® (n -79)	p
Mode of identification	lateral	2 (2.78%)	4 (5.26%)	0.444*
	sitting	70 (97.22%)	72 (94.74%)	0.682**
	no data	2	3	

*Chi² Pearson's test; **Fischer's exact test

Table 3. Data on the EDA procedure in both groups

Parameter	Perifix® (n — 74)	Epimatic® (n — 79)	p
LOR depth [cm]	6.13 (0.95)	5.88 (0.79)	0.074
Puncture attempts until EDA [number]	1.29 (0.68)	1.29 (0.67)	0.962
Time to EDA [s]	47.32 (45.48)	53.13 (45.05)	0.436
Time to feeling of LOR [s]	26.59 (37.33)	31.48 (38.18)	0.492

Table 4. Ease of catheterization

Parameter	Perifix® (n — 74)	Epimatic® (n — 79)	p
Very easy	1 (1.37%)	1 (1.35%)	0.096
Easy	61 (83.56%)	68 (91.89%)	
Slight resistance	11 (15.07%)	5 (6.76%)	
No data	1	5	

Table 5. Level of patient discomfort during the procedure

Discomfort	Perifix® (n — 74)	Epimatic® (n — 79)	p
None	35 (48.61 %)	34 (45.33%)	0.539
Slight	37 (51.39%)	40 (53.33%)	
Significant	0 (0%)	1 (1.33%)	
No data	2	4	

cedure were found. Ease of catherization was comparable in both groups (Tab. 4).

The level of patient discomfort during the procedure did not differ significantly between the groups and was reported by the majority of patients to be 'slight' or 'none' (Tab. 5).

Mean pre-epidural NRS score was > 7 points in both groups and no statistically significant differences were found. Mean post-epidural NRS scores were 2 and 2.3 points in the Epimatic® and Perifix® groups, respectively and no statistically significant differences were found (Tab. 6).

As for future consent for repeat procedure, 98.7% and 98.61% of the women from the Epimatic® and the Perifix® groups declared they would consent to the procedure again (Tab. 7). No statistically significant differences were found.

Table 6. Pain assessment using Numerical Rating Scale (NRS)

Pain assessment using NRS	Perifix® (n — 74)	Epimatic® (n — 79)	p
Pre-epidural NRS	7.66 (0.82)	7.63 (0.89)	0.724
Post-epidural NRS	2.3 (1.38)	2 (0.45)	0.062

Table 7. Declaration of future consent to epidural analgesia

Parameter	Perifix® (n — 74)	Epimatic® (n — 79)	p
Yes	71 (98.61%)	74 (98.67%)	0.977*
No	0 (0%)	0 (0%)	
Other	1 (1.39%)	1 (1.33%)	1.00**
No data	2	4	

*Chi² Pearson test **Fischer's exact test

Table 8. Complications after analgesia

Parameter	Perifix® (n — 74)	Epimatic® (n — 79)	p
None	22 (30.14%)	25 (33.33%)	0.675
Regional pain	48 (65.75%)	43 (57.33%)	0.272
Skin irritation (redness)	3 (4.11%)	7 (9.33%)	0.216
Accidental dural puncture, ADP	0 (0%)	0 (0%)	ND
Accidental catheterization of epidural venous plexus	2 (2.7%)	3 (3.8%)	0.702

The most common EDS analgesia-related complication was regional pain. Local redness at the puncture site were also reported, although less often. No statistically significant differences in terms of complication rates were found between both groups (Tab. 8).

DISCUSSION

There is one study available in the literature comparing Epimatic® syringe and conventional LOR technique conducted by Dilish et al [19], which included 40 patients undergoing lumbar epidural anesthesia with no specified procedure type. In contrast to our study, they reported on significantly shorter time to identify the epidural space (8 vs. 35 seconds, respectively, $p < 0.001$) and a difference between the number of attempts (1.25 vs. 1.6, respectively). Similarly to our results, there was no statistically significant difference between groups in regards to the easiness of catheter insertion. There was one accidental dural puncture in the control group [19].

The literature offers reports on 3 other syringes with a similar mechanism of action (constant positive-pressure for the automated EPD identification), namely (i) Episure™ AutoDetect™ syringe, Indigo Orb, Inc., Santa Clara, California, United States [14, 15, 20–22] (ii) Epidrum, Emooor Innovations Ltd. Taunton, United Kingdom [13, 22–28] and (iii) Epi-Jet, Egemen International, Izmir, Turkey [13, 29].

Our study found no difference for the primary outcome, namely the efficacy of EDA measured by NRS. In the literature, the failed analgesia was defined in the study by Habib [21] and Joseph [14] as a need to resit needle due to the failure to obtain sensory blockade after the initial drug dosage and occurred significantly less often in the the Episure™ group compared to controls (0% vs. 3.2%; 0% vs.8.3% respectively). However, findings from Deighan et al. [23] on Epidrum reports on the higher rate of failed analgesia (6% vs. 0%).

Similarly to our study, the majority of studies reported no difference between the number of puncture attempts between the automated and the conventional group [13, 15, 28, 29]. The remaining revealed lower number for the automated groups, in the study of Episure™ the efficacy of the first attempt was 91.6% vs. 76.6% [14] and in the study of Epidrum less than 2 attempts were needed more often for Epidrum (96.3% vs. 85.9%) [25]. Kim et al. [25] defined the procedural failure as the need of more than 4 needle insertion attempts. Their study reported that this outcome was less often in the Epidrum group compared to controls (0% vs. 9.25%) [25].

In our study, time to EDA was slightly shorter in the study group as compared to controls (53 sec. vs. 47 sec., respectively), but the difference was not statistically significant. However, the literature does not confirm this finding. Episure™ is characterized by quicker EDA achievement (32 vs. 39 s) [14], similarly to Epidrum (18.6 vs. 31.5 s) [25]. Findings on Epi-jet are at odds, which may be caused by an operator's experience [13, 29].

Patient satisfaction with the procedure, defined as consent to the same method in the future, was reported by the vast majority of the women (98.6%) and was comparable in both groups. Other authors did not investigate that parameter.

Our results stated that 93.24% of operators found the procedure easy/very easy in the Epimatic® group and 84.83% in the control group, however, those results did not reach statistical significance most probably to the insufficient sample size. In the literature operator's satisfaction was always higher in the automated syringe group [13, 15, 28, 29], except for Epidrum in contrast to control group in the study by Kartal et al. [13] (60.3% vs. 73.8%).

There is the scarcity of information on complication rates in the literature. The accidental dural puncture (ADP) was reported most common. Similar to our study, Riley et al. [15] and Demirel et al. [29] reported on the lack of this complication. The remaining studies revealed that the side effect appeared less commonly in the automated syringe group [14, 21, 22, 25]. Only one study by Habib et al. [21] reported on accidental puncture of the venous plexus, which appeared insignificantly more often in the Episure™ group (5.4% vs. 4.5%). Moreover, an automatic syringe might be applicable for an ultrasound-guided neuraxial block as it allows the anaesthetist to manipulate the ultrasound transducer, requiring the use of only one hand for needle insertion. It has been proven that ultrasound pre-scanning increases first-pass success and decreases the number of needle passes [9, 30].

Additionally, an automatic syringe gives a unique opportunity for an objective assessment by the teacher during procedure conduct by a younger Colleague, which is not possible with the conventional LOR technique [21, 24].

Study limitations

This study has several limitations. First, the study was not planned as double-blinded research because of the impossibility of using the device without operator knowledge. Secondly, the sample size was moderate and not estimated to power the hypothesis testing. Furthermore, our study was conducted by the experienced anaesthesiologist — however, the low learning curve [20] and studies with residents [21] suggest that results may be applicable for less experienced practitioners. There may be an operator bias. Moreover, we identified epidural space within lumbar level and results may not be extrapolated to other vertebral segments.

CONCLUSIONS

1. Safety and efficacy of EDS identification are comparable in both methods.
2. Both methods of EDS identification may be successfully applied in patients during labour analgesia.

Conflict of interests

The authors have no conflict of interest to declare.

Financial disclosure

The cost of syringes was covered using the authors' own research budget.

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Ophthalmological and obstetric management in pregnant women with retinal disorders

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ABSTRACT

Objectives: To analyze the clinical significance of ophthalmological assessment in pregnant women affected with degenerative retinal lesions, and the lesions' clinical relevance in determining the obstetric management and delivery method.

Material and methods: 69 pregnant women affected with retinal degenerative lesions were included in our study. In each patient, the risk of ophthalmological complications during vaginal delivery was evaluated. After the woman's delivery, alignment between the ophthalmological recommendations and the obstetric management were analyzed. Each case where the management plan differed from the clinical proceedings was thoroughly investigated to determine the cause.

Results: In 69 pregnant women the risk of ophthalmological complications was evaluated, and in 24 cases (35%) assessed as low, as medium in 37 cases (54%) and as high in 8 cases (11%). Among the 69 patients, 42 of women delivered vaginally and the remaining 27 underwent caesarean section. In the high-risk group, the rate of caesarean section was 87%, while in both the low- and medium-risk groups the rate of vaginal births was 75%. Two years of postnatal ophthalmological follow-up did not reveal any complications that could have been associated with the delivery.

Conclusions: Every pregnant woman should undergo ophtalmological examination to assess peripartum risk of complications and determine the method of delivery.

Key words: myopia; ophtalmological examination; caesarean section; laser photocoagulation

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INTRODUCTION

The presence of peripheral, degenerative lesions in the retina remains one of the most important risk factors of ophthalmological complications, most notably, retinal detachment. Pathological retinal lesions may be congenital or acquired, and either located in the central or peripheral parts. In some cases, they may involve retinal vessels as well [1]. The most frequent ophthalmological issue during pregnancy remains myopia associated with peripheral degenerative lesions and the risk of retinal detachment [2]. However, in myopic patients with degenerative lesions, retinal detachment is caused by pathology in the vitreous rather than in the retina itself [3]. It is noteworthy that most cases of peripheral lesions in pregnant patients are detected coincidentally. Unfortunately, patients tend to avoid laser photocoagulation during gestation due to fears for the wellbeing of the fetus, about the potential side effects of the intervention, or its impact on the mode of the delivery. Nonetheless, pregnancy is not a contraindication for ophthalmological treatment and every patient diagnosed with peripheral retinal lesions should undergo laser photocoagulation to separate degenerative tissues from the normal retina [4]. According to a consensus among obstetric and ophthalmological recommendations in 2017, laser photocoagulation should be performed at least 4 weeks before the estimated date of delivery to significantly reduce the risk of intrapartum ophthalmological complications [5]. Prior to delivery, the level of risk of ophthalmological complications for vaginal delivery cases may be assessed according to a three-degree severity scale:

— low
— medium
— high

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The low-risk group is defined as having no contraindications for vaginal delivery. On the other hand, in the high-risk category of ophthalmological complications, elective caesarean section should be recommended. In the medium-risk group, the possibility of eyesight deterioration still exists, however it seems that vaginal delivery remains safe. In this group, shortening the second phase of the delivery with vacuum or forceps may be considered; however, these procedures are relatively rare in contemporary obstetrics.

Aim of the study

To analyze the clinical significance of ophthalmological assessment in pregnant women affected with degenerative retinal lesions, and the lesions' clinical relevance in determining the obstetric management and delivery method.

MATERIAL AND METHODS

69 pregnant women diagnosed with degenerative retinal lesions during ophthalmological examinations were included in our study. Lesions were revealed in 121 eyes. Each patient underwent an ophthalmological assessment of the risk of peripartum complications arising during vaginal delivery and each woman was assigned to one of the low-, medium- or high-risk groups. In addition, we analyzed laser photocoagulation throughout the pregnancy or refractive eye surgery in the past. In each case where the method of delivery was different from that of the ophthalmological recommendations we carefully investigated what happened to identify the specific indications.

RESULTS

Of the 69 pregnant women in our study, 24 patients were assigned to the low-risk group (35%), 37 to the medium-risk group (54%) and only 8 to the high-risk group (11%). Overall, the rate of vaginal delivery was 61% compared with 39% for caesarean section.

In high-risk group, 7 patients (87%) underwent caesarean section and only 1 (13%) delivered naturally due to lack of consent for a caesarean section. Out of 37 patients in the medium-risk group, 14 underwent caesarean section (38%) while 23 delivered naturally (62%). Among the medium-risk patients who underwent caesarean section the indications for an operative delivery were: patients' concerns about peripartum ophthalmological complications (50%), obstetricians' concerns about the increased risk of ophthalmological complications (29%), and in 3 cases (21%) caesarean section was performed because of strictly obstetric indications. On the other hand, among 24 patients with a low-risk of ophthalmological complications, 18 delivered naturally (75%) and 6 patients underwent caesarean section due to their concerns about peripartum ophthalmological complications.

From among the whole study group, 43 patients qualified for laser photocoagulation during pregnancy. After the laser procedure their risk was reassessed, with the results that despite the treatment, from 4 patients in the high-risk group (9%), 3 women underwent caesarean section and 1 delivered naturally due to there being no consent for operative delivery. In 6 patients, the risk of ophthalmological complication was assessed as low (14%), and 5 of them delivered naturally while 1 delivered by caesarean section because of obstetric indications. Most of the patients who underwent laser photocoagulation were assessed as a medium risk for ophthalmological complications (77%). Among this group there were 20 natural deliveries (70%) and 13 operative deliveries. In the subgroup of intermediate-risk, 4 patients qualified for caesarean section based on obstetricians' concerns, 6 cases because of patients' concerns, and 3 cases because additional obstetric factors occurred.

In addition, in our study group there was a group of 7 patients who had undergone refractive surgery in the past. In 2 of the pregnant women who delivered naturally the risk was evaluated as low (29%), in 4 as intermediate (49%) and in 1 as high (14%). Of the intermediate- and high-risk groups only 1 patient delivered naturally, and in the remaining 4 cases caesarean section was performed because of obstetricians' concerns.

During two years of postpartum follow-up there were no complications that could have been associated with delivery.

DISCUSSION

The rate of caesarean sections worldwide is growing and has been referred to a "plague" in contemporary obstetrics [6]. Unfortunately, the trend is evident in Poland, as well. In 2016, 43% of all deliveries were concluded by caesarean section [7]. Despite epidural anesthesia and the improving quality of perinatal care, caesarean section continues to be associated with increased risks of both maternal and neonatal complications during delivery [8–10]. On the other hand, when the incidence of caesarean section rises above 20% in the population it is not matched by a corresponding decrease in maternal or neonatal morbidity. The most frequent indications for operative delivery are obstetric ones, however so called non-obstetric indications are also frequent [11, 12]. For many years, the ophthalmological indications for caesarean section were not summarized in any scientific associations' published recommendations. However, in the current situation, there has been a significant improvement thanks to an obstetric and ophthalmological consensus regarding the mode of the delivery in patients with eye disorders that was published in 2017 [5]. Thanks to this publication, those ophthalmological disorders that may be regarded as an indication for caesarean section were precisely identi-

fied. Nowadays, cooperation between ophthalmologists and obstetricians allows practitioners to assess the risk of peripartum complications and to define the appropriate mode of the delivery. It is worth noting that suggestions that eyesight may worsen permanently after delivery, has not been borne out in the studies.

As refractive surgery in patients who are affected with myopia became a frequent procedure in the general population and as many women no longer use lenses or glasses, every pregnant patient should be interviewed by their obstetrician about their past medical history to evaluate the risk of intrapartum ophthalmological complications. Most patients are not aware that though refractive surgical procedures increase the acuteness of vision, they do not however, prevent ophthalmological complications occurring during delivery. Moreover, refractive surgery does not affect the state of retina and other potential pathologies associated with myopia such as glaucoma or dregs in the corpus vitreum. Due to refractive surgery, the thickness of the cornea is reduced, and every patient should undergo pachymetry to assess their corneal thickness after the procedure. The normal central corneal thickness (CCT) across the population is 510-570um. According to the previously mentioned consensus, in cases where the corneal thickness is less than 350 um elective caesarean section should be considered because of the high risk of corneal complications. Separation of the layers of the Descemet membrane or corneal ectasia may cause permanent deterioration in the eyesight and irregular astigmatism. In such cases surgical treatment may be needed [13]. Every patient with a history of ophthalmological complications, e.g. high myopia, diabetic retinopathy, after eye surgery, keratoconus and visual acuity disorders should be examined by an experienced ophthalmologist during the first trimester of the pregnancy. The physician may assess the risk of peripartum complications and the potential need for treatment during the course of the pregnancy [14].

Our analysis shows that most patients with a high risk of ophthalmological complications underwent caesarean section (almost in 90%). On the other hand, over 75% of the patients in the low-risk group delivered naturally when no additional obstetric indications for caesarean section occurred. Nonetheless, the most challenging group, from a clinical perspective were those women with a medium risk of ophthalmological complications. Among those patients, the rate of vaginal compared with operative deliveries were similar (40% vs 60%, respectively). The most frequent indications for caesarean section were obstetricians' or patients' concerns about natural delivery. However, during two years of postnatal follow up with the whole study group, no eye complications occurred that could have been associated with delivery. This fact suggests that in the group of subjects with a medium risk of ophthalmological complications, there may be an unnecessary rise in the number of elective caesarean sections due to either obstetricians' or patients' concerns. Our data suggest that three-degree scale of risk should be revaluated and most likely modified. Recalibrating the risk scale into two risk groups, namely into low-risk and high-risk, may prevent clinical predicaments and facilitate better obstetric management. This variant seems to be clearer and more applicable in clinical practice, wherein low-risk patients may be qualified for vaginal delivery while high-risk patients should be scheduled for elective caesarean section.

CONCLUSIONS

1. Most patients with a high risk of peripartum complications, caesarean section was the main mode of delivery, while in the low-risk group most patients delivered naturally.
2. In medium-risk group rates of natural compared with operative deliveries were similar (40 vs 60%, respectively).
3. Prenatal laser photocoagulation increases the chances for a vaginal delivery.
4. The main cause of obstetric management that differs from the ophthalmological recommendations were the result of the cautious attitudes of obstetrician attitude and patients' concerns about peripartum complications.
5. Two-degree risk scale seems to be more useful in clinical practice than three-degree scale which is currently in use by ophtalmologists.

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Use of holmium laser for OHVIRA syndrome treatment

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Introduction

Herlyn-Werner-Wunderlich syndrome is a rare complex of structural abnormalities of the female urogenital tract. That comprises an obstructed hemivagina in a didelphic uterus with homolateral renal agenesis/ anomaly. Hence, clinical entity are better described by the term OHVIRA — obstructed hemivagina, ipsilateral renal agenesis/anomaly (dysplasia, duplication or crossed fused ectopia) with a uterine anomaly (Fig. 1). According to researches, 36% of Müllerian anomalies occurs with other structural abnormalities and most of these are renal anomalies.

In literature, the age of OHVIRA syndrome diagnosis is ranged from 10-29 years, on average 14 years old and is most commonly encountered 1 to 2 years after menarche. 87% of patients with OHVIRA syndrome after successful surgery will still have successful pregnancy. Therefore, it is sensible to use minimally invasive surgery in cases of OHVIRA syndrome.

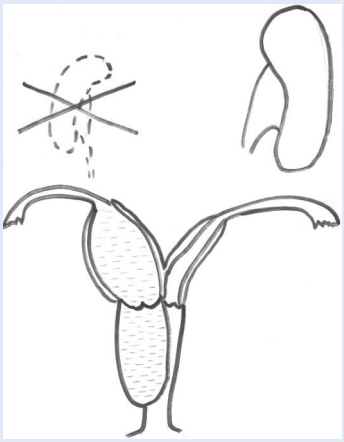

Figure 1. Schematic representation of OHVIRA syndrome

Case report

A 13-year-old girl was referred to our department for evaluation of right-lower quadrant pain during menstruation, suggesting appendicitis. The patient experienced regular cycles since menarche (3 months ago). She reported dysmenorrhea, with right lower abdomen pain. Her medical history was significant for a congenitally absent left kidney as well as right adnexal cyst that was surgically removed at age 3 years. There was severe tenderness in right iliac fossa. Otherwise, her general physical and systemic examination was unremarkable with normal secondary sexual characteristics. Local genital examination revealed normal labia major and minor. Ultrasound of the patient's pelvis showed a 10 cm × 3.3 cm x 3 cm fluid space with internal septation and with spaces of high echogenicity. There was free fluid in pouch of douglas (POD). Transabdominal sonography demonstrated an absent left kidney. We proceeded with diagnostic laparoscopy under general anesthesia which revealed uterus didelphys and enlargement of the right uterus. A subsequent magnetic resonance imaging scan showed uterus didelphys with obstructed right hemivagina and ipsilateral renal agenesis (Fig. 2). The patient was diagnosed with OHVIRA syndrome. After adequate preparation of the patient, a pediatric cystoscope was used to performed bladder examination. Ureteral orifice was not found on the right side. Vaginoscopy revealed left vagina with lateral wall bulge formed from the right vagina. Vaginal septum was completely incised by holmium laser (Ho:YAG) extending to the cervical ostiums while hymen was not injured. Accumulated old menstrual blood was evacuated. The patient's pain was reduced. During the following months, there was still abdominal pain, but less severe, and ultrasonography showed no evidence of blood accumulation in the reproductive system. At the 9-month follow-up the patient was totally symptom-free.

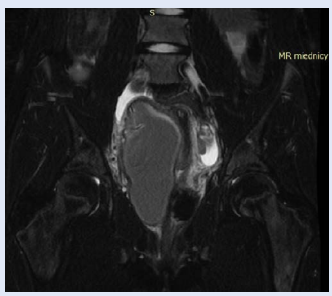

Figure 2. Magnetic resonance image showing uterus didelphys in OHVIRE syndrome

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Conclusion

Due to the rarity and diversification of renal anomaly, a successful diagnosis and treatment of OHVIRA syndrome is challenging for surgeons. Procedure of removal of the septum could be performed in adolescent girls in a minimally invasive way, without damaging the hymen, using a paediatric cystoscope and holmium laser.