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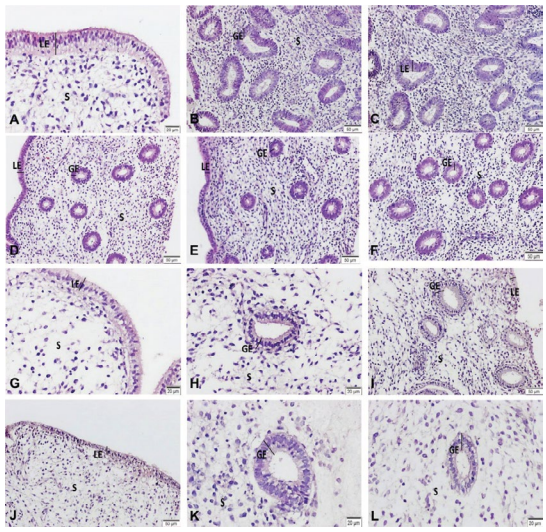

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we WROCŁAWIU
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26.06.2020

Zarząd Główny Polskiego Towarzystwa Ginekologów i Położników

zawiadamia
o zwołaniu Posiedzenia Zarządu Głównego Polskiego Towarzystwa Ginekologów i Położników
oraz
Zwyczajnego Walnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników

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26 czerwca 2020 (piątek) o godzinie 12.00 (I Termin) lub o godz. 12.15 (II Termin).

2. Zwyczajne Walne Zgromadzenie Polskiego Towarzystwa Ginekologów i Położników odbędzie się w dniu
26 czerwca 2020 (piątek) o godzinie 13.30 (I TERMIN)

3. Zwyczajne Walne Zgromadzenie Polskiego Towarzystwa Ginekologów i Położników odbędzie się w dniu
26 czerwca 2020 (piątek) o godzinie 13.45 (II TERMIN)

w Restauracji OKWineBar Między Mostami
ul. Księcia Witolda 1, 50-202 Wrocław

Zakończenie Zwyczajnego Walnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników planowane jest na godz. 15.00

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Porządek zebrania ZG PTGiP dnia **26 czerwca 2020 (piątek) godzina 12.00 (I Termin) oraz godzina 12.15 (II Termin)**

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Walnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników.

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PERINATOLOGIA I GINEKOLOGIA PRZEDŚWIĄTECZNIE

Warszawa

11–12 grudnia 2020 roku

Przewodniczący Komitetu Naukowego:
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Comparison of female sexual function and sexual function of their partners between groups of pregnant and non-pregnant women

Ali Dogukan Angin[1], Enis Özkaya[2], Mehtap Çetin[3], İsmet Gün[4], Onder Sakin[1], Lokman Tekin Ertekin[1], Ramazan Denizli[1], Kazibe Koyuncu[1], Emine Eda Akalin[1]

[1]Department of Obstetrics and Gynecology, Health Sciences University Kartal Training and Research Hospital, İstanbul, Turkey
[2]Department of Obstetrics and Gynecology, Health Sciences University Zeynep Kamil Women and Children's Diseases Training and Research Hospital, İstanbul, Turkey
[3]Department of Obstetrics and Gynecology, Burhaniye State Hospital, Balıkesir, Turkey
[4]Department of Obstetrics and Gynecology, Near East University, Faculty of Medicine, Nicosia, Cyprus

ABSTRACT

Objectives: To compare the female sexual function index and sexual function of their partners between groups of pregnant and non-pregnant Turkish women.

Material and methods: This was a cross-sectional study of 321 women, including 252 healthy pregnant and 69 healthy nonpregnant women. Assessment of female sexual function index (FSFI), ARIZONA scores of their partners were compared in relation to some of the sociodemographic characteristics and pregnancy trimesters.

Results: Comparison of the groups revealed a significantly higher FSFI score in the non-pregnant group whereas the ARIZONA score was significantly higher in the pregnant group ($p < 0.001$). Age, gravidity, parity and smoking rate adjusted mean differences of scores remained statistically significant ($p < 0.001$). Higher ARIZONA (> 11) score rate was significantly higher in pregnant groups (55.6% vs 23.2%, $p < 0.001$). Pregnancy was a risk factor for high ARIZONA score [OR: 4.1 (95% CI 2.2–7.6, $p < 0.001$)]. Lower FSFI score rate was significantly higher in the pregnant group (26.4% vs 69.4%, $p < 0.001$). Pregnancy was a risk factor for low FSFI score [OR: 6.4 (95% CI 3.5–11.7, $p < 0.001$)].

Conclusions: Both female sexual function index and ARIZONA scores of their partners were found to be significantly different between groups of pregnant and nonpregnant Turkish women which indicated altered sexual function of couples during pregnancy.

Key words: female sexual function index; ARIZONA score; pregnancy; partner

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INTRODUCTION

The female sexual response cycle is divided into four stages, including desire, arousal (excitement), orgasm, and dissolution [1]. Regarding these stages, women may experience different forms of sexual dysfunction such as lack of sexual desire, aroused arousal, and inaccessibility to orgasm and pain during sexual activity [1]. It is a multifactorial and underestimated problem with a prevalence of 20–50% in general [2]. Cayan et al. [3] evaluated women aged 18–65 years and found that the prevalence of sexual dysfunction in Turkish women was 46.9%. In literature, a significant decrease in sexual activities has been shown during pregnancy with increased gestational weeks [4]. The reasons suggested by this decline in sexual activity during pregnancy are physical discomfort, fear of harm to the baby, loss of interest, physical oddity, painful coitus, and lack of perceived attraction [5]. On the other hand in a previous study from Turkey, it was shown that The FSFI total scores were not significantly different between the pregnant and nonpregnant women. The study showed significant correlations between the total testosterone and androstenedione levels and sexual function [6]. The sexual function of male

Corresponding author:
Ali Doğukan Anğın
Department of Obstetrics and Gynecology, Health Sciences University Kartal Dr. Lütfi Kırdar Training and Research Hospital, İstanbul, Turkey
e-mail: dralidogukan@gmail.com
phone: +905056232027

partners has been assessed by the ARIZONA scoring questionnaire, the reliability and validity of the ARIZONA scoring in the Turkish language were displayed by Soykan et al. [7]. The Turkish version of the ASEX-Female was shown to have good validity and reliability with good internal consistency (Cronbach's alpha = 0.89). The correlation coefficient was 0.53 for the validity analysis and the cutoff point was 11 for the ROC analysis, which is highly discriminative in terms of validity criteria.

Female sexual dysfunction (FSD) is thought to be a public health problem affecting couples' quality of life. Pregnancy is a special time that involves physical, psychological and hormonal changes that affect women's sexual lives. However, sexual changes during pregnancy and their relationship to their partners' sexual quality require further investigation.

Therefore, the aim of this study was to compare the female sexual function index and sexual function of their partners.

MATERIAL AND METHODS

Subjects

This was a cross-sectional study of 321 women, including 252 healthy pregnant and 69 healthy nonpregnant Turkish women. Female sexual function index (FSFI) and ARIZONA score of their partners were compared in relation to some of the sociodemographic characteristics that were assessed at the Department of Obstetrics and Gynecology in Kartal Training and Research Hospital Obstetrics and Gynecology Clinics and Burhaniye State Hospital between February 2018 and April 2019. The study was conducted with sexually active participants aged 18-41 (married and reported having had sexual intercourse during the previous 4 weeks). The main exclusion criterion is the exclusion of pregnant women with abnormal continuing pregnancies, including the risk of miscarriage, preterm labor, and hypertensive disorder. Pregnant women were informed about pregnancy sexuality in the pregnancy education outpatient clinic in the same hospital and their questions were answered by a midwife. They were informed that sexual intercourse is safe during pregnancy, except in cases of pain, cramping, unexplained vaginal bleeding, early cervical dilatation, and early membrane rupture. Patient information forms and self-reported questionnaires were given to the patients who wanted to participate.

Design

This is a cross-sectional observational study in which the author collected data over a period of 15 months. Participants filled out self-reported questionnaires, including the Female Sexual Function Index (FSFI), and questions about their sociodemographic data and the ARIZONA survey was completed by the male partner of each female participant. Answers of the questionnaire on sociodemographic data were obtained regarding educational background, occupational status, income, medical history and gravity, parity, abortion, vaginal births, and cesarean section. Education was classified as years 8 years (primary and secondary) and more than 8 years (high school and university). All participants were married. Ethical approval was given by the Kartal Education and Research Hospital of Health Sciences University.

Table 1. Coefficients for each item of FSFI scoring system

Domain	Item number	Coefficient
Desire	1, 2	0.6
Arousal	3, 4, 5, 6	0.3
Lubrication	7, 8, 9, 10	0.3
Orgasm	11, 12, 13	0.4
Satisfaction	14, 15, 16	0.4
Pain	17, 18, 19	0.4

Main Outcome Measures

Sexual function was measured by FSFI, a 19-item self-administered questionnaire that assessed sexual function with six domains over the past four weeks: desire, arousal, lubrication, orgasm, satisfaction, and pain. Rosen et al. [8] developed a self-reported questionnaire to assess female sexual function. Turkey's FSF verification was done previously [9]. Questions 1, 2, 15 and 16 are scored between 1 and 5, while all other questions are scored between 0 and 5. The total score of each area obtained from the related questions is multiplied by the coefficient factor (Tab. 1). The total score of all women with a total score below 25 were considered to have sexual dysfunction [10]. To assess sexual dysfunction in the male partner, we used the Arizona Sexual Experience Scale (ASEX), an approved five-item self-assessment scale that measures five major aspects of sexuality: 1) sex drive, 2) sexual arousal, 3) vaginal lubrication (in women) or penile erection. (males), 4) ability to reach orgasm and 5) satisfaction with orgasm. Each item gets scores between 1 and 6, total scores 5–30 and higher results indicate more sexual dysfunction. Total score > 11 was considered to be sexual dysfunction [11]. Pregnancy was grouped as the first (0–13 weeks), second (14–26), and third (27–40 weeks) trimesters.

Statistical Analysis

The statistical parameters were computed using the Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). The continuous variables were ex-

pressed as the mean ± standard deviation. The categorical variables were expressed as the number and percentage. The Mann-Whitney U test was used in the comparison between the averages of two groups. The Kruskal-Wallis test was used to compare more than two continuous variables. Adjusted means were compared by ANCOVA. Multivariate regression analysis was used to assess adjusted associations. Statistical significance was defined as $p < 0.05$.

RESULTS

There was a significant difference between groups in terms of mean age of female partners ($p < 0.05$) however mean BMI, money income and mean age of male partners were similar ($p > 0.05$) (Tab. 2). Gravidity (0.5 vs 1.8, $p < 0.001$) and parity (0.4 vs 0.6, $p = 0.028$) were significantly different between the groups. No difference was determined between the two groups in terms of rates of the route of previous deliveries ($p > 0.05$). No difference was observed between the two groups in terms of systemic disorder ($p > 0.05$). Educational status was also similar between pregnant and nonpregnant groups and groups of women with and without FSFI < 25 ($p > 0.05$). The smoking rate was significantly higher in the non-pregnant group (17.4% vs 8.3%, $p = 0.028$). Adjusted and unadjusted means of ARIZONA and FSFI scores were shown in Table 2. A comparison of the groups revealed a significantly higher FSFI score in the non-pregnant group whereas the ARIZONA score was significantly higher in the pregnant group ($p < 0.001$) (Tab. 3). Age, gravidity, parity and smoking rate adjusted mean of scores remained statistically significant ($p < 0.001$). Higher ARIZONA (> 11) score rate was significantly higher in pregnant groups (55.6% vs 23.2%, $p < 0.001$). Pregnancy was a risk factor for high ARIZONA score [OR: 4.1 (95% CI 2.2–7.6, $p < 0.001$)]. Lower FSFI score rate was significantly higher in the pregnant group (26.4% vs 69.4%, $p < 0.001$). In multivariate regression analysis pregnancy was found to be significantly associated with FSFI score < 25 (beta coefficient = 0.321, $p < 0.001$) after adjustment for the age, gravidity and smoker rates. Pregnancy was a risk factor for low FSFI score [OR:6.4 (95% CI 3.5–11.7, $p < 0.001$)]. A comparison of scores in relation to the three trimesters revealed no statistically significant difference ($p > 0.05$) (Tab. 4).

DISCUSSION

In the current study, which was performed with a sample of pregnant women, we found that the male partner sexual dysfunction (ARIZONA > 11) rate was higher in the pregnant group (55.6% vs 23.2%, $p < 0.001$). Pregnancy was a risk factor for high ARIZONA score [OR: 4.1 (95% CI 2.2–7.6, $p < 0.001$)]. Lower FSFI score rate was significantly higher in the pregnant

Table 2. Comparison of demographic characteristics of pregnant and non-pregnant women

	Groups	N	Mean	Std. Deviation	p value
Age [years]	Non-pregnant	69	28.4	6.01	
	Pregnant	252	26.6	5.06	0.013
BMI [kg/m^2]	Non-pregnant	69	24.7	4.9	
	Pregnant	252	26.4	12.6	0.26
Income	Non-pregnant	69	1.8	0.7	
	Pregnant	252	1.9	0.6	0.177
Age of partner [years]	Non-pregnant	69	31.8	6.8	
	Pregnant	252	30.7	5.3	0.235

Table 4. Comparison summary of mean score values of among different trimesters of pregnancies

Trimesters		N	Mean	Std. Deviation	p value
FSFI Total Score	First	72	19.4	9.8	
	Second	96	19.4	8.9	0.141
	Third	84	16.4	10.5	
	Total	252	18.4	9.8	
ARIZONA Total Score	First	72	12.6	4.3	
	Second	96	11.9	3.6	0.446
	Third	84	11.8	3.1	
	Total	252	12.1	3.7	

Table 3. Comparison summary of adjusted and unadjusted mean score values of pregnant and non-pregnant women

	Groups	N	Mean	Std. Deviation	p value
FSFI Total Score	Non-pregnant	69	27.4	4.7	
	Pregnant	252	18.5	9.8	< 0.001
Age, Gravidity, Smoker Rate Adjusted FSFI Total Score	Non-pregnant	69	27.5	1.3 (SE)	
	Pregnant	252	18.5	0.6 (SE)	< 0.001
ARIZONA Total Score	Non-pregnant	69	9.2754	2.71642	
	Pregnant	252	12.1349	3.72106	< 0.001
Age, Gravidity, Smoker Rate Adjusted Arizona Total Score	Non-pregnant	69	9.4	0.5 (SE)	
	Pregnant	252	12.1	0.2 (SE)	< 0.001

group (26.4% vs 69.4%, p < 0.001). Pregnancy was a risk factor for low FSFI score [OR: 6.4 (95% CI 3.5–11.7, p < 0.001)]. A comparison of scores in relation to the three trimesters revealed no statistically significant difference. In our literature search, we encountered several studies and reviews on this issue, in one of these reviews, one hundred thirty-five studies were systematically reviewed. Ninety-five of these studies were evaluated in more detail in a meta-analysis. The prevalence of female sexual dysfunction in premenopausal women was estimated to be 40.9%. The prevalence rates of individual sexual disorders range from 20.6% (lubrication difficulties) to 28.2% (hypoactive sexual desire disorder). The results show that female sexual dysfunction is a major public health problem affecting 41% of premenopausal women in the world [12]. On the other hand, there are also several studies on specific populations especially the pregnant women, Ninivaggio et al. evaluated the sexual function of 623 nulliparous pregnant women using FSFI in the first, second and early third trimesters [13]. Authors reported sexual dysfunction rates of 36.3% in the first trimester, 36.8% in the second trimester, and 57% in the third trimester, and reported that mean FSFI scores decreased as the pregnancy progressed. The higher rate was reported in another study, Seven et al. [14] assessed pregnant Turkish women, sexual dysfunction rate was 77.6% in their study. In another study on Turkish pregnant women, Eryilmaz et al. showed 81.5% of sexual dysfunction during pregnancy. In their study with 238 Turkish pregnant women, significant relationships between changes were reported in sexual life during pregnancy and marriage duration, educational level, parity, and gravidity [15]. Consistently, Erol et al. [16] and Çorbacıoğlu et al. [17] conducted their studies on In Turkish pregnant women, both studies noted lower sexual function scores in women in the third trimester of their pregnancies compared with those in their first two trimesters of pregnancy. Pregnancy, especially in the third trimester, was found to have an impact on sexual health and decreased sexual function during pregnancy [16, 18]. No differences were determined among different pregnancy trimesters in terms of either score in our study, higher mean total FSFI scores were observed in the first and second trimester but the difference did not reach statistical significance (19.5, 19.5 and 16.5 respectively).

Educational status was found to have a significant impact on sexual function, women having been trained for more than 8 years the low risk of sexual dysfunction compared to women who have been trained for 8 years or less [6]. Educational status was similar between pregnant and nonpregnant groups and groups of women with and without FSFI < 25 or with and without ARIZONA score > 11 (p > 0.05) in our study population. Based on knowledge about sexual dysfunction in the third trimester, postpartum sexual function was assessed in a study; breastfeeding and poor partnership quality have emerged as important risk factors for postpartum sexual dysfunction problems. Depressive symptoms with cesarean section and high maternal education were correlated with dysfunctional problems in many sub-areas. The findings showed that women at risk for female sexual dysfunction were significantly different in terms of partnership quality, breastfeeding, mode of delivery, maternal education and depressive symptoms [19]. The pool of data showed us that, sexual function decreases during pregnancy and worsens as the pregnancy progresses. This process is influenced by many factors such as socio-cultural factors, age, parity, breastfeeding, depression, fatigue, sexual inactivity during the first trimester, postnatal body image, re-conception concerns, and concomitant urinary tract infections. There was no clear evidence that there is a relationship between the mode of delivery and changes in sexual function. Authors of this review pointed out that; sexual quality of life should be part of history due to possible sequelae of pregnancy and childbirth [20]. Symptoms of sexual dysfunction during pregnancy may have a negative impact on the quality of life of women and affect couples' relationships, therefore some interventions may be required, one thousand thirty-seven articles were taken into consideration in a previous review on this issue, four were selected for full-text reading, and two randomized trials (159 participants) were included. Based on this review, due to the heterogeneity between the studies, the results could not be combined. Based on the findings of this review, it is not possible to make a clear and conclusive recommendation on the effectiveness and safety of interventions used in the treatment of symptoms of sexual dysfunction in pregnancy [21]. Therefore preventive measure have great importance on this issue, for this reason, risk factors have been assessed in the literature to introduce some measures, in the cross-sectional study including 286 pregnant women, being a partner at an advanced age, a history of miscarriage, a history of previous health problems and a high level of anxiety were found to be negative factors affecting sexual function. The authors of this study suggested that health professionals should be aware of a number of risk factors that may contribute to sexual dysfunction in pregnant women [22]. In another study, sexually active 246 pregnant women were included in this cross-sectional controlled study and a total of 210 non-pregnant women were used as controls. Groups were compared in terms of age, gestational age, incontinence, body mass index and obstetric history. Mean total FSFI scores were significantly lower in pregnant women than in non-pregnant women. In addition, the rate of sexual dysfunction in pregnant women was significantly higher than in non-pregnant women. However, no significant difference was found in the rate of sexual dysfunction in pregnant women compared to trimesters. In addition, gravidity and parity showed negative effects on sexual functions. However, the number of abortions did not affect sexual function. These data show that pregnancy sig-

nificantly reduces sexual function in women [23]. This study by Aydin et al. was conducted on Turkish women and consistent with our results, pregnancy was found to be a risk factor for sexual dysfunction, no data regarding partner's sexual function was presented in the above-mentioned study.

Obesity and excess weight are increasing worldwide and can jeopardize the sexual functions of women. A previous study aimed to compare the sexual functions of normal and overweight women during pregnancy. A cross-sectional study on 105 overweight and 118 normal weights pregnant women in the 2nd and 3rd pregnancy trimesters was conducted. Female Sexual Function Index (FSFI) was used to assess sexual function. It was found that, in the second trimester, the mean total FSFI scores were similar to those of overweight and normal-weight women. While in the third trimester, the total FSFI scores of overweight women were significantly lower than those of normal-weight women. In the third trimester, the mean scores of overweight women in the areas of desire, arousal, lubrication, orgasm, and dyspareunia were significantly lower. The authors of this study concluded that overweight women in the third trimester of pregnancy had weaker sexual functions compared to normal-weight women [24]. No difference was determined between pregnant and nonpregnant groups and groups of women with and without FSFI < 25 ($p > 0.05$) or with and without ARIZONA score > 11.

Limitations of this study, it was a cross-sectional study and was not prospective, on the other hand, comparisons were made between different women, not with the same women before and after pregnancy and to the best of our knowledge, this is the first study, which assessed sexual function for both partners.

CONCLUSIONS

In conclusion, both female sexual function index and ARIZONA scores of their partners were found to be significantly different between groups of pregnant and nonpregnant Turkish women which indicated altered sexual function of couples during pregnancy.

Acknowledgments

The authors have nothing to declare. Informed consent was obtained from each participant. The study was approved by the institutional ethics committee. "All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards".

REFERENCES

1. Sexual Dysfunctions. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. , doi: 10.1176/appi.books.9780890425596.125889.
2. Garcia S, Moreno S, Aponte H. Prevalence of sexual dysfunction in female outpatients and personnel at a Colombian hospital: correlation with hormonal profile. J Sex Med. 2008; 5(5): 1208–1213, doi: 10.1111/j.1743-6109.2007.00718.x, indexed in Pubmed: 18221292.
3. Cayan S, Akbay E, Bozlu M, et al. The prevalence of female sexual dysfunction and potential risk factors that may impair sexual function in Turkish women. Urol Int. 2004; 72(1): 52–57, doi: 10.1159/000075273, indexed in Pubmed: 14730166.
4. Sydow Kv. Sexuality during pregnancy and after childbirth. Journal of Psychosomatic Research. 1999; 47(1): 27–49, doi: 10.1016/s0022-3999(98)00106-8.
5. Orji EO, Ogunlola IO, Fasubaa OB. Sexuality among pregnant women in South West Nigeria. J Obstet Gynaecol. 2002; 22(2): 166–168, doi: 10.1080/01443610120113319, indexed in Pubmed: 12521698.
6. Astepe BS, Köleli I. A cross-sectional study of female sexual dysfunction among Turkish pregnant and nonpregnant women: correlation with hormone profile. The European Research Journal. 2018, doi: 10.18621/eurj.432490.
7. Soykan A. The reliability and validity of Arizona sexual experiences scale in Turkish ESRD patients undergoing hemodialysis. Int J Impot Res. 2004; 16(6): 531–534, doi: 10.1038/sj.ijir.3901249, indexed in Pubmed: 15175639.
8. Rosen R, Brown C, Heiman J, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000; 26(2): 191–208, doi: 10.1080/009262300278597, indexed in Pubmed: 10782451.
9. Öksüz E, Malhan S. Reliability and validity of the female sexual function index in Turkish population. Sendrom. 2005; 17: 54–59.
10. Oksuz E, Malhan S. Prevalence and risk factors for female sexual dysfunction in Turkish women. J Urol. 2006; 175(2): 654–8; discussion 658, doi: 10.1016/S0022-5347(05)00149-7, indexed in Pubmed: 16407018.
11. Soykan A. The reliability and validity of Arizona sexual experiences scale in Turkish ESRD patients undergoing hemodialysis. Int J Impot Res. 2004; 16(6): 531–534, doi: 10.1038/sj.ijir.3901249, indexed in Pubmed: 15175639.
12. McCool ME, Zuelke A, Theurich MA, et al. Prevalence of Female Sexual Dysfunction Among Premenopausal Women: A Systematic Review and Meta-Analysis of Observational Studies. Sex Med Rev. 2016; 4(3): 197–212, doi: 10.1016/j.sxmr.2016.03.002, indexed in Pubmed: 27871953.
13. Ninivaggio C, Rogers RG, Leeman L, et al. Sexual function changes during pregnancy. Int Urogynecol J. 2017; 28(6): 923–929, doi: 10.1007/s00192-016-3200-8, indexed in Pubmed: 27889829.
14. Seven M, Akyüz A, Güngör S. Predictors of sexual function during pregnancy. J Obstet Gynaecol. 2015; 35(7): 691–695, doi: 10.3109/01443615.2015.1006596, indexed in Pubmed: 25710683.
15. Eryilmaz G, Ege E, Zincir H. Factors affecting sexual life during pregnancy in eastern Turkey. Gynecol Obstet Invest. 2004; 57(2): 103–108, doi: 10.1159/000075582, indexed in Pubmed: 14673220.
16. Erol B, Sanli O, Korkmaz D, et al. A cross-sectional study of female sexual function and dysfunction during pregnancy. J Sex Med. 2007; 4(5): 1381–1387, doi: 10.1111/j.1743-6109.2007.00559.x, indexed in Pubmed: 17651387.
17. Esmer AC, Akca A, Akbayir O, et al. Female sexual function and associated factors during pregnancy. J Obstet Gynaecol Res. 2013; 39(6): 1165–1172, doi: 10.1111/jog.12048, indexed in Pubmed: 23718891.
18. Pauleta JR, Pereira NM, Graça LM. Sexuality during pregnancy. J Sex Med. 2010; 7(1 Pt 1): 136–142, doi: 10.1111/j.1743-6109.2009.01538.x, indexed in Pubmed: 19845548.
19. Wallwiener S, Müller M, Doster A, et al. Sexual activity and sexual dysfunction of women in the perinatal period: a longitudinal study. Arch Gynecol Obstet. 2017; 295(4): 873–883, doi: 10.1007/s00404-017-4305-0, indexed in Pubmed: 28251311.
20. Yeniel AO, Petri E. Pregnancy, childbirth, and sexual function: perceptions and facts. Int Urogynecol J. 2014; 25(1): 5–14, doi: 10.1007/s00192-013-2118-7, indexed in Pubmed: 23812577.
21. Ribeiro MC, Nakamura MU, Torloni MR, et al. Treatments of Female Sexual Dysfunction Symptoms during Pregnancy: A Systematic Review of the Literature. Sex Med Rev. 2014; 2(1): 1–9, doi: 10.1002/smrj.18, indexed in Pubmed: 27784539.
22. Seven M, Akyüz A, Güngör S. Predictors of sexual function during pregnancy. J Obstet Gynaecol. 2015; 35(7): 691–695, doi: 10.3109/01443615.2015.1006596, indexed in Pubmed: 25710683.
23. Aydin M, Cayonu N, Kadihasanoglu M, et al. Comparison of Sexual Functions in Pregnant and Non-Pregnant Women. Urol J. 2015; 12(5): 2339–2344, indexed in Pubmed: 26571317.
24. Ribeiro MC, Nakamura MU, Torloni MR, et al. Maternal overweight and sexual function in pregnancy. Acta Obstet Gynecol Scand. 2016; 95(1): 45–51, doi: 10.1111/aogs.12796, indexed in Pubmed: 26456082.

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The correlation between unexplained infertility and exosomes

Yıldız Uyar, Melike Özgül, Sevtap Gökalp, Gülay Ok, Ayça Tan, Hafize Seda Vatansever

Manisa Celal Bayar University Medical Faculty, Manisa, Turkey

ABSTRACT

Objectives: Endometrial receptivity plays the most important role for successful implantation. Increasing endometrial receptivity may improve infertility and increase Assisted Reproductive Technologies success. The aim of this study was to investigate the effect of exosome specific markers CD63 and CD9 which are promising molecules in the pathogenesis and treatment of many diseases on endometrial receptivity in women with unexplained infertility.

Material and methods: This prospective study was conducted between November 2015 and March 2017. Proliferation and secretion periods of endometrial samples from fertile and infertile cases were collected. The paraffin-embedded tissue sections were stained with hematoxylin-eosin for the immunohistochemical analysis distributions of CD63 and CD9.

Results: The results of this study demonstrated that the CD63 immunoreactivity was higher in both luminal and glandular epithelium of infertile patients when compared with fertile patients during the proliferative phase ($p = 0.009$, $p = 0.008$). In the infertile proliferation phase, endometrium CD9 immunoreactivity was rarely detected in both the luminal and glandular epithelium. In the secretion phase of endometrium, CD9 immunoreactivity was mild in fertile patients, the increased immunoreactivity of CD9 was observed in both luminal and glandular epithelium of infertile patients ($p = 0.037$, $p = 0.037$).

Conclusions: Increased levels of CD63 in infertile proliferation phase endometrium should represent an unfavorable signaling. Moreover, the increased levels of CD9 in infertile secretion phase endometrium could be used as a biomarker to evaluate endometrial receptivity. These exosome-specific markers can be considered as potential molecular markers of infertility.

Key words: exosomes, endometrium, fertility, infertility, embryo implantation

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INTRODUCTION

Infertility is a condition that has psychological, economic and medical effects which cause trauma and stress especially on the people who desire to have children [1]. Infertility is a reproductive system disease defined by the inability to achieve clinical pregnancy after unprotected sexual intercourse for 12 months or more [2, 3]. Unexplained infertility is the absence of identifiable causes for infertility [3].

Endometrial receptivity plays a critical role for successful implantation, and impaired endometrial receptivity may lead to subfertility and limit the success of Assisted Reproductive Technologies (ART) [4]. Implantation, a critical step in the establishment of pregnancy, ensues under the uterine fluid microcirculation signals which contain various proteins, lipids and other molecules secreted from the endometrium and possibly from the fallopian tubes and blastocyst (such as hCG) [5]. Unexplained infertility has been suggested to be caused by a disorder in molecular and cellular biomarkers in endometrial receptivity [6]. In studies on unexplained infertility, which is explained as a high failure of implantation after ART, pro-inflammatory factors and interleukins have been shown in uterine wall invasion [7].

Extracellular vesicles contain exosomes, microvesicles and apoptotic bodies and they are also a heterogeneous group of particles defined by their size, composition and density. The smallest extracellular vesicles are exosomes (30–100 nm) released by plasma membrane fusion of multivesicular bodies containing intraluminal vesicles [8, 9].

Exosomes which are nanoparticles capable of specifically transferring small RNAs and messenger RNA (mRNAs) through the extracellular medium to cells located in distant regions can be isolated from culture supernatants of cell

Corresponding author:
Yıldız Uyar
Manisa Celal Bayar University Medical Faculty, Manisa, Turkey
e-mail: yildizuyar86@outlook.com

lines or from various body fluids [10]. According to cells, the exosomal content may vary. However, different cell-based exosomes also express certain common exosome specific proteins. Among these proteins are CD9, CD37, CD53, CD63, CD81 and CD82 tetraspanin molecules [10]. Exosomes can be characterized and purified via their specific cell surface markers like tetraspanins CD63 and CD9 which are responsible for exosome formation. Moreover, exosomes present signal transduction (EGFR), antigen presentation (MHC I and MHC II) and other transmembrane proteins (LAMP1) on their surface [10, 11]. Exosome secretion has been demonstrated in a number of cell types, including embryonic stem cells and in vitro produced embryos [12]. Some studies have shown that exosomes in the uterine cavity or slightly larger microvesicles (100–300 nm) are released from the endometrial epithelium. These exosomes or microvesicles contain specific miRNAs so that these miRNAs can be transferred to the trophectoderm cells or endometrial epithelial cells to promote implantation [13, 14].

Objectives

The aim of this study was to investigate the distribution of exosomes in endometrial samples taken during proliferation and secretion periods from fertile and unexplained infertile patients and their effects on implantation in unexplained infertility.

MATERIAL AND METHODS

Study Design and Experimental Groups

The study was conducted using fertile (n = 5 proliferation phase and n = 5 secretion phase) and infertile (n = 5 proliferation phase and n = 5 secretion phase) patients who were between 25- and 38-years age. Endometrial specimens were obtained by probe curettage or by pipelling during proliferation and secretion period of the menstrual cycle from both fertile and infertile patients. Menstrual cycle phase was confirmed by histological dating [15]. Prior to commencing with study, ethical permission was taken from Health Science Ethics Committee and written informed consent was obtained from all participants.

Selection of Patient Groups

The current study involved female participants aged between 25–38 years. Participants were selected from amongst patients that were admitted to our hospital Obstetrics and Gynecology Clinic.

Participants in the fertile group had at least one child, did not have any uterine disease, had dysfunctional uterine bleeding, and had to undergo curettage during a non-hemorrhagic period. The infertile group consisted of participants who had no children, had been married for at least 1 year and were not able to achieve clinical pregnancy after unprotected sexual intercourse for 12 months. Infertile cases were determined based on clinical diagnosis, anamnesis, radiological and ultrasound assessments.

Either endometrial evaluation for diagnosis or treatment, curettage materials from infertile and fertile patients were taken under anesthesia via probe curettage or pipelined endometrial sampling. Samples were collected from patients that were clinically diagnosed as fertile or infertile with dysfunctional uterine bleeding.

In the current study, the menstrual cycle-related intake of the samples is essential. Thus, any other organic endometrial changes outside the dysfunctional uterine bleeding were deactivated and standard sample uptake was achieved. Patients with a pathologic diagnosis of an organic cause such as hyperplasia, neoplasia or polyps, or an organic lesion such as myoma in the examination and who had been using steroid hormones for at least six months prior to the study were not included in this study.

As a result of endometrial sampling, patients with endometrium in the proliferation phase and secretion phase were chosen among the patients who admitted to our gynecology outpatient clinic with the complaint of abnormal bleeding and diagnosed with dysfunctional uterine bleeding excluded from organic reasons and whose BHCG negative on day of endometrial sampling included in our study.

The current study consists of two groups of patients, fertile and infertile. The endometrial samples taken from both patient groups were divided further into fertile proliferation (Group 1), infertile proliferation (Group 2), fertile secretion (Group 3) and infertile secretion (Group 4) groups.

Histological Evaluation

The endometrium tissue samples were fixed in 10% neutral formalin and then embedded in paraffin using standard protocols. After processing, samples were embedded in paraffin and 5 µm sections were taken. Sections were used for both histochemical and immunohistochemical analyses.

Histochemical Analyses

For morphological evaluation, sections taken from the paraffin blocks at 5 µm thickness with a rotary microtome were deparaffinized overnight at 60°C (Nuve, FN 400), followed by chemical deparaffinization with xylene for 1 hour. The sections were passed through the decreasing alcohol series (95%, 80%, 70% and 60% alcohol series) for 2 minutes each and the sections were treated for 6 minutes with Hematoxylin (Leica, 3801562E) solution. Sections were washed for 5 minutes under water and then stained with Eosin (Bio-Optica, 380610) for 1.5 minutes after

differentiating with 1% acid-alcohol solution. Sections were taken up into xylene by passing through increasing alcohol series (80% and 95%). The sections which were left in xylene for 1 hour were covered with cover glasses (Marienfeld, 01 01060) using entellan solution (Merck, UN 1866) and examined under a light microscope (Olympus BX43).

Immunohistochemical Analyses

For immunohistochemical evaluation of the CD63 and CD9, avidin-biotin-peroxidase indirect immunohistochemistry method was used. After deparaffinization of sections as above, they were washed with phosphate buffered saline (PBS) and 3% H_2O_2 (H1009, Sigma-Aldrich, USA) was applied. They were then washed with PBS again. For antigen retrieval, they were incubated with trypsin for 10 minutes in 37°C and then washed with PBS. Sections were then incubated with blocking serum for 1 h and, anti-CD63 (sc-5275, lot #D0115, Santa Cruz, USA), and anti-CD9 (sc-13118, lot #K1814, Santa Cruz, USA) primer antibodies in a 1/50 dilution for both antibodies were added and incubated at 4°C for overnight. After the washing step with PBS, the secondary antibodies biotin (30 min) and streptavidin (30 min) were applied respectively (Histostain®-Plus Bulk Kit Cat No: 85-9043- Invitrogen, Carlsbad, California, United States). Immunoreactivity was visualized by the application of 50 µl DAB chromogen (Histostain-Plus IHC Kit, DAB, broad spectrum Cat No. 859643, Invitrogen, Carlsbad, California, United States). After washing with distilled water, they were counter stained with Mayer's hematoxylin and covered with immunohistochemistry mounting medium. Immunohistochemical process was repeated 3 times. Immunohistochemical staining was scored using a semi-quantitative analysis is based on the calculation of HSCORE = ∑Pi(i+1) (i = intensity of staining and Pi is the percentage of positively stained cells for each intensity) formula after evaluation of intensities as 1 (mild), 2 (moderate) or 3 (strong) [16–19]. For each antibody, five different fields were evaluated by at least two investigators independently under light microscope (BX43, Olympus), blinded to the source of the samples as well as to each other's results and the average score was then utilized. Results were given as median after analyzing statistically with Mann-Whitney U test for differences among groups. When the p value was < 0.05, the data was considered significant.

RESULTS

Histological Results

After hematoxylin and eosin staining, endometrial luminal and glandular epithelium were observed in all groups. The luminal epithelium was a single-layered columnar epithelium during proliferation phases of both the fertile and infertile patients. The glands in the connective tissue of the lamina propria were round in shape and had single-layered columnar epithelium in both fertile and infertile patients in proliferative phase (Fig. 1A–F). In the secretory phase of the fertile and infertile patients' endometrium, the luminal epithelium was a single-layered columnar epithelium and the gland structures appeared as glycogen vacuoles in the basal parts of the glandular columnar epithelium (Fig. 1G–L).

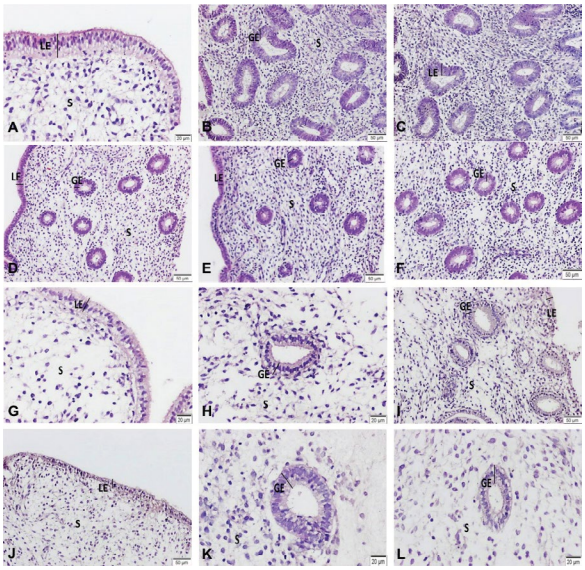

Figure 1. Hematoxylin and eosin staining of endometrium. Proliferative phase of fertile patients (A–C), proliferative phase of infertile patients (D–F), secretion phase of fertile patients (G–I), secretion phase of infertile patients (J–L). (A, G, H, K, L Scale Bars: 20 µm) (B, C, D, E, F, I and J Scale Bars: 50 µm)

Immunohistochemical Results

In the proliferative phase of the fertile patients' endometrium, weak immunoreactivity of CD63 was detected in both the luminal (HSCORE = **190**) (min–max; 170–200) and glandular epithelium (HSCORE = **170**) (min–max; 150-205), and also mild CD63 immunoreactivity was detected in the stroma (Fig. 2A, B). In the proliferative phase of the infertile patients' endometrium, increased (mild/strong) CD63 immunoreactivity was detected in both the luminal (HSCORE = **310**) (min–max; 300–325) and glandular epithelium (HSCORE = **315**) (min–max; 310–320) when compared to the fertile group, they were statistically significant ($p = 0.009$, $p = 0.008$) (Tab. 1 and Fig. 3). Intensity of CD63 immunoreactivity in stroma was seen similar in both fertile and infertile group (Fig. 2C, D). In the secretion phase of the fertile patient's endometrium, weak and mild immunoreactivity of CD63 was detected in the luminal (HSCORE = **175**) (min–max; 165–175) and glandular epithelium (HSCORE = **285**) (min–max; 255–305) respectively, mild CD63 immunoreactivity was also observed in the stroma (Fig. 2E, F). In the secretion phase of the

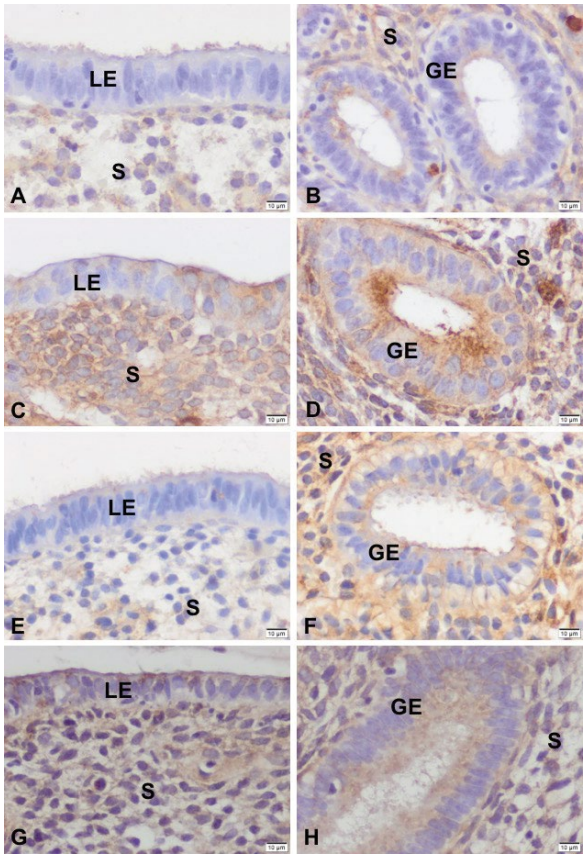

Figure 2. Immunohistochemical distribution of CD63. Scale Bars: 10 µm

infertile patient's endometrium, increased (mild/moderate) CD63 immunoreactivity was detected in both luminal (HSCORE = **200**) (min–max; 200–200) and glandular epithelium (HSCORE = **250**) (min–max; 250–250) when compared with fertile group, however, this intensity was slightly less than the proliferative phase of the infertile endometrium (Fig. 2G, H) and when compare the results it was statistically significant (p = 0.034, p = 0.037) (Tab. 1 and Fig. 3). In addition, intensity of CD63 in the stroma of the infertile secretion phase of the endometrium (mild) decreased when compared with other groups (Fig. 2).

CD9 immunoreactivity was observed as very weak in the infertile proliferation phase of endometrial luminal (HSCORE = **130)** (min–max; 115–135) and glandular epithelium (HSCORE = **140**) (min–max; 100–160) (Fig. 4C, D). This immunoreactivity was weaker then fertile proliferation luminal epithelium (HSCORE = **150**) (min–max; 130–170) (p = 0.025). In contrast to that, CD9 immunoreactivity was not observed in the fertile proliferation glandular epithelium (Fig. 4A, B). It was evident that there were significant differences between fertile and infertile proliferation gland epithelium (p = 0.005) (Tab. 1 and Fig. 5). In stroma, CD9 immunoreactivity was weak in fertile proliferation endometrium, but not observed in infertile proliferation tissue samples. In the infertile secretion phase, CD9 immunoreactivity was weak/moderate in the luminal (HSCORE = **220**) (min–max; 220–220) and the glandular epithelium (HSCORE = **220**) (min–max; 220-220) (Fig. 4G, H), whereas CD9 immunoreactivity of fertile secretion luminal (HSCORE = **150**) (min–max; 130–170) and glandular epithelium (HSCORE = **160**) (min–max; 160–170) was weak (Fig. 4E, F). It was clearly observed that there were significant differences between fertile and infertile secretion phase epitheliums (p = 0.037, p = 0.037) (Tab. 1 and Fig. 5). In stroma, CD9 immunoreactivity was very weak in the fertile secretion tissues, but not observed in infertile secretion tissues.

Table 1. p values of statistical comparisons of CD63 and CD9 HSCORE results

CD63	
Compared Groups	**p Value**
Fertile proliferation vs infertile proliferation (luminal epithelium)	p = 0.009
Fertile proliferation vs infertile proliferation (glandular epithelium)	p = 0.008
Fertile secretion vs infertile secretion (luminal epithelium)	p = 0.034
Fertile secretion vs infertile secretion (glandular epithelium)	p = 0.037
CD9	
Compared Groups	**p Value**
Fertile proliferation vs infertile proliferation (luminal epithelium)	p = 0.025
Fertile proliferation vs infertile proliferation (glandular epithelium)	p = 0.005
Fertile secretion vs infertile secretion (luminal epithelium)	p = 0.037
Fertile secretion vs infertile secretion (glandular epithelium)	p = 0.037

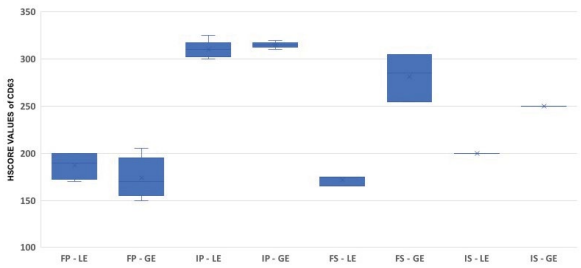

Figure 3. HSCORE values of CD63 immunoreactivities. FP — Fertile Proliferation; IP — Infertile Proliferation; FS — Fertile Secretion; IS — Infertile Secretion; LE — Luminal Epithelium; GE — Glandular Epithelium

DISCUSSION

In our study, we showed that there were differences in the release of CD63 and CD9 membrane surface proteins

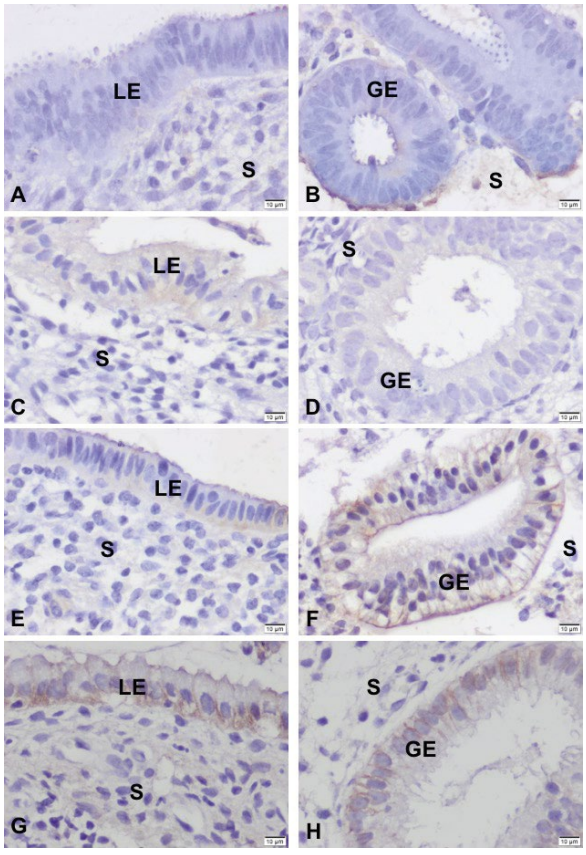

Figure 4. Immunohistochemical distribution of CD9. Scale Bars: 10 µm

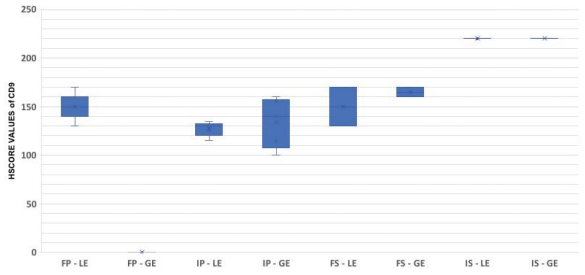

Figure 5. HSCORE values of CD9 immunoreactivities. FP — Fertile Proliferation; IP — Infertile Proliferation; FS — Fertile Secretion; IS — Infertile Secretion; LE — Luminal Epithelium; GE — Glandular Epithelium

used to characterize exosomes in luminal and glandular epithelium of the endometrium between the unexplained infertility and the fertile women.

To date, many researchers have been stated that it is well known the endometrial epithelium is the first maternal surface to interact with the implanting embryo. And they also stated that the inclusion of extracellular vesicles in the implantation process is a relatively new phenomenon [14, 20–24]. In many studies; extracellular vesicles containing specific RNAs, including microRNAs and proteins, have been released into the uterine cavity and transferred to trophoblast cells or endometrial epithelial cells where they promote implantation [13, 14, 21, 25]. Ng YH et al. [14] showed for the first time that the tetraspanins, CD9 and CD63 used as cell surface markers of exosomes that exist on the surface of fertile endometrial epithelial cells and they contain specific miRNAs in the endometrial epithelium and stated that the exosomes and/or exosome-derived miRNA could be used as biomarkers for endometrial receptivity.

Our results demonstrated that CD63 expression in the secretion phase endometrium of fertile group was higher in the glandular epithelium compared to the luminal epithelium, and this intensity was statistically significant. It was observed that intensity of CD63 in luminal and glandular epithelial in the infertile proliferation phase group was higher when compared to the fertile proliferation phase group, and also this increased immunoreactivity was statistically significant. However, significantly decreased CD63 expression was detected during infertile secretion phase compared to the infertile proliferation phase.

Ng YH et al. [14]; in their study, showed that CD63 and CD9 had strong apical staining in the luminal and glandular epithelial cells of fertile women, and interestingly, this staining for CD63 reached the highest levels in the mid-secretory period, which is the time of endometrial receptivity and they said it might be important in implantation. Therefore, they stated that enough secretion of increased exosome production in the mid-secretory period was needed for the implantation window and if this process could not proceed properly, result with implantation failure. As a result, Ng YH et al. [14]; have expressed that exosomes modulate the behavior of the immune system and cancer cells and because the embryo implantation is in common with the behavior of immune system and cancer cells, the clarification of the function of the exosomes in the uterine cavity will extend our understanding of the endometrial-embryo cross talk and infertility.

In our study, we found that CD63 expression was similar and weak in the glandular and luminal epithelium of the fertile proliferative phase endometrium's, whereas CD63 expression in the glandular epithelium in the fertile secretion phase increased significantly. It is thought that during implantation, CD63 has an especially increased in glandular epithelium, and the luminal secretion of CD63 is similar in the proliferation and secretion phases of fertile group, so that CD63 secreted from the glandular epithelium may have a controlling role in implantation.

According to the results of our study, CD63 could not be accepted as an indicator or biomarker of endometrial signaling pathways in the infertile group due to the CD63 expression levels in the infertile group during the proliferation phase, furthermore the CD63 secretion was slightly increased in luminal epithelium but decreased

in glandular epithelium in the infertile secretion phase compared to the fertile secretion phase. However, the observed increase of proliferation phase CD63 in the infertile group compared to the fertile group suggests that exosomal trafficking increases in the endometrial epithelium and it may cause negative effects in order to control different signal pathways before implantation.

Burnett LA et al. [26] stated in their review that several studies have demonstrated that interactions between the embryo and the uterine microenvironment are important for successful implantation and healthy pregnancy [12, 14, 27, 28]. In their studies, Rosenbluth EM et al. [12], have shown that some exosomal miRNAs can be secreted by human embryos in IVF cycles and that these exosome-derived miRNAs can be secreted into the IVF culture environment that can be used as a biomarker in predicting IVF success and outcome of pregnancy.

Our study showed that CD9 immunoreactivity in the glandular epithelium in the fertile proliferation phase was significantly different compared to the luminal epithelium. Thus, CD9 expression was thought to be secreted mainly in the luminal epithelium of fertile proliferative phase. However, in the luminal epithelium of infertile proliferative phase, the CD9 immunoreactivity decreased and it was statistically significant compared to the fertile group. In the infertile proliferative phase, CD9 immunoreactivity was significantly expressed in the glandular epithelium.

It was clearly seen that the CD9 expression in the glandular epithelium of fertile secretion group increased compared to the fertile proliferation phase significantly, however the increase of CD9 immunoreactivity in the infertile secretion phase compared to both fertile groups and infertile proliferation phase group is higher. CD9 immunoreactivity was increased in the infertile secretion phase compared to the fertile secretion phase and this increase was statistically significant. The distribution of CD9 in both luminal and glandular epithelium of infertile secretion phase was also found to be higher than the infertile proliferation phase and it was statistically significant.

Expression of CD9 in the fertile secretion phase supports the presence of exosomes for endometrial regulation before implantation in both luminal and glandular epithelium. In contrast, in the infertile secretion phase, the CD9 increase in both luminal and glandular epithelium significantly increased, suggesting that the exosomal traffics were greater in both epithelium of infertile endometrium may be cause of unexplained infertility.

Iwai et al. [29] showed that changes in CD9 localization due to cellular activity in mice and human uterine secretions and stated that these changes may be related to infertility. Chaudhari-Kank MS et al. [30] showed that expression of CD9 had decreased in endometrial stromal cells of infertile women. They also stated that this decrease in CD9 expression could cause to infertility by impairing implantation. In the study of Chaudhari-Kank MS et al. [30], all cases were only in the secretory period. In our study, proliferation and secretion periods of infertile cases were evaluated and CD9 in stroma, on this subject in both phases expression was observed similar to the results of Chaudhari-Kank MS et al. study which is one of the most recent studies.

According to our study's results, we haven't thought that CD63 could be used as a biomarker in infertility. However, due to the significant increase of CD9 in the secretory phase of infertile group, it may be possible to use CD9 to assess infertility related issues or as a biomarker in terms of endometrium.

CONCLUSIONS

Based on the findings of this study increased CD9 reactivity in infertile secretion phase endometrium could be used as a biomarker which could be an unfavorable signaling of infertility or increased expression for recovery. In addition the increase of CD63 in the infertile proliferation group suggested that exosomal expression in the endometrial epithelium before implantation was changed and may have negative effects.

It can be stated that endometrial differences of exosomes may affect implantation and the results of our study may shed light on future studies. To predict the use of exosomes in infertility, particularly CD9 as a biomarker, further studies are needed to be done.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

1. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015; 8(4): 191–196, doi: 10.4103/0974-1208.170370, indexed in Pubmed: 26752853.
2. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology, World Health Organization, International Committee for Monitoring Assisted Reproductive Technology, World Health Organization. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod. 2009; 24(11): 2683–2687, doi: 10.1093/humrep/dep343, indexed in Pubmed: 19801627.
3. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017; 108(3): 393–406, doi: 10.1016/j.fertnstert.2017.06.005, indexed in Pubmed: 28760517.
4. Cakmak H, Taylor HS, Cakmak H, et al. Molecular mechanisms of treatment resistance in endometriosis: the role of progesterone-hox gene interactions. Semin Reprod Med. 2010; 28(1): 69–74, doi: 10.1055/s-0029-1242996, indexed in Pubmed: 20104430.
5. Salamonsen LA, Edgell T, Rombauts LJF, et al. Proteomics of the human endometrium and uterine fluid: a pathway to biomarker discovery. Fertil Steril. 2013; 99(4): 1086–1092, doi: 10.1016/j.fertnstert.2012.09.013, indexed in Pubmed: 23043689.
6. Sharkey AM, Smith SK. The endometrium as a cause of implantation failure. Best Pract Res Clin Obstet Gynaecol. 2003; 17(2): 289–307, doi: 10.1016/s1521-6934(02)00130-x, indexed in Pubmed: 12758101.

7. Ozkan ZS, Deveci D, Kumbak B, et al. What is the impact of Th1/Th2 ratio, SOCS3, IL17, and IL35 levels in unexplained infertility? J Reprod Immunol. 2014; 103: 53–58, doi: 10.1016/j.jri.2013.11.002, indexed in Pubmed: 24368037.
8. Dear JW, Street JM, Bailey MA. Urinary exosomes: a reservoir for biomarker discovery and potential mediators of intrarenal signalling. Proteomics. 2013; 13(10-11): 1572–1580, doi: 10.1002/pmic.201200285, indexed in Pubmed: 23129434.
9. Stahl PD, Raposo G. Extracellular Vesicles: Exosomes and Microvesicles, Integrators of Homeostasis. Physiology (Bethesda). 2019; 34(3): 169–177, doi: 10.1152/physiol.00045.2018, indexed in Pubmed: 30968753.
10. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013; 200(4): 373–383, doi: 10.1083/jcb.201211138, indexed in Pubmed: 23420871.
11. Abels ER, Breakefield XO. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol. 2016; 36(3): 301–312, doi: 10.1007/s10571-016-0366-z, indexed in Pubmed: 27053351.
12. Rosenbluth EM, Shelton DN, Wells LM, et al. Human embryos secrete microRNAs into culture media--a potential biomarker for implantation. Fertil Steril. 2014; 101(5): 1493–1500, doi: 10.1016/j.fertnstert.2014.01.058, indexed in Pubmed: 24786747.
13. Tannetta D, Dragovic R, Alyahyaei Z, et al. Extracellular vesicles and reproduction-promotion of successful pregnancy. Cell Mol Immunol. 2014; 11(6): 548–563, doi: 10.1038/cmi.2014.42, indexed in Pubmed: 24954226.
14. Ng YH, Rome S, Jalabert A, et al. Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for embryo-endometrial cross talk at implantation. PLoS One. 2013; 8(3): e58502, doi: 10.1371/journal.pone.0058502, indexed in Pubmed: 23516492.
15. Noyes RW, Hertig AT, Rock J, et al. Dating the endometrial biopsy. Am J Obstet Gynecol. 1975; 122(2): 262–263, doi: 10.1016/s0002-9378(16)33500-1, indexed in Pubmed: 1155504.
16. Thike AA, Chng MJ, Fook-Chong S, et al. Immunohistochemical expression of hormone receptors in invasive breast carcinoma: correlation of results of H-score with pathological parameters. Pathology. 2001; 33(1): 21–25, indexed in Pubmed: 11280603.
17. Ishibashi H, Suzuki T, Suzuki S, et al. Sex steroid hormone receptors in human thymoma. J Clin Endocrinol Metab. 2003; 88(5): 2309–2317, doi: 10.1210/jc.2002-021353, indexed in Pubmed: 12727990.
18. Hirsch FR, Varella-Garcia M, Bunn PA, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol. 2003; 21(20): 3798–3807, doi: 10.1200/JCO.2003.11.069, indexed in Pubmed: 12953099.
19. John T, Liu G, Tsao MS. Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. Oncogene. 2009; 28 Suppl 1: S14–S23, doi: 10.1038/onc.2009.197, indexed in Pubmed: 19680292.
20. Altmäe S, Koel M, Võsa U, et al. Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers. Sci Rep. 2017; 7(1): 10077, doi: 10.1038/s41598-017-10098-3, indexed in Pubmed: 28855728.
21. Vilella F, Moreno-Moya JM, Balaguer N, et al. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. Development. 2015; 142(18): 3210–3221, doi: 10.1242/dev.124289, indexed in Pubmed: 26395145.
22. Machtinger R, Laurent LC, Baccarelli AA. Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. Hum Reprod Update. 2016; 22(2): 182–193, doi: 10.1093/humupd/dmv055, indexed in Pubmed: 26663221.
23. Saadeldin IM, Oh HJu, Lee BC. Embryonic-maternal cross-talk via exosomes: potential implications. Stem Cells Cloning. 2015; 8: 103–107, doi: 10.2147/SCCAA.S84991, indexed in Pubmed: 26185458.
24. Evans J, Salamonsen LA, Winship A, et al. Fertile ground: human endometrial programming and lessons in health and disease. Nat Rev Endocrinol. 2016; 12(11): 654–667, doi: 10.1038/nrendo.2016.116, indexed in Pubmed: 27448058.
25. Burns G, Brooks K, Wildung M, et al. Extracellular vesicles in luminal fluid of the ovine uterus. PLoS One. 2014; 9(3): e90913, doi: 10.1371/journal.pone.0090913, indexed in Pubmed: 24614226.
26. Burnett LA, Nowak RA. Exosomes mediate embryo and maternal interactions at implantation and during pregnancy. Front Biosci (Schol Ed). 2016; 8: 79–96, doi: 10.2741/s448, indexed in Pubmed: 26709898.
27. Kropp J, Salih SM, Khatib H. Expression of microRNAs in bovine and human pre-implantation embryo culture media. Front Genet. 2014; 5: 91, doi: 10.3389/fgene.2014.00091, indexed in Pubmed: 24795753.
28. Baig S, Lim JY, Fernandis AZ, et al. Lipidomic analysis of human placental syncytiotrophoblast microvesicles in adverse pregnancy outcomes. Placenta. 2013; 34(5): 436–442, doi: 10.1016/j.placenta.2013.02.004, indexed in Pubmed: 23465879.
29. Iwai M, Hamatani T, Nakamura A, et al. Membrane protein CD9 is repositioned and released to enhance uterine function. Lab Invest. 2019; 99(2): 200–209, doi: 10.1038/s41374-018-0145-1, indexed in Pubmed: 30401958.
30. Chaudhari-Kank M, Zaveri K, Antia V, et al. Comparison of CD9 & CD146 markers in endometrial stromal cells of fertile & infertile females. Indian Journal of Medical Research. 2018; 147(6): 552, doi: 10.4103/ijmr.ijmr_1186_16.

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Folate receptor-mediated cervical staining as an adjunct to colposcopy which can improve the diagnostic accuracy of detecting high grade squamous intraepithelial lesions

Wojciech Homola, Michal Pomorski, Aleksandra Zimmer, Pawel Baranski, Mariusz Zimmer

2nd Department and Clinic of Gynaecology, Obstetrics and Neonatology, Wroclaw Medical University, Wroclaw, Poland

ABSTRACT

Objectives: Cervical cancer is rated fourth in terms of incidence and cancer-related mortality in women. Cytology-based screening programs and colposcopy provided insufficient rates of detecting cervical intraepithelial neoplasia (CIN) prompting researchers to develop new tools. The aim of this study was to evaluate whether folate receptor-mediated staining is useful in detecting CIN2+ during gynecological examination with colposcopy.

Material and methods: In total 96 women with abnormal cytology findings were enrolled. The study was conducted on the Polish population. The diagnostic process consisted of colposcopy, receptor-mediated diagnosis (FRD), and histopathology examination. All women were subjected to the same diagnostic procedure.

Results: The patient mean age of 96 women was 38 ± 14.5 years. On colposcopy, high-grade lesions were detected in 83 women. The FRD gave positive results in 63 women. Histopathology revealed 1 case of carcinoma plano epithelial akeratodes, 21 cases of high-grade squamous intraepithelial lesions, 13 cases of low-grade squamous intraepithelial lesions. A total of 61 cases presented no pathology. FRD as an adjunct to colposcopy gave the following test results in detecting CIN2+ lesions: sensitivity — 94.29%, specificity — 46.67%, PPV — 50.77%, NPV — 93.33%, and accuracy — 64.21%. Using both techniques provided better results than using each of the tests alone.

Conclusions: FRD is a promising test for the diagnosing CIN2+ cervical pathologies because it can increase the probability of detecting CIN2+ without any additional burden posed on patients. Further studies should be conducted on large and various populations to complement current evidence.

Key words: cervical neoplasia; cervical cancer; folate receptor-mediated cervical staining; FRD

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INTRODUCTION

Cervical cancer is a considerable problem for women's health. The estimated number of new cancer cases reached 569,800, and the estimated number of deaths was 311,400 in 2018 worldwide, which placed cervical cancer at the fourth position in terms of incidence and cancer-related mortality in women [1]. Increasing awareness and introduction of screening programs allowed to decrease morbidity and mortality rates due to cervical cancer [2]. In high-income countries, indices for cervical cancer are much lower than in low-income countries. For this reason, cervical screening is one out of three elements of the WHO global strategy towards eliminating cervical cancer worldwide as a public health problem [3].

Currently, many screening programs are based on cytology [4]. However, this method is considered to be of insufficient and differentiated accuracy. An 11-year retrospective analysis of 999 cases published by Kang et al. reported a sensitivity of 97.14% and specificity of 85.58% for detecting high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) [5]. But the study by Wojciech et al. [6] on patients with histologically confirmed cervical intraepithelial neoplasia (CIN) showed a sensitivity of 58.02% and specificity of 63.28% in detecting CIN. For this reason, other noninvasive methods such as based on electrical impedance spectroscopy, folic acid receptor-mediated diagnosis (FRD) method, or those employing arti-

Corresponding author:
Wojciech Homola
2nd Department and Clinic of Gynaecology, Obstetrics and Neonatology, Wroclaw Medical University, Wroclaw, Poland
e-mail: wojtek.homola@gmail.com

ficial intelligence are of great interest [7–9]. The method based on FRD was developed after it had been discovered that the expression of the folate receptor α is increased not only in certain cells but also in many epithelial cancers [10]. Despite the FRD staining being a new method, the evidence is growing rapidly. Preliminary results show that this method is distinguished by simplicity, rapid provision of results, and effectiveness. Therefore, we undertook this study to add evidence on FRD. The aim of this study was to evaluate whether folate receptor-mediated staining is useful in detecting CIN2+ during gynecological examination with colposcopy.

MATERIAL AND METHODS

Women with abnormal cytology findings were enrolled in this study. All women were referred to our institution — an outpatient colposcopy clinic in a tertiary gynecology and obstetrics department. The study was conducted in the Department of Gynecology and Obstetrics, Wrocław Medical University, Wroclaw, Poland. Women who suffered from cancer of the cervix, had a vaginal bleeding or active menstruation, had used vaginal contraceptives and vaginal medications up to 2 days before the enrollment were not included. All women gave the written informed consent for participation in the study and taking part in the diagnostic process to confirm or exclude CIN2+. The study was approved by the Commission of Bioethics at Wroclaw Medical University.

The diagnostic process consisted of colposcopy, FRD, and histopathology examination. Two experienced gynecologists were responsible for gynecology examinations and collection of specimens and data. One histopathologist with experience in the assessment of cervical pathology reviewed and interpreted biopsies. All study participants underwent the same diagnostic process.

For the conduct of colposcopy, the Videocolposcope HD-1000 with the IRIS software (Medicom, Wroclaw, Poland) was used. Images of the cervix were video recorded, both before and after the application of 3% acetic acid. FRD staining was done before visual inspection with acetic acid (VIA). For staining, the folate receptor-mediated staining solution (Shaanxi Gaoyuan Medical Equipment Service Co., Ltd., Shaanxi, China) was used. A single-use foam applicator soaked in the FRD solution was placed on the cervix for 30 seconds and next, in the FRD colour changes on the applicator were assessed in comparison with FRD colour changes sheet. Results were classified as CIN2+ negative: for brown or green colors, while CIN2+ or worse for blue, dark blue or black. At each examination, 2–3 targeted biopsies from the most suspected areas were taken. When there were no FRD induced colors changes, random biopsies were taken.

In the overall assessment, the case was considered to be negative for High-Grade Squamous Intraepithelial Lesions (HG-SIL) when the result of colposcopy was normal without any acetowhitening changes or any worse abnormalities; the FRD staining was brown or green, and histopathology samples were negative for HG-SIL (below CIN II).

Collected data were statistically analyzed. Categorical variables were presented as numbers and percentages, while continuous data were presented as means with a standard deviation. Results between the two diagnostic methods (classical colposcopy and colposcopy + FRD staining) were compared with the Fisher's exact test. To evaluate diagnostic tests, the following results were calculated: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy.

RESULTS

Overall, 96 women were eligible for the study. The patient population mean age was 38 years old with an SD of 14.5 and range from 24 to 86 years. All study participants had abnormal cytology results which are summarized in Table 1. On colposcopy, high-grade lesions were detected in 83 women. The FRD gave positive results in 63 women. Table 2 shows the results of the histopathological examination.

Positive and negative results of colposcopy, FRD and histopathology (Tab. 3) were used to calculate sensitivity, PPV, NPV, and accuracy (Tab. 4).

Table 1. Cytology results of referred women (n = 96)

	Cytology diagnosis	No of patients
1.	Squamous cell carcinoma (SCC)	1
2.	Atypical squamous cells cannot exclude HSIL (ASC-H)	14
3.	AGC-US	6
4.	Atypical squamous cells of undetermined significance (ASC-US)	18
5.	High-grade squamous intraepithelial lesion (HSIL)	15
6.	AIS	1
7.	Low-grade squamous intraepithelial lesions (LSIL)	36
8.	Negative for intraepithelial lesion or malignancy (NILM)	5

Table 2. Histopathological results of the study group (n = 96)

Lp.	Histological diagnosis	No of patients
1.	Carcinoma plano epithelial akeratodes (G1)	1
2.	High-grade squamous intraepithelial lesion (HSIL)	21
4.	Low-grade squamous intraepithelial lesion (LSIL)	13
5.	Normal	61

Table 3. Test calculations

Results of the test		Histopathology results	
		Positive	Negative
Colposcopy	Positive	n = 13	n = 0
	Negative	n = 22	n = 61
FRD	Positive	n = 30	n = 33
	Negative	n = 5	n = 28
Colposcopy + FRD	Positive	n = 33	n = 32
	Negative	n = 3	n = 28

Table 4. Results of test evaluation

	Colposcopy	FRD	Colposcopy + FRD
Sensitivity	37.14% (95% CI: 21.47–55.08)	85.71% (95% CI: 69.74–95.19)	94.29% (95% CI: 80.84%- 99.30)
Specificity	100.00% (95% CI: 94.13–100.00)	45.90% (95% CI: 33.06–59.15)	46.67% (95% CI: 33.67–60.00)
Positive Predictive Value	100.00%	47.62% (95% CI: 41.02–54.30)	50.77% (95% CI: 44.53–56.98)
Negative Predictive Value	73.49% (95% CI: 68.25–78.15)	84.85% (95% CI: 70.41–92.95)	93.33% (95% CI: 78.01–98.22)
Accuracy	77.08% (95% CI: 67.39–85.05)	60.42% (95% CI: 49.92–70.25)	64.21% (95% CI: 53.72–73.79)

CI — confidence interval

DISCUSSION

Our study showed that colposcopies with an adjunct FRD have a sensitivity of 94.29%, specificity of 46.67%, PPV of 50.77% and NPV of 93.33%. Using both techniques leads to posing less destress on patients that would have a false-positive result with a colposcopy alone and limiting further unnecessary testing. Adding FRD to colposcopies elevates sensitivity of the examination from 37.14% to 94.29% increasing the probability that a test result will be positive when the disease is present. Our results may differ from those obtained in other studies because the study group consisted of women with abnormal cytology result referred to a tertiary diagnostic center which specializes in diagnosis and treatment of more complex conditions.

FRD is a novel technique employed in oncology. It is based on the discovery that the folate receptor (FR) expression is highly elevated in certain cells, including a variety of cancers [11]. Parker at al. [12] found positive expression of FR in certain types of ovarian carcinomas as well as in kidney, endometrium, lung, breast, bladder, and pancreatic cancers. Also, normal human tissues showed to have variability in FR expression. Normal human ovaries had a negligible expression of FR while this expression was high in normal human lung tissue. Liu et al. [13] investigated the expression of FRα and the role of FRα in the regulation of the ERK signaling pathway. They found that FRα expression was progressively increasing along with the progression of cervical lesions. In squamous cell carcinoma of the cervix, expression of proteins of ERK signaling pathway correlated with the expression of FRα. They concluded that expression of FRα is associated with the progression of cervical cancer and can regulate cervical cancer cells growth.

Since the discovery of the link between FR and cervical cancer, evidence on the potential role of FR in the detection of cervical metaplasia is growing rapidly. Several studies conducted in the real clinical practice settings were published recently. Lu et al. [14] conducted a study on 169 women and compared the results of FRD and cytology testing. They reported a sensitivity of 71.93%, specificity of 66.07%, PPV of 51.90%, and NPV of 82.22% of FRD in the diagnosis of cervical cancer and considered this result to be comparable to cytology. Li et al. [15] conducted the largest study up until now. They recruited 14,344 women from rural areas of China. In detecting CIN2+, FRD showed a sensitivity of 85.7%, specificity of 76.4%, PPV of 61.3%, and NPV of 92.5%. Authors concluded that FRD had a moderate agreement with cytology in detecting atypical squamous cells, was unable to exclude high-grade intraepithelial lesions, but was more sensitive than cytology. Xiao et al. [16] examined 404 women using FRD to screen them for high-grade cervical lesions. They found that the sensitivity of FRD in detecting CIN2+ was 80.00%, specificity was 51.92%, PPV was 24.19% and NPV 93.12%. Dai et al. [17] included 216 women and subjected to FRD, human papillomavirus testing and ThinPrep cytology test. They reported the following test results for FRD: sensitivity — 80.41%, specificity — 68.29%, PPV — 60%, NPV — 85.5%. They concluded that FRD had significantly higher specificity than HPV testing and TCT, but no differences were noted in specificity. The recent study of Zhao et al. [18] recruited 1,504 patients with abnormal cytology and/or positive human papillomavirus (HPV) testing at primary screening. In this study, the sensitivity of FRD was 77.72% and specificity of FRD was 60.02%. It is worth noting that the rate of detection of pathological lesions increased with the greater severity of the disease. FRD detected 45.45% of CIN1, 66.93% of CIN2, 84.44% of CIN3, and 98% of carcinomas.

The results of our study are in line with the above-discussed reports from the literature. The advantage of our study lies in the evaluation of the benefit of FRD as an added value to the standard of care diagnosis in our institution, while most studies focus on presenting results of FRD alone in comparison to other diagnostic methods. Nevertheless, further studies should be conducted to further investigate the usefulness of FRD in detecting CIN2+ in clinical practice

settings. FRD is a simple technique with a rapid result which should be considered when adding this examination to the current standard of care.

CONCLUSIONS

FRD is a promising test for the diagnosing CIN2+ cervical pathologies because it can increase the probability of detecting CIN2+ without any additional burden posed on patients. Further studies should be conducted on large and various populations to complement current evidence.

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019; 144(8): 1941–1953, doi: 10.1002/ijc.31937, indexed in Pubmed: 30350310.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394–424, doi: 10.3322/caac.21492, indexed in Pubmed: 30207593.
3. Canfell K, Kim JJ, Brisson M, et al. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet. 2020; 395(10224): 591–603, doi: 10.1016/S0140-6736(20)30157-4, indexed in Pubmed: 32007142.
4. Lees BF, Erickson BK, Huh WK. Cervical cancer screening: evidence behind the guidelines. Am J Obstet Gynecol. 2016; 214(4): 438–443, doi: 10.1016/j.ajog.2015.10.147, indexed in Pubmed: 26519782.
5. Kang M, Ha SY, Cho HY, et al. Comparison of papanicolaou smear and human papillomavirus (HPV) test as cervical screening tools: can we rely on HPV test alone as a screening method? An 11-year retrospective experience at a single institution. J Pathol Transl Med. 2020; 54(1): 112–118, doi: 10.4132/jptm.2019.11.29, indexed in Pubmed: 31964113.
6. Wojciech R. [The diagnostic value of cytology and colposcopy in women with cervical intraepithelial neoplasia]. Ginekol Pol. 2011; 82(8): 607–611, indexed in Pubmed: 21957606.
7. Homola W, Fuchs T, Baranski P, et al. Use of electrical impedance spectroscopy as an adjunct to colposcopy in a pathway of cervical intraepithelial neoplasia diagnostics. Ginekol Pol. 2019; 90(11): 628–632, doi: 10.5603/GP.2019.0107, indexed in Pubmed: 31802462.
8. Hu L, Bell D, Antani S, et al. An Observational Study of Deep Learning and Automated Evaluation of Cervical Images for Cancer Screening. J Natl Cancer Inst. 2019; 111(9): 923–932, doi: 10.1093/jnci/djy225, indexed in Pubmed: 30629194.
9. Li K, Cai H, Shen H. Application of FRD Epithelial Tissue Special Staining Solution in Screening of Cervical Lesions. Journal of Guangxi Agricultural and Biological Science. 2016; 7: 1584–1588.
10. Lewis CM, Smith AK, Kamen BA. Receptor-mediated folate uptake is positively regulated by disruption of the actin cytoskeleton. Cancer Res. 1998; 58(14): 2952–2956, indexed in Pubmed: 9679952.
11. Carron PMc, Crowley A, O'Shea D, et al. Targeting the Folate Receptor: Improving Efficacy in Inorganic Medicinal Chemistry. Curr Med Chem. 2018; 25(23): 2675–2708, doi: 10.2174/0929867325666180209143715, indexed in Pubmed: 29424300.
12. Parker N, Turk MJo, Westrick E, et al. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. Anal Biochem. 2005; 338(2): 284–293, doi: 10.1016/j.ab.2004.12.026, indexed in Pubmed: 15745749.
13. Liu C, Ding L, Bai L, et al. Folate receptor alpha is associated with cervical carcinogenesis and regulates cervical cancer cells growth by activating ERK1/2/c-Fos/c-Jun. Biochem Biophys Res Commun. 2017; 491(4): 1083–1091, doi: 10.1016/j.bbrc.2017.08.015, indexed in Pubmed: 28782518.
14. Lu MH, Hu LY, Du XX, et al. An special epithelial staining agents: folic acid receptor-mediated diagnosis (FRD) effectively and conveniently screen patients with cervical cancer. Int J Clin Exp Med. 2015; 8(5): 7830–7836, indexed in Pubmed: 26221336.
15. Li D, Chen L, Wang H, et al. Clinical application of a rapid cervical cancer screening method: Folate receptor-mediated staining of cervical neoplastic epithelia. Asia Pac J Clin Oncol. 2017; 13(1): 44–52, doi: 10.1111/ajco.12573, indexed in Pubmed: 27739250.
16. Xiao S, Xie H, Zhu X, et al. Study on the Significance of Folate Receptor-Mediated Staining Solution (FRD) Staining in Screening High Grade Cervical Lesions. Med Sci Monit. 2019; 25: 2792–2801, doi: 10.12659/MSM.911402, indexed in Pubmed: 30990212.
17. Dai Y, Wang L, Li D. Effectiveness of novel folate receptor-mediated staining solution detection (FRD) for cervical cancer screening. Medicine (Baltimore). 2018; 97(34): e11868, doi: 10.1097/MD.0000000000011868, indexed in Pubmed: 30142780.
18. Zhao Y, Li M, Li Y, et al. Evaluation of Folate Receptor-Mediated Cervical Dyeing as a Method for Detection of Cervical Lesions. J Low Genit Tract Dis. 2019; 23(2): 133–137, doi: 10.1097/LGT.0000000000000411, indexed in Pubmed: 30817686.

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Circulating vaspin levels and nutritional status and insulin resistance in polycystic ovary syndrome

Grzegorz Franik[1], Ryszard Plinta[2], Pawel Madej[1], Aleksander Owczarek[3], Maria Bozentowicz-Wikarek[4], Jerzy Chudek[4,5], Violetta Skrzypulec-Plinta[6], Magdalena Olszanecka-Glinianowicz[7]

[1]Department of Gynecological Endocrinology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Poland
[2]Chair of Physiotherapy, School of Health Science in Katowice, Medical University of Silesia, Poland
[3]Department of Statistics, Department of Instrumental Analysis, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Poland
[4]Pathophysiology Unit, Department of Pathophysiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Poland
[5]Department of Internal Medicine and Oncological Chemotherapy, School of Medicine in Katowice, Medical University of Silesia, Poland
[6]Women's Health Chair, School of Health Science in Katowice, Medical University of Silesia, Poland
[7]Health Promotion and Obesity Management Unit, Department of Pathophysiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Poland

ABSTRACT

Objectives: The study aimed to assess the associations between circulating vaspin levels and nutritional status (assessed on tha basis of BMI) as well as insulin resistance in PCOS.

Material and methods: Eighty-seven PCOS women, 48 obese and 39 normal weight, were enrolled in the cross-sectional study. Seventy-two Non-PCOS women, 41 obese and 31 normal weight, constituted a control group. Body mass, height and waist circumference as well as body composition by bioimpedance were measured. In the morning (16h after the last meal) we determined: serum glucose, insulin, androgens, gonadotropin (LH, FSH) and sex hormone-binding globulin (SHBG) as well as plasma vaspin levels. Standard HOMA-IR formula was used to assess insulin resistance (IR).

Results: Plasma vaspin levels were significantly lower in PCOS, both normal weight and obese, than in Non-PCOS groups. Vaspin levels were similar in normal weight and obese PCOS subgroups. There was no association between plasma vaspin levels and anthropometric parameters in PCOS group. While in Non-PCOS group a negative correlation between plasma vaspin levels and body mass ($r = -0.26$; $p < 0.05$) was found. We did not observe correlations between plasma vaspin levels and serum glucose and insulin concentrations as well as HOMA-IR values, however, in multivariable, stepwise backward regression waist circumference and HOMA-IR values explained 18.0% of plasma vaspin levels variability in the study subjects.

Conclusions: PCOS occurrence is associated with decreased vaspin levels. The influence of nutritional status on vaspin level observed in Non-PCOS is abolished in PCOS women, possibly by more severe insulin resistance.

Key words: vaspin; insulin resistance; nutritional status; PCOS

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INTRODUCTION

Vaspin is an adipokine, the member of the serine protease inhibitors family (serpin) [1]. Vaspin mRNA expression was found in subcutaneous and visceral adipose tissue, liver, pancreas, stomach and skin [2–5].

The experimental studies have shown that vaspin increased insulin sensitivity and glucose tolerance as well as decreased food intake [4, 6]. Expression of vaspin mRNA in rats visceral adipose tissue increased with an excess of body mass and insulin resistance [1]. In addition, factors

Corresponding author:
Magdalena Olszanecka-Glinianowicz
Health Promotion and Obesity Management Unit, Department of Pathophysiology, Medical University of Silesia, 18 Medyków St, 40–752 Katowice, Poland
e-mail: magols@esculap.pl

stimulating vaspin expression were leptin and metformin and inhibiting night break in food intake [7].

In the human expression of vaspin mRNA in visceral adipose tissue was proportional to BMI value and body fat percentage. In turn, this expression in subcutaneous adipose tissue was proportional to WHR values and fasting serum insulin levels [2]. The association between plasma vaspin levels and BMI values and insulin resistance was also shown in another study [8]. Plasma vaspin levels were higher in women than in men [8]. It is suggested that this sex difference develops during puberty [3]. However, in subjects with metabolic and cardiovascular disturbances the association between circulating vaspin levels and BMI values, sex and age were not observed [9, 10].

It has been suggested that increase vaspin mRNA expression and its secretion are a compensatory mechanism that delays insulin resistance development [2, 8, 11]. However, the results of other studies did not confirm this hypothesis [10–13].

Several projects assessing vaspin levels in PCOS showed inconclusive results. Higher vaspin levels were observed in normal-weight PCOS than in Non-PCOS women [14–16]. On the other hand, there were no differences between overweight/obese PCOS and Non-PCOS women [16]. In addition, serum testosterone levels, FAI values and mean ovary volume or the number of follicles were proportional to vaspin levels. While serum FSH and SHBG levels, as well as insulin sensitivity, were inversely related to vaspin levels [16]. The highest plasma vaspin levels were shown in the phenotype A, thus this parameter is considered as the marker of the severity of PCOS [17]. The lack of differences between plasma vaspin levels in adolescents with or without PCOS has also been shown [18]. Similarly, vaspin levels did not differ between normal-weight PCOS and Non-PCOS women in Iranian population, despite higher insulin levels in PCOS group [19]. In turn, treatment with metformin and improvement of the insulin sensitivity caused a decrease in vaspin levels [14]. However, Koiou et al. [16] have shown only a slight effect of treatment with metformin on vaspin levels in normal-weight PCOS women and lack of the impact of moderate weight loss on vaspin levels in overweight and obese PCOS women.

The study aimed to assess the associations between circulating vaspin levels and nutritional status (assessed on the basis of BMI) as well as insulin resistance in PCOS.

MATERIAL AND METHODS

Eighty-seven PCOS (diagnosed on the basis of Rotterdam ESHRE/ASRM criteria [20]) women, 48 obese and 39 normal-weight, were enrolled in the cross-sectional study. Seventy-two Non-PCOS women, 41 obese and 31 normal weight, constituted the control group. The exclusion criteria included any pharmacotherapy, alcohol and nicotine addiction and changes of body mass over 2 kg during the last 3-months. The written informed consent was obtained from all subjects. The Bioethical Committee of the Medical University of Silesia approved the study protocol.

Nutritional status was diagnosed on the basis of BMI values in accordance with World Health Organization criteria. Table 1 presents the characteristics of the study and control groups.

The venous blood samples (15 mL) for laboratory tests were collected in the morning 16 hours after the last meal

Table 1. Patients characteristics'

	PCOS			non-PCOS		
	All (n = 87)	**Normal weight (n = 39)**	**Obese (n = 48)**	**All (n = 72)**	**Normal weight (n = 31)**	**Obese (n = 41)**
Age [years]	25.4 ± 5.5	23.7 ± 4.5^{++}	26.8 ± 5.8	26.4 ± 5.5	23.8 ± 4.3$^{\$\$\$}$	28.4 ± 5.6
Body mass [kg]	79.4 ± 26.4	56.9 ± 11.7^{***+++}	97.7 ± 20.2$^{\&\&\&}$	78.7 ± 20.4	59.8 ± 7.1$^{\$\$\$}$	93.1 ± 14.6
BMI [kg/m^2]	28.6 (20.8–35.7)	20.6^{***+++} (19.6–22.7)	35.1$^{\&\&\&}$ (31.3–40.2)	28.5 (22.9–33.5)	22.4$^{\$\$\$}$ (21.0–24.0)	32.9 (30.3–36.7)
Body fat [kg]	30.2 (15.4–42.6)	15.0^{**++} (12.6–19.7)	40.6$^{\&\&}$ (33.4–56.3)	33.3 (19.1–50.4)	18.1$^{\$\$}$ (14.8–20.6)	49.4 (37.5–50.2)
Body fat [%]	38.1 (27.5–45.7)	26.5^{***+++} (24.2–31.0)	44.8$^{\&\&\&}$ (41.9–51.1)	40.6 (30.4–48.5)	30.0$^{\$\$\$}$ (26.8–33.9)	46.8 (42.3–51.4)
Waist circumference [cm]	89.8 ± 18.7	72.6 ± 7.3^{***+++}	103.7 ± 12.3$^{\&\&\&}$	87.9 ± 18.2	70.5 ± 8.3$^{\$\$\$}$	101.0 ± 11.3
Total cholesterol [mg/dL]	176.3 ± 34.0	167.7 ± 28.1*	183.2 ± 37.0	174.6 ± 30.6	169.1 ± 33.3	178.8 ± 27.5
LDL- cholesterol[mg/dL]	105.4 ± 38.3	93.8 ± 27.5**	115.1 ± 37.6	100.3 ± 27.1	90.3 ± 31.9	106.4 ± 21.9
HDL- cholesterol [mg/dL]	45.7 ± 14.1$^{\%\%\%}$	48.1 ± 15.1$^{*\#\#}$	43.8 ± 12.9$^{\&\&\&\wedge\wedge\wedge}$	57.1 ± 15.2	60.1 ± 16.3$^{\$}$	54.8 ± 14.2
Triglycerides [mg/dL]	100.7 ± 55.2	73.0 ± 31.7$^{**\#\#}$	121.5 ± 61.4$^{\&\&\wedge\wedge}$	80.1 ± 32.1	67.2 ± 26.7$^{\$\$}$	89.9 ± 32.6
Glucose [mmol/L]	5.1 ± 0.8$^{\%\%\%}$	4.9 ± 0.7$^{\#\#}$	5.3 ± 0.9$^{\&\&\wedge\wedge}$	4.7 ± 0.4	4.7 ± 0.5	4.7 ± 0.4
Insulin [µIU/mL]	10.6$^{\%\%}$ (7.8–15.1)	8.4** (6.0–10.6)	12.9$^{\&\&\wedge\wedge}$ (9.7–18.6)	7.4 (5.9–9.5)	6.8 (5.6–8.7)	7.8 (6.3–10.0)
HOMA-IR	2.3$^{\%\%}$ (1.6–3.2)	1.8** (1.2–2.3)	2.8$^{\&\&\wedge\wedge}$ (1.2–4.1)	1.5 (1.2–2.0)	1.5 (1.1–1.9)	1.7 (1.4–2.2)

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ normal weight PCOS vs obese PCOS; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ normal weight PCOS vs normal weight non-PCOS; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$ normal weight PCOS vs obese non-PCOS; $^{\&}p < 0.05$; $^{\&\&}p < 0.01$; $^{\&\&\&}p < 0.001$ obese PCOS vs normal weight non-PCOS; $^{\wedge}p < 0.05$; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ obese PCOS vs obese non-PCOS; $^{\$}p < 0.05$; $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$ normal weight non- PCOS vs obese non-PCOS; $^{\%}p < 0.05$; $^{\%\%}p < 0.01$; $^{\%\%\%}p < 0.001$ all PCOS vs all non-PCOS

between 3–5 days of the menstrual cycle. Height, body mass and waist circumference were measured. BMI was calculated. The bioimpedance method (Bodystat 1500, Douglas, Isle of Man) was used to the assessment of body composition.

Serum and plasma samples were stored frozen in –70°C.

Laboratory procedures

Calorimetric methods (kits made by Roche, Switzerland) were used to determine serum glucose and lipids. Fasting serum insulin levels were measured by enzyme-linked immunosorbent assay — ELISA (DRG Instruments GmbH, Marburg, Germany) with a lower limit of detection of 1.76 μIU/mL. The insulin resistance was assessed on the basis of HOMA-IR = fasting concentration of insulin (μIU/mL) × fasting concentration of glucose (mmol/L)/22.5.

The ELISA (DRG Instruments GmbH, Marburg, Germany) method was also used to determine concentrations of gonadotropin (FSH, LH), prolactin (PRL), estradiol (E_2), androgens (testosterone, free testosterone, androstenedione, DHEA-S) as well as plasma vaspin levels (BioVendor, Brno, The Czech Republic) with the lower limit of detection of 0.01 ng/mL and intra-assay coefficient variation 7.6% and inter-assay coefficients variation 7.65%.

In addition, with the standard formula, the free androgen index (FAI) was calculated.

Statistic analysis

STATISTICA 9.0 PL (StatSoft Poland) software and R software environment were used for statistical analysis. There was no missing data in the database. The mean values ± standard deviation and median with upper and lower quartiles were used for the presentation of the results. The D'Agostino-Pearson test was used to assess the distribution of variables. The Levene test was used to assess the homogeneity of variances. Two-way multivariable analysis of variances with Duncan *post-hoc* test was used for comparison of quantitative variables. The multivariable linear regression with the backward stepwise procedure was used to assess the associations between variables. Cook's distance values were used for identification of outliers. Testing the residuals for heteroskedasticity was performed using the Cook-Weisberg test. Models calculation was performed including evaluation of multicollinearity, which was assessed with the variance inflation factor (VIF below 5). Additionally, how well it fit in the obtained model was also assessed with the F test and determination coefficient R^2. Values below 0.05 were considered statistically significant.

RESULTS

Body mass and BMI values were similar in the corresponding PCOS and Non-PCOS subgroups. Significantly higher glucose and insulin levels as well as HOMA-IR and FAI values and lower HDL cholesterol and SHBG levels were found in PCOS in comparison to Non-PCOS group and obese than normal-weight PCOS subgroups (Tab. 1 and 2).

Plasma vaspin levels were significantly lower in PCOS than in Non-PCOS group. The lower plasma vaspin levels were shown in both PCOS and in the corresponding Non-PCOS subgroups. Similar plasma vaspin levels were found in normal weight and obese PCOS subgroups, while

Table 2. Serum concentrations of hormones and plasma vaspin levels in analyzed groups of PCOS and non-PCOS

	PCOS			non-PCOS		
	All (n = 83)	**Normal weight (n = 39)**	**Obese (n = 48)**	**All (n = 72)**	**Normal weight (n = 31)**	**Obese (n = 41)**
FSH [mIU/mL]	5.7 (4.4–7.4)	5.5 (4.5–6.6)	5.9 (4.4–8.5)	5.4 (3.9–6.8)	5.4 (3.5–6.5)	5.5 (4.3–7.1)
LH [mIU/mL]	10.0$^{\%\%}$ (6.6–14.4)	8.2 (5.2–12.8)	10.9$^{\&\&}$ (8.2–15.6)	8.0 (6.0–12.2)	7.7 (6.4–9.2)	9.1 (4.0–15.4)
LH/FSH	1.7$^{\%}$ (1.1–2.5)	1.6* (1.0–2.4)	1.7$^{\wedge}$ (1.2–2.6)	1.5 (0.9–2.5)	1.5 (1.1–2.5)	1.5 (0.6–2.5)
PRL [ng/mL]	5.9$^{\%\%\%}$ (4.1–8.3)	4.6$^{\#\#++}$ (3.5–7.7)	6.8 (4.9–8.5)	8.1 (5.2–10.7)	7.8 (6.2–12.0)	8.4 (4.7–10.2)
Androstendione [ng/mL]	2.2 (1.5–3.3)	2.8$^{**\#\#++}$ (2.1–3.6)	1.9 (1.3–2.8)	1.9 (1.4–2.8)	2.1 (1.4–3.1)	1.8 (1.4–2.6)
DHEA-S [μg/mL]	2.7 (2.1–3.5)	2.7 (2.2–3.5)	2.8 (2.1–3.4)	2.6 (1.8–3.5)	2.7 (2.1–3.9)	2.4 (1.7–3.3)
Total testosterone [ng/mL]	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.6 (0.5–0.8)	0.6 (0.4–0.8)	0.6 (0.4–0.8)	0.6 (0.4–0.8)
Free testosterone [pg/mL]	1.8$^{\%}$ (1.1–2.8)	2.1$^{*\#+}$ (1.4–3.1)	1.6 (1.1–2.6)	1.4 (0.8–2.3)	1.4 (0.9–2.3)	1.4 (0.8–2.3)
Estradiol [pg/mL]	41.9 (29.6–66.7)	43.6 (32.7–72.6)	40.0 (27.1–64.1)	56.5 (29.7–90.7)	56.8 (34.4–82.7)	54.9 (24.6–111.4)
SHBG [nmol/l]	23.0$^{\%\%}$ (14.8–37.3)	34.2^{**++} (17.3–49.9)	19.0$^{\&\&}$ (10.8–26.7)	30.7 (20.4–49.0)	38.6$^{\$\$}$ (28.2–63.6)	22.9 (16.5–47.3)
FAI	3.1$^{\%\%}$ (1.7–4.9)	2.3$^{**\#+}$ (1.2–4.4)	3.3$^{\&}$ (2.0–5.1)	1.7 (1.1–3.1)	1.5$^{\$}$ (0.9–2.2)	2.0 (1.3–4.4)
Vaspin [ng/mL]	0.13 (0.03–0.87)	0.13$^{\#\#+++}$ (0.04–0.87)	0.13$^{\&\&\wedge\wedge\wedge}$ (0.03–0.53)	0.18 (0.03–2.07)	0.16$^{\$}$ (0.03–1.38)	0.19 (0.06–2.07)

*p < 0.05; **p < 0.01; ***p < 0.001 normal weight PCOS vs obese PCOS; $^{\#}$p < 0.05; $^{\#\#}$p < 0.01; $^{\#\#\#}$p < 0.001 normal weight PCOS vs normal weight non-PCOS; $^{+}$p < 0.05; $^{++}$p < 0.01; $^{+++}$p < 0.001 normal weight PCOS vs obese non-PCOS; $^{\&}$p < 0.05; $^{\&\&}$p < 0.01; $^{\&\&\&}$p < 0.001 obese PCOS vs normal weight non-PCOS; $^{\wedge}$p < 0.05; $^{\wedge\wedge}$p < 0.01; $^{\wedge\wedge\wedge}$p < 0.001 obese PCOS vs obese non-PCOS; $^{\$}$p < 0.05; $^{\$\$}$p < 0.01; $^{\$\$\$}$p < 0.001 normal weight non- PCOS vs obese non-PCOS; $^{\%}$p < 0.05; $^{\%\%}$p < 0.01; $^{\%\%\%}$p < 0.001 all PCOS vs all non-PCOS

lower plasma vaspin levels were observed in normal-weight than in obese Non-PCOS subgroups (Tab. 2).

Correlation between plasma vaspin levels and anthropometric parameters and insulin resistance

We did not observe any association between plasma vaspin levels or any anthropometric parameters in all study groups and the PCOS group. While in Non-PCOS group the negative correlation between plasma vaspin levels and body mass ($r = –0.26$; $p < 0.05$).

There was no correlation between plasma vaspin levels and serum glucose and insulin concentrations as well as HOMA-IR values in both all study groups and both PCOS and Non-PCOS groups analyzed separately.

Multivariable regression analyses

Multivariable, stepwise backward regression analyses revealed that waist circumference but no other anthropometric parameters, as well as HOMA-IR values, explained 18.0% of plasma vaspin levels variability.

DISCUSSION

The results presented in this study demonstrate lower vaspin levels in PCOS women in corresponding, according to nutritional status, Non-PCOS subgroups. In addition, vaspin levels were affected by measures of nutritional status, but only in Non-PCOS subgroup. They were inversely proportional to body mass, and lower in normal weight than obese Non-PCOS.

It should be noted, that it is the first study that showed lower vaspin levels in PCOS women independently from nutritional status. In four published studies higher vaspin levels in PCOS women were described [14–17] and in two there were no differences between PCOS and Non-PCOS subjects [18, 19]. In addition, we found striking differences in vaspin levels in previously published studies. Only in one study performed in 12 subjects the median of vaspin levels was similar to obtained in our study [14]. While in Turkish subjects mean vaspin levels were more than two times higher in PCOS group and in the control group almost five times lower than in our study [15]. Similar, differences were observed in Greek cohorts [16].

Contrary, to previously published studies [17] in multiple regression analysis we observed an inverse association between vaspin levels and waist circumference in PCOS subjects. In addition, contradictory to the other studies, we did not observe an association between vaspin levels and BMI values [8, 17]. These differences are difficult to explain. On the one hand, it may be a result of small study group sizes [14, 15], the differences in nutritional status between study and control groups (mean BMI 5 kg/m^2 lower in control group) [16, 17] and race. On the other hand, it may be the result of different ELISA kits used to vaspin levels measurements, produced by different manufacturers. The lack of studies comparing specificity of kits produced for vaspin levels assessment should be raised.

Furthermore, we did not observe the association between serum glucose and insulin concentrations as well as HOMA-IR values and circulating vaspin levels. It is opposite to some published studies that show that HOMA-IR values were proportional to vaspin levels in PCOS women [17–19] but in accordance with the results of other studies [15, 17]. The association between insulin resistance or glucose metabolism and circulating vaspin levels is not clear. It should be noted that only Youn et al. [8] shown an association between HOMA-IR and vaspin levels in group with normal glucose tolerance. In addition, Tan et al. [14] found that vaspin synthesis is stimulated by glucose in omental adipocytes. Moreover, vaspin mRNA expression but not its circulating levels were proportional to insulin resistance. Therefore, we hypothesized that increased vaspin levels may be a compensatory mechanism in the development of the early stage of insulin resistance, that run out over time. This hypothesis is partially supported by the observed lower vaspin levels in PCOS subjects and its higher levels in obese than normal-weight Non-PCOS women. However, further studies with follow-up are necessary to confirm our hypothesis.

The main limitations of the study are the small sizes of study subgroups and the lack of the assessment of the body fat visceral and subcutaneous using DEXA or CT scanner. Additionally, only selected adipokine vaspin was analyzed thus the assessment of the association between hormonal disturbances of adipose tissue and it's inflammation was not possible.

CONCLUSIONS

PCOS occurrence is associated with decreased vaspin levels in young women. The influence of nutritional status on vaspin level observed in Non-PCOS is abolished in PCOS women, possibly by the coexisting insulin resistance.

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REFERENCES

1. Hida K, Wada J, Eguchi J, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci U S A. 2005; 102(30): 10610–10615, doi: 10.1073/pnas.0504703102, indexed in Pubmed: 16030142.
2. Klöting N, Berndt J, Kralisch S, et al. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Biophys Res Commun. 2006; 339(1): 430–436, doi: 10.1016/j.bbrc.2005.11.039, indexed in Pubmed: 16298335.
3. Körner A, Neef M, Friebe D, et al. Vaspin is related to gender, puberty and deteriorating insulin sensitivity in children. Int J Obes (Lond). 2011; 35(4): 578–586, doi: 10.1038/ijo.2010.196, indexed in Pubmed: 20856257.

4. Klöting N, Kovacs P, Kern M, et al. Central vaspin administration acutely reduces food intake and has sustained blood glucose-lowering effects. Diabetologia. 2011; 54(7): 1819–1823, doi: 10.1007/s00125-011-2137-1, indexed in Pubmed: 21465327.
5. Meyer-Hoffert U. Reddish, scaly, and itchy: how proteases and their inhibitors contribute to inflammatory skin diseases. Arch Immunol Ther Exp (Warsz). 2009; 57(5): 345–354, doi: 10.1007/s00005-009-0045-6, indexed in Pubmed: 19688185.
6. Wada J. Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs. 2008; 17(3): 327–333, doi: 10.1517/13543784.17.3.327, indexed in Pubmed: 18321232.
7. González CR, Caminos JE, Vázquez MJ, et al. Regulation of visceral adipose tissue-derived serine protease inhibitor by nutritional status, metformin, gender and pituitary factors in rat white adipose tissue. J Physiol. 2009; 587(Pt 14): 3741–3750, doi: 10.1113/jphysiol.2009.172510, indexed in Pubmed: 19470778.
8. Youn BS, Klöting N, Kratzsch J, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes. 2008; 57(2): 372–377, doi: 10.2337/db07-1045, indexed in Pubmed: 17991760.
9. Aust G, Richter O, Rohm S, et al. Vaspin serum concentrations in patients with carotid stenosis. Atherosclerosis. 2009; 204(1): 262–266, doi: 10.1016/j.atherosclerosis.2008.08.028, indexed in Pubmed: 18848328.
10. Handisurya A, Riedl M, Vila G, et al. Serum vaspin concentrations in relation to insulin sensitivity following RYGB-induced weight loss. Obes Surg. 2010; 20(2): 198–203, doi: 10.1007/s11695-009-9882-y, indexed in Pubmed: 19506980.
11. Zvonic S, Lefevre M, Kilroy G, et al. Secretome of primary cultures of human adipose-derived stem cells: modulation of serpins by adipogenesis. Mol Cell Proteomics. 2007; 6(1): 18–28, doi: 10.1074/mcp.M600217-MCP200, indexed in Pubmed: 17018519.
12. Suleymanoglu S, Tascilar E, Pirgon O, et al. Vaspin and its correlation with insulin sensitivity indices in obese children. Diabetes Res Clin Pract. 2009; 84(3): 325–328, doi: 10.1016/j.diabres.2009.03.008, indexed in Pubmed: 19356820.
13. Gulcelik NE, Karakaya J, Gedik A, et al. Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. Eur J Endocrinol. 2009; 160(1): 65–70, doi: 10.1530/EJE-08-0723, indexed in Pubmed: 18952766.
14. Tan BK, Heutling D, Chen J, et al. Metformin decreases the adipokine vaspin in overweight women with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diabetes. 2008; 57(6): 1501–1507, doi: 10.2337/db08-0127, indexed in Pubmed: 18375437.
15. Cakal E, Ustun Y, Engin-Ustun Y, et al. Serum vaspin and C-reactive protein levels in women with polycystic ovaries and polycystic ovary syndrome. Gynecol Endocrinol. 2011; 27(7): 491–495, doi: 10.3109/09513590.2010.501874, indexed in Pubmed: 20626239.
16. Koiou E, Tziomalos K, Dinas K, et al. The effect of weight loss and treatment with metformin on serum vaspin levels in women with polycystic ovary syndrome. Endocr J. 2011; 58(4): 237–246, doi: 10.1507/endocrj.k10e-330, indexed in Pubmed: 21325745.
17. Koiou E, Dinas K, Tziomalos K, et al. The phenotypes of polycystic ovary syndrome defined by the 1990 diagnostic criteria are associated with higher serum vaspin levels than the phenotypes introduced by the 2003 criteria. Obes Facts. 2011; 4(2): 145–150, doi: 10.1159/000327935, indexed in Pubmed: 21577021.
18. Cekmez F, Cekmez Y, Pirgon O, et al. Evaluation of new adipocytokines and insulin resistance in adolescents with polycystic ovary syndrome. Eur Cytokine Netw. 2011; 22(1): 32–37, doi: 10.1684/ecn.2011.0279, indexed in Pubmed: 21411410.
19. Akbarzadeh S, Ghasemi S, Kalantarhormozi M, et al. Relationship among plasma adipokines, insulin and androgens level as well as biochemical glycemic and lipidemic markers with incidence of PCOS in women with normal BMI. Gynecol Endocrinol. 2012; 28(7): 521–524, doi: 10.3109/09513590.2011.650747, indexed in Pubmed: 22309615.
20. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19(1): 41–47, doi: 10.1093/humrep/deh098, indexed in Pubmed: 14688154.

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Nasal bone in screening for Trisomy 18 and 13 at 11–13 + 6 weeks of gestation — own experiences

Bartosz Czuba[1], Marek Maczka[2], Wojciech Cnota[1], Agata Wloch[1], Agnieszka Jagielska[1], Anna Niesluchowska-Hoxha[1], Dariusz Borowski[3]

[1]Department of Obstetrics and Gynecology in Ruda Slaska, Medical University of Silesia, Ruda Slaska, Poland
[2]Center for Prenatal Diagnostic, Opole, Poland
[3]Faculty of Health Sciences, Department of Obstetrics, Nicolaus Copernicus University in Torun, Collegium Medicum, Bydgoszcz, Poland

ABSTRACT

Objectives: The objective of the paper is the suitability assessment of screening for Trisomy 18 and 13 on the basis of NT measurement, FHR, double test and assessment of Nasal Bone.

Material and methods: The study was performed in 6,661 singleton pregnancies. In each fetus NT, FHR, DV-PIV were examined. Double test from maternal blood was examined. These ultrasound and biochemical factors were in combined screening investigated. Additional ultrasound marker — Nasal Bone was and its impact on Trisomies 18 and 13 screening was examined.

Results: Two groups of patients were compared — with chromosomal normal and chromosomal abnormalities — Trisomy 18 and 13. Detection Rate of Trisomies 18 and 13 at the risk cutoff 1/300 using combined screening was 84.1% and FPR was 7.1%. Detection Rates of examined chromosomal abnormalities using screening with additional marker — NB was 93.2% and False Positive Rate — 5.6%.

Conclusions: It should be noted that the qualitative analysis of the assessment of NB in the first trimester significantly influences the improvement of screening values focusing on Trisomy 18 and 13 detection. In summary, our research indicates a more effective type of Trisomy 13 and 18 screening using NT, double test, maternal age, CRL and FHR as well as nasal bone presence and absence.

Key words: combined test Trisomy 18 Trisomy 13; first trimester nuchal translucency thickness; Nasal Bone; Serum free β-hCG; Pregnancy-associated plasma protein A

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INTRODUCTION

The main objective of screening tests in the first trimester of gestation is to assess the risk of chromosomal (most often Trisomy 21, 13 and 18) based on ultrasound examination (nuchal translucency [NT] assessment, fetal heart rate [FHR]). What is more, the assessment of the double test (the level of the free β- subunit of human chorionic gonadotropin and pregnancy plasma protein type A-PAPP-A are assessed) as well as maternal age [1, 2] were also taken into consideration.

By means of this methodology, the detection rate (DR) for Trisomy 21 is 87% at FPR (False-positive Results) 5.3%, whereas 90% at FPR 3.1% [1, 3]. The detectability of Trisomy 18 and 13 is 97% and 94%, respectively, at FPR 3.1% [1, 3]. Additional markers are used in screening for Trisomy 21: assessment of the presence of nasal bone, which increases detection of Trisomy 21 by about 5% in the case of FPR from 4.8 to 3.4% [4].

The objective of this retrospective study was

The work is a retrospective study assessing the use of an additional ultrasound marker in the prenatal examination of the first trimester (11^{+0}–13^{+6} weeks of gestation), which is the nasal bone, in the analysis of chromosomal aberration screening: Trisomy 18 and 13.

MATERIAL AND METHODS

We had 6,844 pregnant women included in the study with single pregnancies who underwent prenatal screening

Corresponding author:
Bartosz Czuba
Medical University of Silesia, 2 Lipa St, 41–712 Ruda Śląska, Poland
e-mail: bczuba@o2.pl

of 1st trimester assessing the risk of occurrence of Trisomy 18 and 13 in the years 2014–2019. It should be stressed that the fetuses with other syndromes (Trisomy 21 — 84 , Turner Syndrome — 14, Tetraploidy — 5, Unbalanced translocations — 6 cases respectively) and structural defects with normal karyotype, such as: heart defects — Hypoplastic Left Heart Syndrome — 13 , Atrioventricular Septal Defects — 7, Tetrallogy of Fallot — 4 and Transposition of the Great Arteries — 2 cases were excluded from the research. Other structural fetal defects excluding fetuses from the study were: Fetal Hydrops — 15, Spina bifida — 11, Hydrocephalus — 7, Palate or upper lips cleft — 7, Omphalocele — 4 and Gastroschisis — 4 cases. The study was performed in pregnancies scanned in the Department of Obstetrics and Gynecology in Ruda Śląska, Outpatient 'Sonomedico' Żory and in Outpatient Clinic 'GENOM' in Ruda Śląska. The risk was calculated using the Fetalmedicine Foundation (FMF) certified program The First Trimester Screening Program or Astraia (Astraia Software G.m.b.H., Munich, Germany). These pregnant women were retrospectively divided into two groups: patients with a low risk of chromosomal defects, who gave birth to healthy children were included in the control group (6617 pregnant), whereas, the study group included patients (44 pregnant women) whose fetuses were diagnosed with Trisomy 18 and 13.

All patients who were at increased risk of developing Trisomy 18 or 13 made decisions to undergo invasive diagnostics. Edwards Syndrome (Trisomy 18) and Patau Syndrome (Trisomy 13) were found after amniocentesis and cytogenetic tests. Amniotic fluid obtained during amniocentesis after genetic consultation and obtaining written consent of the patient for amniocentesis were used for these tests. Invasive diagnostics were performed in pregnant women with increased risk of Trisomy 18 and 13 in age-related risk.

Ultrasound examination during the first trimester was performed between 11^{+0} and 13^{+6} weeks of gestation according to the recommendations of FMF and the Ultrasonography Section of the Polish Gynaecological Society [3]. During the ultrasound examination, the following parameters were evaluated: crown-rump length (CRL), fetal heart rate (FHR), nuchal translucency (NT) and, additionally, nasal bone (NB), (nasal bone — its presence or absence). A Double Marker Test was performed, and the mother's age at the time of examination was noted. The first trimester ultrasound was performed by specialists in obstetrics and gynaecology certified by the Fetal Medicine Foundation and the Ultrasound Section of the Polish Society of Gynaecologists and Obstetricians. The ultrasound examination was performed with Volusion 730 Expert, Volusion E6, E8 and E10 (GE Healthcare). Each patient was serum examinated level to a double test on the day of ultrasound examination: assessment of the β-hCG free subunit level (human β- chorionic gonadotropin free subunit) and PAPP-A level (plasma protein A during pregnancy) in venous blood. Levels of the tested substances were individually calculated for each patient on MoM (multiples of the median). Biochemical tests were carried out using the Kryptor method of the Brahms company (Kryptor, Brahms Diagnostica GmbH, Berlin, Germany) and Delfia Express-Perkin Elmer USA.

The results were analysed using the PQStat statistical package ver. 1.4.2.324. The results of the analyses were presented in the tables of descriptive statistics and two-split tables and on the charts. The results of the β-hCG MoM, PAPP-A MoM scales depending on the group (testing and control) were compared using the Mann-Whitney U test. The presence and absence of the nasal bone in healthy fetuses and sick fetuses were analysed by determining detection rates and the number of false-positive results along with confidence intervals and using ROC analyzes. The risk of Trisomy 18 and 13 divided into > 1/300 > 1/200.1 > 100 > 1/50 and with coincident consideration of the presence or absence of the nasal bone of healthy fetuses and afflicted fetuses was analysed by estimating detection rates and the number of false positive results with confidence intervals. The risk of Trisomy of 18 and 13 was also analysed using ROC curve. The test probability on the level of $p < 0.05$ was considered statistically important and noticeable.

RESULTS

The control group included 6,617 pregnant women aged between 14 to 46 years of age (mean age of pregnant women was 31 years), ultrasound examination was performed between 11^{+0} and 14th weeks of gestation (on mean 12^{+3} weeks of gestation). The CRL size of the fetuses ranged from 45 to 84 mm (mean CRL of fetuses was 63.7mm). The mean nuchal translucency in the control group was 1.8 mm (min. 0.8 mm, max. 13.1 mm). In 200 (3.02%) of fetuses from the control group, the nasal bone was not present (Tab. 1, 4).

Fourty-four pregnant women aged between of 20–41 were included in the study group (the mean age of pregnant women was 29). Ultrasound examination was performed between 11^{+0} and 13^{+6} weeks of gestation (on mean 12^{+2} weeks of gestation). CRL size of fetuses ranged from 47 mm to 81.1 mm (mean size of fetuses was 58.8 mm) The mean nuchal translucency in the study group was 5.1 mm (min 1.7 mm, max 8.3 mm). In the examined group nasal bone was found in 14 fetuses — in 8 fetuses with Edwards syndrome and in 6 fetuses with Patau syndrome. In 30 (68.18%) fetuses with Trisomy 18 and 13 the nasal bone was not found during ultrasound examination (Tab. 2, 4).

The differences in NT thickness, MoM β-hCG and MoM PAPP-A as well as the percentage of presence or absence of nasal bone in the control and study group was statistically significant (Tab. 3).

Table 1. Control group

	mean	SD	min.	max.	median	25th percentile (lower quartile)	75th percentile (upper quartile)
age [years]	31	5.54	14	46	31	27	36
CRL [mm]	63.7	8.47	45	84	63.4	57.8	69.5
NT [mm]	1.8	0.56	0.8	13.1	1.8	1.5	2
FHR [/min]	160	6.25	131	206	160	156	164
β-hCG MoM	1.238	0.814	0.08	9.181	1.023	0.702	1.538
PAPP-A MoM	1.076	0.576	0.052	6	0.958	0.671	1.354

CRL — crown-rump length; FHR — fetal heart rate; MoM — multiples of the median; NT — nuchal translucency; SD — standard deviation

Table 2. Study group

	mean	SD	min.	max.	median	25th percentile (lower quartile)	75th percentile (upper quartile)
age [years]	29	6.08	20	41	28	25	33
CRL [mm]	58.8	8.9	47	81.1	56.5	51.3	63.6
NT [mm]	5.1	1.66	1.7	8.3	5.7	3.9	6.4
FHR (/min)	159	11.7	135	179	158	150	170
β-hCG MoM	1.238	0.814	0.08	9.181	1.023	0.702	1.538
PAPP-A MoM	0.533	0.663	0.1	2.995	0.295	0.242	0.48

CRL — crown-rump length; FHR — fetal heart rate; MoM — multiples of the median; NT — nuchal translucency; SD — standard deviation

Table 3. Both groups selected parameters comparison

	Healthy fetuses	Trisomy 18 and 13 fetuses	p value
NT [median]	1.8 mm	5.7 mm	p < 0.001
β-hCG MoM [median]	1.023	0.46	p < 0.001
PAPP-A MoM [median]	0.958	0.295	p < 0.001
Percentage of nasal bone presence	93.98%	31.82%	p < 0.001

NT — nuchal translucency; MoM — multiples of the median

Table 4. Assessment of DR and FPR depending on the cut-off point for Trisomy 18 and 13

	DR		FPR	
	NT + PAPP-A	NT + NB + PAPP-A	NT + PAPP-A	NT + NB + PAPP-A
1:300	84.1%	93.2%	7.1%	5.6%
1:200	81.8%	90.9%	4.5%	3.8%
1:100	68.2%	81.8%	2.2%	2.2%
1:50	56.8%	75.0%	1.1%	1.1%

DR — detection rate; FPR — fetal heart rate; NB — nasal bone; NT — nuchal translucency

Table 5. The assessment of nasal bone presence

	All patients	Present nasal bone		Absent nasal bone	
The number of examined patients	6661	6431	95.54%	230	3.43%
Healthy fetuses	6617	6417	96.98%	200	3.02%
Trisomy 18 and 13 fetuses	44	14	31.82%	30	68.18%

As a result of further analysis, patients were divided into 4 groups depending on the level of risk of Trisomy 18 and 13: the first group with a risk above 1:300 and the second with a risk above 1:200, the third with a risk above 1:100 and the fourth group with a risk above 1:50 occurrence of Trisomy 18 and 13. DR and FPR as well as positive and negative ratio (LH + and LH-) were assessed in each group. Each group was divided into two subgroups, in one of them the risk of Trisomy 18 and 13 was assessed basing on the patient age, NT, FHR, and double PAPP-A test. In the second group, the same fetuses had an estimated risk based on the patient's age, NT, FHR, double test and nasal bone assessment. The highest DR was in the risk group > 1:300 and was 84% for fetuses in which the nasal bone was left beyond the scope of the study and 93% for fetuses in which the presence or absence of the nasal bone was assessed. In this group, we obtained a decrease in FPR from 7.1% (group without nasal bone assessment) to 5.6% (in the group with nasal bone assessment). The lowest DR was found in the group at risk > 1:50 i.e. 56% (screening without nasal bone assessment) and 75% (screening with nasal bone assessment) with a similar FPR of 1.1% (Tab. 5).

Table 6. Nuchal Translucency above 95th percentile in study group

	Control group	Edward's Syndrome	Patau Syndrome
NT percentage above 95th percentile	4.9%	81%	91%

NT — nuchal translucency

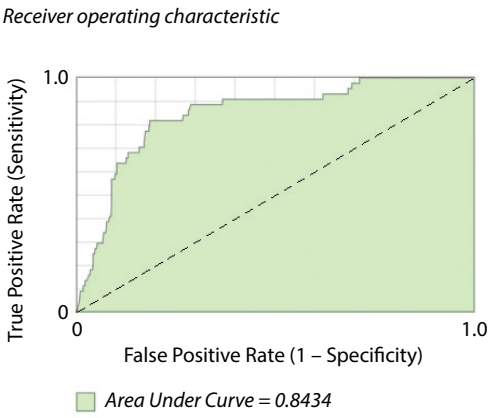

Figure 1. ROC curve for Trisomy 18 and 13 prediction using risk 18 and 13 in the group in which the patient's age, NT FHR and PAPP-A test were assessed

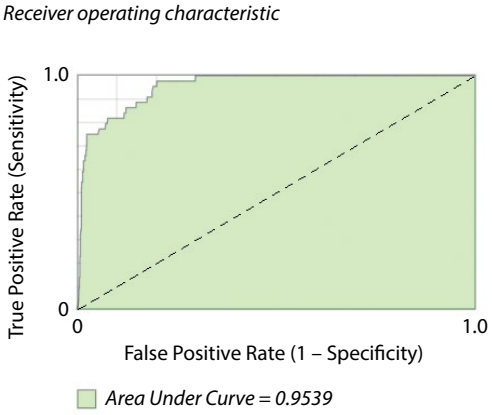

Figure 2. ROC curve for Trisomy prediction 18 and 13 using risk 18 and 13 in the group in which the patient's age, NT, FHR, NB and PAPP-A test were assessed

In every the assessed groups according to risk (> 1:300, > 1:200, > 1:100, > 1:50) an increase in the study's correctness and a decrease in FPR after including the nasal bone screening was obtained: for groups at risk > 1:300 DR increased from 84% to 93% with a decrease in FPR from 7.1% to 5.6%; and for the group at risk > 1:50 DR increased from 56% to 75% at the FPR level of 1.1% for both groups (Tab. 4).

The risk of Trisomy 18 and 13 is in relation to maternal age, CRL, nuchal translucency scan and fetal heart rates, as well as a double test. In our studies, we additionally evaluated the presence or absence of the nasal bone. Thanks to the evaluation of the nasal bone, we have obtained both an increase in the sensitivity of the examination as well as a decrease in FPR (Chart 1, 2).

Nuchal translucency was above 95th percentile in 4.9% of cases in the control group. In fetuses with Trisomy 18 in 81% and in fetuses with Trisomy 13 in 91% of cases. (Tab. 6).

In the group in which the nasal bone was not assessed, but only the nuchal translucency and the double test (control group) were evaluated, the area under the curve in the ROC analysis was 0.8434. On the contrary, the area under the curve in the group with nasal bone assessment (study group) in the ROC analysis was 0.9539. The absence of a nasal bone is a very good marker of Trisomy 18 and 13 because the area under the curve in ROC analysis when the screening nasal bone assessment is included increased from 0.8434 to 0.9593 (Fig. 1, 2).

DISCUSSION

The most frequent human chromosomes are Down syndrome (Trisomy 21) — 1 case for 800 live births, Edwards Syndrome (Trisomy 18 — 1 case for 3,500 to 8,000 live births) and Patau Syndrome (Trisomy 13) — 1 case out of 6,500 live births [5, 6]. Most fetuses with genetic abnormalities die intrauterine at various stages of development, and the incidence of genetic is estimated to be significantly higher [6]. The risk of Edwards and Patau Syndrome depends on the age of the mother and increases with it [7]. However, about 80% of fetuses with genetic abnormalities occur in women under 35 years of age [8]. According to Kroes [1], 69% of mothers whose fetuses had a Trisomy were under 35 years old.

Screening tests used to assess the risk of Trisomy of 21, 18 and 13 are performed between 11^{+0} and 13^{+6} weeks of gestation. The detection of Trisomy 21, 18 and 13 depends on the test method, i.e. the number of ultrasound markers evaluated, and biochemical tests performed. The basic ultrasound marker for chromosomal aberration assessment is nuchal translucency and fetal heart rate (FHR) [7]. Nuchal translucency above the 95th percentile occurs in approximately 72% of Down Syndrome cases [8], while in Edwards and Patau Syndrome only in 66% and 44% of cases assessed in prenatal tests during the first trimester of pregnancy respectively [9]. In our study, nuchal translucency above the 95th percentile occurred in 81% of Edwards' Syndrome and 91% of Patau Syndrome.

Detection (DR) of Trisomy 18 and 13 in screening tests in the first trimester of pregnancy, i.e. based on basal markers (maternal age, NT, fetal heart rate and assessing the double test) is approximately 87% at FPR 5.3% [4]. According to Kagan et al. [13], when examining mother's age, nuchal

translucency and performing a biochemical test (double test), the detection of Trisomy 18 and 13 is 97% and 94% respectively at FPR 3.1%. In our studies, assessing maternal age, nuchal translucency, fetal heart function and performing a double test at 4.5% FPR a detection rate (DR) of 81% for Trisomy 18 and 13 was obtained (Tab. 4).

The nasal bone is an additional ultrasound indicator assessed in screening tests in the first trimester of pregnancy to assess the risk of Trisomy 21, 18 and 13 [11–13]. The nasal bone does not occur in approximately 0.1–2.8% of healthy fetuses [14]. Absence of the nasal bone in healthy fetuses depends on the mother's ethnicity [12]: most often it does not occur in African-Americans (10.4%), less often in Asians (6.8%), while the most rarely absence of the nasal bone is found in Caucasians (2.8%) [15]. In our material, no nasal bone in 3.02% of healthy foetuses was found. The nasal bone is more often absent in the Trisomy 21 than in Trisomy 18 and 13 [12, 14]. In Edwards' Syndrome, the nasal bone is absent in about 52–80% of cases [15, 16], while in Patau's Syndrome in about 31–67% of cases [8, 12, 15]. Our results show similar values — in 68% of fetuses with Trisomy 18 and 13 we did not find the presence of nasal bone.

In our study, after including the nasal bone assessment into the screening, the sensitivity of the examination in each case was increased by about 10–20% (DR for risk > 1: 300 from 84.1% to 93.2%, for risk > 1: 200 from 81.8% to 90.9%, for risk > 1: 100 from 68.2% to 81.8%, and for risk > 1:50 from 56.8% to 75%) . At the same time the false positive results were reduced FPR from 7.1% at cut-off point for risk > 1: 300 to 1.1% at cut-off point for risk > 1:50 (Tab. 4).

After including the presence or absence of the nasal bone assessment into the screening Kagan et al. [13] did not observe in their studies changes in the detection of Trisomy 18 and 13 — at a cut-off point for 1:100 the detection of Trisomy 18 and 13 remained on this level (DR for Trisomy 18:92%; DR for Trisomy 13:83%) with an FPR of 2.5%. In our study, for a cut-off point of > 1:100 taking into account the assessment of the nasal bone compared to the test without assessment of the nasal bone, the sensitivity increased from 68% to 81%, with the same level of FPR — 2.2% .

We stated that the absence of a nasal bone was a very accurate additional marker of Trisomy 18 and 13. The area under the curve in the ROC analysis is statistically important and noticeable ($p < 0.0001$). After including the nasal bone screening an increase in DR was obtained with a decrease in FPR. Therefore, we believe that the assessment of the risk of Trisomy 18 and 13 should be carried out not only in scope of mother's age, fetal heart rate and nuchal translucency thickness but also using an additional ultrasound marker which is the presence or absence of the nasal bone. For this reason, we agree with other authors [17] that nasal bone assessment is a good marker of Trisomy 18 and 13, but we disagree that it should not be considered in the trisomy risk assessment algorithm.

CONCLUSIONS

Concluding, it should be noted that the qualitative analysis of the assessment of the nasal bone in the first trimester significantly influences the improvement of screening values focusing on Trisomy 18 and 13 detection. In summary, our research indicates a more effective type of Trisomy 13 and 18 screening using NT, double test, maternal age, CRL and FHR as well as nasal bone presence and absence.

REFERENCES

1. Brown R. Trisomy 13: 13+ Syndrome (Patau Syndrome). Encyclopedia of Special Education. 2014, doi: 10.1002/9781118660584.ese2442.
2. Ghaffari SR, Tahmasebpour AR, Jamal A, et al. First-trimester screening for chromosomal abnormalities by integrated application of nuchal translucency, nasal bone, tricuspid regurgitation and ductus venosus flow combined with maternal serum free β-hCG and PAPP-A: a 5-year prospective study. Ultrasound Obstet Gynecol. 2012; 39(5): 528–534, doi: 10.1002/uog.10051, indexed in Pubmed: 21793085.
3. zespół z. Polish Gynecological Society – Ultrasound Section Guidelines on ultrasound screening in gynecology – 2015. Polish Gynaecology. 2015; 86(8): 635–639, doi: 10.17772/gp/58975.
4. Nicolaides K, Węgrzyn P. Nicolaides K, Węgrzyn P, Badanie ultrasonograficzne między 11+0-13+6 tygodniem ciąży, Fetal Medicine Foundation, London. ; 2004.
5. Adiego B, Martinez-Ten P, Illescas T, et al. First-trimester assessment of nasal bone using retronasal triangle view: a prospective study. Ultrasound Obstet Gynecol. 2014; 43(3): 272–276, doi: 10.1002/uog.12525, indexed in Pubmed: 23733531.
6. Kozlowski P, Knippel AJ, Froehlich S, et al. Additional performance of nasal bone in first trimester screening. Ultraschall Med. 2006; 27(4): 336–339, doi: 10.1055/s-2005-858880, indexed in Pubmed: 16596511.
7. Cicero S, Longo D, Rembouskos G, et al. Absent nasal bone at 11-14 weeks of gestation and chromosomal defects. Ultrasound Obstet Gynecol. 2003; 22(1): 31–35, doi: 10.1002/uog.170, indexed in Pubmed: 12858299.
8. Karadzov-Orlic N, Egic A, Filimonovic D, et al. Screening for aneuploidies by maternal age, fetal nuchal translucency and maternal serum biochemistry at 11-13+6 gestational weeks. Srpski arhiv za celokupno lekarstvo. 2012; 140(9-10): 606–611, doi: 10.2298/sarh1210606k.
9. Springett AL, Morris JK. Antenatal detection of Edwards (Trisomy 18) and Patau (Trisomy 13) syndrome: England and Wales 2005-2012. J Med Screen. 2014; 21(3): 113–119, doi: 10.1177/0969141314543128, indexed in Pubmed: 24993362.
10. Maiz N, Valencia C, Kagan KO, et al. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol. 2008; 32(4): 488–492, doi: 10.1002/uog.6123, indexed in Pubmed: 18726925.
11. Kagan KO, Wright D, Valencia C, et al. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. Hum Reprod. 2008; 23(9): 1968–1975, doi: 10.1093/humrep/den224, indexed in Pubmed: 18544579.
12. Borenstein M, Persico N, Kagan KO, et al. Frontomaxillary facial angle in screening for trisomy 21 at 11 + 0 to 13 + 6 weeks. Ultrasound in Obstetrics and Gynecology. 2008; 32(1): 5–11, doi: 10.1002/uog.5334.
13. Kagan KO, Cicero S, Staboulidou I, et al. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol. 2009; 33(3): 259–264, doi: 10.1002/uog.6318, indexed in Pubmed: 19248005.
14. Zoppi MA, Ibba RM, Axiana C, et al. Absence of fetal nasal bone and aneuploidies at first-trimester nuchal translucency screening in unselected pregnancies. Prenat Diagn. 2003; 23(6): 496–500, doi: 10.1002/pd.628, indexed in Pubmed: 12813765.
15. Cicero S, Spencer K, Avgidou K, et al. Maternal serum biochemistry at 11-13(+6) weeks in relation to the presence or absence of the fetal

nasal bone on ultrasonography in chromosomally abnormal fetuses: an updated analysis of integrated ultrasound and biochemical screening. Prenat Diagn. 2005; 25(11): 977–983, doi: 10.1002/pd.1211, indexed in Pubmed: 16245371.
16. Czuba B, Cnota W, Wloch A, et al. Frontomaxillary facial angle measurement in screening for trisomy 18 at 11 + 0 to 13 + 6 weeks of pregnancy: a double-centre study. Biomed Res Int. 2013; 2013: 168302, doi: 10.1155/2013/168302, indexed in Pubmed: 24195065.
17. Wojda KM, Moczulska H, Sieroszewski PJ. The absence of fetal nasal bones in ultrasound examination between 11 + 0 and 13 + 6 weeks of gestation versus the occurrence of trisomies 21, 18, and 13. Ginekol Pol. 2019; 90(10): 604–606, doi: 10.5603/GP.2019.0104, indexed in Pubmed: 31686418.

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Decorin levels in early- and late-onset preeclampsia

Gülten Özgen, Gültekin Adanaş Aydın

Bursa Yüksek İhtisas Training and Research Hospital Department of Gynecology and Obstetrics, Bursa, Turkey

ABSTRACT

Objectives: Preeclampsia (PE) is a pregnancy complication caused by typically limited proliferation, apoptosis, migration, and invasion of extra-trophoblast cells. Decorin (DCN) is a decidua-derived transforming growth factor (TGF)-binding proteoglycan which exerts multiple physiological functions such as collagen fibrillogenesis, myogenesis, angiostasis, and restraining placental invasiveness by adversely regulate proliferation, migration, and invasiveness of human extravillous trophoblast cells. Preeclampsia is mainly classified as early- and late-onset PE according to the timing of the disease onset. In the present study, we aimed to investigate the DCN levels in early-onset PE (EOPE, < 34 weeks) and late-onset severe PE (LOPE, ≥ 34 weeks) and uncomplicated pregnancies.

Material and methods: In this case-control study, serum samples were obtained from 21 pregnant women with EOPE and 29 pregnant women with LOPE, as well as from 38 healthy controls (n = 12 early-onset controls and n = 26 late-onset controls) with uncomplicated pregnancies.

Results: The mean DCN level was statistically significantly higher in the early-onset PE controls than late-onset PE controls (p = 0.040). Although the mean DCN level was higher in the early-onset PE controls than EOPE and LOPE groups, it did not reach statistical significance (p = 0.119 and p = 0.117, respectively).

Conclusions: Although DCN has been thought to play a role in the pathophysiology of PE, our study results show that DCN is not a useful predictive marker of EOPE and LOPE. Further large-scale studies are needed to draw a definitive conclusion.

Key words: early-onset; late-onset; preeclampsia; decorin

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INTRODUCTION

Hypertensive disorders in pregnancy are a major health problem worldwide and preeclampsia (PE) is the most common complication [1]. Preeclampsia accounts for 3 to 5% of all pregnancies and is one of the leading causes of maternal, fetal, and neonatal mortality and morbidity [2]. It is mainly classified as early-onset (< 34 weeks) and late-onset (≥ 34 weeks) [3]. Although initial symptoms are similar in both conditions, they have unique biomarkers, genetic risk factors, prognosis, and clinical characteristics [4].

Decorin (DCN), an extracellular matrix protein, is a small leucine-rich proteoglycan expressed in connective tissue. It contains a protein core and a single chondroitin/dermatan sulfateglycosaminoglycan chain bound at the *N-terminal* extension. Previous studies have shown that DCN plays a role in the cell proliferation and formation of collagen fibers and modulates certain cell functions (*i.e.*, proliferation, dissemination, migration, and differentiation) acting as a critical modulator of inflammation. In addition, DCN is a molecule which is highly expressed in reproductive tissues [5–7].

Decorin binds to the transforming growth factor-beta (TGF-β) and activates signaling pathways. The TGF-β binds to its own receptor and induces phosphorylation of the Smad family, which is one of the transcription factors, thereby, modulating the transcription of collagen, matrix metalloproteinases (MMPs), and metalloproteinase tissue inhibitors [8]. Irrespective of these mechanisms, DCN stimulates phosphorylation of vascular endothelial growth factor (VEGF) and insulin-like growth (IGF) receptor expressed by extra-villous trophoblasts [9].

In the literature, alterations in the DCN levels have been shown to be associated with PE. In a study, Gogiel et al. [10] reported increased DCN levels of the umbilical cord vein wall in patients with PE. Similarly, Siddiqui et al. [11] found that increased DCN levels were predictors of PE even before the onset of clinical symptoms. The link between DCN and PE can be attributed to the impaired proliferation and migra-

Corresponding author:
Gültekin Adanaş Aydın
Bursa Yüksek İhtisas Training and Research Hospital Department of Gynecology and Obstetrics, Bursa, Turkey
e-mail: gadanas@gmail.com

tion of trophoblasts and endothelial dysfunction, which are thought to be responsible for adverse pregnancy outcomes (APOs). On the other hand, there is a limited number of studies showing the relationship between DCN and APOs with controversial results.

Based on the pathophysiological mechanisms of PE, we hypothesized that DCN would be useful in the diagnosis of PE, particularly in early-onset PE. In the present study, we, therefore, aimed to investigate the DCN levels in early-onset PE (EOPE) and late-onset PE (LOPE) and uncomplicated pregnancies.

MATERIAL AND METHODS

This prospective, case-control study was carried out at Bursa Yüksek Ihtisas Training and Research Hospital, Obstetrics and Gynecology outpatient clinics between January 2019 and March 2019. A total of 88 participants aged between 18 and 35 years (n = 50 PE and n = 38 healthy controls) were included in the study. The patient group was classified as EOPE (n = 21) and LOPE (n = 29). The control group consisted of healthy women with singleton pregnancy with similar gestational weeks who were under follow-up in our outpatient clinics with uncomplicated pregnancies. Patients with chronic hypertension, thyroid dysfunction, renal or cardiovascular disease, and multiple pregnancy were excluded from the study. Of the control group, 12 were in the < 34th week of pregnancy (early-onset PE controls) and 26 were in the ≥ 34th week of pregnancy (late-onset PE controls). A written informed consent was obtained from each participant. The study protocol was approved by the institutional Ethics Committee (2011-KAEK-25 2019/02-10). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data including demographic data of the patients, maternal age, parity/gravida, last menstrual period, gestational age, body weight and height, and systolic and diastolic blood pressure were recorded. In those with unknown last menstrual period, the gestational age was calculated based on the crown-rump length as assessed by ultrasound in the first trimester.

The diagnosis of PE was based on a systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, measured twice in 4 to 6-hour intervals while resting, after the 20th gestational week accompanied by 300 mg/dL proteinuria in a 24-hour urine sample, or more than +1 proteinuria in spot urine specimens. Early-onset PE was defined as the onset before 34 weeks of pregnancy, while late-onset PE was defined as the onset after 34 weeks of pregnancy. The presence of intrauterine growth retardation (IUGR) defined as an estimated fetal weight below the 10th percentile for the gestational age birth.

All patients were followed during pregnancy. Data including birth data, birth weight, and type of labor were recorded.

Biochemical Analyses

A 5-mL venous blood sample was drawn from each patient during their ward stay and from each healthy control during outpatient visit. The samples were centrifuged at 3,500 rpm for 10 min and kept at –80º until analysis. Serum DCN levels were analyzed using the enzyme-linked immunosorbent (ELISA) method.

Complete blood count and biochemical parameters were analyzed. Complete blood count was analyzed using the Roche SYSMEX analyzer (Roche Diagnostics, Basel, Switzerland). In addition, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total bilirubin, hemoglobin, creatinine, uric acid, and urinalysis were examined using the Synchron LX20 system (Beckman Coulter Diagnostics, CA, USA).

Statistical Analysis

Statistical analysis was performed using the SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), quartile (25th, 50th, and 75th), and number and frequency. The Kolmogorov-Smirnov test was used to test normal distribution of continuous variables. The Kruskal-Wallis test was used to analyze significant differences between non-normally distributed variables. The *post-hoc* Dunn test was performed to identify groups with significant differences. The Fisher-Freeman-Halton exact test was used to examine distribution of categorical variables. The Spearman's correlation analysis was carried out to examine the relationship between DCN levels and other variables. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 88 participants including 50 patients with PE and 38 healthy controls were included in this study. Of the patients, 21 had EOPE and 29 had LOPE. Of the healthy controls, 12 were early-onset PE controls and 26 were late-onset PE controls. Demographic and clinical characteristics and biochemical analyses are shown in Table 1.

Although the mean body weight (p = 0.001), body mass index (p = 0.006), systolic (p = 0.001) and diastolic blood pressure (p = 0.001), ALT (p = 0.001), hemoglobin(p = 0.016), and creatinine (p = 0.001) levels did not significantly differ between the either control group, these levels were significantly lower in the control groups than EOPE and LOPE groups. The mean AST level was similar between the control groups and in the LOPE group, but was significantly lower than the EOPE group (p = 0.001). On the other hand, there was no significant difference in the platelet counts between the control groups; however, the mean platelet count was significantly higher than the EOPE and LOPE groups. In addition, the mean platelet count was significantly lower in the EOPE group than the LOPE group (p = 0.001)

Table 1. Demographic and clinical characteristics and biochemical analyses

			EOPE	LOPE	Late-onset PE controls	Early-onset PE controls	p
Age, [year]	N		21	29	26	12	0.082
	Mean		29.76	30.72	26.62	26.42	
	SD		7.293	6.403	6.682	6.735	
	Percentiles	25th	23.50	25.50	21.75	22.00	
		Median	29.00	32.00	25.00	23.50	
		75th	37.00	36.00	31.00	32.00	
Weight, [kg]	N		21	29	26	12	0.001
	Mean			90.48[a]	76.73[b]	70.58[b]	
	SD			20.373	10.452	11.889	
	Percentiles	25th	76.00	74.00	69.50	61.50	
		Median	80.00	90.00	79.00	68.00	
		75th	87.50	101.00	85.00	79.50	
Height, [cm]	N		21	29	26	12	0.302
	Mean			163.41	162.88	159.25	
	SD			5.172	6.755	6.930	
	Percentiles	25th	159.00	160.00	159.50	154.00	
		Median	162.00	165.00	164.00	160.00	
		75th	166.00	166.50	167.25	165.00	
BMI, [kg/m^2]	N		21	29	26	12	0.006
	Mean		31.8625[a]	33.9284[a]	28.9815[b]	27.8364[b]	
	SD		5.55135	7.76778	4.10810	4.43420	
	Percentiles	25th	29.1279	28.3595	26.7589	24.2936	
		Median	31.6337	34.8944	28.3356	26.1656	
		75th	34.4410	37.5954	32.0019	32.3027	
SBP, mmHg	N		21	29	26	12	0.001
	Mean		162.86[a]	157.59[a]	110.00[b]	110.00[b]	
	SD		18.205	19.208	10.583	8.528	
	Percentiles	25th	150.00	150.00	100.00	100.00	
		Median	160.00	150.00	110.00	110.00	
		75th	175.00	165.00	120.00	120.00	
DBP, mmHg	N		21	29	26	12	0.001
	Mean		104.76[a]	98.97[b]	65.77[c]	66.67[c]	
	SD		8.136	10.805	7.575	8.876	
	Percentiles	25th	100.00	90.00	60.00	60.00	
		Median	100.00	100.00	70.00	70.00	
		75th	110.00	100.00	70.00	70.00	
Gravida	N		21	29	26	12	0.876
	Mean		2.43	2.72	2.23	2.33	
	SD		1.720	1.771	0.863	1.073	
	Percentiles	25th	1.00	1.00	2.00	1.00	
		Median	2.00	2.00	2.00	3.00	
		75th	3.00	4.00	3.00	3.00	
Parity	N		21	29	26	11	0.788
	Mean		1.10	1.10	1.15	1.36	
	SD		1.221	1.263	0.881	1.206	
	Percentiles	25th	0.00	0.00	0.75	0.00	
		Median	1.00	1.00	1.00	2.00	
		75th	2.00	2.00	2.00	2.00	

→

Table 1. Demographic and clinical characteristics and biochemical analyses (continued)

			EOPE	LOPE	Late-onset PE controls	Early-onset PE controls	p
Abortus	N		21	29	26	12	0.057
	Mean		0.33	0.62	0.08	0	
	SD		0.796	1.115	.272	0	
	Percentiles	25th	0	0	0	0	
		Median	0	0	0	0	
		75th	0	1.00	0	0	
Live birth	N		21	29	26	12	0.635
	Mean		1.10	1.10	1.15	1.50	
	SD		1.221	1.263	0.881	1.243	
	Percentiles	25th	0	0	0.75	0	
		Median	1.00	1.00	1.00	2.00	
		75th	2.00	2.00	2.00	2.75	
PLT, [10^3/mL]	N		21	29	26	12	0.001
	Mean		109.52[a]	146.93[b]	264.73[c]	272.00[c]	
	SD		42.666	63.837	62.332	38.657	
	Percentiles	25th	83.50	98.50	197.00	248.25	
		Median	96.00	140.00	275.00	263.00	
		75th	148.00	181.00	313.00	282.50	
WBC, [10^3/mL]	N		21	29	25	12	0.021
	Mean		15.457[a]	14.345[a]	12.992[ab]	10.950[b]	
	SD		5.6085	4.0497	3.4938	1.5193	
	Percentiles	25th	10.800	11.200	10.350	9.525	
		Median	17.800	13.500	12.100	11.200	
		75th	19.650	17.750	16.350	11.600	
Hb, [g/dL]	N		21	29	26	12	0.016
	Mean		10.6000[a]	10.6586[a]	11.5769[b]	11.5250[b]	
	SD		1.44948	1.18609	1.18061	1.25200	
	Percentiles	25th	9.6000	9.8000	10.9000	10.4250	
		Median	10.9000	10.9000	11.8000	11.3000	
		75th	11.6000	11.5500	12.5000	12.6000	
AST, [IU/L]	N		21	29	26	12	0.001
	Mean		115.7619[a]	61.3448[b]	20.2308[b]	19.4167[b]	
	SD		66.05067	40.55972	8.18441	4.01040	
	Percentiles	25th	50.0000	27.5000	13.5000	16.2500	
		Median	121.0000	46.0000	18.5000	19.0000	
		75th	152.0000	93.5000	25.0000	22.0000	
ALT, [U/L]	N		21	29	26	12	0.001
	Mean		111.3810[a]	53.3207[b]	16.2692[c]	12.6667[c]	
	SD		76.59209	48.74625	9.15885	3.65148	
	Percentiles	25th	26.0000	21.0000	9.7500	10.2500	
		Median	117.0000	35.0000	13.5000	12.0000	
		75th	154.0000	70.0000	23.0000	15.7500	
Urea, [mg/dL]	N		21	29	26	12	0.001
	Mean		20.8095[a]	15.3310[b]	8.6231[c]	6.4333[d]	
	SD		6.87836	5.97764	2.37627	2.45872	
	Percentiles	25th	14.7000	10.7000	7.1750	4.6000	
		Median	21.7000	15.0000	8.4500	6.1000	
		75th	25.7000	18.3000	10.0500	8.0000	

Table 1. Demographic and clinical characteristics and biochemical analyses

			EOPE	LOPE	Late-onset PE controls	Early-onset PE controls	p
Creatinine, [mg/dL]	N		21	29	26	12	0.001
	Mean		1.0648[a]	.9500[a]	.6688[b]	.6558[b]	
	SD		.33898	.31988	.14605	.10757	
	Percentiles	25th	0.7450	0.7100	0.5950	0.6025	
		Median	1.1000	0.8300	0.6300	0.6750	
		75th	1.2650	1.2000	0.7000	0.7175	
Gestational week	N		21	29	26	12	0.001
	Mean		30.48[a]	35.90[b]	37.12[c]	37.42[c]	
	SD		2.462	1.566	1.532	2.065	
	Percentiles	25th	28.50	35.00	36.00	37.00	
		Median	32.00	36.00	37.00	38.00	
		75th	32.00	37.00	38.25	39.00	
Birth weight, [g]	N		20	29	26	12	0.001
	Mean		1239.25[a]	2572.45[b]	3044.23[c]	3003.33[c]	
	SD		475.448	722.840	581.914	622.405	
	Percentiles	25th	847.50	1965.00	2460.00	2912.50	
		Median	1215.00	2530.00	3160.00	3150.00	
		75th	1527.50	3275.00	3490.00	3350.00	
Decorin, [pg/mL]	N		21	29	26	12	0.040
	Mean		10.8524	11.0276	9.9750	14.4250	
	SD		4.34714	3.86577	4.56240	5.24632	
	Percentiles	25th	7.4000	8.4500	8.0000	10.8250	
		Median	10.2000	9.5000	10.1000	13.8000	
		75th	15.0000	13.8000	11.8750	19.5000	

EOPE — early-onset preeclampsia; LOPE— late-onset preeclampsia; PE— preeclampsia; SD— standard deviation; BMI— body mass index; SBP— systolic blood pressure; DBP— diastolic blood pressure; PLT— platelet; WBC— white blood cell; Hb— hemoglobin; AST— aspartate aminotransferase; ALT— alanine aminotransferase

The mean DCN level was statistically significantly higher in the early-onset PE controls than late-onset PE controls ($p = 0.040$). Although the mean DCN level was higher in the early-onset PE controls than the EOPE and LOPE groups, it did not reach statistical significance ($p = 0.119$ and $p = 0.117$, respectively). However, based on the p values of these variables, the difference between the groups may be of biological relevance, although not statistically significant.

DISCUSSION

Preeclampsia is one of the complications of pregnancy and is mainly classified into two types according to the time of occurrence: early-onset PE (< 34th week of pregnancy) and late-onset PE (≥ 34th week of pregnancy). 'Early-onset PE occurs in about 10% of all preeclamptic cases and has a complex pathophysiology, the main cause being abnormal placentation with maternal predictive factors. It seems, therefore, reasonable to gain a better understanding of the underlying angiogenic imbalance in early- and late-onset PE and to identify and treat candidate patients at the end of the first trimester, as the incidence of maternal vascular malperfusion and placental vascular lesions are higher in early-onset PE [12].

Failed trophoblast invasion has been proposed the main pathogenetic mechanism in PE. Previous studies have demonstrated that PE is a two-stage disorder: abnormal placentation with reduced placental perfusion in the first stage and maternal systemic pathophysiological changes in the second stage. However, the exact underlying mechanism of the lack of invasion of extravillous trophoblasts in PE remains to be elucidated [13].

Implantation and placentation are essential components of pregnancy which thoroughly rely upon fundamental biological processes invasive trophoblasts, growth factors, growth factor binding proteins, proinflammatory cytokines, proteoglycans, and including highly MMPs. The regulation of MMP activity is the mainstay of these critical processes. Dysregulation of these delicate processes may result in a broad range of pregnancy abnormalities such as PE, IUGR, preterm labor, and miscarriage [14].

In the early period of pregnancy, the fetoplacental development is mediated by a complex cascade system containing growth factors, cytokines, and transcription factors [15]. Decorin is a product of both fetal mesenchymal cells within the placenta and decidual cells in the endometrium. Currently, the role of DCN in stem cell regulation and in the underlying pathogenesis of PE and IUGR has not been fully elucidated. During a recent study, Siddiqui et al. [11] investigated the relation of DCN overexpression in the chorionic villi and/or basal decidua with PE. They reported that basal decidual cell-induced DCN overexpression was related to hypoinvasive phenotype with poor endovascular trophoblast cell differentiation in PE. In addition, the authors found no significant change in DCN levels depending on gestational age during the second trimester in PE patients, although there was an inverse association between the plasma DCN levels and body mass index or body weight. Based on these findings, the authors concluded that increased plasma DCN level might be a predictor of PE before the onset of clinical signs. In another study, Siddiqui et al. [11] found that DCN messenger ribonucleic acid (mRNA) expression at the cellular level showed significantly increased expression in basal plate decidual cells within the placentas from PE (23 to 40 weeks of gestation) patients than controls at all gestational age. Similarly, Nandi et al. [16] found a significant difference in the DCN staining of placental tissues between the PE and control groups. However, at the tissue level, DCN mRNA expression in chorionic villi was similar. In another study, Nandi et al. [17] reported that elevated DCN levels in the maternal blood could be a predictive biomarker for PE.

For a healthy pregnancy, the maternal blood vessel remodeling is driven by the extravillous cytotrophoblasts rather than maternal endothelium. Reduced interstitial invasion and endovascular cytotrophoblasts are associated with IUGR. In their study, Weber et al. [18] described a variety of trophoblast stem cell and pluripotency marker staining patterns based on gestational age and placenta-associated pregnancy complications. The authors concluded that PE, IUGR, and combined PE + IUGR are separate entities based on the differential expression patterns within the placentas complicated with placenta-associated pregnancy complications. We believe that reduced DCN may lead to uncontrolled proliferation and inadequate differentiation of cytotrophoblasts, thereby, resulting in impaired ion-nutrition exchange and decreased hormonal synthesis. More importantly, differentiation of cytotrophoblasts is the cornerstone of healthy placental development in human [15].

In a study, Tan et al. [19] found that abnormal differentiation of trophoblast stem cells was likely to be associated with IUGR. Since certain types of IUGR and PE share a common placental pathology, the authors concluded that overexpression of DCN in the placenta/decidua led to poor trophoblast differentiation in an IUGR subgroup.

Caglar et al. [20] compared DCN levels between pregnancies complicated by idiopathic IUGR and uncomplicated pregnancies and examined the possible relationship between DCN levels and clinical parameters. They found significantly higher maternal serum DCN levels in complicated pregnancies by IUGR and an about 8-times higher risk of high maternal serum DCN levels in complicated pregnancies.

In a study, Murthi et al. [21] collected first trimester tissues via chorionic villus sampling and investigated the temporal relationship between subsequent development of small for gestational age (SGA) and altered DCN expression. The DCN mRNA were determined via using real-time polymerase chain reaction (PCR) and DCN proteins via immunoblotting. The authors showed that DCN mRNA and protein significantly decreased in the placentas from the first-trimester SGA-pregnancies. The aforementioned study is to the first to report a temporal relationship between subsequent development of SGA and altered placental DCN expression in the literature. Similarly, in a previous study of the same researchers, the DCN expression significantly reduced in IUGR compared to gestation-matched controls [22].

The mean DCN level was statistically significantly higher in the early-onset PE controls than late-onset PE controls ($p = 0.040$). Although the mean DCN level was higher in the early-onset PE controls than EOPE and LOPE groups, it did not reach statistical significance ($p = 0.119$ and $p = 0.117$, respectively). However, based on the p values of these variables, we suggest that the difference between the groups may be of biological relevance, although not statistically significant.

Nonetheless, there are some limitations to this study. First, due to the prospective design of the study and termination of the data cut-off date, the number of patients in the control group cannot be increased. Second, we were unable to perform immunohistochemical staining for DCN expression of placental tissues. Despite the lack of any statistically significant difference in the maternal serum DCN samples between the early-onset and late-onset PE groups, no data are available whether there is a significant difference in the DCN level of placental tissues due to the lack of immunohistochemical stating.

In conclusion, although DCN has been thought to be involved in the pathophysiology of PE, our study results show that DCN is not a useful predictive marker of EOPE and LOPE. However, these results might have been yielded due to small sample size of our study. Therefore, further large-scale studies are needed to draw a definitive conclusion. Furthermore, it would be more helpful to gain an insight into the role of DCN in the pathophysiology of PE by measuring the DCN mRNA expression in the basal plate decidual cells within the placenta with immunohistochemical staining.

Conflict of interest

The authors declare no conflict of interest. The authors are solely responsible for the content and writing of the paper.

Financial disclosure

The authors receive no financial support for the study conduct.

Ethical disclosure

A written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee of Bursa YüksekİhtisasTraining and Research Hospital. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Confidentiality of data

All authors of this manuscript declare that they have followed the protocols of publication of patient's data. All caregivers of the participants were informed in detail about the research and signed patient informed consent.

REFERENCES

1. Wallis AB, Saftlas AF, Hsia J, et al. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. Am J Hypertens. 2008; 21(5): 521–526, doi: 10.1038/ajh.2008.20, indexed in Pubmed: 18437143.
2. Saleem S, McClure EM, Goudar SS, et al. Global Network Maternal Newborn Health Registry Study Investigators. A prospective study of maternal, fetal and neonatal deaths in low- and middle-income countries. Bull World Health Organ. 2014; 92(8): 605–612, doi: 10.2471/BLT.13.127464, indexed in Pubmed: 25177075.
3. Lisonkova S, Sabr Y, Mayer C, et al. Maternal morbidity associated with early-onset and late-onset preeclampsia. Obstet Gynecol. 2014; 124(4): 771–781, doi: 10.1097/AOG.0000000000000472, indexed in Pubmed: 25198279.
4. Stepan H, Unversucht A, Wessel N, et al. Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion. Hypertension. 2007; 49(4): 818–824, doi: 10.1161/01.HYP.0000258404.21552.a3, indexed in Pubmed: 17261644.
5. Krusius T, Ruoslahti E. Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. Proc Natl Acad Sci U S A. 1986; 83(20): 7683–7687, doi: 10.1073/pnas.83.20.7683, indexed in Pubmed: 3484330.
6. Schaefer L, Iozzo RV. Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction. J Biol Chem. 2008; 283(31): 21305–21309, doi: 10.1074/jbc.R800020200, indexed in Pubmed: 18463092.
7. Reed CC, Iozzo RV. The role of decorin in collagen fibrillogenesis and skin homeostasis. Glycoconj J. 2002; 19(4-5): 249–255, doi: 10.1023/A:1025383913444, indexed in Pubmed: 12975602.
8. Kinsella MG, Bressler SL, Wight TN. The regulated synthesis of versican, decorin, and biglycan: extracellular matrix proteoglycans that influence cellular phenotype. Crit Rev Eukaryot Gene Expr. 2004; 14(3): 203–234, doi: 10.1615/critreveukaryotgeneexpr.v14.i3.40, indexed in Pubmed: 15248816.
9. Iacob D, Cai J, Tsonis M, et al. Decorin-mediated inhibition of proliferation and migration of the human trophoblast via different tyrosine kinase receptors. Endocrinology. 2008; 149(12): 6187–6197, doi: 10.1210/en.2008-0780, indexed in Pubmed: 18703624.
10. Gogiel T, Galewska Z, Romanowicz L, et al. Pre-eclampsia-associated alterations in decorin, biglycan and versican of the umbilical cord vein wall. Eur J Obstet Gynecol Reprod Biol. 2007; 134(1): 51–56, doi: 10.1016/j.ejogrb.2006.10.003, indexed in Pubmed: 17097211.
11. Siddiqui MF, Nandi P, Girish GV, et al. Decorin over-expression by decidual cells in preeclampsia: a potential blood biomarker. Am J Obstet Gynecol. 2016; 215(3): 361.e1–361.e15, doi: 10.1016/j.ajog.2016.03.020, indexed in Pubmed: 27001218.
12. van der Merwe JL, Hall DR, Wright C, et al. Are early and late preeclampsia distinct subclasses of the disease--what does the placenta reveal? Hypertens Pregnancy. 2010; 29(4): 457–467, doi: 10.3109/10641950903572282, indexed in Pubmed: 20701467.
13. Xu G, Guimond MJ, Chakraborty C, et al. Control of proliferation, migration, and invasiveness of human extravillous trophoblast by decorin, a decidual product. Biol Reprod. 2002; 67(2): 681–689, doi: 10.1095/biolreprod67.2.681, indexed in Pubmed: 12135914.
14. Zhu JY, Pang ZJ, Yu YH. Regulation of trophoblast invasion: the role of matrix metalloproteinases. Rev Obstet Gynecol. 2012; 5(3-4): e137–e143, indexed in Pubmed: 23483768.
15. Huppertz B, Frank HG, Kingdom JC, et al. Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. Histochem Cell Biol. 1998; 110(5): 495–508, doi: 10.1007/s004180050311, indexed in Pubmed: 9826129.
16. Nandi P, Siddiqui MF, Lala PK. Restraint of Trophoblast Invasion of the Uterus by Decorin: Role in Pre-eclampsia. Am J Reprod Immunol. 2016; 75(3): 351–360, doi: 10.1111/aji.12449, indexed in Pubmed: 26554635.
17. Nandi P, Lim H, Torres-Garcia EJ, et al. Human trophoblast stem cell self-renewal and differentiation: Role of decorin. Sci Rep. 2018; 8(1): 8977, doi: 10.1038/s41598-018-27119-4, indexed in Pubmed: 29895842.
18. Weber M, Göhner C, San Martin S, et al. Unique trophoblast stem cell- and pluripotency marker staining patterns depending on gestational age and placenta-associated pregnancy complications. Cell Adh Migr. 2016; 10(1-2): 56–65, doi: 10.1080/19336918.2016.1142035, indexed in Pubmed: 26914354.
19. Tan KH, Tan SS, Ng MJ, et al. Extracellular vesicles yield predictive pre-eclampsia biomarkers. J Extracell Vesicles. 2017; 6(1): 1408390, doi: 10.1080/20013078.2017.1408390, indexed in Pubmed: 29296254.
20. Cağlar M, Yavuzcan A, Göksu M, et al. Decorin: a possible marker for fetal growth restriction. Gynecol Endocrinol. 2014; 30(2): 141–144, doi: 10.3109/09513590.2013.860125, indexed in Pubmed: 24256371.
21. Murthi P, van Zanten DE, Eijsink JJH, et al. Decorin expression is decreased in first trimester placental tissue from pregnancies with small for gestation age infants at birth. Placenta. 2016; 45: 58–62, doi: 10.1016/j.placenta.2016.07.008, indexed in Pubmed: 27577711.
22. Swan BC, Murthi P, Rajaraman G, et al. Decorin expression is decreased in human idiopathic fetal growth restriction. Reprod Fertil Dev. 2010; 22(6): 949–955, doi: 10.1071/RD09240, indexed in Pubmed: 20591329.

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Evaluation of predictive value of biochemical markers for adverse obstetrics outcomes in pregnancies complicated by cholestasis

Milena Guszczynska-Losy[1], Przemyslaw K. Wirstlein[2], Ewa Wender-Ozegowska[2], Malgorzata Kedzia[2]

[1]Gynaecological and Obstetrical University Hospital in Poznan, Poland
[2]Department of Reproduction, Chair of Obstetrics, Gynaecology, and Gynaecological Oncology, Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Objectives: Intrahepatic cholestasis of pregnancy (ICP) is significantly more often associated with an abnormal perinatal outcome compared to a group of healthy pregnant women.
The aim of the study was to analyse the correlation between the adverse perinatal outcome and the biochemical parameters in pregnancy complicated by cholestasis, and to assess their predictive value for neonatal complications.

Material and methods: Eighty-six patients with ICP were divided into 3 groups according to their fasting serum bile acid level [group I n = 60, 10–39.90 µmol/L; group II n = 20, 40–99.90 µmol /L; group III n = 6, TBA (total bile acids) ≥ 100.00 µmol/L]. Linear regression models were created to determine the relation of serum TBA, ALT, and AST concentration with total adverse perinatal outcome, defined as an occurrence of at least one perinatal outcome: stillbirth, preterm birth, spontaneous and iatrogenic preterm birth, presence of meconium in amniotic fluid, Apgar score (< 7 in 5th min), pH from umbilical artery (< 7.1), necessity for NICU admission, the presence of breathing disorders, and the need to perform phototherapy.

Results: TBA ≥ 40.00 µmol/L is connected to an elevated risk of the occurrence of total adverse perinatal outcome (OR = 4.17, p = 0.0037, AUC = 0.62, p = 0.046). TBA ≥ 40.00 µmol/L is a predictor of preterm birth (OR 2.3, p = 0.0117), iatrogenic preterm birth (OR 2.5, p = 0.006), admission to NICU (OR 2.38, p = 0.0094), intubation or assisted ventilation (OR 2.16, p = 0.0301), and phototherapy (OR 2.0, p = 0.0438). The threshold value of TBA for the need for phototherapy was 52.7 µmol/L (AUC = 0.67, p = 0.0089) and for preterm birth, 32.1 µmol/L (AUC = 0.62, p = 0.0251).

Conclusions: Pregnant women with ICP and TBA serum level over 40.00 µmol/L have a worse prognosis regarding obstetric outcomes. The concentration of bile acids is a predictor of the occurrence of adverse perinatal outcomes, although the concentration of ALT and AST failed to show such a connection.

Key words: cholestasis; bile acids; adverse obstetric outcomes

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INTRODUCTION

The birth of a healthy, full-term newborn is the most important goal for every obstetrician. This task becomes a challenge especially if the pregnant woman, who was completely healthy, becomes ill during pregnancy. Intrahepatic cholestasis of pregnancy (ICP) is an illness that emerges in 1% of pregnant women in the second or at the beginning of the third trimester [1]. Cholestasis of pregnancy is the most common liver disorder occurring during pregnancy, with symptoms most commonly subsiding shortly after birth [2]. It is a benign liver condition, however, due to the itching that occurs at night, it can be troublesome for a pregnant woman [3]. Cholestasis is manifested by elevated serum bile acid (TBA, total bile acids) and aminotransferases levels. In fewer than 10% of cases, cholestasis is accompanied with jaundice [4]. Although the disease is benign for the pregnant woman, it may be very dangerous for the foetus, because it is significantly more often associated with an abnormal perinatal outcome, including stillbirth as the most serious one, compared to a group of healthy pregnant women [5].

Corresponding author:
Przemyslaw K. Wirstlein
Department of Reproduction, Chair of Obstetrics, Gynaecology, and Gynaecological Oncology, Poznan University of Medical Sciences, 33 Polna St., 60–535 Poznan, Poland
e-mail: abys@wp.pl

Among other negative events for the newborn resulting from cholestasis, we observe spontaneous and iatrogenic preterm birth, a worse postnatal condition evaluated on the basis of the pH of the umbilical cord blood and Apgar score, hypoxia, the presence of meconium in the amniotic fluid, and admission to a neonatal intensive care unit (NICU) [6]. Despite numerous hypotheses (placenta microstructure disorders, foetal arrhythmia), the mechanism increasing the risk of such complications, including stillbirth in cholestasis patients, has not been identified yet. Also, no therapies preventing this complication exist [7–9]. The meta-analyses carried out so far have led to a conclusion that the occurrence of adverse obstetrics outcomes, including stillbirth, is associated with the concentration of bile acids in the pregnant woman [10]. Kawakita et al. [11] demonstrated that a concentration of TBA ≥100 μmol/L is correlated with the risk of stillbirth, and a concentration ≥ 40.0 μmol/L is correlated with the presence of meconium in the amniotic fluid. Based on the meta-analysis carried out by Glanza et al., it can be concluded that cholestasis with a concentration of bile acids < 40.00 μmol/L will not impact the increase in risk of foetal complications [12]. However, Chen et al. [13] have shown that an adverse obstetric outcome is affected by a concentration of TBA ≥ 57.55 μmol/L. The mentioned neonatal complications occur unpredictably, without any perceivable preceding symptoms. Currently, the only tool for assessing the risk of an abnormal perinatal outcome is the evaluation of the concentration of bile acids in the pregnant woman's serum.

Aim of the study

The aim of the study was to analyse the correlation between the adverse perinatal outcome and the biochemical parameters in pregnancy complicated by cholestasis, and to assess their predictive value for neonatal complications.

MATERIALS AND METHODS

Patients

The analysis included 86 patients with diagnosed Intrahepatic Cholestasis of Pregnancy (ICP), hospitalised in the Gynaecological and Obstetrics Clinical Hospital of Poznan University of Medical Science (GPSK). The research was conducted from January 2017 until December 2018. The protocol of the study was approved by the Bioethics Committee of the Poznan University of Medical Sciences of Karol Marcinkowski in Poznań (1062/16/01.12.2016 and 197/18/01.02.2018). An informed written consent was obtained from each of the patients participating in the study.

The intrahepatic cholestasis of pregnancy was diagnosed on the basis of clinical symptoms (presence of itchiness without skin changes) and abnormal laboratory tests results: elevated concentration of TBA in the serum measured in fasting blood ≥ 10.00 μmol/L, and concentration of aminotransferases: alanine aminotransferase (ALT) > 33 U/L, aspartate aminotransferase (AST) > 32.00 U/L [14]. The exclusion criteria included: other conditions causing pruritus, chronic/acute liver and bile duct diseases (viral or autoimmune hepatitis, primary biliary cholangitis, acute fatty liver, obstructive jaundice, cholecystolithiasis), pre-eclampsia, HELLP syndrome. Each of the women who qualified to participate in the study were Caucasian.

The pregnant women with ICP were divided into three groups, based on the fasting bile acid levels in the serum, reflecting the severity of the illness. Group I, with TBA concentration of 10–39.90 μmol/L, n = 60 (69.77%) with mild cholestasis, group II — TBA 40–99.90 μmol/L, n = 20 (23.25%) presenting ICP with medium severity, and group III — TBA ≥ 100.00 μmol/L, n = 6 (6.98%), comprised of patients suffering from severe cholestasis (Tab. 1) [5].

Treatment

After the diagnosis of cholestasis, all patients were treated with ursodeoxycholic acid (UDCA), starting with a dose of 250 mg three times a day. The medicine's dose was modified subject to the lack of therapeutic effect when treated with a minimal dose (intensified itchiness reported by the patient, elevated TBA level), every few days. The maximal applied dose did not exceed 1500 mg/day.

The concentration of bile acids and aminotransferases was monitored twice a week, or daily, in selected cases.

Every pregnant woman had cardiotocography done four times a day and ultrasonography, along with evaluation of blood flows in the foetal vessels once or twice a week.

Blood from pregnant women suspected of cholestasis was collected from the ulnar vein. The evaluation of bile acids, aminotransferases and bilirubin was performed in GPSK Central Laboratory.

Termination of pregnancy was planned based on TBA levels and the week of pregnancy when cholestasis was diagnosed. In the case of mild cholestasis (TBA < 40.00 μmol/L), the birth took place following the recommendations of PTGiP (the Polish Society of Gynaecologists and Obstetri-

Table 1. Characteristics of patients with cholestasis of pregnancy

Analysed variable	Examined group
Quantity	86
Age [years]	30 (22–46)[a]
Gravidity (number of past pregnancies)	1 (1–6)[a]
TBA 10–39.9 μmol/L	n = 60 (69.77%)
TBA 40–99.9 μmol/L	n = 20 (23.25%)
TBA ≥ 100 μmol/L	n = 6 (6.98%)

[a]median (range)

Table 2. Demographic and laboratory characteristic of pregnant women with mild and severe cholestasis

	TBA < 40.0 µmol/L n=60	TBA ≥ 40 µmol/L n=26	p value
Age [years] mean ± SD	31 ± 4	31 ± 5	0.904
Gravidity, median (range)	1 (1–6)	2 (1–4)	0.287
Gestational age at diagnosis [weeks], median (range)	33 (20–39)	33 (13–39)	0.723
TBA at diagnosis [µmol/L], median (range)	17.1 (7.9–37.3)	66.3 (40.1–171.3)	**< 0.001**
AST at diagnosis [U/L], median (range)	97.95 (16.7–339.2)	167.9 (28.7–695.2)	**0.026**
ALT at diagnosis [U/L], median (range)	183.4 (13.5–620.8)	276.7 (29.6–1228.9)	**0.034**

cians), after the foetus' lungs have matured after the 38th week of pregnancy [15]. With TBA ≥ 100.00 µmol/L, the termination of pregnancy took place following the stimulation of maturity of the foetus' lungs, after the 34th week of pregnancy, and with TBA 40–99.9 µmol/L — after the 36th week of gestation. Depending on the level of cervix maturation, labour was preinduced with 3 g of dinoprostone in cervical gel or a Foley's catheter, while oxytocin in infusion pump was used for induction, as per GPSK scheme (5 IU oxytocin with 49 ml of solvent (0.9% NaCl or 5% glucose), infusion starts at 3 mL/h flow, increased by 0.5 mL/h every 30 minutes, up to 6 mL/h).

In the absence of favourable prognostic conditions for natural labour, the pregnancy was ended by caesarean delivery.

Laboratory examination

The concentration of aminotransferases and the total serum bile acid levels were measured using the electrochemiluminescence method on a Cobas 6000 apparatus (Roche, Basel, Switzerland).

Analysed perinatal outcomes

The following obstetrics outcomes were analysed for the specified groups of patients: the total adverse perinatal outcome, stillbirth, preterm birth, spontaneous preterm birth, iatrogenic preterm birth, presence of meconium in the amniotic fluid, Apgar score (< 7 in 5th minute), pH from the umbilical artery (< 7.10), the necessity for NICU admission, the presence of breathing disorders, and the need to perform phototherapy.

The occurrence of at least one of the above analysed perinatal outcomes was considered a total adverse perinatal outcome.

Statistical analysis

For ROC curve analysis, MedCalc Software (Ostend, Belgium) was used. SigmaStat version 3.5 software (Systat Software, Inc., Point Richmond, CA, USA) was used for statistical analysis. The results were analysed using the Mann-Whitney rank sum test for variables with a non-parametric distribution. Linear regression models were created to determine the relationship of serum TBA, ALAT, AspAT concentration with selected obstetric failures. The Chi-square test and the Fisher Exact Test were used for the assessment of the distribution of the tested characteristics. $P < 0.05$ was considered statistically significant.

RESULTS

Perinatal outcomes

The analysed groups of pregnant women did not differ in terms of demographics. The average time in which cholestasis occurred, both mild and severe, was the 33rd week of pregnancy. However, biochemical parameters, such as TBA, AST and ALT concentration, differed significantly statistically between the groups of pregnant women with a mild form and those with a severe form of cholestasis. (Tab. 2)

The patients with TBA ≥ 40.00 µmol/L gave birth on average two weeks earlier in relation to women with mild cholestasis, which was a statistically significant difference (36th vs 38th week, $p = 0.0087$). No statistically significant differences in the manner of pregnancy termination, the percentage of multiple pregnancies, birth weight in the percentage of newborns, whose weight was lower than the 10th and the 3rd percentile were found in both groups of patients. In the examined group of pregnant women with cholestasis, 67 had a single pregnancy, 18 had a twin pregnancy and one had a triplet pregnancy.

Total adverse perinatal outcome

Among the analysed group of 86 pregnant women with intrahepatic cholestasis, an adverse perinatal outcome was found in 50 women, which included 31 of 60 women (52%) with mild cholestasis, and 19 of 26 women (73%) with a severe form of the illness. This difference was statistically significant ($p = 0.0076$). An adverse obstetrics outcome applied to 42 of 72 (58%) newborns from mothers with TBA < 40.00 µmol/L, and 26 of 34 (76%) newborns from mothers with TBA concentrations ≥ 40.00 µmol/L.

No stillbirths and no newborn deaths occurred with any of the patients in the examined groups.

In the group with severe cholestasis, pregnancies ended prematurely significantly more often than in the group with

Table 3. Perinatal outcomes of patients with mild and severe cholestasis

	TBA < 40.0 µmol/L; n = 60	TBA ≥ 40 µmol/L; n = 26	p value
Gestation age at delivery [weeks], median (range)	38 (31–41)	36 (29–39)	**0.0087[a]**
Multiple pregnancy, n (%)	12 (20%)	7 (27%)	0.669[b]
Birthweight [g], mean ± SD	2942 ± 639	2688 ± 771	0.0770[c]
Birthweight (percentile) < 10 percentile	6 (8%)	2 (6%)	0.6687[b]
< 3 percentile	4 (5%)	0	N/A
Route of delivery, n (%)			
Vaginal	21 (35%)	15 (58%)	0.0501[b]
Caesarean section	33 (55%)	10 (38%)	0.1589[b]
Vacuum	6 (10%)	1 (4%)	0.3378[b]
Total adverse perinatal outcome (n)	31 patients (52%) 42 newborns (58%)	19 patients (73%) 26 newborns (76%)	**0.0076[b]** 0.0581[b]
Stillbirths	0	0	
Preterm delivery < 37th week of gestation (n) (%)	14 (23%)	14 (54%)	**0.0055[b]**
Spontaneous (n) (%)	5 (38%)	2 (14%)	0.3845[d]
Iatrogenic (n) (%)	9 (62%)	12 (86%)	
NICU admission (n) (%),	23 (32%)	17 (50%)	0.0660[b]
NICU length of stay [days], median (range)	4 (3–42)	5 (3–90)	**0.0472 [a]**
Ventilation (n) (%) or intubation	11 (15%)	10 (29%)	0.082[b]
Phototherapy (n) (%)	11 (15%)	13 (38%)	**0.0066[b]**
Breathing problems (n) (%)	14 (19%)	11 (32%)	0.1337[b]
Presence of meconium — stained amniotic fluid (n) (%)	7 (10%)	8 (24%)	0.0531[b]
Apgar score < 7 at 5th min. after birth (n) (%)	2 (3%)	1 (3%)	0.9531[b]
Umbilical arterial pH < 7.10 (n) (%)	1 (1%)	1 (3%)	0.2111[b]

[a]Mann-Withney Rank Sum Test; [b]chi Sqare; [c]Student U-test; [d]2 × 2 Fisher Exact test

mild cholestasis (54% vs 23%, p = 0.0055). In both groups, the decision to terminate gestation before term was more frequent than the occurrence of premature spontaneous delivery, and was 62% and 86%, respectively, for patients with a TBA concentration of < 40.00 µmol/L and ≥ 40.00 µmol/L. The newborns from mothers with severe cholestasis were hospitalised in NICU compared significantly to the newborns of mothers with mild cholestasis (5 days vs 4 days, p = 0.0472) and required phototherapy more often (38% vs 15%, p = 0.0066). However, the necessity to apply ventilation, the occurrence of breathing disorders, the presence of meconium in the amniotic fluid, the Apgar score in 5th minute, and the number of newborns born with a pH from umbilical artery of < 7.10 did not differ between the groups (Tab. 3).

Biochemical markers

Total adverse perinatal outcome

The average TBA concentration in the group of pregnant women with an adverse perinatal outcome was statistically significantly higher in relation to the women with a normal obstetrics outcome, and was, respectively, 45.2 ± 40.5 vs. 25.7 ± 15.3; p = 0.0028. This correlation was confirmed for single pregnancies p = 0.042. No statistical differences were found in the Aspat and Alat concentrations between women with adverse and normal obstetrics outcomes.

Based on the conducted analysis, it was found that, in the group with more severe cholestasis (TBA ≥ 40.00 µmol/L), the chance of the occurrence of a total adverse perinatal outcome is 4.17 times higher than in relation to the group with mild cholestasis (p = 0.037) (Tab. 4). This relationship was separately confirmed for single pregnancies (OR 3.79, p = 0.0127), but no dependence was found for multiple pregnancies (p = 0.9838) (Tab. 4a). The predictive accuracy of the TBA concentration (> 40.1 µmol/L) for a total adverse perinatal outcome was confirmed by means of a ROC curve (p = 0.046) (Tab. 5).

Preterm births

The analysis of the relation of elevated TBA (≥ 40.00 µmol/L) with the occurrence of preterm (iatrogenic and spontaneous) birth shown a statistically significant relationship (OR 2.3, p = 0.0117) in relation to pregnant women

Table 4. OR for predictors of adverse perinatal outcomes in intrahepatic cholestasis of pregnancy

Adverse perinatal outcome	Predictor	OR	95% CI	p value
Total adverse perinatal outcome	**TBA[a]**	**4.17**	**1.59–10.93**	**0.0037**
	AST[b]	0.71	0.48–1.39	0.4468
	ALT[c]	0.74	0.39–1.40	0.3509
Preterm births	**TBA[a]**	**2.3**	**1.21–4.49**	**0.0117**
	AST[b]	0.84	0.39–1.79	0.649
	ALT[c]	0.78	0.44–1.40	0.4048
Iatrogenic preterm births	**TBA[a]**	**2.5**	**1.30–4.85**	**0.006**
	AST[b]	0.95	0.42–2.11	0.8922
	ALT[c]	0.90	0.50–1.64	0.7423
Admission to NICU	**TBA[a]**	**2.38**	**1.24–4.58**	**0.0094**
	AST[b]	1.46	0.63–3.39	0.3815
	ALT[c]	2.72	0.90–2.99	0.4723
Ventilation	**TBA[a]**	**2.16**	**1.08–4.34**	**0.0301**
	AST[b]	1.28	0.44–3.76	0.6512
	ALT[c]	1.34	0.61–2.91	0.4647
Phototherapy	**TBA[a]**	**2.00**	**1.02–3.93**	**0.0438**
	AST[b]	1.06	0.41–2.75	0.9014
	ALT[c]	0.89	0.47–1.71	0.7338
Breathing disorders	TBA[a]	1.88	0.97–3.68	0.0634
	AST[b]	1.22	0.47–3.21	0.6805
	ALT[c]	1.23	0.61–2.45	0.5612
Presence of meconium in amniotic fluid	TBA[a]	1.98	0.81–4.86	0.1361
	AST[b]	2.27	0.37–14.06	0.3791
	ALT[c]	2.49	0.62–10.05	0.2005

[a]Reference category is 0–39.9 µmol/L; [b]Reference category is 0 ≤ 40 IU/L; [c]Reference category is 0 ≤ 40 IU/L

with a lower TBA concentration. The chance of preterm labour for a single pregnancy was 2.9 (p = 0.0259), but this dependence was not confirmed for multiple pregnancies (p = 0.3041) (Tab. 4a). Based on the ROC curve analysis, it was found that a TBA concentration of > 32.0 µmol/L is an optimal predictive factor of preterm labour (p = 0.0251) (Tab. 5).

Iatrogenic preterm birth

Higher concentration of TBA (≥ 40.00 µmol/L) was a significant predictive factor for iatrogenic preterm birth, OR 2.5, p = 0.006 (Tab. 4). This correlation was confirmed for single pregnancies OR 4.2, p = 0.0082, whereas a higher TBA concentration was not a predictor of pregnancy ending in multiple pregnancies (p = 0.3082) (Tab. 4a).

Newborn admission to NICU

For the group with the most severe cholestasis, TBA was a predictor for admitting the newborn to the neonatal intensive care unit (OR 2.38, p = 0.0094) (Tab. 4). The chance of admission of a neonate born by a mother with a TBA of ≥ 40.00 µmol/L to NICU for a single pregnancy was 2.6 times higher than for newborns born by mothers with a low TBA concentration (p = 0.0373). This dependence was not confirmed with multiple pregnancies p = 0.2026 (Tab. 4a).

The use of intubation or ventilation

The correlation of TBA concentration with the use of ventilation showed a statistically significant difference between the analysed groups (p = 0.0301). For newborns born by mothers with more severe cholestasis, the chances of the necessity of ventilation were almost twice as high OR 2.16 (Tab. 4).

The use of phototherapy

The chances for the necessity of phototherapy for newborns from patients with a higher TBA concentration was twice as high as for mothers with a mild form of cholestasis OR 2.0, which was a statistically significant correlation p = 0.0438 (Tab. 4). Based on the ROC curve analysis, it was found that a concentration of TBA ≥ 52.70 µmol/L is a predictive factor of phototherapy p = 0.0089 (Tab. 5).

Breathing disorders

TBA concentration was not a predictor of the occurrence of breathing disorders, p = 0.0634 (Tab. 4).

Presence of meconium in amniotic fluid

There was no correlation between the concentration of TBA and the presence of meconium in amniotic fluid, p = 0.1361 (Tab. 4).

The concentration of neither AST nor ALT was a predictive factor of any of the analysed adverse perinatal outcomes.

DISCUSSION

In the present study, the correlation of bile acid and aminotransferases levels with an adverse perinatal outcome in 86 pregnant women with intrahepatic cholestasis of pregnancy was analysed. Our study has shown that pregnant women with a TBA of ≥ 40.00 µmol/L serum level experienced an adverse obstetrics result significantly more often than pregnant women with a lower TBA concentration.

In the group of patients suffering from severe cholestasis, over 70% of the women and 76% of the newborns experienced adverse perinatal outcomes, including preterm labour, the presence of meconium in the amniotic fluid, admission of the newborn to NICU, the necessity to intubate, and the use of phototherapy. Our results are consistent with the data presented by other authors. In 2017, Cui et al. presented a meta-analysis of 1,928 patients with cholestasis of pregnancy, in which they assessed the relationship between

Table 4a. OR for predictors of adverse perinatal outcomes in intrahepatic cholestasis divided on single and multile of pregnancy

Adverse perinatal outcome	Predictor	Single pregnancy			Multiple pregnancy		
		OR	95% CI	P value	OR	95% CI	P value
Total adverse perinatal outcome	**TBA[a]**	**3.79**	**1.33–10.82**	**0.0127**	1.01	0.32–4.57	0.9838
	AST[b]	0.44	0.17–1.13	0.0884	n/a[d]		
	ALT[c]	0.60	0.3–1.21	0.1551	n/a[d]		
Preterm births	**TBA[a]**	**2.91**	**1.17–7.40**	**0.0259**	2.17	0.46–9.50	0.3041
	AST[b]	**0.39**	**0.16–0.94**	**0.0364**	n/a		
	ALT[c]	0.53	0.24–1.18	0.1212	0.59	0.12–2.77	0.4998
Iatrogenic preterm births	**TBA[a]**	**4.20**	**1.45–12.12**	**0.0082**	1.73	0.60–4.93	0.3082
	AST[b]	0.50	0.19–1.31	0.1565	n/a[d]		
	ALT[c]	0.90	0.35–2.33	0.8296	0.29	0.06–1.38	0.1196
Admission to NICU	**TBA[a]**	**2.60**	**1.06–6.38**	**0.0373**	2.09	0.67–6.52	0.2026
	AST[b]	1.14	0.42–3.06	0.7957	0.3705	0.09–1.44	0.1536
	ALT[c]	1.30	0.57–3.2	0.5032	n/a[d]		
Ventilation	TBA[a]	**2.16**	**1.08 – 4.34**	**0.0301**	0.80	0.11–5.96	0.8298
	AST[b]	1.28	0.44 – 3.76	0.6512	0.59	0.15–2.31	0.4509
	ALT[c]	1.34	0.61 0–2.91	0.4674	1.02	0.52–4.12	0.9825
Phototherapy	TBA[a]	1.05	0.4–2.8	0.916	2.24	0.83–6.0	0.1095
	AST[b]	0.99	0.37–2.64	0.9833	1.37	0.41–4.54	0.604
	ALT[c]	0.63	0.3–1.32	0.2239	n/a[d]		
Breathing disorders	TBA[a]	1.60	0.58–4.46	0.3671	1.96	0.75–5.16	0.1717
	AST[b]	0.95	0.31–2.87	0.9245	0.58	0.03–10.08	0.7108
	ALT[c]	1.24	0.47–3.27	0.6695	0.96	0.31–2.96	0.9424
Presence of meconium in amniotic fluid	TBA[a]	1.7	0.33–6.47	0.6089	2.55	0.66–9.76	0.1732
	AST[b]	n/a[d]			0.33	0.02–1.19	0.4611
	ALT[c]	1.84	0.27–2.37	0.5294	3.36	0.38–30.08	0.2779

[a]Reference category is 0–39.9 µmol/L; [b]Reference category is 0 ≤ 40 IU/L; [c]Reference category is 0 ≤ 40 IU/L; [d]not applicable. All patients represented the same category

Table 5. Results of ROC curves analysis of TBA predictive values for negative neonatal outcomes

Adverse perinatal outcome	AUC [95% CI]	Cut Off [µmol/L]	Sensitivity (true positive rate) [95% CI]	Specificity (true negative rate) [95% CI]	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value	p
Total adverse perinatal outcome	0.62 [0.51–0.71]	40.1	41.43 [29.8–53.8]	87.88 [71.8–96.5]	3.42	0.67	87.9	41.4	**0.046**
Phototherapy	0.67 [0.58–0.76]	52.7	54.17 [32.8–74.4]	84.34 [74.7–91.4]	3.46	0.54	50.0	86.4	**0.0089**
Preterm births	0.62 [0.53–0.72]	32.1	55.56 [40.0–70.3]	72.58 [59.8–83.1]	2.03	0.61	59.5	69.2	**0.0251**

the TBA serum level and the risk of an abnormal perinatal result. The authors concluded that an increase of the TBA level to ≥ 40.00 µmol/L is related to a significantly increased risk of the occurrence of a total abnormal perinatal outcome, preterm labour, the presence of meconium in amniotic fluid, hypoxia, and breathing disorders in newborns [6].

In our study, we found a significantly higher percentage of preterm births in the group of patients with more severe cholestasis. In a study of 106 pregnant women with cholestasis, Chen et al. showed that a concentration of TBA ≥ 40.15 μmol/L is connected to an almost fourfold increased risk of preterm delivery as compared to pregnant women whose TBA concentration is lower than this value. In pregnancies complicated with cholestasis, the number of preterm births grows with the increase of the concentration of bile acids [16]. The results from collective data from meta-analyses also indicate an elevated risk of spontaneous preterm delivery (OR = 3.47) and iatrogenic preterm delivery in pregnant women with cholestasis [5].

In our research, the high percentage of preterm births was due to the high rate of occurrence of iatrogenic preterm labours for both TBA concentration ranges, and was, respectively, 62% and 68% for the group with mild and severe cholestasis. Such high percentages of interventions result from the high proportion of multiple pregnancies (20% and 27%, respectively, in the group with mild and severe cholestasis), and, or primarily to avoid the most serious complication of cholestasis, which is intrauterine foetal death. None of the patients with cholestasis who gave birth in GPSK during this period had intrauterine foetal death or neonatal death. In 2019, Ovadia et al. published a meta-analysis of data 5557 patients with intrahepatic cholestasis of pregnancy, concerning the relationship of bile acid serum concentration with stillbirth. Interpretation of the results obtained allowed the formulation of conclusions that only a concentration of bile acids of over 100.00 μmol/L is related to an increased risk of stillbirth [5]. The risk of intrauterine foetal death increases regardless of pregnancy advancement. The concentration of bile acids does not exceed 100.00 μmol/L in most of the pregnant women suffering from cholestasis. In this group of patients, the risk of stillbirth is comparable with the risk for the general population of pregnant women, in both ranges, 40–100 μmol/L, and < 40 μmol/L.

The group of 6 pregnant women whose bile acid concentrations exceeded 100.00 μmol/L seems, from a clinical perspective, the most interesting one. The average age in that group was 29.6 years (28–32 years). For five of them, it was the second pregnancy, and only one woman had cholestasis in the previous pregnancy. In this group, two patients were in twin pregnancy. Two patients gave birth in completed the 37th week of pregnancy, and four prematurely (respectively, in the 31st, 33rd, 34th, and 36th week of pregnancy). In this group of patients, the average TBA serum concentration was 135.20 μmol/L (102.00–171.30 μmol/L), AST 230.77 U/L (36.00–350.60 U/L), ALT 356.72 U/L (52.30––521.90 U/L). Caesarean section was performed on 5 of the 6 patients. Although the presence of meconium in the amniotic fluid was present twice, all newborns had normal pH in the umbilical cord blood (> 7.3) and the Apgar score in 5th minute ranged from 7 to 10. Three newborns were admitted to NICU: the twins born in the 31st week due to breathing disorders and the necessity to apply mechanical ventilation, and one of the twins from the 33rd week for the same reason. These three newborns also required phototherapy. In both twin pregnancy cases, the decision to terminate the pregnancy was based on very high concentrations of bile acids, which increased despite treatment. In the pregnancy which ended in the 31st week of pregnancy, HELLP syndrome developed additionally.

In our study, neonatal problems significantly more often concerned babies of mothers with severe cholestasis. These newborns were significantly more frequently prematurely born, stayed longer in NICU and required phototherapy more often. Similar results were presented by Garcia-Flores et al. [17] who, in a group of 52 newborns from 47 pregnant women with cholestasis, found significantly more frequent adverse neonatal outcomes, including the presence of meconium in the amniotic fluid, admission to NICU, and neonatal global morbidity.

Our analysis of the relation between adverse obstetrics outcomes and serum bile acids and aminotransferases level showed that a TBA concentration of over 40.00 μmol/L is connected to an elevated risk of the occurrence of total adverse perinatal outcome (OR = 4.17, p = 0.0037, AUC 0.62, p = 0.046). A TBA of ≥ 40.00 μmol/L was also a predictor of preterm labour, iatrogenic preterm labour, admission to NICU, intubation, assisted ventilation, and phototherapy. The correlation was confirmed for single pregnancies, but was not visible in multiple pregnancies, probably due to the small number of patients within the group. In the paper from 2017, Chen et al. [13] showed that a concentration of TBA ≥ 57.55 μmol/L was a significant predictor of the occurrence of an adverse obstetrics outcome (OR = 3.214). The results obtained by Celik et al. [18] show that both preterm births and admission to NICU take place significantly more often if the concentration of TBA in the serum exceeds 34.00 μmol/L. Our analysis of ROC curves showed that a TBA of > 32.00 μmol/L was a significant predictor of preterm delivery, while a TBA of > 52.70 μmol/L was a significant predictor for phototherapy. The above-cited authors showed that the presence of meconium in the amniotic fluid, and foetal distress occur significantly more often if cholestasis occurs before the 34th week of pregnancy, regardless of the concentration of bile acids [18]. Our study fails to confirm the results obtained by the above authors. In our material, the average time of onset of both mild and severe cholestasis was the 33rd week of pregnancy. A study published by Oztas et al. showed that a TBA concentration of ≥ 51.00 μmol/L is a predictor of a low Apgar score in pregnant women with cholestasis. We did not confirm this relationship [19].

Unfortunately, adverse outcomes of pregnancy complicated by cholestasis may occur despite the treatment and reduction of the bile acid serum level [20]. Based on a randomised controlled trial (PITCHES), whose objective was to evaluate whether the application of ursodeoxycholic acid reduces the percentage of adverse perinatal outcomes in women with intrahepatic cholestasis of pregnancy, it was found not to have such an impact. Serious adverse events took place in both the treated group and the placebo group. In both groups, intrauterine foetal death occurred (twice in the placebo group and once in the UDCA- treated group). The stillbirths took place in the 35th and the 37th weeks of pregnancy [21].

The monitoring of pregnant woman with cholestasis should include systematic tests of bile acid serum level and active proceedings, involving elective early termination of pregnancy, in particular with high (> 100.00 µmol/L) concentrations of bile acids. Therefore, these women should be hospitalised in centres where bile acid concentrations are routinely tested, ready for immediate termination of pregnancy and specialist care for premature newborns.

The analysis we presented confirms that a higher TBA concentration is connected with adverse obstetric result in ICP patients. It may be concluded, following the analysis, that pregnant women with a TBA serum level over 40.00 µmol/L, have a worse prognosis regarding obstetric outcomes. The concentration of bile acids is a predictor of the occurrence of adverse perinatal outcomes, although the concentration of ALT and AST failed to show such a connection.

REFERENCES

1. McIlvride S, Dixon PH, Williamson C. Bile acids and gestation. Mol Aspects Med. 2017; 56: 90–100, doi: 10.1016/j.mam.2017.05.003, indexed in Pubmed: 28506676.
2. Yeap SP, Harley H, Thompson R, et al. Biliary transporter gene mutations in severe intrahepatic cholestasis of pregnancy: Diagnostic and management implications. J Gastroenterol Hepatol. 2019; 34(2): 425–435, doi: 10.1111/jgh.14376, indexed in Pubmed: 29992621.
3. Geenes V, Williamson C. Intrahepatic cholestasis of pregnancy. World J Gastroenterol. 2009; 15(17): 2049–2066, doi: 10.3748/wjg.15.2049, indexed in Pubmed: 19418576.
4. Kenyon AP, Piercy CN, Girling J, et al. Obstetric cholestasis, outcome with active management: a series of 70 cases. BJOG. 2002; 109(3): 282–288, doi: 10.1111/j.1471-0528.2002.01368.x, indexed in Pubmed: 11950183.
5. Ovadia C, Seed PT, Sklavounos A, et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. Lancet. 2019; 393(10174): 899–909, doi: 10.1016/S0140-6736(18)31877-4, indexed in Pubmed: 30773280.
6. Cui D, Zhong Y, Zhang L, et al. Bile acid levels and risk of adverse perinatal outcomes in intrahepatic cholestasis of pregnancy: A meta-analysis. J Obstet Gynaecol Res. 2017; 43(9): 1411–1420, doi: 10.1111/jog.13399, indexed in Pubmed: 28691322.
7. Geenes VL, Lim YH, Bowman N, et al. A placental phenotype for intrahepatic cholestasis of pregnancy. Placenta. 2011; 32(12): 1026–1032, doi: 10.1016/j.placenta.2011.09.006, indexed in Pubmed: 22015023.
8. Ibrahim E, Diakonov I, Arunthavarajah D, et al. Bile acids and their respective conjugates elicit different responses in neonatal cardiomyocytes: role of Gi protein, muscarinic receptors and TGR5. Sci Rep. 2018; 8(1): 7110, doi: 10.1038/s41598-018-25569-4, indexed in Pubmed: 29740092.
9. Güven D, Altunkaynak BZ, Altun G, et al. Histomorphometric changes in the placenta and umbilical cord during complications of pregnancy. Biotech Histochem. 2018; 93(3): 198–210, doi: 10.1080/10520295.2017.1410993, indexed in Pubmed: 29366351.
10. Diken Z, Usta IM, Nassar AH. A clinical approach to intrahepatic cholestasis of pregnancy. Am J Perinatol. 2014; 31(1): 1–8, doi: 10.1055/s-0033-1333673, indexed in Pubmed: 23359238.
11. Kawakita T, Parikh LI, Ramsey PS, et al. Predictors of adverse neonatal outcomes in intrahepatic cholestasis of pregnancy. Am J Obstet Gynecol. 2015; 213(4): 570.e1–570.e8, doi: 10.1016/j.ajog.2015.06.021, indexed in Pubmed: 26071912.
12. Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology. 2004; 40(2): 467–474, doi: 10.1002/hep.20336, indexed in Pubmed: 15368452.
13. Chen H, Zhou Y, Deng DR, et al. Intrahepatic cholestasis of pregnancy: biochemical predictors of adverse perinatal outcomes. J Huazhong Univ Sci Technolog Med Sci. 2013; 33(3): 412–417, doi: 10.1007/s11596-013-1133-8, indexed in Pubmed: 23771669.
14. Morton A, Laurie J. The biochemical diagnosis of intrahepatic cholestasis of pregnancy. Obstet Med. 2019; 12(2): 76–78, doi: 10.1177/1753495X18795979, indexed in Pubmed: 31217811.
15. Bomba-Opoń D, Drews K, Huras H, et al. Polish Gynecological Society Recommendations for Labor Induction. Ginekol Pol. 2017; 88(4): 224–234, doi: 10.5603/GP.a2017.0043, indexed in Pubmed: 28509326.
16. Pata O, Vardareli E, Ozcan A, et al. Intrahepatic cholestasis of pregnancy: correlation of preterm delivery with bile acids. Turk J Gastroenterol. 2011; 22(6): 602–605, doi: 10.4318/tjg.2011.0427, indexed in Pubmed: 22287405.
17. Garcia-Flores J, Cañamares M, Cruceyra M, et al. Clinical value of maternal bile Acid quantification in intrahepatic cholestasis of pregnancy as an adverse perinatal outcome predictor. Gynecol Obstet Invest. 2015; 79(4): 222–228, doi: 10.1159/000370003, indexed in Pubmed: 25720981.
18. Çelik S, Çalışkan CS, Çelik H, et al. Predictors of adverse perinatal outcomes in intrahepatic cholestasis of pregnancy. Ginekol Pol. 2019; 90(4): 217–222, doi: 10.5603/GP.2019.0039, indexed in Pubmed: 31059115.
19. Oztas E, Erkenekli K, Ozler S, et al. Can routine laboratory parameters predict adverse pregnancy outcomes in intrahepatic cholestasis of pregnancy? J Perinat Med. 2015; 43(6): 667–674, doi: 10.1515/jpm-2014-0207, indexed in Pubmed: 25294714.
20. Grymowicz M, Czajkowski K, Smolarczyk R. Pregnancy course in patients with intrahepatic cholestasis of pregnancy treated with very low doses of ursodeoxycholic acid. Scand J Gastroenterol. 2016; 51(1): 78–85, doi: 10.3109/00365521.2015.1064990, indexed in Pubmed: 26152830.
21. Chappell LC, Bell JL, Smith A, et al. PITCHES study group. Ursodeoxycholic acid versus placebo in women with intrahepatic cholestasis of pregnancy (PITCHES): a randomised controlled trial. Lancet. 2019; 394(10201): 849–860, doi: 10.1016/S0140-6736(19)31270-X, indexed in Pubmed: 31378395.

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Ovarian preservation and prognosis in adnexal torsion surgery — a retrospective analysis

Xiulan Weng[1], Xi Xie[1], Chaobin Liu[1], Xinru Hong[2], Jingsong Yi[1]

[1]Department of Gynaecology, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

[2]Department of Obstetrics and Gynaecology, 900 Hospital of the Joint Logistics Team, Fuzhou, China

ABSTRACT

Objectives: This study aims to analyze the conditions of ovarian preservation during adnexal torsion surgery, and safety of ovarian preservation.

Material and methods: A retrospective analysis of 130 patients, who underwent surgery for ovarian benign tumor pedicle torsion in Fujian Provincial Maternal and Child Health Hospital from June 2013 to June 2018, was conducted. This study analyses the possible risk factors affecting the operation method using multiple logistic regression and analyses the complications and the recovery of ovarian function after the treatment of the ovarian preservation.

Results: Among these patients, 58 received ovarian cystectomy, while 72 received ovariectomy. There was no significant difference in terms of age, preoperative blood, operation time and surgical bleeding volume between the two groups ($p > 0.05$). However, there was a significant difference in preoperative adnexal blood flow, abdominal pain to the surgical interval, and a collection of torsion cycles ($p < 0.05$). There was an increased risk of ovarian resection in patients whose blood flow of the annex disappeared, whose time of abdominal pain was long, and whose number of twists were significant. For the preservation group, there were no increases in postoperative complications.

Conclusions: According to clinical indicators, such as preoperative adnexal blood flow, abdominal pain to the interval of surgery and the number of torsion cycles, it was determined whether it was feasible to keep the ovary. Retaining the ovary is safe, effective and feasible in adnexal torsion.

Key words: appendix torsion; ovarian preservation; postoperative follow-up; surgical management

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INTRODUCTION

The torsion of female fallopian tubes, ovaries, or both is called adnexal torsion (AT), which account for 2.7% of all gynecologic acute abdomen cases [1], while 71% of cases occur in women of childbearing age [2]. In traditional surgery, the affected adnexa should be resected to prevent the risk of acute pulmonary embolism. The removal of adnexal masses may effect female fertility and endocrine function [3]. With the development of medicine, conservative surgery for maintaining ovarian preservation in young women has been given increasing attention in recent years, but it remains difficult to solve the problem on how to avoid thromboembolism and ensure the recovery of ovarian blood supply function [4]. The present study retrospectively classifies 130 cases of adnexal torsion cases in Fujian Provincial Maternal and Child Health Hospital from June 2013 to June 2018. Furthermore, the possible conditions for ovarian preservation and the follow-up records of the prognosis of patients were analyzed and summarized.

MATERIAL AND METHODS

1.1 General information

From June 2013 to June 2018, Fujian Maternal and Child Health Hospital admitted 130 cases of adnexal torsion, who were within the 12–45 years old age range (after menarche-before menopause). These patients underwent routine preoperative examination to eliminate surgical contraindications. Furthermore, adequate information was collected from all patients prior to the surgery. Patients who needed ovarian preservation are willing to bear the risk

Corresponding author:
Xi Xie
Department of Gynaecology, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China
e-mail: xiexi668@sina.com
phone: 0086-591-87279323, +86-18965912989

Table 1. Comparison of the general information for the preservation group and resection group (mean ± SD)

Group	Cases	Age [y]	Mean diameter of tumor [cm]	Duration of Torsion [h]	Intraoperative blood loss [mL]
Preservation	58	27.05 ± 7.37	7.475 ± 2.40	31.40 ± 24.03	60.00 ± 35.06
Resection	72	30.74 ± 8.64	8.384 ± 2.58	42.65 ± 41.10	51.20 ± 40.10
t		–2.227	–1.816	–1.100	0.615
p		0.068	0.894	0.288	0.195

Table 2. Analysis of other factors in the preservation group and resection group

Factors	β	p	OR	95% CI	
Preoperative blood picture	0.156	0.764	1.169	0.421	3.244
Operation time	–0.110	0.754	0.896	0.451	1.782
Interval from abdominal pain to operation	–0.016	0.017	0.984	0.971	0.997
Intraoperative blood loss	0.001	0.821	1.001	0.994	1.008
Twists number	–0.576	0.037	0.562	0.372	0.967
Attachment blood flow	–1.160	0.000	0.313	0.181	0.542

B — Regression coefficients; p — Significance (p-value); OR — Odds ratio; CI — confidence interval

Table 3. Comparison of general conditions after the operation between the two groups (mean ± SD)

Group	Cases	WBC (^109/L)	Max T [°C]	Days in hospital
Preservation	58	10.09 ± 3.46	37.29 ± 0.42	5.9 ± 2.01
Resection	72	9.68 ± 3.09	37.30 ± 0.34	8.7 ± 2.07
t		0.637	–0.159	–2.525
p		0.921	0.094	0.029

WBC — white blood cell count; Max T — Postoperative maximum body temperature

of postoperative thromboembolic disease, ovarian re-distortion, necrosis, infection and secondary surgery. Among these patients, 58 received ovarian cystectomy (preservation group) and 72 received adnexal resection (resection group). Table 1 presents the overall situation of the two groups.

1.2.1 General treatment

Patients in both groups, who provided adequate information prior to surgery, received laparotomy or laparoscopic surgery. All pathological findings were benign. Each patient received anti-inflammatory symptomatic treatment and subcutaneous injection of low molecular weight heparin of 2,500–5,000 units daily, until discharge.

1.2.2 Follow-up

Postoperative follow-ups were conducted to determine whether there are any complications in patients with ovarian preservation, such as thrombosis, concurrent infection and secondary surgery. In order to evaluate the recovery of ovarian function, these patients were followed up for two years after surgery for menstruation, restoration of blood supply, menstruation and dominant follicles that affect the adnexa, and basic endocrine. The fertility status of women with fertility needs were also determined.

1.2.3 Statistical methods

SPSS 23.0 software was used for the statistical analysis. Data were analyzed by t-test, unconditional single factor, and multi-factor logistic regression. $P < 0.05$ was considered statistically significant.

RESULTS

1. There were no statistically significant differences in terms of age at onset, time of operation, blood loss during the operation and tumor size between the two groups (Tab. 1). However, there were statistically significant differences in the number of torsion cycles, the interval from onset to the operation, and in the adnexal blood flow signals before the operation (Tab. 2).
2. There were no statistically significant differences in blood picture and maximum temperature between the resection group and preservation group, while the difference in time of postoperative hospitalization was statistically significant (Tab. 3).
3. Laparoscopic surgery was performed for 39 patients (39/58) in preservation group and 26 patients (26/72) in the resection group. In the preservation group, there were no serious complications, such as septicemia thromboembolic events and infarction. At one month after the operation, the color doppler ultrasonography examination indicated that the blood flow of the affected side was restored. After 2–3 months,

the average levels of basic hormones were as follows: E2 of 30.21 ± 5.78 pg/mL, FSH of 4.68 ± 0.66 IU/L, LH of 5.01 ± 0.98 IU/L. Furthermore, the menstruation recovered within three months, and dominant follicles were observed in 2–6 months by color Doppler ultrasonography. Among these cases, 33 had a successful pregnancy and the delivery was within two years after the operation. Furthermore, no abortion or premature delivery occurred.

DISCUSSION

Pedicle torsion of ovarian cyst often takes ovarian ischemia as the main pathophysiological change, accompanied by necrosis and infection [5]. Since the torsion of the ovarian cyst pedicle has a higher risk of rupture, embolism and even death, it is clinically suggested that the operation should be performed as soon as possible after the diagnosis, and that the adnexectomy should be performed after clamping the pedicle of the tumor [6]. With the aggravation of the tendency of younger onset of ovarian cysts in recent years, the ovarian preservation in the pedicle torsion of ovarian cysts has gained increasing attention, while the conditions for ovarian preservation have been rarely discussed [7]. In the present study, it was found that the number of torsion cycles, preoperative blood flow of the adnexa, and the interval from onset time to operation were lesser, better and shorter in the preservation group, when compared to the resection group. Studies have shown that when the onset time is short, the number of torsion cycles is few. Furthermore, the preoperative color Doppler ultrasonography indicated that there were blood flow signals in the adnexa, and that young patients, excluding malignant risk, could receive adnexal preservation operation, while postoperative complications should be closely followed up. Once torsion of the adnexal cyst is diagnosed, emergency surgery is required in principle. Meanwhile, in order to prevent the thrombus from falling off and leading to thromboembolism, the operator usually adopts the resection of the affected adnexa as a traditional method [6]. During the operation, the pedicle of the torsion is clamped and removed, which is not feasible for torsion reduction [8]. However, in recent years, with the increasing awareness of the protection of ovarian function, the torsion of the adnexa has been increasingly preserved during the operation. Recent reports have indicated that thromboembolism complications (such as pulmonary embolism) does not increase after the reduction of adnexal torsion [9, 10]. In fact, the incidence of pulmonary embolism in the case of adnexal torsion is very low (some studies report an incidence of 0.2%) [11], and thromboembolic events after conservative surgery only rarely occur. In the present study, the preservation surgery of the adnexa was performed on 58 patients, and the reduction of the torsion followed by cyst removal was performed during the operation. There were no serious complications caused by the thrombus detachment. Furthermore, there were no significant signs of infection in any of the cases after the operation, and there were no statistically significant differences in the highest postoperative temperature and white blood cell count between the preservation group and resection group. However, the length of stay was significantly lower in the preservation group than in the resection group, and the difference was statistically significant. Therefore, it can be considered that the reduction of torsion does not necessarily lead to complications, such as thromboembolism and serious infection.

With the deepening of the understanding of this disease, more and more scholars have tended to perform ovarian cyst resection and reduction. Some scholars have considered that the removal of the ovarian reduction cyst can be performed after the arteriovenous ligation of the affected side. On the one hand, this can prevent the venous thrombosis from falling off and causing an embolism [12]. On the other hand, this preserves the patient's adnexa. However, this destroys the main blood vessels of the ovaries, and has a significant impact on the ovarian function of the affected side. In the present study, the surgery in the preservation group was successfully performed, and the blood vessels were not damaged. Furthermore, the postoperative basal endocrine hormone level did not exhibit a decline in ovarian function. Those who had fertility needs, excluding other factors that may cause infertility, had a successful pregnancy within two years after the operation. These results show that conservative surgery of the adnexa torsion can better protect the fertility and endocrine function of patients.

In the past, the laparotomy exploration was a standard surgical procedure for adnexal torsion. In recent years, with the development of laparoscopy, and the advantages of rapid postoperative recovery, most scholars have supported laparoscopic surgery to rescue the torsion of the ovary [13]. In the present study, laparoscopic surgery was performed on 39 patients (39/58) in the preservation group and on 26 patients (26/72) in the resection group, and the average length of hospital stay was significantly shorter, when compared to the laparotomy. The difference was statistically significant (5.03 ± 2.62 vs 6.82 ± 3.33, $p = 0.020$). Therefore, the preferred surgical procedure for ovarian benign tumor pedicle torsion is laparoscopic exploration [14].

In summary, although surgeries, such as the torsion reduction of adnexa cysts, cannot be considered completely safe, as far as the existing clinical studies are concerned, it can be considered that this type of surgery is of low risk and controllable. The number of torsion cycles, the onset time and the signal of preoperative blood flow are the key reference factors that allowed the investigators to make these clinical decisions. For patients with fertility desires, the func-

tion of the affected adnexa should be preserved as much as possible. However, the article also has its limitations. For example, retrospective studies cannot analyze causality and the sample size is limited. So more strictly designed, rigorously controlled, and large-scale multi-center clinical trials are still needed to determine how to effectively distinguish reversible ischemic adnexa from irreversible necrotic adnexa and identify laboratory indicators that can objectively reflect the vitality of adnexal torsion with good applicability.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Spinelli C, Piscioneri J, Strambi S. Adnexal torsion in adolescents: update and review of the literature[J]. Curr Opin Obstet Gynecol. 2015; 27(5): 320–325.
2. Fujishita A, Araki H, Yoshida S, et al. Outcome of conservative laparoscopic surgery for adnexal torsion through one-stage or two-stage operation. Journal of Obstetrics and Gynaecology Research. 2014; 41(3): 411–417, doi: 10.1111/jog.12534.
3. Moribata Y, Kido A, Yamaoka T, et al. MR imaging findings of ovarian torsion correlate with pathological hemorrhagic infarction. Journal of Obstetrics and Gynaecology Research. 2015; 41(9): 1433–1439, doi: 10.1111/jog.12717.
4. Yan X, Xianling Z, Ting Y. Comparison of clinical features of ovarian cyst torsion in postmenopausal and childbearing age patients . Chinese Journal of Women and Children Health Research. 2016; 27(11): 1399–1401.
5. Childress K, Dietrich J. Pediatric Ovarian Torsion. Surgical Clinics of North America. 2017; 97(1): 209–221, doi: 10.1016/j.suc.2016.08.008.
6. Ashwal E, Krissi H, Hiersch L, et al. Presentation, Diagnosis, and Treatment of Ovarian Torsion in Premenarchal Girls. Journal of Pediatric and Adolescent Gynecology. 2015; 28(6): 526–529, doi: 10.1016/j.jpag.2015.03.010.
7. Rastogi D, Yadav A, Hariprasad S, et al. Neonatal ovarian cyst with torsion – A case report. Current Medicine Research and Practice. 2015; 5(1): 26–28, doi: 10.1016/j.cmrp.2015.02.001.
8. Santos X, Cass D, Dietrich J. Outcome Following Detorsion of Torsed Adnexa in Children. Journal of Pediatric and Adolescent Gynecology. 2015; 28(3): 136–138, doi: 10.1016/j.jpag.2014.04.002.
9. Chu K, Zhang Q, Sun N, et al. Conservative laparoscopic management of adnexal torsion based on a 17-year follow-up experience. Journal of International Medical Research. 2018; 46(4): 1685–1689, doi: 10.1177/0300060517754025.
10. Spinelli C, Buti I, Pucci V, et al. Adnexal torsion in children and adolescents: new trends to conservative surgical approach – Our experience and review of literature. Gynecological Endocrinology. 2012; 29(1): 54–58, doi: 10.3109/09513590.2012.705377.
11. Parelkar SV, Mundada D, Sanghvi BV, et al. Should the ovary always be conserved in torsion? A tertiary care institute experience. J Pediatr Surg. 2014; 49: 465–8.
12. Bin Z, Yan G, Jingjing Li. Report of 62 cases of ovarian cyst pedicled to preserve ovarian laparoscopic surgery[J] . Chinese Journal of Minimally Invasive Surgery. 2014; 7(14): 600–602.
13. Spinelli C, Buti I, Pucci V, et al. Adnexal torsion in children and adolescents: new trends to conservative surgical approach – Our experience and review of literature. Gynecological Endocrinology. 2012; 29(1): 54–58, doi: 10.3109/09513590.2012.705377.
14. Kives S, Gascon S, Dobuc E, et al. Diagnosis and Management of Adnexal Torsion in Children, Adolescents, and Adults. J Obstet Gynaecol Can. 2017; 39(2), doi: 10.1016/j.jogc.2016.10.001.

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Application of medical simulation in the education of medical students in the area of gynecology and obstetrics

Tomasz Klosiewicz[1,2], Radoslaw Zalewski[1], Joanna Faferek[3], Agnieszka Zawiejska[4]

[1]Poznan University of Medical Sciences, Department of Medical Rescue, Poznan, Poland
[2]Polish Society of Medical Simulation, Poland
[3]Department of Medical Education, Jagiellonian University Medical College, Cracow, Poland
[4]Department of Reproduction, Poznan University of Medical Sciences, Poland

ABSTRACT

The education of new generations of doctors faces major challenges. The education system should ensure access to modern and effective educational techniques. Medical simulation is a method that is developing very dynamically. Currently, every medical university in Poland has access to the facilities of a Medical Simulation Centre. Many types of simulations can be used. The variety of techniques is considerable. Starting from simple trainers, through advanced patient simulators to hybrid simulation or virtual reality. Thanks to their use, it is possible to teach basic medical procedures in a safe way, without compromising the patient's intimacy. An additional advantage is the possibility to train in an interdisciplinary team. The aim of this work was to present the possibility of using medical simulation as a method of effective and interesting teaching of medical students in the field of gynaecology and obstetrics. The authors described different techniques and levels of simulation sophistication. The basic tasks of the teacher were also described. The paper may be an interesting complement to the knowledge of education for each physician involved in the work with students.

Key words: high fidelity simulation training; medical education; patient safety; quality of health care; gynaecology

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INTRODUCTION

In interventional specialties, including obstetrics and gynaecology, being on call is necessary to gain skills in dealing with medical emergencies. Young doctors very often simultaneously take shifts in accidents & emergency (A&E) department or ambulance. Shifts like this, often called "a school of life", are necessary to gain technical, communicative and decision making skills.

In 2016, according to the Central Statistics Office, 122,000 patients in A&E where receiving obstetrics and gynaecology treatment. In 2017 this number increased to 127,900 [1]. A good example of this can be hypertensive emergencies. The number of women in Poland suffering from pregnancy-induced hypertension (PIH) is estimated on the level of 30 000 per year. Pre-eclampsia is being diagnosed in around 2–3% of those patients, but there is still a high risk of death for both mother and a child. On the world scale according to World Health Organization (WHO) 12% of all maternal deaths is connected to eclampsia. [2, 3]. Placenta previa is one of the most common causes of perinatal complications [4]. Over 50% of infants delivered in out-hospital setting receive less than 10 points in APGAR scale [5]. Breast cancer is the most frequently recognized cancer among Polish women and is in second place as a cause of death among this group of diseases. In 2012 17,000 of new cases were recognized [6]. The above statistics point out that the ability to recognize maternal or neonatal lethal conditions as well as correct treatment is a real challenge for every doctor, regardless of specialty. The question is if the medical education system is capable to teach students practical skills, which will help them to conquer this challenge?

The main task of medical simulation is to create realistic working conditions, so the student can perform proce-

Corresponding author:
Tomasz Klosiewicz
Poznan University of Medical Sciences, Department of Medical Rescue, Poznan, Poland; Polish Society of Medical Simulation, Poland
e-mail: klosiewicz.tomek@gmail.com

dures in a real-time and real-conditions. Clinical education is an inevitable part of medical education but has many limitations. It is not always possible for all students to perform all necessary procedures. Moreover, if a patient is in deteriorating condition, the treatment is often given by the most experienced specialist. As a result, students have limited ability to make their own decisions. It turns out that less than 4% of residents of obstetrics and gynaecology are confident in their ability to perform procedures without further training. It is estimated that only 28% of people responsible for training residents in this area believe in the effectiveness of learning using phantoms [7].

The purpose of this paper is to familiarize the reader with the medical simulation as an effective teaching method, that can be used in the process of education of medical students.

MODERN FORMS OF SIMULATION

The history of using simulation in obstetrics goes back to the late 19th century. Several types of labour simulators were created then, but one of them — the Budin-Pinard phantom was recommended by J. Whitridge Williams in the paper presented at the Congress of the American Medical Colleges. Since then, the use of simulators has become less popular because an increasing number of women were giving birth in hospitals, which gave doctors the opportunity to train skills in real conditions [8].

Although simulation is often associated with teaching resuscitation, its possibilities go much further. It is currently considered one of the most effective methods of education at the pre- and post-graduate level [9, 10]. The dynamic development of technology has brought new opportunities to use simulations in obstetrics and gynaecology. The degree of the realism of performed activities and environmental conditions is called fidelity of simulation. Fidelity is affected by physical and technical conditions, as well as psychological and environmental factors. In the simplest division we can distinguish the simulation of low and high fidelity.

Low fidelity simulation

Low fidelity simulation is most frequently used to practically perform a short fragmentary action or procedure. A couple examples of simple evaluations used in gynaecology and obstetrics are breast or gynaecology examination simulators (pelvic). They enable the simulation of appropriate pathology and the analysis of anatomically correct patients. Thanks to such simple verification, the student focuses on the examination itself without the need to communicate with the patient. These simulators are usually made of silicone. The possibilities are wide ranging: from setting the weight of the breast through various types of pressure sensors testing the pressure on the glands. Simulators for gynaecological examination can be complete phantoms of a woman or only a part of her body. Thanks to such solutions, not only gynaecological examination can be practiced, but also swab collections, performing vaginal ultrasound, learning the correct anatomical structures and various types of pathologies. Simple standards can also be used in conjunction with a standardized patient. This allows creating an advanced and sophisticated scenario, that helps to provide patient-doctor interaction.

There is a wide range of simulators and standards available on the market. The construction of the appropriate phantom and its technological advancement can be selected depending on the level of experience and skills of the students.

Endoscopy is often used in gynaecology. The ability to operate the endoscope especially under the control of a camera image is often used in simulation. These devices allow to acquire psychomotor skills that are necessary to perform procedures, that is why they can be used at various stages of education. A study on the effectiveness of such a method was carried out. A virtual patient was used in resecting the uterine myoma and evaluating the entire procedure. This study showed a significant skills improvement in all training's participants [11].

High fidelity simulation

High fidelity simulation provides a high level of interactivity and realism for the learner. Nowadays advanced patient simulators are available on the market (HPS, Human Patient Simulator), which in very realistic way can mimic more adequately very specific functions such as physiological and pathological reactions. Modern HPS can imitate presence of the pulse, allows to measure blood pressure, to perform electrocardiography and ultrasonography, to auscultate the chest, examine the abdomen, check pupillary reaction. Seizures, bleeding, sweating, speech, cry, cough or change of the skin colour to cyanotic, pale or yellow can be presented. Neonatal simulators can mimic body movement and muscle tension. Moreover, different pathologies from massive haemorrhage, through eclampsia to cardiac arrest can be presented. There are also complex labour simulators. They have various options for setting and programming scenarios. Focusing only on a delivery, physiological and pathological delivery can be programmed. A child may be born in various positions. Figures 1–3 show various trainers and simulators used to teach the delivery. Adequate simulation type should be chosen based on recipients' knowledge and skills. Introducing very complex simulation cases on the early stages of education, may discourage students from participating in this form of learning. After passing through various stages of teaching activities, knowledge and skills can be combined using increasingly advanced scenarios by moving to high fidelity simulations. Crofts et al. compared

Figure 1. Childbirth trainer for hybrid simulation

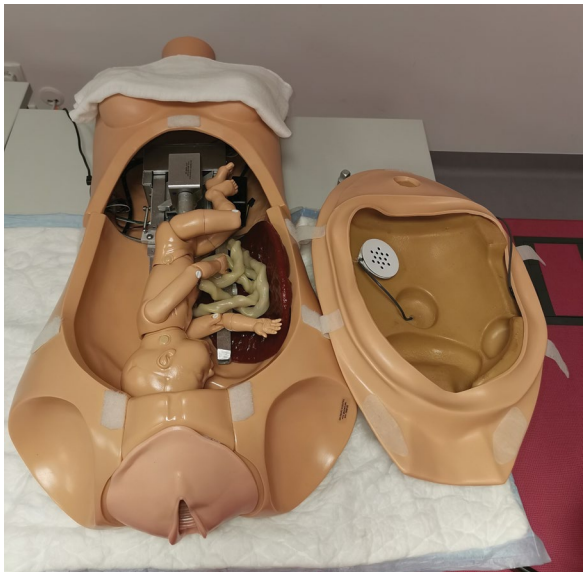

Figure 2. Simple mechanical childbirth trainer

simulation performed with low and high fidelity. Healthcare workers were supposed to deal with shoulder distortion during labour simulation. Both methods improved the results of shoulder dystocia treatment. The use of an advanced simulator gave additional benefits resulting from the possibility of assessing the force used to perform the manoeuvre, as well as communication with the patient [12].

There are also reports of using medical simulation as a tool to check the correct functioning of multi-stage complex medical procedures. Polish research team Puślecki et al. for the first time in Europe, used this method to test the possibility of using extracorporeal membrane oxygenation (ECMO) therapy in a patient after cardiac arrest, respiratory failure and cardiotoxic substances poisoning. In this experiment, one simulator was used at the pre-hospital stage, early hospital care and the operating room. The scope of activities

Figure 3. Human Patient's Simulator

was very wide – from chest compressions to deep veins cannulation. This example shows that simulation can go far beyond the walls of teaching rooms [13].

One of the simulation techniques that most faithfully reflects the doctor's working conditions is the use of a standardized patient (SP). His role is performed by an actor who plays the role of a patient. SP can be both volunteers and full-time employees. Some universities organize training, during which SP work with actors, psychologists and healthcare professionals. SP should be prepared to fulfil the role of the patient and therefore must have appropriate knowledge about the patient's life, current chief complaints and past medical history. Introducing SP into the simulation gives the opportunity to teach not only the correct diagnosis of disease entities, but also a professional approach to the patient, verbal and non-verbal communication, the ability to break bad news, and to deal with a difficult patient.

TEACHER PARTICIPATION IN SIMULATION

The role of the teacher leading the scenario varies depending on the technique or method chosen. In low fidelity simulation, the teacher's task is to present the activity and supervise their correct performance by each student. Therefore, the lecturer must be familiar with the equipment used, the type of trainer and the procedure itself. High fidelity simulation is a bit more demanding because it involves the need to operate the simulator. Learning objectives are achieved by creating a scenario according to which the exercise will run. The duration of the scenario varies from 10–20 minutes. Depending on the learning objectives, it is possible to create an environment for the patient's room, operating theatre, delivery room, ambulance, apartment or street. Simulation centres are most often equipped with properly prepared rooms imitating real conditions. The benefit of these classes is to familiarize the students with the conditions in which he or she will find himself performing professional activities.

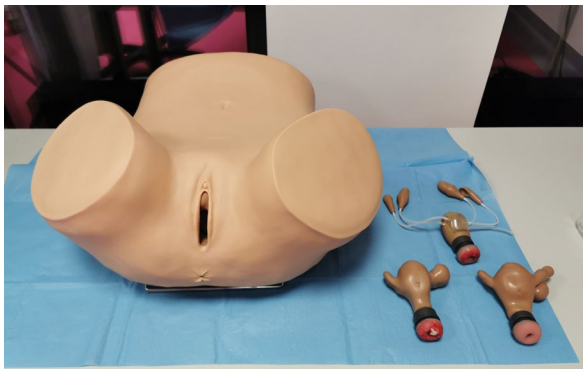

Figure 4. Speculum examination trainer

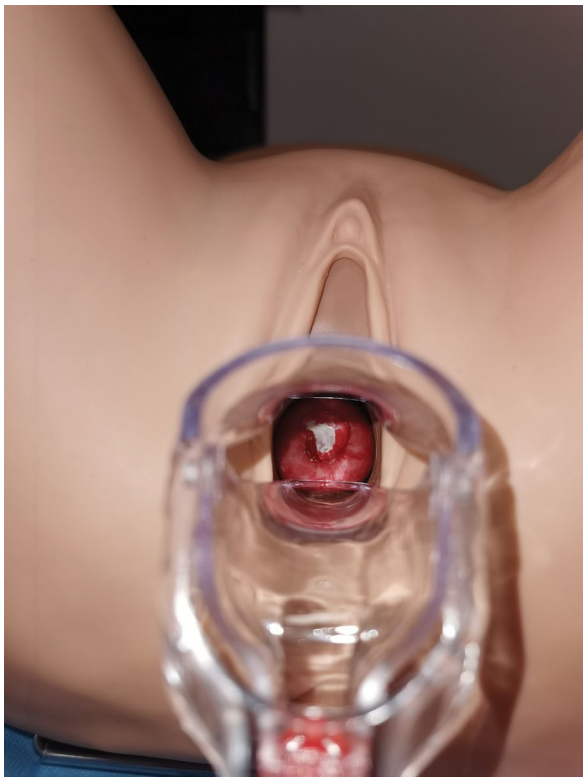

Figure 5. Speculum examination trainer — interior view

Student's work in the simulation room should take place without the teacher's direct presence. The person running the scenario should be in the control room and react to the student's activities by changing the simulator's vital signs. Possible negative consequences resulting from a mistake should occur during the scenario, so that the student is aware of them and has a chance to correct them. Concerning students' decisions, the priority is to make the scenario the basis for further reflection. Safe ways of making mistakes in simulated conditions without risking the health and life of patients is impossible in teaching at the patient's bedside.

Each scenario may be recorded and then presented to students during debriefing. This is the most important part of the simulation. Students together with the teacher analyse their activities. During debriefing the good and bad elements of the procedure should be discussed. The student should draw conclusions for the future. It is possible only when, independently or guided by the teacher, using clinical reasoning, he or she finds the essence of the problem. An important task of the lecturer is to create safe conditions both during training and in the following debriefing.

PATIENT EXAMINATION

The advantage of the simulation method is the ability to learn how to perform the correct examination before the start of clinical classes. There are papers presenting that 33% of medical graduates have never conducted a gynaecological examination [14]. According to patients, the consent or refusal of a vaginal examination was influenced by factors such as: gender (with a predominance of women), age (for the benefit of older students), less formal behaviour and past gynaecological examination experience [15].

Dinh et al. demonstrated the benefits of teaching medical students to perform ultrasound examinations using a standardized patient [16]. Whereas Nitsche et al. [17] concluded that 73% of third-year students were able to correctly assess the degree of cervical dilatation with an accuracy of 1 cm after simulation training with the use of a trainer. A trainer for teaching *per vaginam* examination was presented on figures 4 and 5.

The best method to learn breast examination is to use hybrid simulation. It is a combination of the two methods described earlier, where the standardized patient puts on a breast trainer. A student not only performs palpation, but also interacts with a patient. The superiority of this method over testing the trainer alone was demonstrated both in terms of change detection and student satisfaction [18].

LEARNING TEAM-WORK AND INTERPERSONAL COMMUNICATION

Patient safety during life-threatening situations largely depends on the effectiveness of interdisciplinary teams. In 1999, a landmark report was published, which highlighted medical errors and patient safety. The committee that issued this document states that those responsible for the organization of healthcare must develop programs based on team training, especially when thinking about medics working in critical care [19].

Such a team cannot work effectively based only on the procedures, excellent equipment and technical skills of team members. It is important that people with different medical background participate in one training. Australian researchers have shown a significant improvement in

test results after joint classes preceded by a lecture and instructional video. This form of training was very positively assessed by students. On the other hand, in the opinion of working medics, it allows them to better treat obstetric emergencies [20, 21].

In one of the papers published in 2018, the authors indicated that simulation allows better separation of roles in an interdisciplinary team and acquisition of good practices such as mutual respect [22]. Researchers from the Netherlands found that thanks to the creation of interdisciplinary teams and training based on simulation, the quality of life, quality of health education as well as the quality of medical care during pregnancy are increased [23]. It has also been proven that simulation-based training is an effective strategy for improving communication skills between team members and between the doctor and the patient's family [24].

Draycott et al. assessed the impact of joint training of anaesthesiologists, obstetricians and midwives on reduction in perinatal asphyxia and neonatal hypoxic-ischemic encephalopathy. Infants born with 5-minute Apgar score of ≤ 6 decreased from 86.6 to 44.6 per 10,000 births [25]. The above paper indicates the indisputable value of joint exercise of students from various fields of study already at the stage of undergraduate education.

SIMULATION IMPACT ON PATIENT TREATMENT RESULTS

Medical simulation, especially low-fidelity, allows learning how to perform medical procedures in comfortable conditions and without a risk for patients. Mannell et al. [26] found that the use of high-fidelity simulation significantly increased the absorption of knowledge and practical skills related to receiving physiological delivery among medical students. Other authors specify that already two or three 45-minutes sessions during which each student had the opportunity to receive delivery twice in a session was adequate to obtain minimum competence 6 months after the training. As a result of this form of education, it is also possible to learn more effectively how to deal with shoulder distortion or the management of postpartum haemorrhage [27–29].

After training medical staff from the delivery ward, which used the method of medical simulation, the percentage of neonates requiring assisted ventilation fell from 7.3 to 5.9. At the same time, 24-hour mortality decreased from 11.1/1000 to 7.2/1000 [30]. Moreover, previous simulation training improved management of postpartum haemorrhage reduced the incidence of this complication from 2.1% to 1.3% [31].

Two eight-hour simulation trainings conducted at one-year intervals resulted in a decrease in the number of patients requiring transfusion of five or more blood units [32].

Draycott et al. showed that the development of good training based on medical simulation has a direct impact on the safety of patients in delivery wards. They created a curriculum that aimed to focus on reducing the frequency of neonatal injuries during delivery. It turned out that the incidence of perinatal injuries decreased from 9.3% to 2.3% [33].

LOSS OF KNOWLEDGE

The human mind is not perfect. The skills acquired disappear over time. After one-day simulation training in the field of emergency obstetrics, outcomes fell after about three months and after a year there were no significant changes compared to the group that did not participate in training [34].

On the other hand, the skills of emergency management, both in obstetrics and resuscitation of neonates, acquired during training decreased at different intervals between 6 and 12 months. Adding simulation exercises immediately before students begin clinical classes, improves their ability to assimilate knowledge, slows their loss compared to the group that starts classes in a traditional way [35–36].

SUMMARY AND CONCLUSIONS

Medical simulation is a good method of teaching basic skills as well as expanding knowledge in gynaecology and obstetrics among medical students. It gives a wide range of possibilities from learning to perform simple manual activities, through more complex procedures, to managing available equipment and personnel. It should be used at the beginning of education and precede classes with the participation of patients. The greatest benefits are obtained when classes using medical simulation are repeated regularly. Starting clinical classes with a one-day course based on this method, repeating it at the end of the class and then after a year could bring positive teaching results.

REFERENCES

1. Emergency aid and medical rescue in 2016, Information note. General Statistical Office, 2017. https://stat.gov.pl/files/gfx/portalinformacyjny/pl/defaultaktualnosci/5513/14/1/1/pomoc_dorazna_i_ratownictwo_medyczne_w_2016_r.pdf (5.03.2019).
2. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013; 122(5): 1122–1131, doi: 10.1097/01.AOG.0000437382.03963.88, indexed in Pubmed: 24150027.
3. World Health Organization. The World Health Report 2005 — make every mother and child count. Geneva, 2005.
4. Fan D, Wu S, Wang W, et al. Prevalence of placenta previa among deliveries in Mainland China: A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore). 2016; 95(40): e5107, doi: 10.1097/MD.0000000000005107, indexed in Pubmed: 27749592.
5. Gonda J, Kleszczynski J, Szarpak L, et al. Childbirth in the emergency medical services practice. Am J Emerg Med. 2016; 34(9): 1888, doi: 10.1016/j.ajem.2016.06.068, indexed in Pubmed: 27352983.
6. Zatoński W, Sulkowska U, Didkowska J. Kilka uwag o epidemiologii nowotworów w Polsce. Nowotwory. Journal of Oncology. 2015; 65(3): 179–196, doi: 10.5603/njo.2015.0041.
7. Sheth SS, Fader AN, Tergas AI, et al. Virtual reality robotic surgical simulation: an analysis of gynecology trainees. J Surg Educ. 2014; 71(1): 125–132, doi: 10.1016/j.jsurg.2013.06.009, indexed in Pubmed: 24411435.

8. Owen H, Pelosi MA. A historical examination of the Budin-Pinard phantom: what can contemporary obstetrics education learn from simulators of the past? Acad Med. 2013; 88(5): 652–656, doi: 10.1097/ACM.0b013e31828b0464, indexed in Pubmed: 23524924.
9. Beal MD, Kinnear J, Anderson CR, et al. The Effectiveness of Medical Simulation in Teaching Medical Students Critical Care Medicine: A Systematic Review and Meta-Analysis. Simul Healthc. 2017; 12(2): 104–116, doi: 10.1097/SIH.0000000000000189, indexed in Pubmed: 28704288.
10. Russell E, Hall AK, Hagel C, et al. Simulation in Canadian postgraduate emergency medicine training - a national survey. CJEM. 2018; 20(1): 132–141, doi: 10.1017/cem.2017.24, indexed in Pubmed: 28511730.
11. Elessawy M, Skrzipczyk M, Eckmann-Scholz C, et al. Integration and Validation of Hysteroscopy Simulation in the Surgical Training Curriculum. J Surg Educ. 2017; 74(1): 84–90, doi: 10.1016/j.jsurg.2016.06.007, indexed in Pubmed: 27567366.
12. Crofts JF, Bartlett C, Ellis D, et al. Training for shoulder dystocia: a trial of simulation using low-fidelity and high-fidelity mannequins. Obstet Gynecol. 2006; 108(6): 1477–1485, doi: 10.1097/01.AOG.0000246801.45977.c8, indexed in Pubmed: 17138783.
13. Puślecki M, Ligowski M, Dąbrowski M, et al. The role of simulation to support donation after circulatory death with extracorporeal membrane oxygenation (DCD-ECMO). Perfusion. 2017; 32(8): 624–630, doi: 10.1177/0267659117716533, indexed in Pubmed: 28653554.
14. Bhoopatkar H, Wearn A, Vnuk A. Medical students' experience of performing female pelvic examinations: Opportunities and barriers. Aust N Z J Obstet Gynaecol. 2017; 57(5): 514–519, doi: 10.1111/ajo.12634, indexed in Pubmed: 28488309.
15. Armitage AJ, Cahill DJ. Medical students and intimate examinations: What affects whether a woman will consent? Med Teach. 2018; 40(12): 1281–1286, doi: 10.1080/0142159X.2018.1428736, indexed in Pubmed: 29385938.
16. Dinh ViAm, Frederick J, Bartos R, et al. Effects of ultrasound implementation on physical examination learning and teaching during the first year of medical education. J Ultrasound Med. 2015; 34(1): 43–50, doi: 10.7863/ultra.34.1.43, indexed in Pubmed: 25542938.
17. Nitsche JF, Shumard KM, Fino NF, et al. Effectiveness of Labor Cervical Examination Simulation in Medical Student Education. Obstet Gynecol. 2015; 126 Suppl 4: 13S–20S, doi: 10.1097/AOG.0000000000001027, indexed in Pubmed: 26375554.
18. Nassif J, Sleiman AK, Nassar AH, et al. Hybrid Simulation in Teaching Clinical Breast Examination to Medical Students. J Cancer Educ. 2019; 34(1): 194–200, doi: 10.1007/s13187-017-1287-3, indexed in Pubmed: 29019167.
19. Kohn LD, Corrigan JM, Donaldson MS. To Err Is Human: Building a Safer Health System. Committee on Quality of Health Care in America, Institute of Medicine. 2000.
20. Kumar A, Nestel D, East C, et al. Embedding assessment in a simulation skills training program for medical and midwifery students: A pre- and post-intervention evaluation. Aust N Z J Obstet Gynaecol. 2018; 58(1): 40–46, doi: 10.1111/ajo.12659, indexed in Pubmed: 28656616.
21. Störr A, König-Bachmann M, Schwarz C. [Simulation Training in Obstetrics: Survey of participants in a low-fidelity training]. Z Geburtshilfe Neonatol. 2017; 221(3): 137–144, doi: 10.1055/s-0043-110055, indexed in Pubmed: 28666306.
22. Ruyak SL, Migliaccio L, Levi A, et al. Role development in midwifery education: A place for simulation. Midwifery. 2018; 59: 141–143, doi: 10.1016/j.midw.2018.01.021, indexed in Pubmed: 29427726.
23. Truijens SEM, Banga FR, Fransen AF, et al. The Effect of Multiprofessional Simulation-Based Obstetric Team Training on Patient-Reported Quality of Care: A Pilot Study. Simul Healthc. 2015; 10(4): 210–216, doi: 10.1097/SIH.0000000000000099, indexed in Pubmed: 26222503.
24. Dadiz R, Weinschreider J, Schriefer J, et al. Interdisciplinary simulation-based training to improve delivery room communication. Simul Healthc. 2013; 8(5): 279–291, doi: 10.1097/SIH.0b013e31829543a3, indexed in Pubmed: 23842120.
25. Draycott T, Sibanda T, Owen L, et al. Does training in obstetric emergencies improve neonatal outcome? BJOG. 2006; 113(2): 177–182, doi: 10.1111/j.1471-0528.2006.00800.x, indexed in Pubmed: 16411995.
26. Mannella P, Antonelli R, Montt-Guevara M, et al. Simulation of childbirth improves clinical management capacity and self-confidence in medical students. BMJ Simulation and Technology Enhanced Learning. 2018; 4(4): 184–189, doi: 10.1136/bmjstel-2017-000259.
27. Nitsche JF, Butler TR, Shew AW, et al. Optimizing the amount of simulation training used to teach vaginal delivery skills to medical students. Int J Gynaecol Obstet. 2018; 140(1): 123–127, doi: 10.1002/ijgo.12329, indexed in Pubmed: 28941280.
28. Kordi M, Erfanian F, Fakari FR, et al. The comparison the effect of training by means of simulation and oral method on midwives' skill in management of shoulder dystocia. J Educ Health Promot. 2017; 6: 50, doi: 10.4103/jehp.jehp_115_15, indexed in Pubmed: 28616417.
29. Amod H, Brysiewicz P. Developing, implementing and evaluating a simulation learning package on post-partum haemorrhage for undergraduate midwifery students in KwaZulu-Natal*. Health SA Gesondheid. 2017; 22: 194–201, doi: 10.4102/hsag.v22i0.993.
30. Mduma E, Ersdal H, Svensen E, et al. Frequent brief on-site simulation training and reduction in 24-h neonatal mortality--an educational intervention study. Resuscitation. 2015; 93: 1–7, doi: 10.1016/j.resuscitation.2015.04.019, indexed in Pubmed: 25957942.
31. Nelissen E, Ersdal H, Mduma E, et al. Clinical performance and patient outcome after simulation-based training in prevention and management of postpartum haemorrhage: an educational intervention study in a low-resource setting. BMC Pregnancy Childbirth. 2017; 17(1): 301, doi: 10.1186/s12884-017-1481-7, indexed in Pubmed: 28893211.
32. Egenberg S, Øian P, Eggebø TM, et al. Changes in self-efficacy, collective efficacy and patient outcome following interprofessional simulation training on postpartum haemorrhage. J Clin Nurs. 2017; 26(19-20): 3174–3187, doi: 10.1111/jocn.13666, indexed in Pubmed: 27874995.
33. Draycott TJ, Crofts JF, Ash JP, et al. Improving neonatal outcome through practical shoulder dystocia training. Obstet Gynecol. 2008; 112(1): 14–20, doi: 10.1097/AOG.0b013e31817bbc61, indexed in Pubmed: 18591302.
34. van de Ven J, Fransen AF, Schuit E, et al. Does the effect of one-day simulation team training in obstetric emergencies decline within one year? A post-hoc analysis of a multicentre cluster randomised controlled trial. Eur J Obstet Gynecol Reprod Biol. 2017; 216: 79–84, doi: 10.1016/j.ejogrb.2017.07.020, indexed in Pubmed: 28738295.
35. Nelissen E, Ersdal H, Mduma E, et al. Helping Mothers Survive Bleeding After Birth: retention of knowledge, skills, and confidence nine months after obstetric simulation-based training. BMC Pregnancy Childbirth. 2015; 15: 190, doi: 10.1186/s12884-015-0612-2, indexed in Pubmed: 26303614.
36. Carolan-Olah M, Kruger G, Brown V, et al. Communicating out loud: Midwifery students' experiences of a simulation exercise for neonatal resuscitation. Nurse Educ Pract. 2018; 29: 8–14, doi: 10.1016/j.nepr.2017.10.027, indexed in Pubmed: 29144999.

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GC-MS as a tool for reliable non-invasive prenatal diagnosis of Smith-Lemli-Opitz syndrome but essential also for other cholesterolopathies verification

Aleksandra Jezela-Stanek[1], Anna Siejka[2], Ewa M. Kowalska[2], Violetta Hosiawa[3], Malgorzata Krajewska-Walasek[1,4]

[1]*Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland*
[2]*Department of Biochemistry, Radioimmunology and Experimental Medicine, The Children's Memorial Health Institute, Warsaw, Poland*
[3]*Department of Gynecologic Oncology, Jagiellonian University Medical College, Cracow, Poland*
[4]*Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland*

ABSTRACT

Rare multiple congenital malformations/developmental disorders are challenging in clinical diagnosis. The introduction of next-generation sequencing (NGS) has revolutionized this diagnostic by offering multigene panels or whole-exome sequencing. However, if there is no possibility to perform NGS or if we are facing prenatal ultrasound results, clinical diagnostics is even more difficult. For a selected group of congenital metabolic disorders, resulting from defects in cholesterol biosynthesis (called cholesterolopathies), application of gas chromatography-mass spectrometry (GS-MS) may provide or orientate diagnostics. The most common of these is Smith-Lemli-Opitz syndrome (SLOS), but in this publication, we also want to introduce other cholesterolopathies and draw attention to the possibility of non-invasive prenatal diagnosis of SLOS.

Key words: prenatal diagnosis; GC-MS; prenatal ultrasound; Smith-Lemli-Opitz; cholesterol biosynthesis

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INTRODUCTION

Cholesterol (cholest-5-en-3beta-ol) is a chemical compound belonging to specific lipids called steroids, which common feature is the presence of a carbon skeleton, composed of four coupled rings (steran) (Fig. 1).

In the human body, it occurs in plasma, blood and tissues in both free and fatty acid esterified forms. Endogenous cholesterol is mainly synthesized in the liver, intestines and skin, as well as in the central nervous system [1]. The human brain contains 23% of the cholesterol circulating in the body. Most of it is synthesized in oligodendrocytes and accumulated in myelinated axon sheaths, but it is also found in the cells of neurons and astrocytes. The blood-brain barrier prevents the penetration of cholesterol from the bloodstream, which excludes the regulation of cholesterol levels in the brain through supplementation [1]. The optimal amount of cholesterol in the human body is determined by the correct course of the process of its biosynthesis, which may be disturbed in discussed below syndromes.

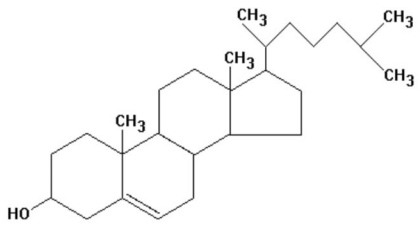

Figure 1. Cholesterol ($C_{27}H_{46}O$) with marked side groups (hydroxyl and methyl)

Although the developing fetus attempts to synthesize cholesterol, in the first weeks of pregnancy, it uses mainly maternal cholesterol. Its transport through the **membranes of the secondary yolk sac (during the first eight weeks of gestation), and later the placenta (when trophoblast takes over its nutritional role) to the fetal circulation**, determines the proper development of the embryo and

Corresponding author:
Aleksandra Jezela-Stanek
Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, 26 Plocka St, 01–138 Warsaw, Poland
e-mail: jezela@gmail.com

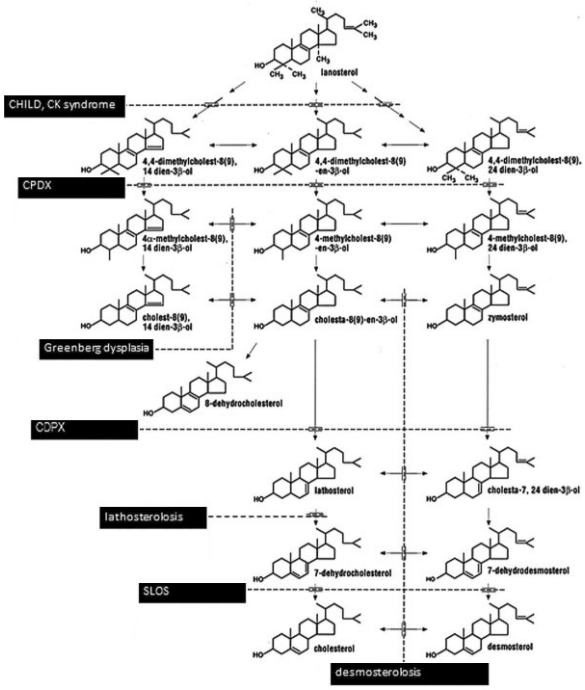

Figure 2. Part of the cholesterol biosynthesis pathway (modified from Nowaczyk MJM, Cunniff C. 2012. Smith–Lemli–Opitz syndrome and other disorders of cholesterol biosynthesis: An introduction. Am J Med Genet Part C Semin Med Genet 160C: 239–241).

the development of most organs. Cholesterol molecules collected by the embryo participate in signalling pathways crucial for embryonic development — they regulate the function of sonic hedgehog (SHH) proteins [2]. These proteins determine the survival and migration of nerve cells and important nuclear receptors, such as the transcription factor for alpha-fetoprotein, which, by binding to DNA GATA sequences, enables the transcription of numerous genes involved in the development of crucial organs [3].

Congenital defects in enzymes of the cholesterol biosynthesis pathway have recently emerged as significant causes of congenital anomalies. Patients with these metabolic diseases present with different malformations that involve many organs and systems [4–6]. To date, nine disorders due to enzymatic defects in post-squalene cholesterol biosynthesis have been identified (Fig. 2). These are:

1. Smith–Lemli–Opitz syndrome (SLOS, OMIM: 270400),
2. X-linked dominant chondrodysplasia punctata type 2 (CDPX2, OMIM: 302960) and MEND (male EBP disorder with neurological defects; OMIM: 300960),
3. Congenital hemidysplasia with ichthyosiform erythroderma and limb defects syndrome (CHILD syndrome, OMIM: 308050),
4. Sterol-C4-methyloxidase-like deficiency (SC4MOL deficiency, OMIM: 607545),
5. CK syndrome [named for the initials of the original proband] (OMIM: 300831),
6. Greenberg/HEM dysplasia (hydrops-ectopic calcification-moth-eaten skeletal dysplasia, OMIM: 215140), (this phenotype is likely due to a laminopathy, but is usually discussed with inborn errors of cholesterol synthesis, see Table 1 for causative details),
7. Antley–Bixler syndrome with ambiguous genitalia (cytochrome P450 oxidoreductase deficiency, POR deficiency, OMIM: 201750),
8. Desmosterolosis (OMIM: 602398),
9. Lathosterolosis (OMIM: 607330).

SMITH-LEMLI-OPITZ SYNDROME

The best-known among the listed diseases is Smith–Lemli–Opitz syndrome caused by a low activity of 3β-hydroxysteroid-D7-reductase (7-dehydrocholesterol reductase, DHCR7). Its role is to convert 7-dehydrocholesterol (7DHC) into cholesterol in the Kandutsch-Russell pathway and 7-dehydrodesmosterol into desmosterol in the Bloch pathway (Fig. 3).

Desmosterol is mainly present in the brain [7]. SLOS, first described in 1964, is also the first multiple malformation syndrome attributed to an inborn error of sterol synthesis [8, 9]. The consequence of a malfunctioning enzyme is primarily the accumulation of 7-DHC in blood and tissue and probably 7-dehydrodesmosterol in the brain [10]. Besides, acting in cells Δ7,Δ8-isomerase converts 7-dehydrocholesterol into 8-dehydrocholesterol, and ,similarly, 7-dehydrodesmosterol in 8-dehydrodesmosterol) [11]. Studies have shown that patients with milder symptoms have normal cholesterol levels in the membranes of nerve cells, which is probably due to its local synthesis. At the same time, it seems that disease symptoms may also be caused by the accumulation of 7,8-dehydrodesmosterol or its oxidised metabolites [10].

Although its clinical presentation may vary, SLOS is usually characterized by prenatal and postnatal growth retardation, microcephaly, moderate to severe intellectual disability, and multiple major and minor malformations, including characteristic facial features, cleft palate, cardiac defects, postaxial polydactyly, 2–3 toe syndactyly, hypospadias, and undervirilization of the genitalia in males [9, 12, personal observation]. There are also data suggesting higher intrauterine mortality of SLOS-affected fetuses [13]. The reported manifestations of Smith–Lemli–Opitz syndrome during the prenatal period (presented in Tab. 1) include, among others: intrauterine growth retardation [14, 15], low maternal unconjugated estriol (MSuE3) [16], or a combination of very rare congenital anomalies, such as ulnar hypoplasia, vertebral segmentation anomalies, congenital pulmonary adenomatoid malformation, fused lungs, laparoschisis, holomyelia, and hypothalamic hamartoma [17].

Table 1. Clinical presentation and diagnostic procedures in disorders with impaired cholesterol biosynthesis

Disorder	Possible prenatal; typical postnatal findings	Causative gene/enzyme	Biochemical markers (pre- and postna tal)
Smith–Lemli–Opitz syndrome	increased nuchal translucency (NT > 3 mm); delayed growth, microcephaly, CNS malformations (i.a., holoprosencephaly), cleft palate, cardiac defects (i.a., atrioventricular canal defect, AVCD), renal and genital anomalies (especially sex reversal in 46,XY fetuses!), polydactyly [27]	*DHCR7*/ 7-dehydrocholesterol reductase	elevated 7-dehydrocholesterol and 8-dehydrocholesterol; very low to normal cholesterol; prenatally also: 7-dehydropregnantriol and 8-dehydroestriol in maternal urine
X-linked dominant chondrodysplasia punctata type 2 (CDPX2 or Conradi-Hunermann-Happle syndrome) --- Emopamil binding protein (EBP) deficiency in males (X-linked recessive syndrome of multiple congenital abnormalities and intellectual disability, allelic to X-linked dominant chondrodysplasia punctate); MEND syndrome (male EBP disorder with neurological defects)	affects females; asymmetric shortening of the long bones, stippling of the epiphyses, clubfoot, joint contractures, heart and renal defects [23]; postnatal skin manifestation (ichthyosis following the Blaschko's lines, scarring alopecia), cataract --- 12 cases reported; CNS malformations (corpus callosum agenesis, Dandy–Walker malformation, hydrocephalus), heart defect, postaxial polydactyly; postnatally facial dysmorphism, epiphyseal stippling [19]	*EBP*/Emopamil binding protein (3b-hydroxysteroid-D8,D7-sterol isomerase) --- Hypomorphic mutations of the *EBP* gene *EBP*/Emopamil binding protein (3b-hydroxysteroid-D8,D7-sterol isomerase)	elevated cholesta-8(9)-en-3β-ol (and 8-dehydrocholesterol) --- elevated cholesta-8(9)-en-3β-ol (and 8-dehydrocholesterol)
Congenital hemidysplasia with ichthyosiform erythroderma and limb defects syndrome (CHILD syndrome)	unilateral limb reduction defects, poly- oligo-, ectrodactyly, unilateral renal malformations, congenital heart defects (10–20% of cases) and punctate epiphyseal calcifications [28]	*NSDHL*/3β-hydroxysteroid dehydrogenase	elevated 4α-monomethyl and 4,4'α-dimethyl sterols or elevated 4α-carboxymethylcholest-8(9)-en-3β-ol (not in plasma, only tissue)
Sterol-C4-methyloxidase–like (SC4MOL) deficiency	5 cases reported; microcephaly in 4 [29]	*MSMO1(SC4MOL)*/Methylsterol monooxygenase 1 (sterol-C4-methyloxidase)	elevated 4α-monomethyl and 4,4'α-dimethyl sterols; low to normal cholesterol (postnatally)
CK syndrome	24 affected males from three unrelated families; microcephaly in all; postnatally: facial dysmorphism and severe developmental delay [28]	Hypomorphic mutations of the *NSDHL* /3β-hydroxysteroid dehydrogenase	elevated 4α-monomethyl and 4,4'α-dimethyl sterols (to a lower degree than in CHILD s.) (not in plasma, only tissue)
Greenberg dysplasia/ Hydrops-ectopic calcification-moth-eaten dysplasia (HEM dysplasia)	lethal prenatally; hydrops fetalis (non-immune), severe limb shortening with disorganized cartilaginous/bony architecture, small thorax	*LBR*/ Lamin B receptor (has sterol-D14-reductase activity)	elevated cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol
Congenital adrenal hyperplasia due to P450 oxidoreductase deficiency	numerous cases reported (5 in Poland) *skeletal, craniofacial, and urogenital anomalies*: craniosynostosis, exophtalmos, radio-humeral synostosis, bowing of the femur/ulna, multiple contractures (resemblance to Antley-Bixler syndrome due to *FGFR2* mutations), ambiguous genitalia in 46,XX and 46,XY fetuses	*POR*/ P450 oxidoreductase (genetics are summarised by Baronio et al. [30]	elevated lanosterol and dihydrolanosterol + presence of markers of adrenal/gonadal CYP21A2, CYP17A1, CYP19A1 deficiency (elevated serum 17α-hydroksyprogesterone, 21-deoxy-cortisol, corticosterone, progesterone, diminished androgens and estrogens)
Desmosterolosis	10 cases reported; agenesis of the corpus callosum with white matter atrophy with/without ventriculomegaly, retromicrognathia with/without cleft palate, distal arthrogryposis, and delay in growth and development [31]	*DHCR24*/ 3b-hydroxysteroid-D24-reductase	elevated desmosterol (and 7-dehydrocholesterol)
Lathosterolosis	5 cases reported with spectrum of congenital anomalies overlaping with those in Smith–Lemli–Opitz s: microcephaly, postaxial polydactyly, horseshoe kidney (1 case), meningocele (2 case) (summarized by Anderson et al. [24]; 1 another with progressive liver involvement [32])	*SC5DL*/ 3b-hydroxysteroid-D5-desaturase (sterol-C5 desaturase)	elevated lathosterol (and raised concentration of 8(9)-cholestenol)

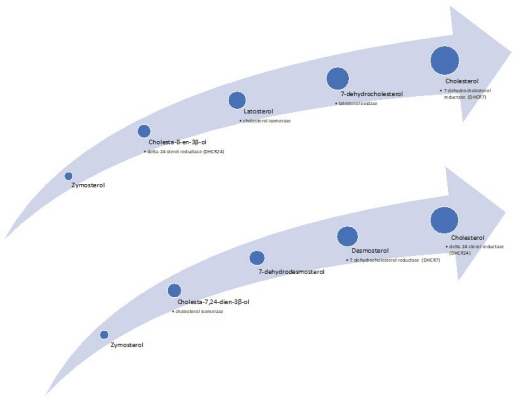

Figure 3. Graphs showing two ways of cholesterol biosynthesis and the most important metabolites of the Kandutsch-Russell (above) and Bloch (below) pathways. Involved enzymes are under the name of a chemical compound

PRENATAL FINDINGS AND POSTNATAL PRESENTATION OF INBORN ERRORS OF CHOLESTEROL BIOSYNTHESIS

Except for Smith–Lemli–Opitz syndrome, other diseases with aberrant cholesterol biosynthesis are rarely diagnosed. Some of them, however, show phenotypic overlap with SLOS, *e.g.*, lathosterolosis [18] or emopamil binding protein (EBP) deficiency in males [19]. Other disorders, *e.g.*, HEM dysplasia, CHILD syndrome, or POR deficiency, may result in specific and somehow recognizable prenatal findings (as noted in Table 1). Since the detailed descriptions of the syndromes mentioned above caused by congenital cholesterol biosynthesis defects have already been discussed in several publications [4, 5, 20–23] reiterating imaging findings in details here is beyond the scope of this manuscript. In Table 1 we listed most significant clinical and detailed biochemical features that are indicative of specific syndromes caused by an inborn error in cholesterol biosynthesis, thus being helpful in prenatal evaluation of fetuses with specific developmental anomalies.

Therefore, we believe that it is worth keeping this group of disorders in mind during prenatal evaluation. It allows ordering the proper biochemical and/or molecular diagnostic tests to establish the diagnosis, verify the carrier status in the couples, specifying the recurrence risk in subsequent pregnancies in the family, as well as implementing adequate support for the child: with a high-cholesterol diet in Smith–Lemli–Opitz syndrome or, as reported just recently, with simvastatin in lathosterolosis [24] as soon as possible. For most rare diseases, including inborn errors of metabolism, before attempting to perform prenatal testing in at-risk families, it is essential to establish the diagnosis in the affected relatives or carrier status in the couples. Nevertheless, as congenital errors of cholesterol biosynthesis may be associated with: a) abnormal ultrasound features (mentioned in Tab. 1), b) a gestational biochemical marker (such as low maternal serum unconjugated estriol, uE_3; abnormal GC-MS results) it is possible, although challenging, to suspect such disorders in the course of a pregnancy, even without a previous index case in the family [18, 26].

LABORATORY DIAGNOSTICS OF INBORN ERRORS OF CHOLESTEROL BIOSYNTHESIS

Cholesterol precursors are specific biochemical markers of its biosynthesis. Hence their quantification in body fluids, amniotic fluid samples or tissues is useful for the diagnosis of cholesterol biosynthesis pathway disorders. The proper prenatal diagnosis of these syndromes is of great value both for the given family (giving a reliable recurrence risk and the possibility to perform prenatal or preimplantation diagnostics) and for scientific studies (by improving current knowledge on human developmental processes).

A rapid and reliable diagnosis may be established using gas chromatography-mass spectrometry (GC-MS). It has been developed and validated for quantitative analysis of five sterols: cholesterol, 7-dehydrocholesterol, desmosterol, lathosterol, and sitosterol in amniotic fluid and plasma [19]. Moreover, reliable prenatal diagnosis of Smith–Lemli–Opitz syndrome may be achieved in a rapid and non-invasive manner by GC-MS analysis of a maternal urine sample [33, 34]. It is based on measurements of specific metabolites, 7-dehydropregnantriol and 8-dehydroestriol, that are **definite** markers for pregnancies with SLOS-affected fetuses. It is noteworthy that Smith–Lemli–Opitz syndrome is the only autosomal recessive (with 25% risk of recurrence), multiple congenital anomaly disorder where such **(urine-based, biochemical)** non-invasive and reliable prenatal diagnosis is available.

Years ago, Department of Biochemistry at CMHI introduced three methods of analysis of SLOS-specific 7- and 8-dehydrometabolites that detect their abnormal concentrations of fetal origin in pregnant women in a single sample of mother's urine, in amniotic fluid and after birth in the child's blood. The first one, as a non-invasive procedure, is clinically very attractive and of great practical value (personal observation). During the analysis the excretion of 7 and 8-dehydropregnanetriol (7-,8-DHPT), pregnantriol (PT), pregnandiol (PD), estriol (E3) and 8-dehydroestriol (8--DHE3) is measured. In the urine of pregnant mothers, high excretion of 7- and 8-DHPT is observed in fetuses affected by SLOS [33] (Fig. 4).

However, the highest diagnostic value of pregnancy affected by SLO syndrome is found in the 7-DHPT/PT and (8-DHE3)/E3 ratios [35]. Increased amounts of 7- and 8-dehydrometabolites of fetal steroid origin expressed in relation to naturally occurring estriol and pregnanetriol in the pregnant mother's urine is a biochemical indicator of a genetic defect

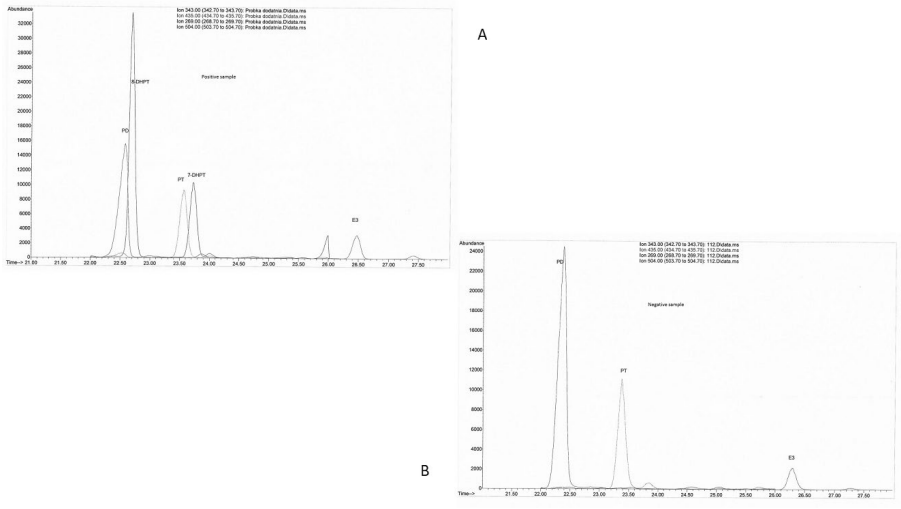

Figure 4. GC/MS results of maternal urine sample in pregnancy; A. affected with Smith-Lemli-Opitz syndrome (note elevated 7- and 8-DHPT); B. healthy child

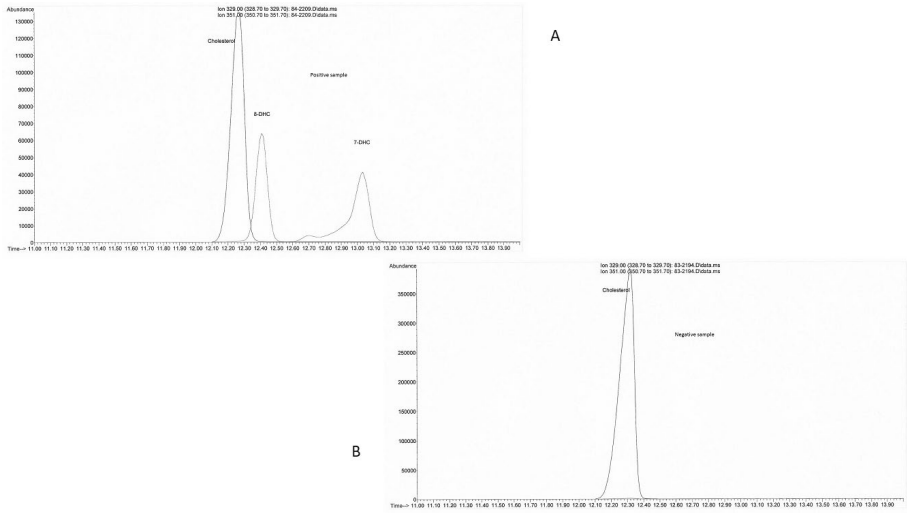

Figure 5. GC/MS of plasma sample from; A. patient affected with Smith-Lemli-Opitz syndrome (note elevated 7- and 8-DHC); B. healthy patients

in the *DHCR7* gene in the fetus. Morning urine samples of pregnant women taken between 13 and 24 weeks of gestation are preferred for analysis.

Another technique to detect DHCR7 deficiency in the fetus is the analysis of amniotic fluid metabolites, where 7- and 8-dehydrocholesterol occurring in trace amounts in children without SLO syndrome are directly quantified. The same chemical compounds are determined in the child's plasma after birth, and their concentration is 50–1000 times higher than the reference norm, which at low cholesterol is a high diagnostic value in SLO syndrome (Fig. 5).

For verification of biochemical results or in cases when prenatal samples are not useful for biochemical tests (borderline normal results or lack of validation for specific markers), molecular analyses may be offered for prenatal detection of the discussed syndromes. They are based on DNA isolated from chorionic villus, amniotic fluid or rarely umbilical blood. Results of biochemical analysis of maternal urine, amniotic fluid and direct CVS analysis are available within 2 or 3 days. Molecular studies of cultured amniocytes or villus cells require 2 to 3 weeks to complete.

CONCLUSIONS

We hope that this article will provide readers with knowledge about a group of disorders characterized by defects in cholesterol biosynthesis, thus increasing awareness in the medical community about their diagnosis, especially in the prenatal period with the application of non-invasive prenatal biochemical test (GC-MS analysis). The validated methodology allows for **cheap (comparing to molecular**

Table 2. Details concerning samples and shipment for testing toward Smith–Lemli–Opitz syndrome to the CMHI

SLOS markers/material	Sample requirements		Necessary forms
7,8-dehydro-cholesterol and cholesterol in **amniotic fluid** (AF); also latosterol, desmosterol (in 13-18 hbd samples)	1 ml AF	protect the tube from sunlight, wrap with dark film	Test Requisition Form available at: www.czd.pl (refer to Pracownia Hormonów Steroidowych i Zaburzeń Metabolizmu)
estriol, 8-dehydroestriol, 7-dehydropregnantriol, pregnantriol, pregnandiol in **maternal urine** + creatinine	single morning urine sample of pregnant women ≥ 13 hbd	as above	as above

testing, including a panel of genes or exome sequencing), fast and reliable diagnosis of some of them. In Poland, it is offered at the Children's Memorial Health Institute, Department of Biochemistry, Radioimmunology and Experimental Medicine. The details of samples and shipment for Smith-Lemli-Opitz syndrome testing are given in the table below.

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Conflict of interest

None.

REFERENCES

1. Miller W, Bose H. Early steps in steroidogenesis: intracellular cholesterol trafficking. Journal of Lipid Research. 2011; 52(12): 2111–2135, doi: 10.1194/jlr.r016675.
2. Wolf G. The function of cholesterol in embryogenesis. J Nutr Biochem. 1999; 10(4): 188–192, doi: 10.1016/s0955-2863(98)00102-8, indexed in Pubmed: 15539288.
3. Baardman ME, Kerstjens-Frederikse WS, Berger RMF, et al. The role of maternal-fetal cholesterol transport in early fetal life: current insights. Biol Reprod. 2013; 88(1): 24, doi: 10.1095/biolreprod.112.102442, indexed in Pubmed: 23153566.
4. Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res. 2011; 52(1): 6–34, doi: 10.1194/jlr.R009548, indexed in Pubmed: 20929975.
5. Herman GE, Kratz L. Disorders of sterol synthesis: beyond Smith-Lemli-Opitz syndrome. Am J Med Genet C Semin Med Genet. 2012; 160C(4): 301–321, doi: 10.1002/ajmg.c.31340, indexed in Pubmed: 23042573.
6. Nowaczyk MJM, Cunniff C. Smith-Lemli-Opitz syndrome and other disorders of cholesterol biosynthesis: An introduction. Am J Med Genet C Semin Med Genet. 2012; 160C(4): 239–241, doi: 10.1002/ajmg.c.31344, indexed in Pubmed: 23042602.
7. DeBarber AE, Eroglu Y, Merkens LS, et al. Smith-Lemli-Opitz syndrome. Expert Rev Mol Med. 2011; 13: e24, doi: 10.1017/S146239941100189X, indexed in Pubmed: 21777499.
8. SMITH DW, LEMLI L, OPITZ JM. A NEWLY RECOGNIZED SYNDROME OF MULTIPLE CONGENITAL ANOMALIES. J Pediatr. 1964; 64: 210–217, doi: 10.1016/s0022-3476(64)80264-x, indexed in Pubmed: 14119520.
9. Nowaczyk MJM, Irons MB. Smith-Lemli-Opitz syndrome: phenotype, natural history, and epidemiology. Am J Med Genet C Semin Med Genet. 2012; 160C(4): 250–262, doi: 10.1002/ajmg.c.31343, indexed in Pubmed: 23059950.
10. Martín MG, Pfrieger F, Dotti CG. Cholesterol in brain disease: sometimes determinant and frequently implicated. EMBO Rep. 2014; 15(10): 1036–1052, doi: 10.15252/embr.201439225, indexed in Pubmed: 25223281.
11. Batta AK, Salen G, Tint GS, et al. Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3 beta-ol) in patients with Smith-Lemli-Opitz syndrome. J Lipid Res. 1995; 36(4): 705–713, indexed in Pubmed: 7616117.
12. Porter FD. Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management. Eur J Hum Genet. 2008; 16(5): 535–541, doi: 10.1038/ejhg.2008.10, indexed in Pubmed: 18285838.
13. Jezela-Stanek A, Ciara E, Małunowicz E, et al. Smith-Lemli-Opitz syndrome Collaborative Group. Differences between predicted and established diagnoses of Smith-Lemli-Opitz syndrome in the Polish population: underdiagnosis or loss of affected fetuses? J Inherit Metab Dis. 2010; 33 Suppl 3: S241–S248, doi: 10.1007/s10545-010-9132-4, indexed in Pubmed: 20556518.
14. Goldenberg A, Wolf C, Chevy F, et al. Antenatal manifestations of Smith-Lemli-Opitz (RSH) syndrome: a retrospective survey of 30 cases. Am J Med Genet A. 2004; 124A(4): 423–426, doi: 10.1002/ajmg.a.20448, indexed in Pubmed: 14735596.
15. Haas D, Haege G, Hoffmann GF, et al. Prenatal presentation and diagnostic evaluation of suspected Smith-Lemli-Opitz (RSH) syndrome. Am J Med Genet A. 2013; 161A(5): 1008–1011, doi: 10.1002/ajmg.a.35837, indexed in Pubmed: 23532938.
16. Shinawi M, Szabo S, Popek E, et al. Recognition of Smith-Lemli-Opitz syndrome (RSH) in the fetus: utility of ultrasonography and biochemical analysis in pregnancies with low maternal serum estriol. Am J Med Genet A. 2005; 138(1): 56–60, doi: 10.1002/ajmg.a.30898, indexed in Pubmed: 16097001.
17. Quélin C, Loget P, Verloes A, et al. Phenotypic spectrum of fetal Smith-Lemli-Opitz syndrome. Eur J Med Genet. 2012; 55(2): 81–90, doi: 10.1016/j.ejmg.2011.12.002, indexed in Pubmed: 22226660.
18. Ho ACC, Fung CW, Siu TS, et al. Lathosterolosis: a disorder of cholesterol biosynthesis resembling smith-lemli-opitz syndrome. JIMD Rep. 2014; 12: 129–134, doi: 10.1007/8904_2013_255, indexed in Pubmed: 24142275.
19. Rossi M, Hall CM, Bouvier R, et al. Radiographic features of the skeleton in disorders of post-squalene cholesterol biosynthesis. Pediatr Radiol. 2015; 45(7): 965–976, doi: 10.1007/s00247-014-3257-9, indexed in Pubmed: 25646736.
20. Amaral C, Gallardo E, Rodrigues R, et al. Quantitative analysis of five sterols in amniotic fluid by GC-MS: application to the diagnosis of cholesterol biosynthesis defects. J Chromatogr B Analyt Technol Biomed Life Sci. 2010; 878(23): 2130–2136, doi: 10.1016/j.jchromb.2010.06.010, indexed in Pubmed: 20630811.
21. Jira P. Cholesterol metabolism deficiency. Handb Clin Neurol. 2013; 113: 1845–1850, doi: 10.1016/B978-0-444-59565-2.00054-X, indexed in Pubmed: 23622407.
22. Kanungo S, Soares N, He M, et al. Sterol metabolism disorders and neurodevelopment-an update. Dev Disabil Res Rev. 2013; 17(3): 197–210, doi: 10.1002/ddrr.1114, indexed in Pubmed: 23798009.
23. Guibaud L, Collardeau-Frachon S, Lacalm A, et al. Antenatal manifestations of inborn errors of metabolism: prenatal imaging findings. J Inherit Metab Dis. 2017; 40(1): 103–112, doi: 10.1007/s10545-016-9992-3, indexed in Pubmed: 27853988.
24. Anderson R, Rust S, Ashworth J, et al. Lathosterolosis: A Relatively Mild Case with Cataracts and Learning Difficulties. JIMD Rep. 2019; 44: 79–84, doi: 10.1007/8904_2018_127, indexed in Pubmed: 30097991.
25. Whittock NV, Izatt L, Simpson-Dent SL, et al. Molecular prenatal diagnosis in a case of an X-linked dominant chondrodysplasia punctata. Prenat Diagn. 2003; 23(9): 701–704, doi: 10.1002/pd.667, indexed in Pubmed: 12975777.
26. Konstantinidou A, Karadimas C, Waterham HR, et al. Pathologic, radiographic and molecular findings in three fetuses diagnosed with HEM/Greenberg skeletal dysplasia. Prenat Diagn. 2008; 28(4): 309–312, doi: 10.1002/pd.1976, indexed in Pubmed: 18382993.
27. Jezela-Stanek A, Małunowicz E, Anna S, et al. Trends in prenatal diagnosis of non-specific multiple malformations disorders with reference to the own experience and research study on Smith-Lemli-Opitz syndrome. Ginekol Pol. 2015; 86(8): 598–602, doi: 10.17772/gp/57851, indexed in Pubmed: 26492708.

28. du Souich C, Raymond FL, Grzeschik KH. et al. NSDHL-Related Disorders. 2011 Feb 1 [Updated 2018 Oct 25]. In: Adam MP, Ardinger HH, Pagon RA. et al. ed. GeneReviews®. University of Washington, Seattle 1993-2019: [Internet].
29. Frisso G, Gelzo M, Procopio E, et al. A rare case of sterol-C4-methyl oxidase deficiency in a young Italian male: Biochemical and molecular characterization. Mol Genet Metab. 2017; 121(4): 329–335, doi: 10.1016/j.ymgme.2017.06.013, indexed in Pubmed: 28673550.
30. Baronio F, Ortolano R, Menabò S, et al. 46,XX DSD due to Androgen Excess in Monogenic Disorders of Steroidogenesis: Genetic, Biochemical, and Clinical Features. Int J Mol Sci. 2019; 20(18), doi: 10.3390/ijms20184605, indexed in Pubmed: 31533357.
31. Rohanizadegan M, Sacharow S. Desmosterolosis presenting with multiple congenital anomalies. Eur J Med Genet. 2018; 61(3): 152–156, doi: 10.1016/j.ejmg.2017.11.009, indexed in Pubmed: 29175559.
32. Prasun P, Ferguson E, Iverson A, et al. Lathosterolosis: An Extremely Rare Inherited Condition Associated With Progressive Liver Disease. J Pediatr Gastroenterol Nutr. 2019; 69(5): e142–e145, doi: 10.1097/MPG.0000000000002434, indexed in Pubmed: 31259789.
33. Shackleton CH, Roitman E, Kratz LE, et al. Midgestational maternal urine steroid markers of fetal Smith-Lemli-Opitz (SLO) syndrome (7-dehydrocholesterol 7-reductase deficiency). Steroids. 1999; 64(7): 446–452, doi: 10.1016/s0039-128x(99)00026-4, indexed in Pubmed: 10443900.
34. Jezela-Stanek A, Małunowicz EM, Ciara E, et al. Maternal urinary steroid profiles in prenatal diagnosis of Smith-Lemli-Opitz syndrome: first patient series comparing biochemical and molecular studies. Clin Genet. 2006; 69(1): 77–85, doi: 10.1111/j.1399-0004.2006.00551.x, indexed in Pubmed: 16451140.
35. Shackleton CH, Roitman E, Kratz L, et al. Dehydro-oestriol and dehydropregnanetriol are candidate analytes for prenatal diagnosis of Smith-Lemli-Opitz syndrome. Prenat Diagn. 2001; 21(3): 207–212, doi: 10.1002/1097-0223(200103)21:3<207::aid-pd27>3.0.co;2-i, indexed in Pubmed: 11260610.

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Right-sided ovarian ectopic pregnancy with Jaydess *in situ*

Lukasz Panasowiec, Maja Kufelnicka-Babout, Piotr Sieroszewski

Fetal Medicine and Gynecology Department, Medical University of Lodz, Poland

ABSTRACT

The estimated prevalence of the ectopic pregnancy (EP) is 1–2% of all pregnancies. Ovarian pregnancy is a rare finding with an incidence rate of 0.15% of all pregnancies and 1–3% of ectopic gestations. The use of intrauterine device (IUD) is a significant risk factor of ectopic pregnancy. Jaydess levonorgestrel intrauterine system (LNG-IUS) is considered as an extremely reliable method of contraception with the cumulative Pearl index of approx. 0.9% after a three-year period of use. This study presents a case of failure of the Jaydess intrauterine device *in situ* in a female patient with positive Beta Human Chorionic Gonadotropin (serum b-HCG) who was diagnosed with right-sided ovarian ectopic pregnancy. Although LNG-IUS represents the group of the most efficient contraception methods, the risks of failure still exist and should be taken into consideration. Before the insertion, every female patient should be fully informed on the potential adverse effects by a health practitioner.

Key words: ovarian ectopic pregnancy; contraception; intrauterine device failure; Jaydess levonorgestrel intrauterine system; life-threatening hemorrhage; natural conception

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INTRODUCTION

Ovarian ectopic pregnancy (OEP) is a rare gynecological condition. It commonly ends with an ovarian rupture before the end of the first trimester [1], which leads to a life-threatening hemorrhage. It is worth mentioning that the incidence of OEP after natural conception oscillates between 1 in 2.000 and 1 in 60.000 deliveries and corresponds with 3% of all ectopic pregnancies. Jaydess intrauterine device system contains 13.5 mg of levonorgestrel. It is inserted in the uterus for a definite time period and works as a long-acting, reversible contraceptive measure. It is considered to be one of the most reliable and safest methods.

CASE REPORT

A patient from our clinic was a 33-year-old woman with three prior natural deliveries. She had an appendectomy as a child and denied suffering from chronic or adnexal diseases. The patient was hemodynamically efficient, with normal skin color and temperature, BP 136/76 mmHg and heart rate (HR) 80/min. She was admitted with severe lower abdominal pain. The pain was persistent for the duration of three days, with a tendency to increase. The patient's last menstrual period (LMP) was nearly 2 weeks before and her menstrual cycles were regular. On admission, blood tests were performed, including serum b-HCG. WBC = 11.5 x 10^3/μL, CRP = 5.5 mg/L, HGB = 13.5 g/dL. The pregnancy test was positive, with increasing serum b-HCG level (739 mLU/mL → 790 mLU/mL). On the physical examination, the abdomen showed no peritoneal symptoms. In the speculum the vaginal shield of the cervix was normal, IUD threads were invisible. There were no signs of bleeding or staining. The vaginal examination showed the uterus in a neutral position, regular in size and painless; there were no palpable adnexal masses; the pouch of Douglas presented no abnormalities or tenderness. Transvaginal sonography revealed the uterus of normal size with the IUD (Jaydess) placed correctly in the uterine cavity, with no gestational sac, the right ovary measuring 40 x 23 mm and the left ovary measuring 36 x 20 mm. TVS showed echogenically dense fluid (4 cm pocket) in the rectouterine pouch. All the clinical signs suggested tubal ectopic pregnancy. The patient was qualified for a laparoscopic surgery which revealed rupture of the right ovary and signs of bleeding. The aborted gestational sac was found in the rectouterine pouch. The intraperitoneal adhesions were released, the rupture of the ovary was surgically corrected and the aborted gestational sac with a volume of about 150 mL of blood was evacuated. The IUD (Jaydess) was removed from the uterine cavity. The patient was discharged from the hospital after 2 days. The histopathological examination confirmed the OEP.

DISCUSSION

According to various studies, other locations of the EP may also apply in 1.3% the abdomen and in 3.2% the ovary [2]. The OEP appears in 1/25.000–40.000 pregnancies and is difficult for clinical as well as ultrasound diagnosing. Some ectopic ovarian pregnancies may result from the insertion of IUDs. Intrauterine contraceptive devices are discovered in about 20% of patients with the non-ovarian EP and are uncovered in 57–90% of patients with primary ovarian pregnancy [3]. It may also be caused by altered tubal motility, which facilitates implantation of pregnancy in the ovary [4]. IUDs do not protect against implantation in the ovary [5]. The OEP can be misinterpreted as a ruptured corpus luteum cyst. Chronic minor pelvic pain is the most common clinical feature of ovarian localization of the pregnancy as it was in the case of our patient. In addition, there may be palpable an adnexal mass upon examination. The diagnosis is frequently established during a surgery and requires histological confirmation. However, the diagnosis of ovarian pregnancy is possible in only about 28% of cases during surgical procedures. Although ultrasonography may suggest the proper diagnosis, surgery remains the best method for diagnosis and management.

To conclude, women with a history of ectopic pregnancy should carefully consider the pros and cons of re-insertion of an IUD. In addition, all women who decide to choose the system should be instructed in detail on the risks of using the LNG-IUS as a method of contraception by a medical practitioner.

REFERENCES

1. Shrestha A, Chawla CD, Shrestha RM. Ruptured Primary Ovarian Pregnancy: A Rare Case Report. Kathmandu University Medical Journal. 2013; 10(3): 76–77, doi: 10.3126/kumj.v10i3.8026.
2. Sergent F, Mauger-Tinlot F, Gravier A, et al. Grossesses ovariennes: réévaluation des critères diagnostiques. J Gynecol Obstet Biol Reprod (Paris. 2002; 31: 741–746).
3. Gaudoin MR, Coulter KL, Robins AM, et al. Is the incidence of ovarian ectopic pregnancy increasing? European Journal of Obstetrics & Gynecology and Reproductive Biology. 1996; 70(2): 141–143, doi: 10.1016/s0301-2115(95)02557-x.
4. Raziel A, Golan A, Pansky M, et al. Ovarian pregnancy: A report of twenty cases in one institution. American Journal of Obstetrics and Gynecology. 1990; 163(4): 1182–1185, doi: 10.1016/0002-9378(90)90685-z.
5. Herbertsson G, Magnusson S, Benediktsdottir K. Ovarian pregnancy and iucd use in a defined complete population. Acta Obstetricia et Gynecologica Scandinavica. 1987; 66(7): 607–610, doi: 10.3109/00016348709022065.

Corresponding author:
Lukasz Panasowiec
Fetal Medicine and Gynecology Department, Medical University of Lodz, Poland
e-mail: lukaszpanasowiec@interia.pl

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