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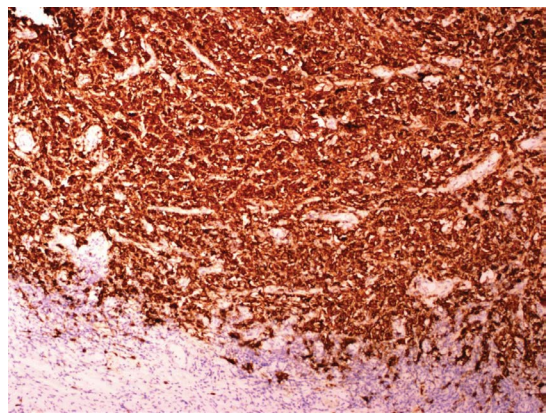

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The impact of competitive sports on menstrual cycle and menstrual disorders, including premenstrual syndrome, premenstrual dysphoric disorder and hormonal imbalances

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ABSTRACT

Objectives: With the considerable increase of female participation in youth sports, it has become crucial for medical professionals, coaches and parents to improve their competitiveness by understanding the conditions for which these females are at elevated risk and mitigating possible health consequences.
The aim of this study was to evaluate the effect competitive sports have on the disorders of the menstrual cycle, to investigate the frequency of PMS (premenstrual syndrome)/PMDD (premenstrual dysphoric order) in professional female athletes and to identify risk factors predisposing for PMS and PMDD. Additionally, the levels of selected hormones such as serum estradiol, FSH, LH and prolactin were investigated to identify any hormonal perturbances that might have influence or be the risk factors for menstrual dysfunctions.

Material and methods: The study group consisted of 75 professional athletes (girls and young women at the age of 16–22) who lived on the territory of Silesia. The control group consisted of 50 girls and young women at the same age, who did not practice any sport. The research tools included daily diary of PMS symptoms created in line with The American College of Obstetricians and Gynecologists (ACOG) recommendations and ICD-10 diagnostic criteria, daily diary of PMDD symptoms created according to DSM-V diagnostic criteria of the American Psychiatric Association (APA) and premenstrual symptoms screening tool (PSST).

Results: The analysis of menstrual cycle disorders showed statistical significance for heavy menstrual bleeding ($p = 0.01$) and longer breaks between menstrual bleeds ($p = 0.01$). PMDD was diagnosed in 8% and PMS in more than 42% of respondents. The incidence of PMDD was not at significant variance between the groups (9.33% in contrast to 6.0%), while incidence of PMS was statistically different in both groups ($p = 0.045$) (49.33% vs 32.0%). A significant correlation between PMS, average age ($p = 0.00001$) and menarche age ($p = 0.03$) in young active athletes has been shown. The risk of PMS increased with age (by 1.71 with each year) ($p = 0.0007$).

Conclusions: A number of other risk factors predisposing for PMS and PMDD has also been identified. The findings of these researches will enable the athletic care network to provide better care for young female athletes.

Key words: menstruation cycle; menstrual disorders; premenstrual syndrome; premenstrual dysphoric disorder; sex hormones

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INTRODUCTION

Over the past several decades there has been a dramatic increase in youth sport participation. The number of female athletes skyrocketed: a 2012 longitudinal national survey found that the number of high school aged girls participating in competitive sport has increased tenfold [1].

Unsurprisingly, girls and young women benefit from engaging in sport activities; they better their health and life skills as well as uncover their passions [2]. It is also important because it increases their self-confidence. The direct relationship between athletics and women in leadership roles has been proved by a 2013 Erst and Young survey of

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821 high-level executives, which demonstrated that 96% of the women in chief executive positions had played sport [3].

However, engaging in sport can also be a risk factor for unhealthy behaviours, high training loads and metabolic disturbances in a subset if vulnerable athletes [4]. An official document entitled Female Athletes Issues for the Team Physician: A Consensus Statement — 2017 Update confirms that female athletes suffer musculoskeletal injuries and medical problems that come from and/or impact athletic activity. It also highlights that team physicians are required to recognize the gender-specific ramifications of a number of issues including menstrual dysfunctions because in the athlete, it is at the minimum two to three times more frequent than in the non-athlete. Female athletes are also at higher risk of primary and secondary amenorrhea [5].

Long-term training periods or participation at competitions also weaken ovarian activity, which can manifest as luteal phase defect, irregular menstruation or amenorrhea. Regular intensive physical activities activate hypothalamic-pituitary-adrenal axis, lower thyroid hormones, leptin, insulin growth factor and the secretion of growth hormone. Stress that usually accompanies competitions is yet another factor which intensifies menstrual disorders in girls and women who train intensively. Stress reaction causes changes in the cyclic and pulsatile secretion of GnRH, lowers the concentration of gonadotropins, raises the level of prolactin, growth hormone, testosterone, ACTH, adrenal steroids and endorphins [6, 7].

Moreover, epidemiologic studies showing that 15–20% of the general population of women of reproductive age meet the criteria of high susceptibility to PMS (premenstrual syndrome) and 3–8% of them meet PMDD (premenstrual dysphoric disorder) exposure conditions [8–10] which indicates the need to investigate whether practicing competitive sport might also be a risk factor for PMS and PMDD. These findings might prove important for athletes, parents, coaches and care providers to recognize and mitigate risks associated with the participation and be aware of the practicality of prevention and early detection of menstrual dysfunction to prevent serious health consequences.

The aim of this study was to evaluate the impact of practicing competitive sports on menstrual cycle disorders, to investigate the frequency of PMS/PMDD in female professional athletes and to identify risk factors predisposing for PMS and PMDD. Additionally, the levels of selected hormones such as serum estradiol, FSH, LH and prolactin were investigated to identify any hormonal perturbances that might have influence or be the risk factors for menstrual dysfunctions. The findings were intended to aid the development of more effective prevention, early detection and treatment strategies for menstrual problems in female athletes.

MATERIAL AND METHODS

Study population

The prospective study involved 125 girls and young women (16–22 years of age). The study group consisted of 75 female professional athletes (girls and young women). The inclusion criteria: obtaining the informed consent for the examination, menstrual bleeds for notes than 2 years, the absence of systemic diseases, including endocrine disorders, depressive, anxiety and personality disorders. Girls and young women were members of sport clubs and/or students of sport schools. They practiced athletics, including medium and long-distance runs. They were subject to two endurance improving training methods: continuous method and interval method, with diversification into light, moderate and heavy training loads and unification of exercise duration.

To be classified as a professional athlete the subject had to be a member of a sport club, take part in championships and attend minimum 4 two-hour trainings per week.

The control group consisted of 50 girls and young women who were not practicing any sports.

The mean age was 18.7 ± 1.9 years in the study group, and 18.5 ± 1.8 years in the control group. The difference was statistically insignificant. The study and control groups were also homogenous in terms of height and menarche age. Body weight was statistically significant ($p = 0.001$) while body mass index (BMI) was statistically insignificant between the groups (Tab. 1).

Research methods

The research tools included a questionnaire to obtain information about social and demographic characteristics, family history, the course of menstruation cycle, obstetrical and gynecological history, gynecological diseases, type of sport practiced, the time and intensity of trainings, life style, diet, stimulants used, present health condition, current medications and the occurrence of PMS and PMDD symptoms.

Daily diary of PMS symptoms has been created in accordance with The American College of Obstetricians and Gynecologists (ACOG) recommendations and ICD-10 diagnostic criteria. ACOG asserts that PMS cannot be diagnosed unless one of the six psychological symptoms (depression, angry outbursts, irritability, anxiety, confusion, social withdrawal) or one of the four physical symptoms (breast tenderness, abdominal bloating, headache, swelling of extremities) is observed. In order to diagnose PMS, the symptoms must present themselves 5 days before the menstruation period in every of the two successive menstrual cycles (prospectively), they must lessen in 4 days following the onset of menstruation and cannot reappear until at least 13th day of menstruation cycle.

Everyday diary of PMDD symptoms has been developed according to diagnostic criteria of the American Psychiatric

Table 1. General characteristics of the study and control groups

Values	Group	Mean	Standard deviation	Median	5th percentile	95th percentile	Mann–Whitney's U test
Age [years]	Studied	18.7	1.9	18.0	16.0	21.3	NS (p = 0.40)
	Control	18.5	1.8	20.0	16.0	20.6	
Height [cm]	Studied	170	6	171	158	178	NS (p = 0.18)
	Control	169	6	170	159	178	
Body weight [kg]	Studied	58.1	6.2	59.0	45.8	68.0	**p = 0.001**
	Control	62.6	8.7	62.5	47.0	73.6	
BMI	Studied	20.01	1.82	19.82	16.96	22.66	NS (p = 0.55)
	Control	21.84	2.64	21.30	18.11	26.49	
Menarche	Studied	13.3	1.4	13.0	11.0	15.0	NS (p = 0.06)
	Control	12.9	1.2	13.0	11.0	15.0	

BMI — body mass index; NS — not significant

Association (APA) — DSM-IV (Diagnostic and Mental Disorders) [11–14].

Each subject had her estradiol, FSH, PH and PRL levels investigated. For this purpose, they had 5–6 mL of peripheral venous blood collected. Serum was separated by centrifugation of venous blood clots collected into a dry tube by a CENTRIFUGE MPW 340 (3000 revolutions, 8 minutes) device. Until biochemical analysis, centrifuged blood was stored in eppendorfs and frozen at (-20°C).To avoid false positive results the blood was collected in the morning, after night rest, between the 2nd and 5th day of menstruation cycle, after fasting and following a 12 hour break from the gymnastic exercises. The stimulation of nipples was avoided 8–12 hours before the test.

The concentration levels of estradiol, FSH, LH and prolactin in accordance with the norm for follicular phase were determined using Abbot ARCHITECT CMIA (chemiluminscencemicroparticle immunoassay) diagnostics. The precision of ARCHITECT Estradiol is ≤ 5 pg/mL for concentrations in the range of the low control (target 45 pg/mL) and ≤ 7% for concentrations in the range of the medium control (target 190 pg/mL) and high control (target 600 pg/mL). The analytical sensitivity of ARCHITECT Estradiol is ≤ 10 pg/mL.

The ARCHITECT FSH test has been developed to ensure the precision expressed by the total value of coefficient of variation of ≤ 10% for concentrations on the range of the low, medium and high control. Analytical sensitivity of Architect FSH is below 0.05 m IU/mL. The precision of ARCHITECT LH is ≤ 10% of the total coefficient of variation. Analytical sensitivity of Architect LH is below 0.07 m IU/mL. Analytical sensitivity of ARCHITECT Prolactin has been evaluated below 0.6 ng/mL.

Statistical analysis

For statistical analysis Excel 2007 and STATISTICA 9.0 were used. Continuous values were described by means of arithmetic mean, standard deviation and median. Statistical comparison of the results Mann-Whitney U test and Kruskal-Wallis test were used. For the evaluation of statistical classification Fisher's exact test, Chi^2 with Yates correction and logistic regression were applied. $P < 0.05$ was assumed as the level of statistical significance.

Ethical statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Medical University of Silesia (KNW/0022/KB1/8/11).

RESULTS

Menstrual cycles

The analysis of menstrual cycle disorders showed statistical significance for heavy menstrual bleeding (p = 0.01) and longer breaks between menstrual bleeds (p = 0.01). The study group was statistically more likely to suffer from hypermenorrhea and longer breaks in menstruation than the control group. For the remaining domains the differences were not statistically significant. The groups were homogenous in terms of the length of menstruation cycle and the severity of menstrual pain (Fig. 1).

Premenstrual syndrome and premenstrual dysphoric disorder

In accordance with APA and ACOG recommendations, PMDD was diagnosed in 8% and PMS in more than 42% of the whole research population. PMDD incidence did not significantly differ between the groups (9.33% in the study group vs 6.0% in control group) as opposed to PMS incidence which was statistically different in both groups (p = 0.045) (49.33% in the study group vs 32.00% in control group) (Fig. 2).

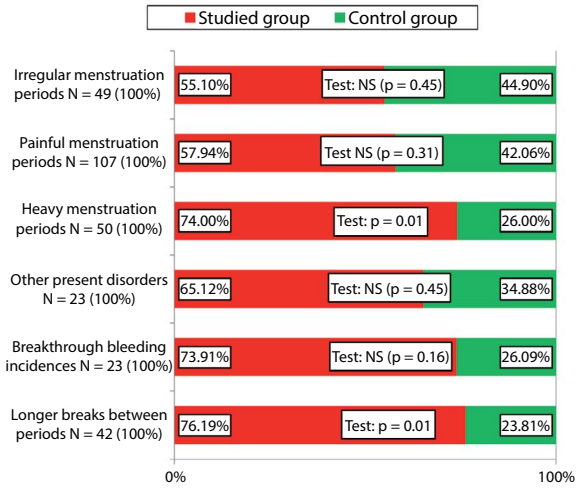

Figure 1. Abnormalities in the menstruation cycle in the study and control groups. NS — not significant

To validate the occurrence of various types of PMS and PMDD a premenstrual symptoms screening tool (PSST) was used. It proved the previous lack of PMS and PMDD in women without any clinical symptoms in both groups and differentiated PMS and PMDD into various scenarios, including mild and severe PMS and PMDD. In a significant percentage of women in the study group (37.33%) and control group (26.00%) a mild PMS was diagnosed. The differences between the groups were statistically insignificant (Fig. 3).

The analysis of selected parameters such as respondents' age, their BMI, age of menarche, years in sport, frequency of training or coffee, alcohol and cigarettes consumption indicates that some might be risk factors for PMS and PMDD.

The first investigated factors were subjects' age, BMI and menarche age. A one-way analysis did not show any significant influence of these variables on PMDD prevalence. However, it presented a significant correlation between PMS, average age ($p = 0.00001$) and menarche age ($p = 0.03$) in girls and young women who were professional athletes. The risk of PMS increased with age (by 1.71 with each year; $p = 0.0007$).

There was nothing statistically significant between the number of years in sport and PMDD prevalence was observed. However, it must be noted that the greatest percentage of professional athletes suffering from PMDD practiced 2 years (28.57%) and 7 years (28.57%) (Fig 4.) (Tab. 2).

The incidence of PMS was, on the other hand, significantly associated with longer professional experience ($p = 0.0001$) in the study group. The highest percentage of girls with PMS diagnosis was observed among women practicing 2 years (18.92%) and 5 years (16.22%) (Fig. 5).

No statistically significant correlation between weekly number of trainings and PMDD prevalence was shown in the studied group while the percentage of subjects with diagnosed PMS was increasing proportionally to the increasing number of trainings, with the highest value in case of 7 trainings per week ($p = 0.004$) (Fig. 6).

There was no statistically significant effect of coffee, alcohol or cigarettes on PMDD incidence in either group

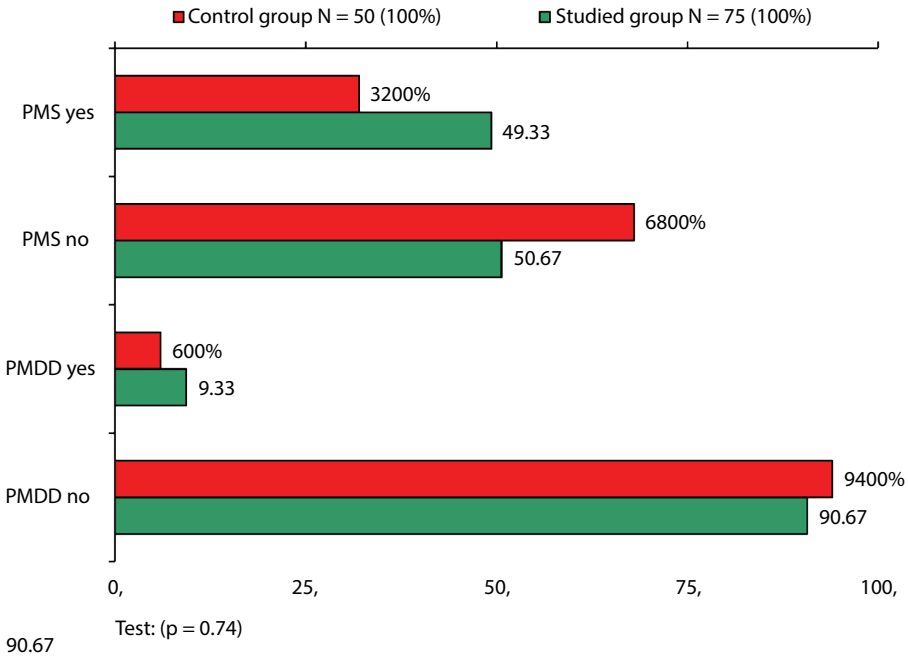

Figure 2. PMS and PMDD diagnosed according American Psychiatric Association and American College of Obstetricians and Gynecologists recommendations in both groups. PMDD — premenstrual dysphoric order; PMS — premenstrual syndrome

(Tab. 3), however, higher frequency of PMS was noted in respondents who took coffee and alcohol more frequently and in greater amount (more than 3 cups of coffee per day and more than 500 mL of beer or 2 glasses of wine of 50 mL of stronger alcohol). No impact of cigarettes has been noted and none of the above correlations have been observed in the control group (Tab. 4).

Neither the regularity of meals nor weight-reducing diet had any statistically significant impact on the prevalence of PMS and PMDD in two groups.

Clinical analysis of selected sex hormones

Long-lasting periods of physical strain in women engaged in competitive sports can temporarily impair the hormonal function of the ovaries. Therefore, the researchers decided to investigate whether excessive physical activity reduced the concentration of gonadotropins, estrogens and prolactin in women.

The study (N = 75) and control (N = 50) groups were homogenous in terms of FSH, LH, prolactin and estradiol levels determined in the first phase of menstrual cycle. Hormone levels in case of PMS were analysed. LH level was statistically different (p = 0.02) in patients with PMS in the control group. However, it does not seem to be of clinical relevance (Tab. 5 and 6).

Statistically significant correlation between the number of trainings and the level of estradiol was shown, with the lowest value in the case of 4 trainings per week (p = 0.03). The level of prolactin was statistically significant for the overall number of training hours per week (p = 0.003). The lowest prolactin level was obtained in girls practicing 12 hours a week. The findings from this study may encourage more

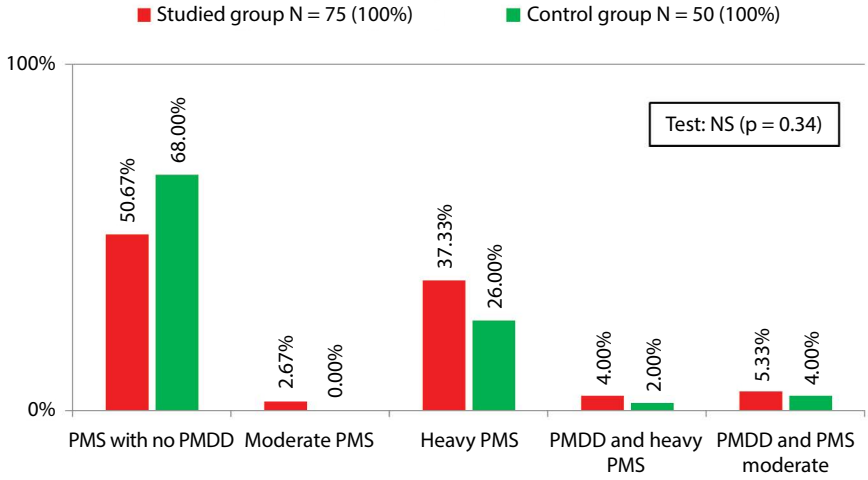

Figure 3. Premenstrual symptoms screening tool results in both groups. NS — not significant; PMDD — premenstrual dysphoric order; PMS — premenstrual syndrome

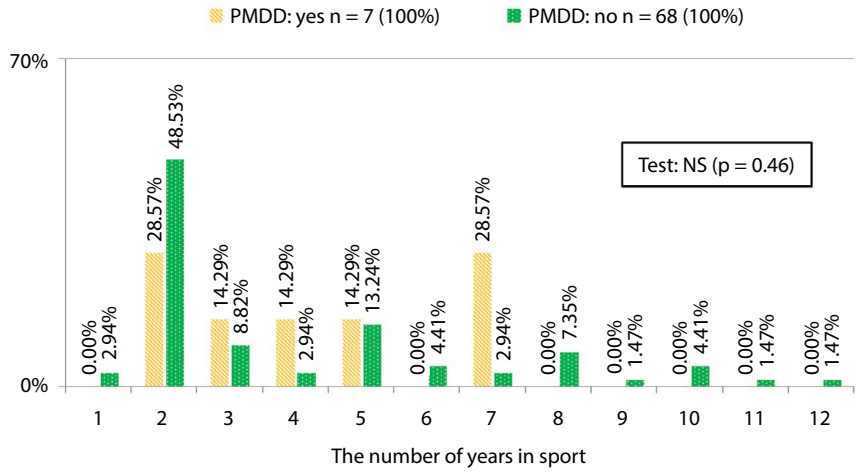

Figure 4. The correlation between PMDD and the number of years in sport. NS — not significant; PMDD — premenstrual dysphoric order

Table 2. Age, BMI and menarche in the study and control groups

Study group							
Values	**PMDD**	**Mean**	**Standard deviation**	**Median**	**5th percentile**	**95th percentile**	**Mann-Whitney's U test**
Age [years]	Yes	19.7	2.0	20.0	17.0	21.7	NS (p = 0.14)
	No	18.5	1.9	17.5	16.0	21.0	
BMI	Yes	20.77	1.97	19.67	18.99	23.49	NS (p = 0.49)
	No	19.93	1.80	19.92	16.84	22.38	
Menarche	Yes	13.4	2.0	13.0	11.3	16.4	NS (p = 0.86)
	No	13.3	1.3	13.0	11.0	15.0	
Control group							
Values	**PMDD**	**Mean**	**Standard deviation**	**Median**	**5th percentile**	**95th percentile**	**Mann-Whitney's U test**
Age [years]	Yes	20.0	0.0	20.0	20.0	20.0	NS (p = 0.20)
	No	18.4	1.8	20.0	16.0	20.7	
BMI	Yes	20.32	1.30	20.98	19.04	21.14	NS (p = 0.29)
	No	21.93	2.68	21.46	18.04	26.51	
Menarche	Yes	13.0	1.0	13.0	12.1	13.9	NS (p = 0.83)
	No	12.9	1.2	13.0	11.0	15.0	
Study group							
Values	**PMS**	**Mean**	**Standard deviation**	**Median**	**5th percentile**	**95th percentile**	**Mann-Whitney's U test**
Age [years]	Yes	19.6	1.7	20.0	17.0	22.0	p = 0.00001
	No	17.7	1.7	17.0	16.0	21.0	
BMI	Yes	19.76	2.18	19.69	16.43	23.91	NS (p = 0.22)
	No	20.24	1.37	20.11	18.19	22.07	
Menarche	Yes	13.7	1.6	13.0	11.8	17.2	p = 0.03
	No	12.9	1.1	13.0	11.0	14.0	
Control group							
Values	**PMS**	**Mean**	**Standard deviation**	**Median**	**5th percentile**	**95th percentile**	**Mann-Whitney's U test**
Age [years]	Yes	18.5	1.8	20.0	16.0	20.0	NS (p = 0.90)
	No	18.5	1.9	20.0	16.0	21.0	
BMI	Yes	21.19	1.39	21.08	18.98	22.84	NS (p = 0.41)
	No	22.14	3.03	21.63	17.79	27.64	
Menarche	Yes	13.8	1.3	13.0	11.8	15.0	NS (p = 0.06)
	No	12.6	1.1	13.0	11.0	14.0	

BMI — body mass index; PMDD — premenstrual dysphoric order; PMS — premenstrual syndrome; NS — not significant

research into other hormones, such as testosterone, which was not investigated for this study, as none of the subjects manifested any androgenized features.

DISCUSSION

Following recent increase in the number of female athletes and expansion of female competitions there has been a great focus on supporting active girls and young women and improve their competitiveness [15]. Without opposition, numerous researchers highlight that a noteworthy percentage of women engaged in various sport disciplines is afflicted by disorders of the menstruation cycle. So far, the greatest number of studies on the frequency of amenorrhea and oligomenorrhea have focused mostly on long-distance runners, marathon runners, gymnasts, swimmers and women whose profession also involved intensive physical activity, such as ballet dancers [16]. In the population of Polish sportswomen, menstruation cycle disorders have been investigated in female sprinters, rowers, canoers, judokas, fencers, basketball and handball players [17, 18].

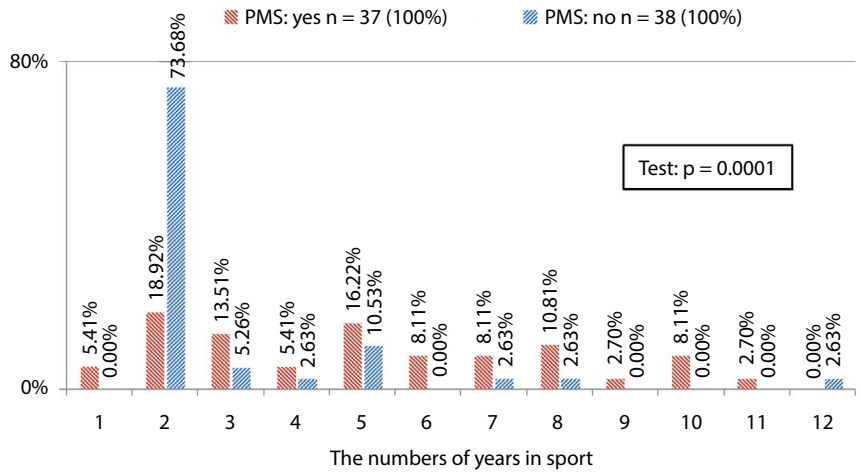

Figure 5. The correlation between PMS and the number of years in sport. PMS — premenstrual syndrome

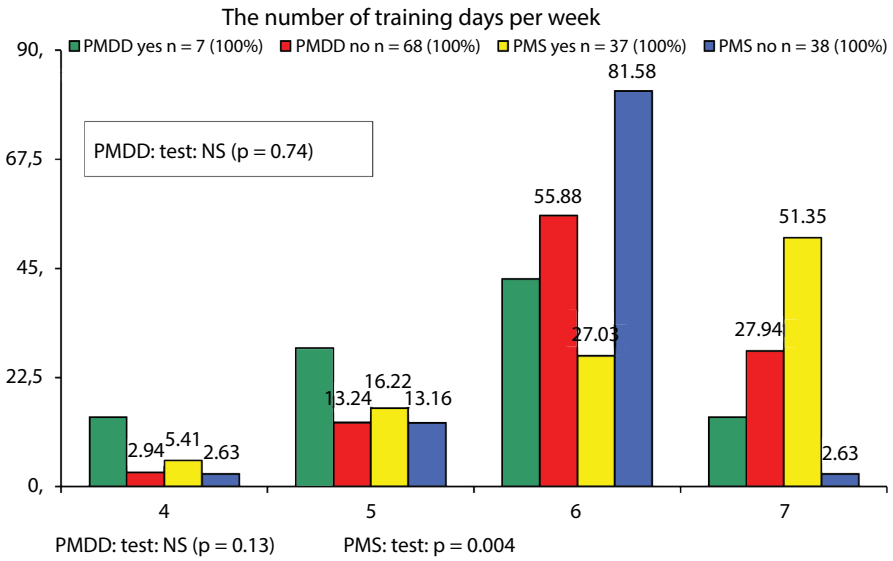

Figure 6. PMS and PMDD versus the frequency of training. NS — not significant; PMDD — premenstrual dysphoric order; PMS — premenstrual syndrome

This research involved professional young female athletes, including medium and long distance-runners. It showed significantly longer breaks between menses (p = 0.01) in women practicing sports as compared to women who didn't practice any sport. The situation was similar in case of hypermenorrhea (p = 0.01). The remaining abnormalities such as irregular and painful menses were specific to more than a half of respondents who were active in sports.

It should be noted that consequences of menstrual dysfunction might manifest as suppression of reproductive hormones and infertility, altered cardio-vascular risk factors (e.g. lipid profile, endothelial function) and lower BMD (bone mineral density). Considering the fact that the consequences of menstrual dysfunction on BMD may not be reversible in every respect, evaluation of bone health should be considered if menstrual disorders are prolonged [19]. Coaches and clinicians should also remember that female youth and adolescent athletes are at greater risk for certain types of injuries, including concussions, musculoskeletal injury and Female Athlete Triad, which consists of low energy availability with or without disordered eating, menstrual dysfunction and low bone mineral density [20]. Therefore, the etiology of menstrual dysfunction must be always considered multi-factorial and thoroughly investigated.

Numerous studies showed fully diagnosed PMDD in 3–8% of women at reproduction age [21–25]. Robinson and Swindle confirmed an even significantly higher percentage

Table 3. The impact of coffee, alcohol and cigarettes on prevalence of PMDD

Type of stimulant	Frequency	Study group			Control group		
		PMDD		Test	PMDD		Test
		No	Yes		No	Yes	
Coffee	Does not drink	41	5	NS (p = 0.70)	18	1	NS (p = 1.00)
	Drinks	27	2		29	2	
Cigarettes	Does not smoke	66	6	NS (p = 0.26)	41	2	NS (p = 0.57)
	Smokes	2	1		6	1	
Alcohol	Does not drink at all	30	2	NS (p = 0.21)	11	0	NS (p = 0.22)
	Drinks but only to give a toast	34	3		28	3	
	Drinks on regular basis	4	2		8	0	

NS — not significant; PMDD — premenstrual dysphoric order

Table 4. The impact of coffee, alcohol and cigarettes on prevalence of PMS

Type of stimulant	Frequency	Study group			Control group		
		PMS		Test	PMS		Test
		No	Yes		No	Yes	
Coffee	Does not drink	28	18	p = 0.03	10	9	NS (p = 0.12)
	Drinks	10	19		24	7	
Cigarettes	Does not smoke	37	35	NS (p = 0.61)	29	14	NS (p = 1.00)
	Smokes	1	3		5	2	
Alcohol	Does not drink at all	21	11	p = 0.03	9	2	NS (p = 0.13)
	Drinks but only to give a toast	16	21		18	13	
	Drinks on regular basis	1	5		7	1	

NS — not significant; PMS — premenstrual syndrome

Table 5. The levels of sex hormones in the studied and control groups depending on PMDD prevalence

Hormone	Group	PMDD	Descriptive statistics					Mann-Whitney's U test
			Mean	Median	SD	5^{th} percentile	95^{th} percentile	
FSH [m IU/mL]	Studied	Yes	4.54	4.30	1.04	2.69	5.70	NS (p = 0.36)
		No	5.01	5.03	1.17	2.66	6.67	
	Control	Yes	5.78	6.06	0.70	4.99	6.30	NS (p = 0.93)
		No	5.65	5.88	1.59	3.21	6.81	
LH [m IU/mL]	Studied	Yes	2.73	2.26	1.41	1.07	5.22	NS (p = 0.30)
		No	3.30	2.48	1.53	1.45	5.29	
	Control	Yes	4.03	2.88	2.06	2.80	6.41	NS (p = 0.90)
		No	3.92	3.31	1.97	1.38	7.81	
Estradiol [pg/mL]	Studied	Yes	43.61	38.10	13.52	33.60	68.30	NS (p = 0.47)
		No	50.28	45.90	28.11	8.80	74.20	
	Control	Yes	45.87	43.60	4.37	43.10	50.90	NS (p = 0.61)
		No	47.35	42.30	16.71	30.40	73.10	
Prolactin [ng/mL]	Studied	Yes	9.48	7.79	3.76	6.27	14.53	NS (p = 0.16)
		No	17.22	10.71	23.49	3.78	40.76	
	Control	Yes	12.49	9.01	7.05	7.86	20.61	NS (p = 0.28)
		No	9.41	7.97	4.69	5.00	17.40	

NS — not significant; PMDD — premenstrual dysphoric order

Table 6. The levels of sex hormones in the studied and control groups depending on PMS prevalence

Hormone	Group	PMS	Descriptive statistics					Mann-Whitney's U test
			Mean	Median	SD	5th percentile	95th percentile	
FSH [m IU/mL]	Studied	Yes	4.85	4.84	1.45	2.28	8.84	NS (p = 0.23)
		No	5.08	5.04	0.79	4.04	6.67	
	Control	Yes	5.73	5.91	0.84	4.08	6.81	NS (p = 0.54)
		No	5.62	5.82	1.79	2.74	7.21	
LH [m IU/mL]	Studied	Yes	2.87	2.34	1.62	0.95	5.43	p = 0.02
		No	3.61	3.36	1.32	1.96	5.29	
	Control	Yes	4.47	4.21	2.10	1.38	7.83	NS (p = 0.22)
		No	3.67	3.11	1.86	0.64	7.67	
Estradiol [pg/mL]	Studied	Yes	49.95	39.8	35.76	4.10	178.0	NS (p = 0.35)
		No	49.37	45.9	15.06	28.00	74.20	
	Control	Yes	46.68	43.60	13.67	28.50	73.10	NS (p = 0.86)
		No	47.53	42.10	17.47	31.20	73.20	
Prolactin [ng/mL]	Studied	Yes	20.27	13.89	30.35	3.78	141.5	NS (p = 0.25)
		No	12.82	10.42	9.35	3.78	40.76	
	Control	Yes	9.02	7.86	3.64	5.18	20.61	NS (p = 0.73)
		No	9.86	7.97	5.31	4.39	22.91	

NS — not significant; PMS — premenstrual syndrome

(exceeding 16%) of women with diagnosed PMDD [21]. Epidemiologic studies indicate that 15–20% of menstruating women meet PMS criteria. The recognition rate is about 50% in women of reproductive age and 50–80% in girls [21, 26, 27]. However, this study is the first to confirm PMS frequency in active sportswomen according to ACOG criteria and to identify risk factors predisposing for both PMS and PMDD.

In accordance with APA and ACOG recommendations, PMDD was diagnosed in 8% and PMS in more than 42% of the whole research population. The study presented a significant correlation between PMS, average age (p = 0.00001) and menarche age (p = 0.03) in girls and young women who were professional athletes. The risk of PMS increased with age (by 1.71 with each year) (p = 0.0007). The incidence of PMS was also significantly associated with longer professional experience (p = 0.0001) in the study group and was significantly increasing proportionally to the increasing number of trainings, with the highest value in case of 7 trainings per week (p = 0.004).Higher frequency of PMS was noted in respondents who drank coffee and alcohol more frequently and in greater amount. These findings may encourage more research into other risk factors such as body composition, nutrition or psychological factors.

Hormone levels in sportswomen have also been evaluated by numerous researchers. The recent studies claim that the concentration of prolactin may rise under the influence of physical effort (as opposed to energetic deficiency) but this phenomenon is not likely to cause menstruation disturbances in physically active women. They also showed that prolactin levels are subject to minor short-term changes throughout the cycle and prolactin concentration in sportswomen with amenorrhea does not significantly differ from the prolactin concentration in menstruating women who do not practice any sport [28–33]. However, studies by both Bielecka et al. and Męczekalski et al. showed an increased prolactin level in female professional athletes, who were exposed to physical and psychological stress during sport competitions [30, 31, 34, 35]. This research showed that study and control groups were homogenous in terms of FSH, LH, prolactin and estradiol levels determined in the first phase of menstrual cycle, however it should be noted that it involved only single determination of prolactin levels. Daily concentration profiles might be more reliable, but they were not carried out in this study.

This study has been also limited by the relatively small number of subjects, thus it cannot be related to the whole population of sportswomen unless further extended and the results are significantly dependent on the specific questionnaire which is based on the respondents' subjective evaluation of the reported symptoms.

However, in none of the previously conducted studies was it ever attempted to verify the effects the physical effort made while engaging in competitive sports on the rate of occurrence of PMS as well as PMDD with accordance to APA and ACOG criteria. That is why the juxtaposition of daily prospective assessment of PMS and PMDD symptoms and

the application of the screening tool for premenstrual symptoms (PSST) prove to be a genuinely innovative approach.

Findings from this research may encourage more research into menstrual dysfunctions in female athletes to increase the awareness of their coaches and clinicians, who can better support them in enjoying full benefits of sport participation.

CONCLUSIONS

Age, menarche age, the number of years in sport and the intensity of trainings increase the risk of PMS occurrence in young female athletes. The risk of PMDD is increasing with the age of girls who train intensively. The amount of consumed alcohol and coffee are significant risk factors for premenstrual tension syndrome and premenstrual dysphoric disorder in female athletes.

Author contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, MC and AD-C; data curation, MC, IG, CG, and BN; writing — original draft preparation, MC, IG, and CG; writing — review and editing, AD-C, MR, and VS-P.

Conflict of interest

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

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Subcutaneous rifampicin versus povidone-iodine for the prevention of incisional surgical site infections following gynecologic oncology surgery — a prospective, randomized, controlled trial

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ABSTRACT

Objectives: Surgical site infection (SSI) following gynecologic oncology surgery is a severe problem for both patient and surgeon in terms of increasing morbidity, length of hospital stay, anxiety, and costs. In this prospective, randomized, controlled study we investigated the effect of subcutaneous rifampicin and povidone-iodine on incisional SSI following gynecologic oncology surgery.

Material and methods: Three hundred patients scheduled for abdominal surgery due to any malign gynecological pathology were randomly assigned into one of three groups of 100 members each, as follows: the subcutaneous tissue was irrigated with saline in Group 1; saline + 10% povidone iodine in Group 2; saline + rifampicin in Group 3. Patients were invited to follow-up once every 10 days in a 30-day period for evaluation. Patients who developed a superficial incisional SSI were recorded.

Results: No significant relationship was observed between the SSI and the subcutaneous agents used ($p = 0.332$). It was observed that there was a statistically significant increase in the rate of incisional surgical site infections as the period of hospitalization ($p = 0.044$), patient's age ($p = 0.003$), existence of comorbidities ($p = 0.001$), and perioperative blood transfusion ($p = 0.021$) increased.

Conclusions: Subcutaneous agents are not effective in preventing surgical site infections after gynecologic oncology surgeries. Further large-scale prospective randomized controlled studies may provide other options to prevent SSIs.

Key words: gynecologic surgical oncology; povidone-iodine; rifampicin; surgical site infection

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INTRODUCTION

The incidence of surgical site infections (SSIs) is 5–16%, and they are observed in 6–15% of gynecologic oncology cases [1, 2]. The infections in this patient group are much more important and riskier than the others because it may increase the patient's morbidity, cause delay in adjuvant therapy and additional treatment due to infection, thereby increasing the cost of care [3].

Chronic diseases, advanced age, smoking, hypoalbuminemia, malnutrition, hypothermia, hyperglycemia, prolonged hospital stay, perioperative blood transfusion, and corticosteroids and other immunosuppressive agents can increase superficial incisional SSI rates by adversely affecting the host defense. At the same time, the architectural characteristics and ventilation of the operating room, surgical clothing, surgical hand washing, preparation of the incision site under appropriate conditions, prophylactic antibiotic use, surgical technique, the materials used during surgery, and length of surgery also affect superficial incisional SSI rates [4, 5].

Despite the developments in the sterilization methods, operation room conditions, and intensive care unit facilities, SSI continues to constitute a serious problem for surgery. In order to establish a diagnosis according to certain criteria and access more accurate statistical data in infections that occur after surgery, Centers for Disease Control and Prevention of the United States of America provided standard definitions, and it was accepted to use the definition "Surgical Site Infection" [6]. According to the standard definitions provided by this center, the SSIs were grouped into two: incisional and organ/site infection. The incisional SSI is observed within 30 days after the operation and constitutes 2/3 of the surgical site infections [7].

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An increase has recently been observed in the incidence of SSI particularly in gynecologic oncology cases due to reasons such as that elderly individuals with chronic diseases are more commonly operated compared to the past and long and complicated operations are performed [8].

The factors that play a role in the occurrence of surgical site infections can be listed as bacteria load and virulence, tissue factor, and other factors associated with the patient. The source of the pathogens are usually the endogenous flora caused by the patient's skin, mucous membranes, or intestinal system. The microorganisms contained in the patient's skin and mucosa are the most significant reservoir of the incisional surgical site infections in particular; therefore, antibiotics prophylaxis, providing preoperative local cleaning, eliminating the debris when the skin incision is closed and washing the wound abundantly, and providing appropriate local antisepsis decreases the risk of incisional wound infection [9]. The World Health Organization recommended 16-item measures for SSI prevention in 2016 [10]. In this package of measures, incisional wound irrigation with antibiotics and povidone-iodine has been recommended along with important preventions like perioperative oxygenation, maintaining normal body temperature, intensive blood glucose control, adequate circulating volume control. The data on the topical antibiotic use to prevent surgical site infections is limited. Rifampicin is a cheap and easily accessible semi-synthetic, high topical effect antibiotic which is very strong bactericidal on many Gram (+) and (–) bacteria including *S. aureus* [11–13]. For these reasons, we have been using rifampicin as a topical antibiotic for many years in our practice.

Objectives

We have previously evaluated the effects of subcutaneous agents used in benign gynecologic cases on SSI formation [14]. Also, there is a limited number of studies in the literature about the SSIs observed after gynecologic oncology cases in particular. Therefore, we compared the efficacy of the subcutaneous rifampicin and 10% povidone-iodine used for preventing the incisional SSI in gynecologic oncology cases in this preliminary prospective randomized controlled study which is the second leg of SSI research in obstetrics& gynecology. Furthermore, the other factors that could cause SSI were evaluated.

MATERIAL AND METHODS

The Ethical Committee of Gaziantep University approved the study protocol (2018/156), and written informed consent was obtained from all patients before enrollment.

Three hundred patients who had no pregnancy and active infection and who were decided to have abdominal surgery due to gynecologic malignancy between February 2018 and July 2019 in the Gynecology and Obstetrics Clinic of Gaziantep University Faculty of Medicine were grouped into 3 groups of 100 people in a randomized manner. Each patient that was operated on between the dates mentioned above was included in the groups in turn. It was randomly specified which subcutaneous agent would be used for which patient. After the abdominal operation was completed and the abdominal fascia was closed, the subcutaneous tissue was irrigated only with saline in Group 1 (control group), saline + 10% povidone-iodine in Group 2, and saline + rifampicin in Group 3. All subcutaneous tissues were irrigated with 250 mL of saline. Then 10 mL of 10 % povidone-iodine in Group 2 and 500 mg/6 mL of rifampicin in Group 3 was applied directly on the subcutaneous tissue without being diluted. The excess liquid was cleaned off and the subcutaneous tissue scrubbed with a gauze. A five-point gynecological perioperative infection prevention bundle was used in all groups [15]. This bundle includes 5 preoperative measures as; chlorhexidine wash using 4% chlorhexidine gluconate wipes, mechanical bowel preparation with oral antibiotics preoperatively, antibiotic and skin preparation administration, adoption of enhanced sterile techniques during intestinal resection and wound closure perioperatively, and strict wound management postoperatively. All groups were operated in the same operating room under the same technical conditions with the same team. The subcutaneous thickness was grouped into 3 according to the measurements; thin ≤ 4 cm, moderate: 5–9 cm, thick ≥ 10 cm. The 10-Fr hemovac drains (400 cc) were inserted subcutaneously and 2/0 polyglactin 910 (Vicryl®) was used as a subcutaneous suture in moderate and thick groups. Subcuticular 3–0 modified glycolic acid (Monosyn®) was used for the skin in pfannenstiel incisions, and subcuticular 3–0 polypropylene (PP, Premilene®) or staple was used for the skin in midline incisions. The patients were discharged with Cefazolin 1 gram/day and Naproxen sodium 550 mg/day and recommendations for the wound site care. The patients' ages, period of hospitalization, comorbidities, laboratory parameters, perioperative bleeding amount, blood transfusion, if any, and smoking habits were recorded. All patients were invited for SSI check with the intervals of 10 days until postoperative day 30. SSI was diagnosed according to the following criteria: purulent discharge (regardless of whether there is positive culture), positive culture of wound or wound discharge, presence of at least one of the following signs: pain, swelling, redness, warmth and wound-opening and surgeon's opinion towards the infection [16]. Wound swabs were taken when clinically indicated. The patients who met one of the diagnosis criteria for surgical site infection within this period were recorded.

The conformity of numerical data with the normal distribution was tested with Shapiro-Wilk test. ANOVA and LSD tests were used for comparing the variables with normal distribution in 3 groups. The relationship between the cat-

egorical variables was tested with the chi-squared test. SPSS 22.0 software pack was used in the analyses. $P < 0.05$ was considered significant.

Sample size estimation

Sample size estimation was performed based on previous study results [14]. To find significant difference between rifampicin and povidone-iodine with effect size of 27% (%5 vs %32) minimum required sample size for each group was calculated as 90 ($\alpha = 0.05$, $1\text{-}\beta = 0.90$). G power package version 3.1.9.2 was used to perform sample size estimation.

RESULTS

The gynecologic cases and their rates were as follows, respectively: 135 patients had endometrial cancer (45%), 94 patients had ovarian cancer (31.3 %), and 71 patients had cervical cancer (23.7%). The main characteristics of the patients are shown in Table 1.

The total number of incisional SSIs in the patients was 28 (9.3%) (Group 1: 12, Group 2: 6, Group 3: 10). It was observed that there was a statistically significant increase in the rate of incisional SSI as the period of hospitalization ($p = 0.044$), patient's age ($p = 0.003$), existence of comorbidities ($p = 0.001$), and perioperative blood transfusion ($p = 0.021$) increased. The risk factors that can play a role in the occurrence of SSI are shown in Table 2.

No significant relationship was observed between the SSI and the subcutaneous agents used ($p = 0.332$) (Fig. 1).

DISCUSSION

The surgical site infection is one of the most important problems of gynecological surgery. Most of the studies previously conducted in the field of gynecology and obstetrics are on SSI after cesarean section [17 ,18] and less benign gynecological cases [19, 20]. There is a limited number of studies on the surgical site infections that occur after gynecologic oncology cases [21].

The factors they play role in the SSI formation can be classified into 3 groups: microbe-related risk factors, with Staphylococcus aureus and Streptococcus pyogenes; host-related risk factors, with morbid obesity, an index of disease severity, old age, protein-calorie malnutrition, steroid use, diabetes, immunodeficiency, cancer, and systemic infection; and operation-related risk factors, including prolonged hospital stay before surgery, duration of the operation, tissue trauma, poor hemostasis, and foreign material in the wound. The performance of an intraabdominal procedure, operation time > 4 hours, a contaminated or dirty-infected operation, and concomitant illness of significance were other important factors [22]. Although it is not possible to fix the factors related to the patient, most of the risk factors related to surgical practice and microbe can be fixed. Therefore, taking measures for all risk factors that result in SSI will decrease the incidence of these infections.

One of the most important methods in reducing the SSIs is the monitoring and evaluation performed by the hospital infection control committees as also applied in our hospital [23]. For this purpose, the rates of SSIs in the hospitals and the active pathogens must be specified, and the appropriate antibiogram charts must be formed for these. The results obtained from these evaluations must be shared with the surgery team, and studies must be conducted for continuous quality improvement for decreasing the infections. Besides appropriate surgical antibiotic prophylaxis is crucial.

Table 1. The baseline characteristics of the patients

Variables	Saline (n = 100)	Saline + Rifampicin (n = 100)	Saline + 10% povidone-iodine (n = 100)	p value
Age† [yr]	59.31 ± 8.81^{A}	55.38 ± 11.18^{B}	59.47 ± 10.48^{A}	0.021*
Hospitalization† [day]	4.09 ± 0.96	4.4 ± 1.26	4.25 ± 1.04	0.353
SC thickness† [cm]	4.9 ± 1.4	4.98 ± 1.87	4.72 ± 1.25	0.789
PO blood loss† [mL]	208.1 ± 147.75^{B}	372.3 ± 275.97^{A}	218.1 ± 197.31^{B}	0.001*
BMI†	22.2 ± 2.72	23.16 ± 3.47^{A}	21.74 ± 2.39^{B}	0.013*
Hemoglobin† [g/dL]	10.78 ± 1.08	10.86 ± 1.21	10.81 ± 1.29	0.961
Comorbid disease‡	14 (14)	14 (14.1)	16 (16)	0.906
SC drain‡	12 (12)^{B}	33 (33)^{A}	18 (18)^{B}	0.001*
SC suture‡	67 (67)	64 (64)	57 (57)	0.324
İncision‡ Pfannenstiel	2 (2)	2 (2)	1 (1)	0.816
Midline	98 (98)	98 (98)	99 (99)	
Smoking‡	26 (26)	30 (30)	22 (22)	0.445
Blood transfusion‡	10 (10)	15 (15)	10 (10)	0.435

†mean ± st.deviation; ‡Count(percent); *Significant at 0.05 level (^{A} is significantly higher than ^{B}); SC — Subcutaneous; PO — Peroperative; BMI — Body Mass Index; CRP — C reactive protein

Table 2. The risk factors that may play a role in SSI formation

Variables	SSI (n = 28)	No SSI (n = 272)	p value
Age† [yr]	58.86 ± 9.27	57.97 ± 10.46	0.003*
Hospitalization† [day]	4.57 ± 0.96	4.21 ± 1.11	0.044*
Sc thickness† [cm]	4.96 ± 1.97	4.86 ± 1.48	0.977
PO blood loss† [mL]	383.93 ± 312.12	254.04 ± 212.1	0.235
BMI†	23.14 ± 3.57	22.29 ± 2.87	0.876
Hemoglobin† [g/dL]	10.89 ± 1.2	10.81 ± 1.19	0.668
Comorbid disease‡	15 (55.6)	29 (10.7)	0.001*
SC drain‡	9 (32.1)	54 (19.9)	0.128
SC suture‡	18 (64.3)	170 (62.5)	0.852
İncision‡ Pfannenstiel Midline	0 (0.0) 28 (100.0)	5 (1.8) 267 (98.2)	0.469
Smoking‡	9 (32.13)	69 (25.4)	0.436
Blood transfusion	7 (25.0)	28 (10.3)	0.021*
Groups‡ Saline Saline + Rifampicin Saline + Povidone-iodine	 12 (42.9) 10 (35.7) 6 (21.4)	 88 (32.4) 90 (33.1) 94 (34.6)	0.332

†mean ± st.deviation; ‡Count (percent); *Significant at 0.05 level; SSI — Surgical Site Infection; SC — Subcutaneous; PO — Peroperative; BMI — Body Mass Index; CRP — C reactive protein

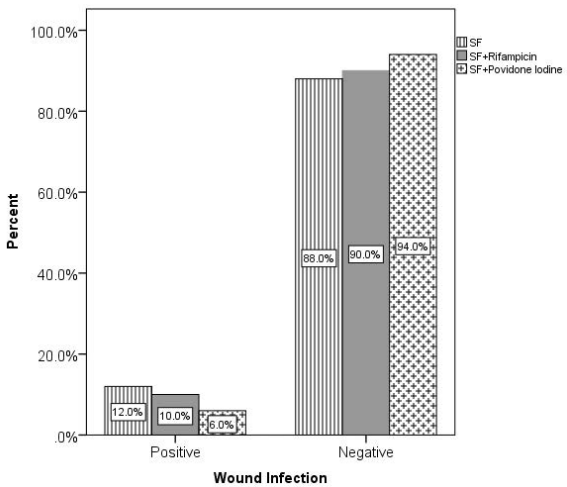

Figure 1. Relationship between subcutaneous agents and SSI

Wound site irrigation with serum saline solution, povidone-iodine, or antibiotics was mentioned among the intraoperative and postoperative recommendations provided by the World Health Organization (WHO) for prevention of the surgical site infection, and it was stated that there was not sufficient evidence demonstrating that irrigation with serum saline solution reduced the risk of infection and that irrigation with povidone-iodine could be helpful in clean and clean contaminated wounds [10].

Subcutaneous antibiotics are more advantageous in terms of side effects, toxicity and efficacy than systemic antibiotics. The agents that could not be used systemically may also be used subcutaneously. There are also disadvantages such as tenderness and contact dermatitis [24].

Subcutaneous antibiotics can be used in different forms like solutions, gels, powders, creams, beads or implants. The frequently used antibiotics are cephalosporins, aminoglycosides, glycopeptides, chloramphenicol, rifampicin and bacitracin [25]. It is not easy to determine using which agent, how much, how long and in what form, for prophylaxis in different types of surgical wounds. For this reason, the efficacy and limits of topical antibiotic use at the surgical site have not been clear enough yet [26]. Besides the topical antibiotics, the efficacy of local anesthetics and local antiseptics have been observed in different studies [27].

It was observed that the use of prophylactic subcutaneous drain did not decrease the rates of subcutaneous hematoma, seroma, and infection even in obese patients [28, 29]. We also did not reveal a positive effect of subcutaneous hemovac drains on the incidence of SSI. At the same time the subcutaneous drains can cause discomfort and excess cost. These results are comparable to studies focusing on other indications like cesarean section [30]. Otherwise, studies demonstrating the benefit of subcutaneous drainage are also available [31]. Therefore, a definite conclusion cannot be obtained about the general use of prophylactic subcutaneous drainage in the surgery.

According to the result of a Cochrane review performed in 2014 about subcutaneous suture, it was stated that subcutaneous tissue closure did not have any effect on the site infection in gynecologic abdominal surgery [32]. We

also did not find any effect of the subcutaneous thickness and the presence of subcutaneous suture on the SSIs. Also, considering the potential negative effects like bacterial contamination or tissue reaction, the subcutaneous sutures should be questioned.

In our study, we observed that the rate of SSI increased as the patient's age increased, and that this outcome was similar to the outcomes of many studies that were previously conducted [33]. This can be related to the decrease in the normal defense mechanisms and increase in the incidence of chronic diseases such as diabetes mellitus in particular with the age. We observed more surgical site infections in the patients with chronic diseases as well. It was an expected outcome to observe an increase in the rate of surgical wound infection as the post-operative period of hospitalization prolonged, because an increase is observed in the microorganisms in the skin flora of the patients who are hospitalized for a long period of time, and this flora can contain the resistant microorganisms observed in the hospitals. Therefore, discharging the patients as early as possible during the post-operative period will decrease the probability of surgical site infection [34]. The increase in the rate of wound infection as the rate of perioperative blood transfusion increases are parallel with the outcome of many studies that were previously conducted on this subject, and perioperative blood transfusion must be avoided unless mandatory [35].

In this study we conducted for comparing the efficacy of surgical wound irrigation performed with saline, 10% povidone-iodine, or rifampicin, SSI was most commonly observed in the saline group and least commonly observed in the povidone-iodine group. Despite the numerical difference between the groups, no statistically significant difference was observed between the agents in terms of preventing the SSI. Perhaps the most striking aspect of this study is the emergence of different result from our previous study on benign gynecological cases [14]. Our previous study revealed that SSI decreased significantly with subcutaneous rifampicin and 10% povidone-iodine irrigation compared to saline alone in benign gynecologic cases This different result between these two studies may suggest that defense mechanisms and risk factors in oncology patients have changed and weakened, also the average age, chronic disease and blood transfusion rates are higher than the previous study. In benign gynecological cases, patients receive 3 doses of one type parenteral antibiotics during their hospital stay, while in oncology cases we give uninterrupted two type parenteral antibiotics until they are discharged. However, oncological cases stay in the hospital longer than other cases, and wound care is performed twice a day during their stay. Such differences between benign cases and oncological cases can lead to different results between the two studies. In addition, although there was no statistical difference, the least infection was in the povidone-iodine group and the most infection was in the saline group. This may indicate the possibility of statistically significant difference when more patients are evaluated in larger studies.

The facts that could not be partially foreseen such as limited number of patients, the possibility of existence of people with active infection in the team, and not having information on the hygienic condition of the patients during the perioperative period are included in the limitations of our study.

This clinic trial is a preliminary study to present the results of the wound irrigation technique with rifampicin or povidone-iodine that we have used in our practice for many years and it may contribute to reaching the sufficient level of evidence on surgical wound infections in gynecologic oncology cases, which are still missing in the literature, and that it may be a guide for the studies that will be conducted on this subject in future.

CONCLUSIONS

Superficial incisional SSI rate in gynecologic oncology patients significantly increases as the period of hospitalization ($p = 0.044$), patient's age ($p = 0.003$), existing comorbidities ($p = 0.001$) and perioperative blood transfusion ($p = 0.021$) increased. However, it does not change when using subcutaneous rifampicin or povidone-iodine. Although there was no statistical difference, the least infection was in the povidone-iodine group and the most infection was in the saline group. This may indicate the possibility of statistically significant difference when more patients are evaluated in larger studies. We believe that broader prospective randomized controlled trials are required to make a definitive comment on this issue.

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The immune complex p53 protein/anti-p53 autoantibodies in the pathogenesis of ovarian serous carcinoma

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ABSTRACT

Objectives: The tests conducted were intended to analyze the concentration of p53 protein and anti-p53 autoantibodies in serum of women with ovarian tumours.

Material and methods: The study included patients with diagnosed ovarian cancer: *Cystadenoma serosum* or *Cystadenocarcinoma papillare serosum* at IIIc stage (including 10 women who had G1, 14 women who had G2 and 30 women who had G3 staging). Concentrations of parameters were measured by ELISA.

Results: The analysis of the obtained results showed statistical significance between the concentration of p53 protein depending on the degree of differentiation of G1 and G3 ($p < 0.001$) and anti-p53 autoantibodies depending on the degree of differentiation of G1 and G2 ($p < 0.05$) as well as G2 and G3 ($p < 0.01$). In addition, the determined p53/anti-p53 autoantibodies ratio was only significant between G1 and G2 ($p < 0.05$), as was the assessment of the percentage of the tested parameters in the immune complex.

Conclusions: Immune system disorders involving the p53 protein and anti-p53 autoantibodies may be one of the immune mechanisms involved in the pathogenesis of ovarian serous cancer.

Key words: autoantibodies anti-p53/ p53 protein; ovarian cancer

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INTRODUCTION

In the course of ovarian carcinoma, an important role plays the immune response directed against neoplastic cells, especially apoptosis. It is one of the key processes leading to initiation of carcinogenesis [1].

In the regulation of apoptosis, a significant role is also performed by protein p53, which participates in the intrinsic pathway of the process, called a p53-dependent mitochondrial pathway. Protein p53, encoded by the gene *TP53* and localized on chromosome 17p13.1, is crucial for a proper course of the cell cycle and therefore, is called a guardian of the genome. One of the major functions of the p53 protein is to activate transcriptions of genes responsible for initiating apoptosis in response to DNA damage, which may be linked to its anti-tumour activity [2, 3]. The loss of protein p53 activity was shown in the pathogenesis of many neoplasms, while the most often mechanism leading to this phenomenon is the point mutation of the gene *TP53* [4, 5].

Mutations in *TP53* head for creating a protein with a decreased activity, or a native protein which is not capable of regulating the cell cycle and effectively fulfilling its function [6, 7]. The mutated p35 protein supports the survival and proliferation of neoplastic cells, inhibits their apoptosis, and promotes resistance to chemotherapy. The altered form of the protein has a longer half-life in the organism, which enables its determination in the extracellular fluid, including blood serum [8].

The studies concerning ovarian neoplasms conducted so far also indicate disturbances in humoral immune response manifesting itself in production of autoantibodies anti-p53 [9, 10]. Detection of anti-p53 autoantibodies in biological fluids are the result of the secondary humoral response directed against the mutated protein p53. They belong to antibodies of IgG class and may be detected even after several years from diagnosing a neoplastic change [11, 12].

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Due to the fact that the systemic changes accompanying ovarian neoplasms and occurring with soluble apoptosis mediators are still not fully known, their fuller evaluation requires further studying. Taking into the account that these changes may perform a crucial function in the pathogenesis of ovarian cancers and that the concentration of the immune system soluble mediators in body fluids may be applied in clinical practice, the primary objective of the paper was to assess the concentration of p53 protein and anti-p53 autoantibodies in the serum of women with ovarian tumours.

MATERIAL AND METHODS

This study had 74 women aged 23 to 70 years (mean age: 37.5 ± 9.42 years), who were diagnosed with ovarian cancer, qualified for the study. In this group, 20 women had *Cystadenoma serosum* and 54 women had *Cystadenocarcinoma papillare serosum* at IIIc stage according to the FIGO classification and, within the group, 10 patients were diagnosed with G1, 14 were diagnosed with G2, and the remaining 30 were diagnosed with G3 staging. Clinical diagnosis of the tumour was confirmed by histopathological examination. Any other reproductive system disease was not found in the examined women. The patients were hospitalized in the Clinical Ward of Obstetrics and Gynecology, Women's Health Chair, Medical University of Silesia in Ruda Śląska. The control group included 34 healthy women aged between 27–60 (mean age: 45.34 ± 19.25 years). No pathological changes in these women's reproductive organs occurred. The research material in all the women was the blood serum. The blood was taken from women after making clinical diagnosis, before surgery. In the morning the blood was taken from the cubital vein, to a clot tube, in order to obtain the serum and 30 minutes after taking the blood, it was centrifuged at 1500 × g for 15 minutes. Until testing was performed, the serum was stored at –80° C. In the control group, the blood was taken while the women came for the check-up and the same procedure was used. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of the studied parameters: p53 Kit Elisa Gen-Probe, Diaclone and anti-p53 autoantibodies ELISA, Steinbeis-Transferzentrum, Angewandte Biologische Chemie. Test sensitivity was the following: 1.5 U/mL (for p53) and 0.07 U/mL (for anti-p53 autoantibodies). All the women, who participated in the study, consented to conducting the research. The approval of the Ethics Committee of the Medical University of Silesia in Katowice was obtained.

Using the computer program Statistica for Windows 10.0 and Microsoft Excel, the results were statistically analyzed. The Shapiro-Wilk test was used in order to verify the distribution of the obtained results. After verification that the received results corresponded to the normal distribution, the arithmetic mean (x) and standard deviation (SD) were calculated for each parameter. The mean values of the studied parameters in the studied group and the control group were compared by means of the Student's t-test, while for nonparametric results: Mann-Whitney's test was used. Correlations were tested by Spearman's rank correlation test and presented as correlation coefficient (r). $P < 0.05$ was considered statistically significant.

RESULTS

In the serum of healthy women belonging to the control group and women with ovarian serous cystadenoma, the concentration of the p53 was below the test sensitivity threshold which amounted to 1.5 U/mL. In the group of women with ovarian cancer, due to the fact that the obtained values did not correspond with the normal distribution, the results are presented as a median and an upper and lower interquartile range (Q1 and Q3) which equalled 22.14 U/mL and 47.71 U/mL, respectively, while the median value was equal to 35.89 U/mL. The conducted analysis of the test results revealed statistical significance between the concentration of the studied parameter depending on the degree of differentiation G1 and G3 ($p < 0.001$). However, no significant differences between the concentration of p53 protein in the serum of women with moderately differentiated (G2) and poorly differentiated (G3) ovarian cancer were shown. The obtained results are shown in Figure 1. There was no correlation between CA125 concentration in the serum of women with ovarian cancer and the concentration of p53 protein in their serum.

Further analysis included the assessment of anti-p53 autoantibodies concentration in the studied women. In the serum of healthy women and women with ovarian serous cystadenoma, anti-p53 autoantibodies were not detected. Whereas in the group of women with ovarian cancer concentration of the parameter did not correspond with the

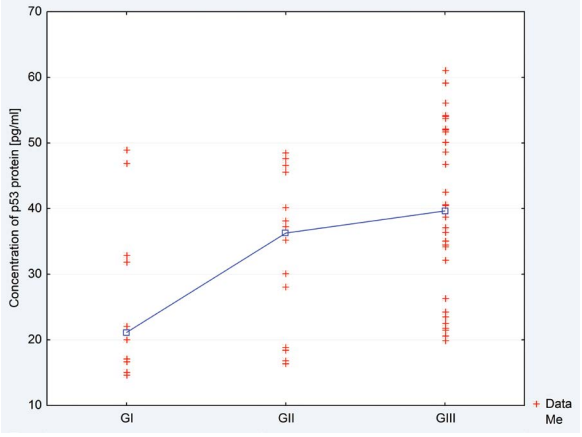

Figure 1. Concentrations of p53 protein in serum of women depending on the degree of differentiation G1, G2 and G3

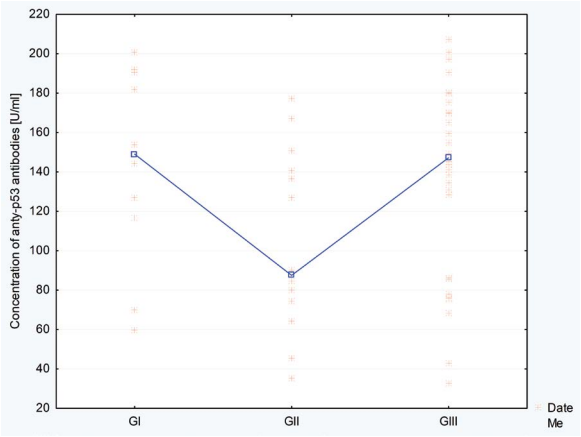

Figure 2. Concentrations of anti-p53 autoantibodies in serum of women depending on the degree of differentiation G1, G2 and G3

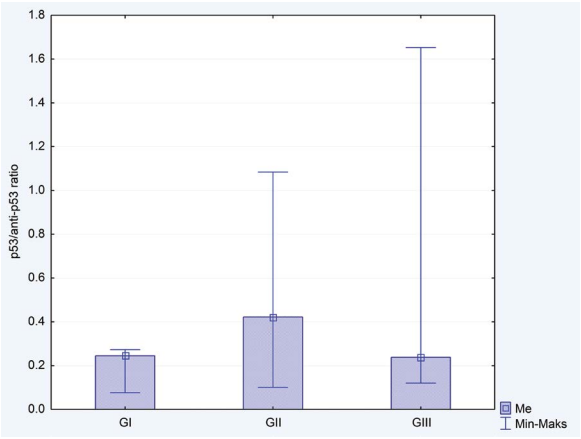

Figure 3. p53/anti-p53 autoantibodies ratio in serum of women depending on the degree of differentiation G1, G2 and G3

normal distribution and therefore the results are presented as a median and an upper and lower interquartile range (Q1 and Q3), amounted to 80.30 U/mL and 170.10 U/mL respectively, while the median value equalled 140.92 U/mL. The conducted analysis of the study results revealed a significance between the concentration of the studied parameter depending on the degree of differentiation G1 and G2 ($p < 0.05$) and G2 and G3 ($p < 0.01$). The obtained results are shown in Figure 2. There was no correlation between the concentration of CA125 in the serum of women with an ovarian neoplasm and anti-p53 autoantibodies concentration in their serum.

Subsequently, the ratio p53/anti-p53 autoantibodies was determined. A statistically significant difference was shown for the value of this coefficient only between G1 and G2 ($p < 0.05$) in Figure 3.

Moreover, since in the serum of women with ovarian cancer, the concentration anti-p53 autoantibodies was

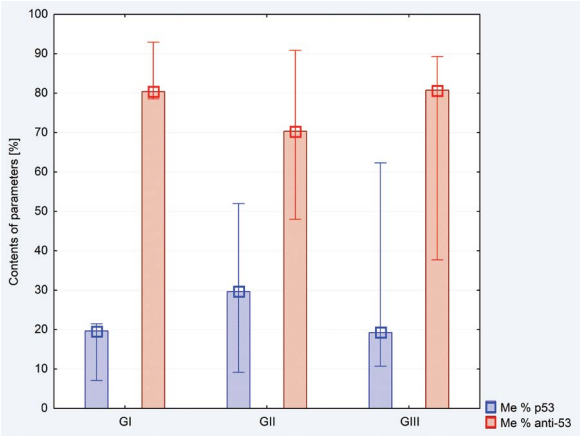

Figure 4. Percentage contents of p53 protein and anti-p53 autoantibodies in serum of women depending on the degree of differentiation G1, G2 and G3

three times higher than the concentration of protein p53, the percentage contents of protein p53 and autoantibodies p53 in the total immune complex was determined. The analysis revealed a statistically significant increase of the percentage contents of p53 protein in the total immune complexes in the serum between G1 and G2, was illustrated in Figure 4.

DISCUSSION

Studies have so far indicated that some neoplastic cells may reveal sensitivity to factors inducing the intrinsic pathway of apoptosis and therefore, it is justifiable to study the markers of this process. It is thanks to its apoptosis conduct that the neoplastic fully transformed cells are eliminated. The imbalance between the number of proliferating cells and those undergoing apoptosis may be caused by the presence of the p53 protein. It influences the decrease of ability to activate the programmed death of the cell which is irreversibly damaged. Its presence may also lead to initiation of the neoplastic process. The altered form of the protein has a longer half-life in the organism which enables its determination [13, 14].

The previous studies concerned mostly the assessment of protein p53 expression in women with ovarian cancer using the immunohistochemical method [15], whereas in this study we tried to assessment of the concentration of this parameter in the serum of women with ovarian cancer.

In our studies, the concentration of protein p53 in the serum of both women with ovarian cancer and from the control group was assessed. The analysis included 74 female patients with a diagnosed serous ovarian cancer at the IIIC stage according to FIGO classification and with ovarian serous cystadenoma. In the serum of healthy women constituting the control group and women with serous

cystadenoma, the concentration of p53 protein was below the sensitivity threshold. However, a significantly increased concentration of protein p53 in the serum of women with ovarian cancer when compared to its concentration assessed in the control group was indicated. The obtained increased concentration of protein p53 points out to impairment of the apoptotic process on the intrinsic pathway. Interesting was the analysis of the concentration of the studied parameter conducted according to the degree of tumour differentiation. The indicated statistical significance between G1 and G3 proves that the impaired apoptosis with protein p53 is associated with the degree of histological differentiation of ovarian cancer.

Very little data are currently available on the prevalence of anti-p53 antibodies in patients with epithelial ovarian cancer [16]. Murphy et al. [17] used ELISA to detect anti-p53 antibody in sera in normal heath individuals and ovarian cancer patients, however, the distribution of anti-p53 antibody in the two groups showed no significant difference. Additionally, Høgdall et al. [18] found that the frequency of anti-p53 antibodies in ovarian cancer patients increased somewhat with increasing clinical stage of disease, but the differences did not reach statistical significance. In contrast, other studies showed opposite results [19–21]. Anti-p53 antibodies are often detected in serum from patients with advanced ovarian carcinoma [22]. Additionally, the preoperative serum anti-p53 antibody status had no prognostic relevance for progression-free survival and survival [23]. However, the generation of a humoral immune response against p53 protein in the close tumour environment, as demonstrated by the occurrence of anti-p53 autoantibodies in the ascitic fluid of ovarian carcinoma patients, is associated with poor disease-free survival [24]. Many researchers have been interested in the use of autoantibodies as serological markers for cancer diagnosis, because of the absence of these autoantibodies in normal individuals and in non-cancer conditions. With the successive addition to study panel, except p53, another autoantigen tumour associate to (surviving, p16, cyclin B1, cyclin D1, cyclin A and cyclin E), there was a stepwise increase in sensitivity of up to 62.5%, and in specificity of 90.2% [19].

Currently, there are many studies conducted aiming at determination of a possible application of anti-p53 autoantibodies in the diagnostics of ovarian cancer. Arjomandi et al. [25] presented an innovative algorithm which could increase the specificity and sensitivity of potential tests for early detection of ovarian cancer. For detection, the authors used the protein p53 complex with selected antigenic epitopes. Based on the conducted analyses, they did not demonstrate the determination of the studied autoantibodies in the screening diagnostics of ovarian cancer. Moreover, according to the authors, assessing the concentration of anti-p53 autoantibodies with CA125 does not constitute a sufficiently sensitive and specific screening test for detection of early lesions in female patients with ovarian cancer.

Anderson et al. [20] observed a significantly increased concentration of autoantibodies in the serum of over 42% of women with serous ovarian cancer, whereas in the case of different histological types of cancer as well as benign tumours, the concentration of antibodies was considerably decreased. In the opinion of researchers, the application of anti-p53 autoantibodies as markers for early detection of ovarian cancer is limited due to a low sensitivity at an early stage of disease and non-specific reactions in the case of neoplasms demonstrating an increased expression of abnormal protein p53.

The assessment of autoantibodies p53 application in monitoring the efficacy of the basic treatment therapy of ovarian cancer was also made by Häfner et al. [26]. The authors demonstrated that antibodies p53 could constitute a more sensitive marker than antigen CA 125 in detecting minimal residual disease remaining after the surgical procedure or chemotherapy. The concentration of antigen CA125, released by neoplastic cells, is associated with the mass of ovarian tumours, while the induction of antibodies p53 takes place in response to the occurrence of single neoplastic cells. According to the authors, assessing p53 antibodies could become useful in monitoring the maintenance and consolidation therapy.

Similar studies were performed out by Dobrzycka et al. [27], who found antibodies p53 in 33,3% of female patients undergoing aggressive treatment of ovarian cancer. A significantly increased concentration of these antibodies was found in the serum of patients with serous ovarian cancer with III and IV clinical stage of tumour. Moreover, as a part of the conducted analysis in female patients with serous cancer anti-p53 autoantibodies positive, they assessed the values for the chance of one-year and five-year survival with a diagnosed cancer which amounted to 86.4% and 72.7% respectively. The median of the overall survival time for women anti-p53 autoantibodies positive was equal to 11 months, while for patients anti-p53 autoantibodies negative, it amounted to 57 months. In the case of women with mucinous cancer they did not demonstrate the correlation between the presence of the determined antibodies in the serum, and the overall survival time [27].

Our date did not demonstrate the presence of antibodies p53 in the serum of healthy women and women with serous cystadenoma. However, there was found a significantly increased concentration of anti-p53 autoantibodies complex in the serum of women with ovarian cancer.

Interestingly, the determined coefficient p53/anti-p53 autoantibodies which was almost 3-times higher in women with ovarian cancer than in the control group,

may indicate impairments in the internalization of the immune complex occurrence in the system which in turn undoubtedly favours the induction of the immune tolerance in response to the growing tumour. On the other hand, the conducted assessment of the percentage contents of p53 protein and anti-p53 autoantibodies in the immune complex proves that patients with ovarian cancer are accompanied by the impairment of immunoprecipitated p53 protein — anti-p53 autoantibodies occurrence, while the excess of free antibodies in the serum may have an influence on a weaker immune reactivity by interfering with some soluble mediators of the immune system. In contrast, the increased levels of p53-AAbs in the serum of women with ovarian cancer indicate an increased effect of secondary humoral response against the mutated p53 protein, and they are also associated with the degree of histological differentiation of the tumour, and their determination may be used in laboratory diagnostics, which requires, however, further research.

CONCLUSIONS

The results of the conducted studies allow to draw the following conclusions:

1. The regulation of the immune response towards suppression and the creation of an environment conducive to the proliferation of cancer cells may involve disorders associated with the p53 protein/anti-p53 autoantibodies system.
2. The occurring systemic changes in the immune system with soluble mediators play an important role in the pathogenesis of ovarian cancer. Intensification of apoptosis and humoral immune response impairment in women with ovarian cancer confirms a considerable involvement of p53 protein and anti-p53 autoantibodies in the pathogenesis of serous ovarian cancer.

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Are neutrophil to lymphocyte ratio and platelet to lymphocyte ratio clinically useful for the prediction of early pregnancy loss?

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ABSTRACT

Objectives: Red cell distribution width (RDW), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have all been identified as systemic inflammatory markers. The aim of this study to investigate whether the use of systemic inflammatory markers can predict early pregnancy loss.

Material and methods: A total of 137 patients with early pregnancy loss was compared with 148 participants in the control group who had given birth at term. In the study group, CBC values were included in the study at the time of referral to the hospital for routine follow-up, while patients did not experience early pregnancy loss. In the control group, CBC values of the patient before the seventh week of pregnancy were included in the study.

Results: There was no significant difference between the two groups in terms of RDW, MPV, PCT and PDW values. The NLR and PLR values were significantly higher in the early pregnancy loss group than the control group ($p < 0.05$).

Conclusion: Our findings suggest that high NLR and PLR values are potent markers for the prediction of early pregnancy loss.

Key words: early pregnancy loss; systemic inflammation; inflammatory markers

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INTRODUCTİON

Early pregnancy loss is described as a nonviable, intrauterine gestation with either an empty gestational sac or an embryo/fetus without heartbeat within the first 12 6/7 weeks of gestation [1]. Approximately 10% of all clinically recognized pregnancies ending in an early loss and about 80% of all pregnancy losses occur in the first trimester [1]. Despite the high frequency of early pregnancy loss, its pathophysiology is still not fully understood [2]. Also, the natural history of early pregnancy loss, including temporal ordering of signs and symptoms in early pregnancy has not to be fully described [3]. The causes of early pregnancy losses have been reported in the literature as genetic causes, infectious causes, immunological causes, implantation abnormalities, anatomic abnormalities and endocrine disorders [4]. However, approximately 40% of early pregnancy losses are categorized as idiopathic [4].

Human pregnancy can be defined as the implantation of the semi-allogeneic fetus into the endometrium [5]. Complicated pregnancies such as hyperemesis gravidarum, preterm delivery, preeclampsia, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, frequently have an excessive inflammatory response that leads to adverse pregnancy outcomes [6]. The role of systemic inflammatory reactions in the pathogenesis of early pregnancy loss has been examined in several studies. In one study, it was stated that the women with euploid miscarriage had significantly higher levels of TNFα, IFNγ, IL-6 and IL-10 compared to normal pregnant controls [7]. However, the technical difficulties and high cost of evaluating inflammatory markers in the blood sample limited the use of these investigations in clinical practice. Parameters such as red cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR), which are readily available as systemic inflammation markers from complete blood count (CBC), are widely used in the diagnosis of many inflammatory diseases and prediction of the complicated pregnancies [8, 9]. The objective of this study to investigate whether the use of systemic inflammatory markers can predict early pregnancy loss.

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MATERIAL AND METHODS

This retrospective study was conducted at the Obstetrics and Gynecology Department of the Gazi Yaşargil Training and Research Hospital, during the period from September 2019 to December 2019. The study was approved by the same hospitals Ethics Committee.

The inclusion criteria of the study group were early pregnancy loss patients with ageing between 18–35 years old. The inclusion criteria of the control group were pregnant women with live birth ≥ 37 weeks and ageing between 18–35 years old. The two groups were matched for age and body mass index (BMI). The exclusion criteria for participation in the study were as follows: women with inadequate data, multiple gestation, molar pregnancy, a history of recurrent miscarriages or infertility, a known thrombophilia or any other medical condition needing chronic drug treatment, complicated pregnancies (preeclampsia, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy), any congenital uterine anomaly, large uterine fibroids and smoking during pregnancy.

Age, gestational week, gravida, parity, body weight and height were obtained by examining the medical records of patients. The gestational week was determined by sonographic measurement. BMI was calculated by dividing the body weight (in kilograms) by the square of the height (m^2).

In the study group, CBC values were included in the study at the time of referral to the hospital for routine follow-up, while patients did not experience early pregnancy loss. In the control group, CBC values of the patient before the seventh week of pregnancy were included in the study. The CBC values of the patients were measured with Mindray BC 6800, an automatic blood counting device using laser and impedance measurement technique. Haemoglobin (Hb), white blood cell count (WBC), neutrophil count, lymphocyte count, platelet (PLT) count, RDW, PDW, MPV, PCT and CRP values were all derived from patient' medical files. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. The PLR was calculated by dividing the platelet count by the lymphocyte count.

Statistical analysis

IBM SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) statistical package program was used for statistical evaluation of our research data. Measured variables were presented as mean±standard deviation (std), and categorical variables were presented as numbers and percentages (%). Kolmogorov-Smirnov test was used to determine whether the numerical data matched the normality distribution. Student's t-test was used to compare the normally distributed data. Mann-Whitney U test was used to compare the non-normally distributed data. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 137 patients with early pregnancy loss was compared with 148 participants in the control group who had given birth at term. The demographic and clinical features of all patients are summarized in Table 1. The median age of the study group was 23, and the median age of the control group was 26. There was no significant difference between the two groups in terms of age, BMI, gravida and parity.

The laboratory values of the groups are shown in Table 2. The haemoglobin, RDW, WBC, PLT count, PCT, MPV, and PDW

Table 1. Demographic and clinical features of the groups

Variables	Early pregnancy loss group (n = 137)	Control group (n = 148)	p value
Age (years)*	23 (18–35)	26 (19–35)	> 0.05
BMI (kg/m^2)**	23.12 ± 3.66	23.78 ± 3.82	> 0.05
Gravida*	3 (1–5)	4 (1–6)	> 0.05
Parity*	1 (0–4)	1 (0–5)	> 0.05

* — median (minimum-maksimum); ** — mean ± standart deviation

Table 2. Laboratory values of the groups

Variables	Early pregnancy loss group (n = 137)	Control group (n = 148)	p value
Haemoglobin (g/dL)*	11.8 (8.6–13.1)	11.4 (9.3–12.7)	> 0.05
RDW (%)**	11.6 ± 1.3	12.2 ± 1.5	> 0.05
WBC (/mm^3 ×10^3)**	9.2 ± 2.6	8.4 ± 2.2	> 0.05
NEU (×10^3/uL)**	4.6 ± 1.4	3.4 ± 1.3	**< 0.05**
LYM (×10^3/uL)*	1.6 (0.4–3.4)	2.3 (0.9–4.2)	**< 0.05**
Platelet (/mm^3 ×10^3)*	264.1 (142.0–431.0)	257.8 (168.0–418.0)	> 0.05
PCT (%)*	0.19 (0.12–0.35)	0.18 (0.13–0.33)	> 0.05
MPV (fL)*	8.8 (6.8–10.9)	8.6 (6.7–10.7)	> 0.05
PDW (%)*	15.8 (15.2–17.4)	15.2 (12.1–16.7)	> 0.05
NLR*	3.5 (1.3–7.1)	1.9 (1.1–4.2)	**< 0.05**
PLR*	150.7 (71.6–339.2)	84.1 (46.4–204.3)	**< 0.05**

* — median (minimum-maksimum); ** — mean ± standart deviation

values were not significantly different between the early pregnancy loss and control groups. The neutrophil count was significantly higher ($p < 0.05$) and the lymphocyte count was significantly lower ($p < 0.05$) in the early pregnancy loss group than the control group. The NLR and PLR values were significantly higher in the early pregnancy loss group than the control group ($p < 0.05$).

DISCUSSION

In this retrospective study, we compared first-trimester systemic inflammatory markers of pregnant women with a live birth at ≥ 37 weeks with those pregnancies ended with an early loss. Our findings indicate that early pregnancy loss has an association with systemic inflammation.

During pregnancy, there is an increase in systemic inflammation [10]. Regulated inflammation is essential in every stage of pregnancy [11]. Physiologic regulation of immune response prevents the rejection of semi-allogeneic fetus, and this regulation is mainly through changes in cytokine levels [10]. Deregulation of this mechanism can cause adverse pregnancy outcomes such as spontaneous or recurrent abortion, preeclampsia, preterm labour, and intrauterine growth restriction [10].

In several studies, it was reported that the cytokine levels are different in women with recurrent miscarriages. In the study of O'Hern Perfetto et al. [12], it was suggested that the lower levels of IL-22 in the uterine decidua in patients with unexplained recurrent pregnancy loss. However, there are conflicting results in the literature about the status of the systemic inflammatory response in spontaneous miscarriage. In a study conducted by Sacerdoti et al. [13], decrease in local vascular endothelial growth factor (VEGF) may contribute to the early pregnancy loss. In contrast, in the study of Ku et al. [14], it was reported that there was no correlation between circulating IL-6 levels with spontaneous miscarriage.

Inflammatory markers, which are associated with early pregnancy loss in various studies, are not available in all centers due to technical difficulties and high costs. The diagnostic value of systemic inflammatory markers such as NLR, PLR, PDW, MPV, PCT, RDW in many diseases such as preeclampsia, coronary artery disease, autoimmune diseases, inflammatory diseases has already been shown in several studies [15, 16]. However, there are few studies and insufficient data in the literature on the relationship between these markers and early pregnancy loss. In this study, we planned our study to evaluate whether these markers, which we can quickly obtain with the complete blood count, have changed in patients before early pregnancy loss.

High RDW values are thought to reflect increased inflammation and oxidative stress [17]. In addition to their central role in hemostasis, studies have shown that platelets are potent immune modulators and effectors [18]. PDW, MPV and PCT are regarded to be markers of platelet activation [19]. It was shown that PLT count, PCT and RDW was significantly higher in patients with recurrent pregnancy loss than in controls [20]. However, in our study, there was no significant difference between the early pregnancy loss and control groups in terms of RDW, PLT count, PCT and MPV values. These results suggest that platelet activation may not have a significant role in the pathogenesis of inflammation in spontaneous early pregnancy loss. These results can also be explained by the exclusion of patients with recurrent abortion or chronic diseases into the study. Studies with a large number of patients needed in this regard.

In many systemic inflammatory diseases and malignancies, the physiological response of the immune system is to increase the neutrophil count and decrease in lymphocyte count, and this has lead to the widespread use of NLR and PLR values in the diagnosis and evaluating the prognosis of inflammatory diseases [21]. Also, in several studies, it was reported that high NLR values during the first trimester were powerful predictors of subsequent complicated pregnancies such as preeclampsia, gestational diabetes and intrahepatic cholestasis of pregnancy [22–24]. However, there are few studies investigating the association between NLR and PLR values and early pregnancy loss. In a study by Christoforaki et al., it was found that NLR does not differ significantly between pregnant women with live birth and those whose pregnancy ended in miscarriage [25]. In contrast, in the study of Bas et al., NLR and PLR values evaluated at the sixth gestational week can be used for the risk assessment of spontaneous abortion. In our study, when the groups were compared, NLR and PLR values were significantly higher in the early pregnancy loss group than the control group.

The strength of the study is that there are few studies in the literature about predicting early pregnancy loss with systemic inflammatory markers. Excluding women with all possible confounding factors that can cause early pregnancy loss, such as advanced maternal age, multiple pregnancies, recurrent miscarriage, chronic diseases is another strength of the study.

There are some limitations to this study. This study has been designed retrospectively and has the potential to contain limitations of such studies. Another limitation is the absence of pro-inflammatory cytokines such as TNF-α, VEGF, IL-6, which have been previously identified with early pregnancy loss. A study by correlating the results of systemic inflammatory markers with these cytokines may provide more insight into the prediction of early pregnancy loss.

CONCLUSİON

The results of this study suggest that NLR and PLR are potent markers in the prediction of early pregnancy loss.

Conflict of interest

The authors declared no conflict of interest.

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Comparison of maternal characteristics, pregnancy course, and neonatal outcome in preterm births with and without prelabor rupture of membranes

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ABSTRACT

Objectives: The aim of this study was to evaluate pregnancy outcome of patients with prelabor rupture of membranes receiving expectant management and giving birth prematurely in comparison to preterm births of patients with intact membranes.

Material and methods: It was a retrospective cohort study comparing maternal and neonatal outcome in two groups of preterm births. The first group included 299 consecutive singleton preterm births complicated by prelabor rupture of membranes. The second group consisted of 349 consecutive singleton preterm births without prelabor rupture of membranes.

Results: Patients without pPROM underwent Caesarean sections more often than women from the pPROM group (65.3% vs 45.2%; $p < 0.001$). No statistically significant differences regarding the gestational age during delivery were identified. Lower birth weight was detected in the group with no history of pPROM ($p < 0.001$).

No differences regarding early-onset sepsis were identified and higher percentage of late-onset infections was observed in infants with no history of pPROM (8.9% vs 4.7%; $p = 0.04$). Pulmonary hypertension was more common in the infants from the pPROM group (4% vs 1.4%; $p = 0.049$). Neonatal respiratory distress syndrome and respiratory failure were more prevalent in cases of no pPROM history — 20% vs 12.7% ($p = 0.02$) and 40% vs 25.8% ($p < 0.001$), respectively.

Conclusions: Development of multiple complications in preterm neonates may be more associated with the management, gestational age at birth, and birth weight than with the occurrence of preterm prelabor rupture of membranes.

Key words: pregnancy complications; pregnancy outcome; premature birth; preterm premature rupture of the membranes

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INTRODUCTION

Prematurity remains the leading cause of neonatal morbidity and mortality worldwide [1]. Preterm prelabor rupture of membranes (pPROM) complicates about 3% of all pregnancies and one-third of pregnancies delivered before term [2]. Even though this complication has been managed for decades, available data on the results of planned early birth compared to expectant management of pPROM do not strongly support any method [3, 4].

Objective

The aim of this study was to evaluate maternal and neonatal outcome of preterm births complicated by pPROM and receiving expectant management in comparison to preterm births with intact membranes.

MATERIAL AND METHODS

Patient population and data collection

It was a retrospective cohort study comparing maternal and neonatal outcome in two groups of preterm births. The first group included 299 consecutive singleton preterm births complicated by preterm prelabor rupture of membranes. The second group consisted of 349 consecutive singleton preterm births without preterm prelabor rupture of membranes. Patients with fetal congenital defects and multiple pregnancies were excluded from the study. Data was collected from

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maternal and neonatal records of patients managed in a tertiary referral obstetric center between October 2016 and December 2018. Maternal obstetric history, comorbidities, pregnancy course, cervical microbiome, applied management, and delivery mode were examined. Neonatal outcome analysis included neonate's condition, birth weight, length of hospitalization, management in the Neonatal Intensive Care Unit (NICU), and type of applied treatment.

Patients' management

Preterm prelabor rupture of membranes was diagnosed with a rapid test detecting insulin-like growth factor binding protein (IGFBP-1) and with ultrasound examination. In case of positive result, expectant management was introduced. Cervical specimen collected on admission was cultured and prophylactic antimicrobial agents were administered. The antimicrobial regimen was established based on the local epidemiological data and consisted of intravenous cefuroxime for 10 days. The medication was adjusted in case of drug allergy or detected antimicrobial resistance. Cervical swabs were repeated every two weeks and further treatment was determined depending on the culture results. If no pathological agents were identified, the antimicrobial treatment was withdrawn after administering the prophylactic dose. Serum inflammatory markers were monitored every 2 days. Amniotic fluid index was measured twice a week.

Both groups — with and without pPROM — were monitored with ultrasound examinations and nonstress tests. In case of contractions before completed 35th gestational week, accompanied by normal levels of inflammatory markers and normal nonstress test results, intravenous tocolysis was introduced. Antenatal corticosteroids were administered for better fetal maturation in case of preterm birth.

Premature infants were screened for early onset infections by monitoring of inflammatory markers, blood culture, and general condition. Prophylactic antimicrobial management included ampicillin and gentamicin for 48–72 hours.

Statistical analysis

Statistical analysis was performed using Statistica 13 (StatSoft. Inc.). T-Student test was used for quantitative data comparison between two groups. Two-sided Fisher's exact test was used for comparison of discrete variables. Logistic regression models were employed for multivariable analysis. P value < 0.05 was considered significant.

RESULTS

Maternal characteristics

Results of compared maternal characteristics are presented in Table 1. No differences between patients with pPROM and with intact membranes regarding maternal age, parity, and gestational age were observed. One of the

Table 1. Maternal characteristics and course of pregnancy in preterm births with and without preterm prelabour rupture of membranes

Parameter	pPROM (n = 299)	No pPROM (n = 349)	No pPROM without hypertension (n = 240)	No pPROM with hypertension (n = 109)	p[1]	p[2]	p[3]	p[4]
Mean age [years]	32.6	31.9	31.5	32.8	0.09	**0.01**	0.79	**0.04**
Primigravid	132 (44.1)	162 (46.4%)	107 (44.4%)	55 (50.5%)	0.58	0.93	0.26	0.35
GDMG[1]	45 (15%)	50 (14.3%)	30 (12.4%)	20 (18.3%)	0.82	0.32	0.45	0.16
GDMG[2]	26 (8.7%)	17 (4.9%)	10 (4.1%)	7 (6.4%)	0.058	**0.04 RR = 2.09 (0.99–4.57)**	0.54	0.42
GDM	71 (23.7%)	67 (19.2%)	40 (16.7%)	27 (24.8%)	0.18	0.053	0.89	0.08
PGDM	20 (6.7%)	31 (8.9%)	16 (6.6%)	15 (13.8%)	0.31	1	**0.02 RR = 0.49 (0.25–0.97)**	**0.04 RR = 2.06 (1–4.23)**
Pregnancy-induced hypertension	9 (3%)	55 (15.6%)			**< 0.001 RR = 0.19 (0.09–0.39)**			
Pregestational hypertension	15 (5%)	54 (15.5%)			**< 0.001 RR = 0.32 (0.18–0.58)**			
Preeclampsia	0	41 (11.7%)			**< 0.001**			
Hypothyroidism	58 (19.4%)	100 (28.7%)	70 (29%)	30 (27.5%)	**0.008 RR = 0.68 (0.50–0.91**	**0.011 RR = 0.67 (0.48–0.91)**	0.1	0.8

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Table 1. Maternal characteristics and course of pregnancy in preterm births with and without preterm prelabour rupture of membranes (continued)

Parameter	pPROM (n = 299)	No pPROM (n = 349)	No pPROM without hypertension (n = 240)	No pPROM with hypertension (n = 109)	p^1	p^2	p^3	p^4
Asthma	9 (3%)	4 (1.2%)	4 (1.7%)	0	0.1	0.4	0.12	0.31
Intrahepatic cholestasis of pregnancy	9 (3%)	18 (5.2%)	13 (5.4%)	5 (4.6%)	0.24	0.19	0.54	1
History of cervical conisation	4 (1.3%)	0	0	0	**0.045**	0.13	0.58	1
Cervical insufficiency	47 (15.7%)	58 (16.6%)	49 (20.3%)	9 (8.3%)	0.59	0.11	0.1	**0.005 RR = 0.4 (0.2–0.81)**
Pessary	32 (10.7%)	20 (5.7%)	16 (6.6%)	4 (3.7%)	**0.03 RR = 1.87 (1.06–3.33)**	0.13	**0.03 RR = 2.92 (1.03–9.63)**	0.33
Cervical cerclage	10 (3.3%)	16 (4.6%)	15 (6.2%)	1 (0.9%)	0.55	0.15	0.3	**0.03 RR = 0.15 (0.01–1.02)**
3rd trimester vaginal bleeding	17 (5.7%)	51 (14.6%)	44 (18.3%)	7 (6.4%)	**< 0.001 RR = 0.39 (0.22–0.67)**	**< 0.001 RR = 0.31 (0.17–0.54)**	0.81	**0.003 RR = 0.35 (0.15–0.77)**
Pathological vaginal microbiome	130 (43.5%)	157 (45%)	108 (44.8%)	49 (45%)	0.75	0.73	0.82	1
Positive amniotic fluid culture[5]	19 (6.4%)	7 (2%)	7 (2.9%)	0	**0.008 RR = 3.17 (1.28–8.22)**	0.07	**0.003**	0.1
Suspected intrauterine infection	24 (8%)	16 (4.6%)	14 (5.8%)	2 (1.8%)	0.07	0.4	**0.02 RR = 4.38 (1.04–26.7)**	0.16
Steroids administration	184 (61.5%)	200 (57.3%)	128 (53.1%)	72 (66%)	0.3	0.07	0.4	**0.03 OR = 1.7 (1.06–2.73)**
Full prophylactic steroids dose (24 mg) administration followed by birth within 10 days	140 (46.8%)	147 (42%)	90 (37.3%)	57 (52.3%)	0.24	**0.04 OR = 1.47 (1.04–2.08)**	0.37	**0.01 OR = 1.83 (1.16–2.89)**
Intravenous tocolysis	105 (35.1%)	83 (23.8%)	74 (30.7%)	9 (8.3%)	**0.002 OR = 1.74 (1.23–2.44)**	**0.31**	**< 0.001 RR = 4.25 (2.22–8.8)**	**< 0.001 RR = 0.29 (0.13–0.52)**
Abnormal NST results	48 (16%)	135 (38.7%)	72 (29.9%)	63 (57.8%)	**< 0.001 OR = 0.3 (0.2–0.44)**	**< 0.001 OR = 0.45 (0.3–0.68)**	**< 0.001 OR = 0.14 (0.09–0.23)**	**< 0.001 OR = 3.2 (2–5.1)**
Stillbirth	2 (0.7%)	13 (3.7%)	11 (4.6%)	2 (1.8%)	**0.02 RR = 0.18 (0.04–0.78)**	**0.004 RR = 0.15 (0.03–0.68)**	0.29	0.36
Caesarean section	145 (45.2%)	228 (65.3%)	139 (57.7%)	89 (81.7%)	**< 0.001 OR = 0.5 (0.36–0.69)**	**0.03 OR = 0.68 (0.49–0.96)**	**< 0.001 OR = 0.21 (0.12–0.36)**	**< 0.001 OR = 3.23 (1.87–5.6)**
Emergency Caesarean section	41 (13.7%)	138 (39.5%)	76 (31.5%)	62 (56.8%)	**< 0.001 OR = 0.24 (0.16–0.36)**	**< 0.001 OR = 0.34 (0.22–0.53)**	**< 0.001 OR = 0.12 (0.07–0.2)**	**< 0.001 OR = 2.85 (1.79–4.54)**
Mean gestational age at birth [weeks + days]	33 + 4	32 + 6	32 + 5	33+0	0.2	0.48	0.1	0.48
Birth before completed 34th gestational week	98 (32.8%)	135 (38.7%)	91 (37.8%)	44 (40.4%)	0.12	0.24	0.16	0.72
Birth after completed 34th gestational week	201 (67.2%)	214 (61.3%)	150 (62.2%)	65 (59.6%)	0.12	0.24	0.16	0.72

pPROM — preterm prelabour rupture of membranes; p^1 — statistical significance concerning comparison between all patients with pPROM and all patients without pPROM; p^2 — statistical significance concerning comparison between all patients with pPROM and patients without pPROM nor hypertension; p^3 — statistical significance concerning comparison between all patients with pPROM and patients without pPROM but suffering from hypertension; p^4 — statistical significance concerning comparison between patients with and without hypertension among the group with no pPROM; GDMG1 — gestational diabetes mellitus treated with diet; GDMG2 — gestational diabetes mellitus treated with insulin; RR — risk ratio; PGDM — pregestational diabetes mellitus; [5]Only the amniotic fluid collected during Caesarean sections was cultured; OR — odds ratio; NST — nonstress test

Table 2. Vaginal microbiome detected in patients from the pPROM and no pPROM group

Pathogen	With pPROM n (%)	Without pPROM n (%)	p
Positive culture	130 (43.5)	157 (45)	0.8
E. coli	43 (14.4)	42 (12)	0.4
S. agalactiae	39 (13)	58 (16.6)	0.2
C. albicans	31 (10.4)	48 (13.8)	0.2
P. bivia	19 (6.4)	19 (5.4)	0.7
E. faecalis	17 (5.7)	14 (4)	0.4
G. vaginalis	7 (2.3)	9 (2.6)	1
K. pneumoanie	5 (1.7)	5 (1.4)	1
E. cloacae	3 (1)	3 (0.9)	1
Ureaplasma	3 (1)	6 (1.7)	0.5
P. melanogenica	2 (0.7)	0	0.2
S. pyogenes	2 (0.7)	0	0.2
B. fragilis	1 (0.3)	0	0.5
B. ovatus	1 (0.3)	0	0.5
C. crusei	1 (0.3)	0	0.5
C glabrata	1 (0.3)	4 (1.1)	0.4
C. lusitaniae	1 (0.3)	1 (0.3)	1
C. freundii	1 (0.3)	0	0.5
C. krosei	1 (0.3)	1 (0.3)	1
H. influenzae	1 (0.3)	0	0.5
P. mirabilis	1 (0.3)	0	0.5

most relevant differences regarded the occurrence of hypertensive disorders — 24 (8%) patients in the pPROM group and 109 (31%) in the second group ($p < 0.001$). Therefore, additional subgroups in the no pPROM group were analyzed depending on the hypertensive status in order to avoid bias resulting from different hypertensive disorders incidence between pPROM and no pPROM group [5].

Patients with pPROM more frequently had undergone cervical conization in the past (1.3% vs 0; $p = 0.045$). Obstetric pessary had been also more often used during earlier stages of pregnancies complicated by pPROM (10.7% vs 5.7%; $p = 0.03$). Occurrence of cervical insufficiency and treatment with cervical cerclage was similar in both pPROM and no pPROM groups. Gestational diabetes treated with insulin occurred more frequently in the pPROM group compared to no pPROM, no hypertension group ($p = 0.04$). Hypothyroidism (28.7% vs 19.4%; $p = 0.008$), 3rd trimester vaginal bleeding (14.6% vs 5.7%; $p < 0.001$), and stillbirth (3.7% vs 0.7%; $p = 0.02$) were more frequent in the preterm pregnancies without pPROM. No differences between pPROM and no pPROM groups regarding the incidence of gestational diabetes treated with diet, asthma, and intrahepatic cholestasis of pregnancy were identified.

Similarly, no statistically relevant differences in the cervical microbiome among two groups were detected. Both the occurrence of positive culture result and the microbiome composition were comparable. Detected pathogens included *E. coli, S. agalactiae, C. albicans, P. bivia, E. faecalis, G. vaginalis, K. pneumoanie, E. cloacae, Ureaplasma, P. melanogenica, S. pyogenes, B. fragilis, B. ovatus, C. crusei, C. glabrata, C. lusitanie, C. freundii, C. krosei, H. influenzae,* and *P. mirabilis.* Detailed information on the microbiome is presented in Table 2.

Management during pregnancy

Distribution of antenatal corticosteroids administration was similar in both groups. However, a difference was identified concerning administration of full prophylactic dose followed by birth within ten days. Patients with pPROM more often received full prophylaxis (46.8%) than patients with preterm birth uncomplicated by pPROM nor hypertension (37.3%) ($p = 0.04$). Intravenous tocolysis (fenoterol, atosiban) was more frequently required and administered in the pPROM patients (35.1% vs 23.8%; $p = 0.002$). Monitoring with the nonstress test resulted in detection of significantly more abnormalities in patients without pPROM than with the amniotic fluid leakage (38.7% vs 16%; $p < 0.001$).

Mode of delivery

No statistically significant differences regarding the gestational age during delivery were identified.

Patients without pPROM more often underwent Caesarean sections than women from the pPROM group (65.3% vs 45.2%; $p < 0.001$). The difference between emergency Cesarean section was even higher. Almost 40% of patients from the no pPROM group underwent emergency Cesarean section — 31.5% in the hypertension negative subgroup and 56.8% in the hypertension positive subgroup. The high Cesarean section rate resulted from high number of medically indicated preterm deliveries in this group.

Every patient undergoing a Cesarean section had her amniotic fluid collected and cultured. Results showed more frequent prevalence of positive amniotic fluid culture in the pPROM group (6.4%) than in the second group (2%) ($p = 0.008$).

Birth weight

There was a difference regarding mean birth weight, prevalence of small for gestational age under 10th percentile (2.7% in pPROM group vs 8.9% in no pPROM group; $p = 0.001$); extremely low birth weight between 750 and 1000 g (4.3% in pPROM group vs 8.6% in no pPROM group; $p = 0.04$), and incredibly low birth weight under 750 g (2.7% in pPROM group vs 8.3% in no pPROM group; $p = 0.002$). Lower birth weight was detected in the group with no history of pPROM ($p < 0.001$). The Ponderal index was not significantly different, however lower values were observed in the pPROM group and the no pPROM hypertension positive subgroup (Tab. 3).

Table 3. Neonatal outcome in preterm births with and without preterm prelabour rupture of membranes

Parameter	pPROM (n = 299)	No pPROM (n = 349)	No pPROM without hypertension (n = 240)	No pPROM with hypertension (n = 109)	p[1]	p[2]	p[3]	p[4]
Female infants	114 (38.1%)	150 (43%)	105 (43.6%)	45 (41.3%)	0.23	0.22	0.57	0.73
1st minute Apgar score between 8–10	225 (73.5%)	212 (60.7%)	147 (61%)	65 (59.6%)	**< 0.001 OR = 1.97 (1.4–2.76)**	**0.001 OR = 1.92 (1.33–2.78)**	**0.003 OR = 2.06 (1.3–3.27)**	0.81
1st minute Apgar score between 4–7	56 (18.7%)	92 (26.4%)	54 (22.4%)	38 (34.9%)	**0.02 OR = 0.64 (0.44–0.94)**	0.3	**0.001 OR = 0.43 (0.26–0.7)**	**0.02 OR = 1.84 (1.12–3.03)**
1st minute Apgar score between 0–3	10 (3.3%)	45 (12.9%)	39 (16.2%)	6 (5.5%)	**< 0.001 RR = 0.26 (0.12–0.52)**	**< 0.001 RR = 0.18 (0.09–0.37)**	0.39	**0.005 RR = 0.34 (0.13–0.8)**
3rd minute Apgar score between 8–10	229 (76.6%)	231 (66.2%)	155 (64.3%)	76 (69.7%)	**0.004 OR = 1.67 (1.18–2.37)**	**0.003 OR = 1.8 (1.23–2.61)**	0.16	0.4
3rd minute Apgar score between 4–7	55 (18.4%)	87 (24.9%)	57 (23.7%)	30 (27.5%)	**0.046 OR = 0.68 (0.46–0.99)**	0.14	0.054	0.51
3rd minute Apgar score between 0–3	7 (2.3%)	31 (8.9%)	28 (11.6%)	3 (2.8%)	**< 0.001 RR = 0.26 (0.11–0.57)**	**< 0.001 RR = 0.2 (0.08–0.47)**	0.73	**0.007 RR = 0.23 (0.06–0.78)**
5th minute Apgar score between 8–10	248 (82.9%)	256 (73.4%)	168 (69.7%)	88 (80.7%)	**0.004 OR = 1.77 (1.2–2.6)**	**< 0.001 OR = 2.08 (1.39–3.14)**	0.66	**0.04 OR = 1.8 (1.04–3.11)**
5th minute Apgar score between 4–7	39 (13%)	65 (18.6%)	47 (19.5%)	18 (16.5%)	0.07	**0.044 OR = 0.62 (0.39–0.98)**	0.42	0.56
5th minute Apgar score between 0–3	5 (1.7%)	27 (7.7%)	25 (10.4%)	2 (1.8%)	**< 0.001 RR = 0.22 (0.07–0.58)**	**< 0.001 RR = 0.16 (0.05–0.43)**	1	**0.004 RR = 0.18 (0.03–0.74)**
10th minute Apgar score between 8–10	258 (83.6%)	274 (78.5%)	180 (74.7%)	94 (86.2%)	**0.01 OR = 1.72 (1.14–2.61)**	**0.001 OR = 2.1 (1.35–3.26)**	1	**0.02 OR = 2.09 (1.13–3.88)**
10th minute Apgar score between 4–7	29 (9.7%)	51 (14.6%)	48 (15.8%)	13 (11.9%)	0.07	**0.001 RR = 0.49 (0.31–0.76)**	0.58	0.07
10th minute Apgar score between 0–3	4 (1.3%)	24 (6.9%)	22 (9.1%)	2 (1.8%)	**< 0.001 RR = 0.2 (0.06–0.58)**	**< 0.001 RR = 0.15 (0.04–0.440**	0.66	**0.01 RR = 0.2 (0.03–0.84)**
Mean infant's hospitalization duration [days]	20.4	26.6	25.5	28.8	**0.03**	0.33	**0.02**	**0.046**
NICU admission	76 (25.4%)	108 (30.9%)	68 (28.2%)	40 (36.7%)	0.14	0.5	**0.04 OR = 0.59 (0.37–0.94)**	0.13
Mean birth weight [g]	2416	2075	2136	1943	**< 0.001**	**< 0.001**	**< 0.001**	**0.03**
Mean length at birth [cm]	49.4	46.9	47.4	45.8	**< 0.001**	**0.006**	**< 0.001**	**0.007**
Mean Ponderal Index at birth	20.3	33.4	39.9	19.7	0.33	0.84	0.07	0.08
LBW[5]	106 (35.5%)	125 (35.8%)	87 (36.1%)	38 (34.9%)	0.94	0.86	1	0.9
VLBW[6]	19 (6.4%)	37 (10.6%)	21 (8.7%)	16 (14.7%)	0.07	0.32	**0.02 RR = 0.43 (0.22–0.86)**	0.13
ELBW[7]	13 (4.3%)	30 (8.6%)	17 (7%)	13 (11.9%)	**0.04 RR = 0.51 (0.25–0.99)**	0.2	**0.01 RR = 0.37 (0.16–0.81)**	0.15

Table 3. Neonatal outcome in preterm births with and without preterm prelabour rupture of membranes (continued)

Parameter	pPROM (n = 299)	No pPROM (n = 349)	No pPROM without hypertension (n = 240)	No pPROM with hypertension (n = 109)	p[1]	p[2]	p[3]	p[4]
ILBW[8]	8 (2.7%)	29 (8.3%)	21 (8.7%)	8 (7.3%)	**0.002 RR = 0.32 (0.13–0.71)**	**0.003 RR = 0.31 (0.13–0.71)**	**0.04 RR = 0.37 (0.13–1.05)**	0.84
Small for gestational age	8 (2.7%)	31 (8.9%)	17 (7%)	21 (19.3%)	**0.001 RR = 0.3 (0.13–0.67)**	**0.02 RR = 0.39 (0.15–0.9)**	**< 0.001 RR = 0.14 (0.06–0.32)**	**0.001 RR = 2.72 (1.43–5.2)**
Early onset sepsis	23 (7.7%)	31 (8.9%)	23 (9.5%)	8 (7.3%)	0.67	0.44	1	0.55
Late onset sepsis	14 (4.7%)	31 (8.9%)	19 (7.9%)	12 (11%)	**0.04 RR = 0.53 (0.27–1)**	0.15	**0.04 RR = 0.43 (0.19–0.96)**	0.42
Pneumonia	13 (4.3%)	22 (6.3%)	15 (6.2%)	7 (6.4%)	0.2	0.34	0.44	1
Adaptive breathing disorders	72 (24%)	83 (23.8%)	55 (22.8%)	28 (25.7%)	1	0.76	0.8	0.59
Pneumothorax	6 (2%)	6 (1.7%)	2 (0.8%)	4 (3.7%)	1	0.31	0.47	0.08
Broncho-pulmonary dysplasia	20 (6.7%)	30 (8.6%)	24 (9.6%)	6 (5.5%)	0.4	0.2	0.82	0.22
Respiratory distress syndrome	38 (12.7%)	70 (20%)	47 (19.5%)	23 (21.1%)	**0.02 OR = 0.58 (0.38–0.89)**	**0.03 OR = 0.6 (0.38–0.95)**	**0.04 OR = 0.54 (0.31–0.97)**	0.77
Pulmonary hypoplasia	2 (0.7%)	0	0	0	0.21	0.51	1	
Pulmonary hypertension	12 (4%)	5 (1.4%)	3 (1.2%)	2 (1.8%)	**0.049 RR = 2.8 (0.93–9.05)**	0.07	0.37	0.66
Respiratory failure[9]	77 (25.8%)	141 (40.4%)	94 (39%)	47 (43.1%)	**< 0.001 OR = 0.51 (0.37–0.72)**	**0.001 OR = 0.67 (0.51–0.85)**	**0.001 OR = 0.46 (0.29–0.72)**	0.56
Circulatory failure	20 (6.7%)	24 (6.9%)	16 (6.6%)	8 (7.3%)	1	1	0.83	0.82
Death	6 (2%)	16 (4.6%)	10 (4.1%)	6 (5.5%)	0.08	0.2	0.09	0.59
Hyperbilirubinemia	182 (60.1%)	180 (51.6%)	122 (50.6%)	58 (53.2%)	**0.02 OR = 1.46 (1.07–2)**	**0.02 OR = 1.5 (1.07–2.12)**	0.17	0.73
Anaemia	49 (16.4%)	102 (29.2%)	64 (26.6%)	38 (34.9%)	**< 0.001 OR = 0.48 (0.32–0.7)**	**0.004 OR = 0.54 (0.35–0.82)**	**< 0.001 OR = 0.37 (0.22–0.6)**	0.13
Hypoglycaemia	51 (17.1%)	47 (13.5%)	26 (10.8%)	21 (19.3%)	0.23	**0.047 OR = 1.7 (1.02–2.8)**	0.66	**0.04 OR = 1.96 (1.05–3.67)**
Intraventricular haemorrhage	20 (6.7%)	44 (12.6%)	30 (12.4%)	14 (12.8%)	**0.01 RR = 0.53 (0.3–0.9)**	**0.03 RR = 0.54 (0.3–0.95)**	0.07	1
Necrotizing enterocolitis	5 (1.7%)	12 (3.4%)	7 (2.9%)	5 (4.6%)	0.23	0.39	0.14	0.53
Retinopathy	7 (2.3%)	22 (6.3%)	16 (6.6%)	6 (5.5%)	**0.02 RR = 0.37 (0.15–0.9)**	**0.02 RR = 0.35 (0.13–0.9)**	0.1	0.8
nCPAP[10]	100 (33.4%)	141 (40.4%)	88 (36.5%)	53 (48.6%)	0.07	0.47	**0.006 OR = 0.53 (0.34–0.83)**	**0.045 OR = 1.64 (1.03–2.59)**
Mechanical ventilation	46 (15.4%)	69 (19.8%)	49 (20.3%)	20 (18.3%)	0.15	0.17	0.54	0.77
Parenteral nutrition	88 (29.4%)	138 (39.5%)	86 (35.7%)	52 (47.7%)	**0.008 OR = 0.64 (0.46–0.89)**	0.1	**0.001 OR = 0.46 (0.29–0.72)**	**0.044 OR = 1.63 (1.03–2.59)**

pPROM — preterm prelabour rupture of membranes; p[1] — statistical significance concerning comparison between all patients with pPROM and all patients without pPROM; p[2] — statistical significance concerning comparison between all patients with pPROM and patients without pPROM nor hypertension; p[3] — statistical significance concerning comparison between all patients with pPROM and patients without pPROM but suffering from hypertension; p[4] — statistical significance concerning comparison between patients with and without hypertension among the group with no pPROM; OR — odds ratio; RR — risk ratio; [5]LBW — low birth weights; [6]VLBW — very low birth weight; [7]ELBW — extremely low birth weight; [8]ILBW — incredibly low birth weight; [9]Defined as persistent hypoxaemia or hypercapnia despite surfactant therapy and "maximal" conventional ventilation, includes both patients with severe adaptive breathing disorders and with respiratory distress syndrome; [10]Treatment with neonatal Continuous Positive Airway Pressure

Infections

On the basis of the nonstress test (fetal tachycardia), elevated maternal body temperature, maternal heart rate and inflammatory markers, patients with pPROM were more often suspected of developing intrauterine infections (8%) compared to the no pPROM group (4.6%), however this difference was not statistically significant (p = 0.07). This observation did not correspond with the results of early or late-onset sepsis rates in the neonates. No differences regarding early-onset sepsis were identified and higher percentage of late-onset infections was observed in infants with no history of pPROM (8.9% vs 4.7%; p = 0.04) (Tab. 3). The largest difference was observed between infants of patients with pPROM and patients without pPROM but with hypertension (4.7% vs 11%). Figures 1 and 2 show the distribution of early and late-onset sepsis depending on the gestational week at birth and pPROM status. No difference in gestational age at birth among mentioned subgroups were identified.

Neonatal respiratory complications

Pulmonary hypertension was more common in the infants from the pPROM group (4% vs 1.4%; p = 0.049). Surprisingly, the distribution of other respiratory complications was similar between the groups or higher in the group with no history of pPROM (Tab. 3). Identified statistically significant differences regarded the occurrence of respiratory failure, which was defined as persistent hypoxemia or hypercap-

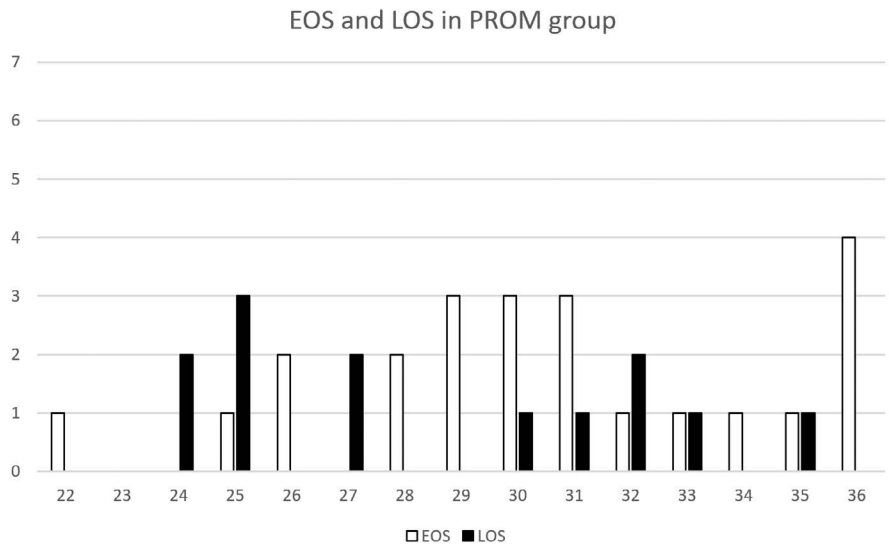

Figure 1. Occurrence of early onset sepsis and late-onset sepsis in the pPROM group depending on the gestational week at birth

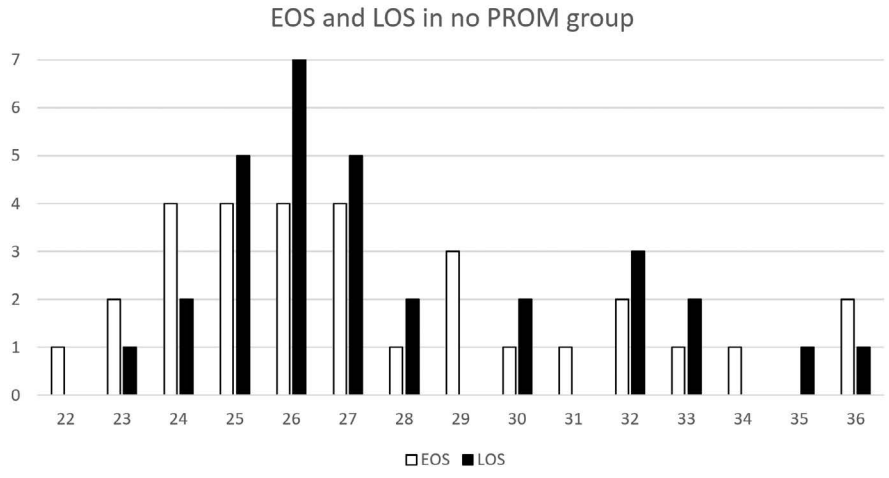

Figure 2. Occurrence of early onset sepsis and late-onset sepsis in the no pPROM group depending on the gestational week at birth

nia despite surfactant therapy and "maximal" conventional ventilation and included both patients with severe adaptive breathing disorders and with respiratory distress syndrome. This condition affected over 40% of infants in the no pPROM group and 25.8% of infants from the pPROM group ($p < 0.001$). Neonatal respiratory distress syndrome was also more prevalent in case of no pPROM history (20% vs 12.7%; $p = 0.02$). The prevalence of neonatal respiratory distress syndrome in both pPROM and no pPROM groups depending on the gestational age at birth is presented in Figure 3 and Figure 4. No statistically significant differences in occurrence of pulmonary hypoplasia, bronchopulmonary dysplasia, use of neonatal continuous positive airway pressure (nCPAP) nor mechanical ventilation between the pPROM and no pPROM group during the observation time were detected.

Other neonatal complications

Preterm neonates of patients without pPROM obtained more frequently lower Apgar score results, more often developed intraventricular hemorrhage ($p = 0.01$), retinopathy ($p = 0.02$), and anemia ($p < 0.001$), required longer infant's hospitalization ($p = 0.03$), and parenteral nutrition ($p = 0.008$) (Tab. 3). Hyperbilirubinemia was more frequently diagnosed in the pPROM group ($p = 0.02$). Figures 5 and 6 show the distribution of neonatal complications depending on the gestational age at birth.

Multivariable analysis

In the bi-variable logistic regression models pPROM was not associated with the occurrence of early-onset sepsis or the late-onset sepsis after adjustment for ges-

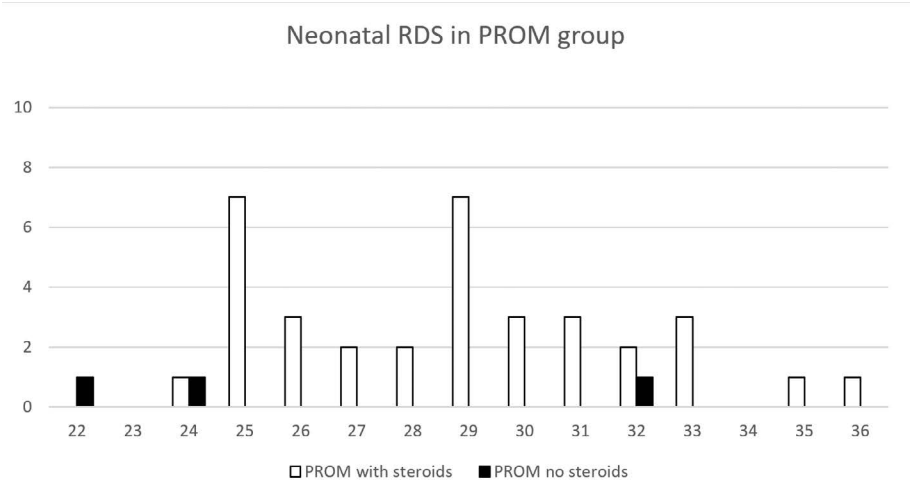

Figure 3. Neonatal Respiratory Distress Syndrome in the pPROM group depending on the gestational week at birth

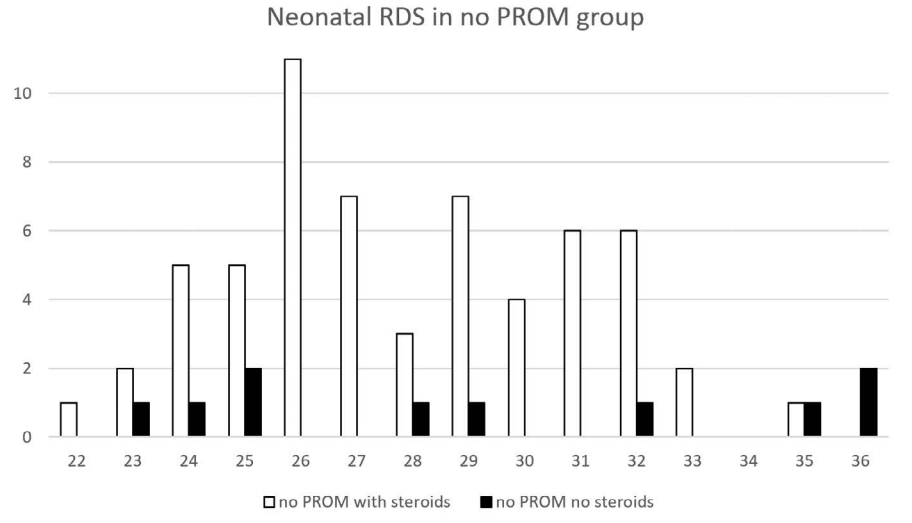

Figure 4. Neonatal Respiratory Distress Syndrome in the no pPROM group depending on the gestational week at birth

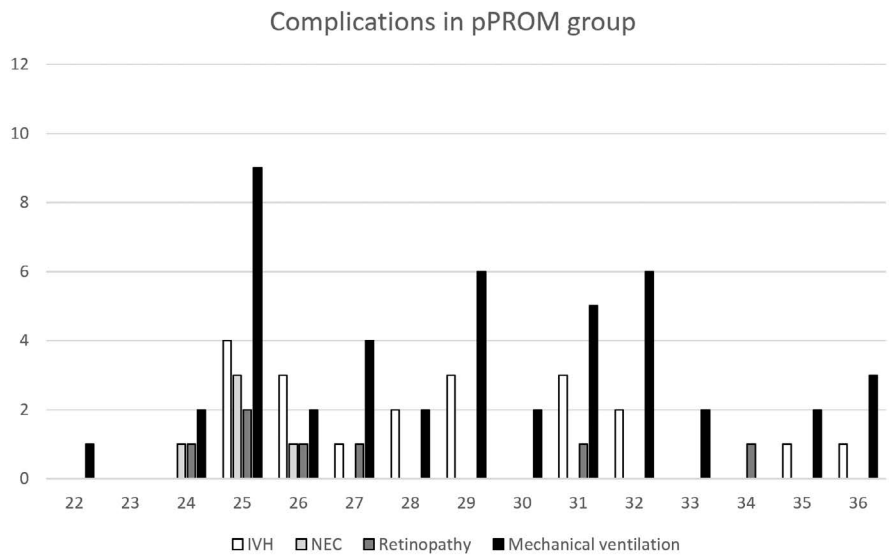

Figure 5. Occurrence of neonatal complications in the pPROM group depending on the gestational week at birth

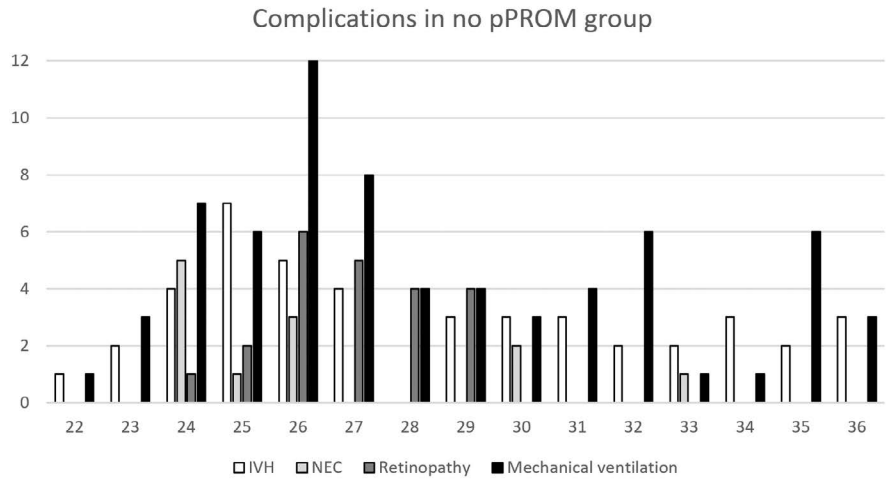

Figure 6. Occurrence of neonatal complications in the no pPROM group depending on the gestational week at birth

tational age at birth ($p < 0.001$) or lower birth weight ($p < 0.001$).

Similarly, the occurrence of the respiratory distress syndrome was independent from pPROM after adjustment for gestational age at birth ($p < 0.001$) or lower birth weight ($p < 0.001$).

PPROM occurrence was associated with reduced probability of respiratory failure (odds ratio 0.44 with 95% confidence interval 0.29–0.66; $p < 0.001$) even after adjustment for gestational age at birth ($p < 0.001$).

DISCUSSION

Certain differences regarding maternal comorbidities and past medical history, pregnancy course, delivery mode, and neonatal complications between patients giving birth before term with pPROM and with intact membranes, were identified in this study.

The largest difference in maternal comorbidities was observed in case of the prevalence of pregnancy-induced hypertension (3% vs 15.6%), pregestational hypertension (5% vs 15.5%), and preeclampsia (0 vs 11.7%), which were less common in the pPROM group. Analogous results were reported by other authors: 5.8% vs 7.6% of hypertensive disorders in the Bouvier et al. study [6] and 14.5% vs 52% of pregnancy-induced hypertension incidence in the pPROM and control groups described by Dannapaneni et al. [7]. Similarly, Pharande et al. observed lower prevalence of hypertensive disease of pregnancy in patients with pPROM

than with the intact membranes (7.6% vs 26.7%) [8]. Hypothyroidism was also more prevalent in the group without pPROM (19.4% vs 28.7%), which stands in contrast to results of other studies. In a systematic review by Maraka et al., the pooled relative risk of prelabor rupture of membranes in women with subclinical hypothyroidism was 1.43% according to data from six randomized trials and cohort studies [9]. In a prospective cohort study by Johnson et al. patients with subclinical hypothyroidism presented significantly more frequent history of pPROM (13%) compared to euthyroid women (1.4%) [10]. However, most studies indicate association between hypothyroidism and increased risk of prematurity, which might explain different proportions in our study focusing exclusively on the analysis of patients with preterm births [11]. Bouvier et al. emphasized the meaning of gestational diabetes in possible pathogenesis of pPROM due to sterile inflammation as they reported both higher rates of gestational diabetes and insulin intake in the pPROM group [6]. Our results confirm these findings only partially as insulin intake was more common among patients with pPROM (8.7%) than among the subgroup without pPROM and without hypertension (4.1%). No other significant differences in gestational diabetes incidence were observed.

Analyzed past medical history included treatment for cervical intraepithelial lesion (CIN) before pregnancy. In a systematic review based on 21 observational studies, the relative risk of pPROM was enhanced in these women (RR 2.36) [12]. Our results confirm this statement as the incidence of conization before gestation was more frequent in the pPROM group. In a recent study by Maina et al. the incidence of pPROM among patients with the history of excisional treatment was also increased (13.13% vs 2.71%) [13]. Another analyzed factor was cervical insufficiency in current pregnancy. Differences in cervical insufficiency and treatment with cervical cerclage were not significant, but the positive history of pessary application in current pregnancy was higher in the pPROM group.

Gestational age at birth did not differ between the groups. However, the group without pPROM was characterized by lower birth weight, higher occurrence of small for gestational age, extremely low birth weight, and incredibly low birth weight. These differences were still significant after subgroup analysis with the consideration of hypertensive disorders distribution. This was not observed in the Pharande et al. study, but comparable results were obtained by other authors [8, 14].

Pathological cervical microbiome was detected in 43.5% patients with pPROM and 45% without pPROM. In a study by Swiatkowska-Freund et al. the incidence of positive cervical swabs in the pPROM group was 49% [15]. As the prevalence concerns almost half of the patients, these results support introduction of standard antimicrobial prophylaxis as prevention of ascending infection route due to ruptured membranes. In this study mentioned prophylaxis was introduced in every patient with pPROM on admission or after pPROM occurrence during earlier hospitalization. Combined with strict monitoring of maternal inflammation parameters and fetal well-being, this early antimicrobial intervention, adjusted when needed after the antibiogram results analysis, could be the reason that the early onset-sepsis (EOS) rates were similarly distributed among the groups with and without pPROM (7.7% vs 8.9%). Similar observations regarding prophylaxis utility can be made based on other studies, in which antimicrobial prophylaxis was introduced in less than 50% or in over 80% of patients and the rates of EOS cases strongly varied [8, 15]. What is more, in a study by Hanke et al., it was proved in a multivariable logistic regression that pPROM is not an independent risk factor for EOS in infants with extremely low birth weight [16]. The proportion of infants exposed to prenatal antibiotic treatment in this study exceeded 80%. The number of neonates exposed to tocolytic treatment of mothers was also relatively high (68.1%) and higher than in the control group. Concerning the low EOS rate and more frequent use of intravenous tocolysis in patients with pPROM than with the intact membranes, our results are consistent with other studies reporting administration of intravenous tocolysis in pPROM [16, 17].

The occurrence of late-onset sepsis (LOS) in our study was higher in the group of premature infants without the history of pPROM. The association between LOS incidence, pPROM occurence, gestational age at birth and birth weight were examined in a multivariable analysis in order to determine LOS risk factors. Eventually, no relation between LOS incidence and pPROM was observed. This indicates more relevant factors associated with LOS other than pPROM. As the mean hospitalization duration of infants from the no pPROM group was significantly longer, it is possible that the risk of LOS depends on the number of required neonatal procedures and time of observation under strict medical supervision.

Regarding the incidence of neonatal respiratory complications, our study showed increased risk of pulmonary hypertension in the pPROM group. As patients with fetal congenital defects were excluded from the study, this result does not represent pulmonary hypertension caused by diagnosed congenital heart disease. This result is also compatible with other studies reporting compromised lung development due to oligohydramnios [8, 18, 19]. Pulmonary hypertension is also associated with prematurity and its treatment side effects e.g. by mechanisms including ventilator-induced lung injury and oxidant stress [20]. On the contrary, the incidence of respiratory distress syndrome (RDS) and respiratory failure was higher in the group with

intact membranes (12.7% vs 20% and 25.8% vs 40.4%, respectively). This result could be associated with lower birth weight and increased cesarean section rate (45.2% vs 65.3%) including emergency cesarean sections (13.7% vs 39.5%) in the group without pPROM [21, 22]. Administration of antenatal corticosteroids, including the full prophylactic dose was comparable between the groups (46.8% vs 42%). Multivariable analysis confirmed higher incidence of RDS associated with lower birth weight and earlier gestational age at birth.

CONCLUSIONS

Development of multiple complications in preterm neonates, including sepsis and respiratory complications, may be more associated with the management, gestational age, and birth weight than with the occurrence of preterm prelabor rupture of membranes.

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Dehiscence of cesarean section scar during pregnancy and delivery — risk factors

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ABSTRACT

Objective: We wanted to identify risk factors for dehiscence of cesarean section (CS) scars in patients undergoing repeated cesarean section.

Material and methods: This was a retrospective case-control study over a 3-year period in our medical center (2011–2014), comparing women who had repeated CS without complications and women diagnosed with dehiscence. Data were collected from medical records and the groups were compared for demographic and obstetrical data.

Results: Dehiscence was identified in 27 women, while 54 women without dehiscence were the control group. Statistically significant differences were found in the need for augmentation, the number of previous cesarean sections, cesarean section in the active phase of labor and length of hospitalization.

Discussion: The need for augmentation of labor, CS in the nonactive stage and more than one cesarean section, all increased the risk of dehiscence. There was no association between dehiscence and scar pain, time elapsed since the previous cesarean section, the method of wound closure or fever.

Key words: cesarean scar dehiscence; augmentation of labor; active phase of labor; scar pain; cesarean section

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INTRODUCTION

Uterine rupture refers to complete disruption of all uterine layers, including the serosa. It is a life-threatening pregnancy complication for both mother and fetus in women undergoing a trial of labor after cesarean section (TOLAC). Other adverse outcomes include complications relating to severe hemorrhage, bladder laceration, hysterectomy, and neonatal morbidity relating to intrauterine hypoxia. Most uterine ruptures in resource-rich countries are associated with TOLAC. In resource-limited countries, many uterine ruptures are due to obstructed labor and lack of access to operative delivery. By comparison, uterine dehiscence generally refers to an incomplete, and frequently clinically occult, uterine scar separation where the serosa remains intact and is not usually associated with hemorrhage or adverse maternal or perinatal outcomes. It is found incidentally in < 2% of prior Caesarean section patients [1]. Madaan et al. [2] (2011) found four cases of uterine rupture out of 300 patients undergoing TOLAC 3 diagnosed during laparotomy for suspected rupture and one case due to severe post-partum hemorrhage. In the same study, six cases of dehiscence were diagnosed, all during repeated cesarean [2]. A study of 188 women attempting vaginal birth after cesarean section (VBAC) found that previous vaginal delivery and non-recurrent indication for the previous cesarean section were good predictors of successful VBAC [3]. This study also found that uterine rupture/dehiscence was 6.5% and 4.8% in recurrent and non-recurrent indication, respectively [3].

Most cases of uterine dehiscence are diagnosed incidentally at repeat cesarean delivery, but some are identified during a prenatal ultrasound examination, sometimes with extrusion of a sac containing fetal membranes and amniotic fluid. A defect in the scar may detected as early as the first trimester, with the possibility of "cesarean scar" pregnancy [4]. Scar defects and uterine windows have also been detected in the non-pregnant uterus [5, 6].

The management of dehiscence during pregnancy is influenced by gestational age (e.g. previable, preterm viable, term), but there is insufficient evidence to make firm recommendations. Near term, repeat cesarean delivery before the onset of labor is probably the safest option to avoid

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progression to rupture. Remote from term, published and anecdotal case reports have described successful outcomes with expectant management, close monitoring, and early delivery [7, 8] and with laparotomy to repair the defect [9]. However, there is no standard or optimal approach: if the pregnancy is continued, the patient should be thoroughly counseled about the potential risks to her and to the fetus.

A large study by Miller et al (1994) of more than 1000 women who had a trial of labor after cesarean (TOLAC) found that the uterine rupture rate was 1.7% in women who had had two or more cesarean sections vs 0.6% in women who had had one caesarean section [10]. A smaller study by Caughey et al. [11] (1999) of 134 women found that 3.7% of women who had had two cesarean sections experienced uterine rupture compared with 0.8% of women who had had one cesarean section.

A retrospective study of signs, symptoms, and complications of partial and complete uterine rupture done between 1987–2008 revealed that 18.5% of the women who had partial rupture complained about abdominal pain vs 32% of the women who had complete rupture, without statistical significance [12]. In another study, only 5% of women who did not receive epidural anesthesia and had a rupture in the uterus experienced scar pain [13], while Johnson et al. [14] (1990), conducted a study of 10.976 women who underwent TOLAC, where only 22% of the women who had uterine rupture had complained of abdominal pain.

Objectives

The purpose of our study was to identify risk factors for scar dehiscence, in order to suggest management of repeat cesarean section and careful evaluation of the cesarean scar.

MATERIAL AND METHODS

This study was approved by the Institutional Review Board (Helsinki Committee) of our medical center. The electronic medical records between 1.1.2011 and 7.7.2014 in our center were retrospectively searched using the terms "dehiscence of scar" or "rupture of the uterus" or "uterine scar".

The control group was selected from women with a repeat cesarean section without evidence of scar dehiscence at the same time period as the women in the study group. For each woman in the study group we matched two women for the control group who delivered by cesarean section immediately after the woman in the study group. If two consecutive women were diagnosed with dehiscence, then four consecutive women were chosen for the control group. All women were healthy and had not undergone any surgery on the uterus after the first cesarean section which may have affected the structure of the uterus.

The following parameters were included in the data collection: age, number of pregnancies, number of births, spontaneous abortions, termination of pregnancy, ectopic pregnancy, number of cesarean sections, number of births after the first cesarean section, the number of pregnancies with dehiscence, cause of first and last cesarean section, complaints about pain in the surgical scar, evidence of infection or fever after the last cesarean section (fever was determined by two temperature measurements above 38 with a range of 6 hours). We also examined whether there was evidence of infection during surgery (according to the surgical report), duration of hospitalization at previous birth, uterine incision closing method in the previous cesarean section (either one or two layers), time elapsed between last birth and present (< or > 18 months), need for and type of augmentation of labor, type of anesthesia and the stage of labor when the cesarean was performed.

Statistical analysis

Quantitative data were described using mean and standard deviation/median and range according to data distribution. Quality data were described using prevalence and percentage**.** For comparison between the research group and the control group, quantitative data were evaluated using the Wilcoxon rank sum test due to their asymmetric distribution. The age of the women was examined using an independent sample t-test. Qualitative data were examined using Chi-square test or Fisher's exact test.

RESULTS

The study group consisted of 27 women diagnosed with dehiscence, while the control group consisted of 54 women. All women had at least one cesarean section.

There was no statistically significant differences between the study group and the control group in age, number of births or pregnancies, number of abortions or termination of pregnancy (TOP), ectopic pregnancy, and number of births after the first cesarean section (Tab. 1). Women in the study

Table 1. Demographic data for each group

Parameter	Case	Control	p
Age (mean ± standard deviation)	30.1 (± 5.4)	30.1 (± 5.3)	NS
Number of pregnancies (median and range)	3 (2–6)	3 (2–11)	NS
Number of births (median and range)	2 (1–4)	2 (1–9)	NS
Spontaneous abortions	11	14	NS
TOP	2	8	NS
Ectopic pregnancy	0	1	NS
Number of births after first cesarean section (2 or more)	3	1	NS

NS — not significant

Table 2. Number of cesarean sections in both groups

Number of cesarean sections	Control	Case	p
One CS	77.80%	55.60%	NS
Two CSs	22.20%	37.00%	0.034
Three or more CSs	0%	7.40%	

NS — not significant; CS — cesarean section

Table 3. Comparison between the two groups

Parameter	Control	Case	p
Time elapsed from previous cesarean section less than 18 months	14.80%	18.50%	NS
Cervical opening in previous cesarean section (active stage of labor)	44.40%	18.50%	0.027
Augmentation	1.90%	22.20%	0.005
Spinal anesthesia	85.20%	77.80%	NS
Epidural anesthesia	9.30%	11.10%	
General anesthesia	5.60%	11.10%	
Closing uterine incision by one layer	79.20%	80.80%	NS
Scar pain	14.80%	18.50%	NS
Fever	5.70%	4%	NS

NS — not significant

group had a higher number of cesarean sections than women in the control group with statistical significance (Tab. 2).

Table 3 summarizes the comparison between the two groups: there were statistically significant differences between the groups in the need for augmentation (more in the study group) and the stage of labor when the last cesarean was performed (more active stage in the control group) while other parameters were not statistically different. In both groups, the most common anesthesia type was spinal anesthesia, 82.7% compared to epidural and general. 34.6% of the women in the study group were hospitalized for more than 5 days compared to 13.0% of the control group. Statistically significant difference p = 0.036 (Fisher's Exact Test).

DISCUSSION

The main purpose of this study was to identify factors that increase the risk of dehiscence to avoid or reduce the risk of this life-threatening complication in future pregnancy.

The study results showed that women in the study group had a higher number of cesarean sections. Many studies have shown that women undergoing cesarean section are at increased risk of maternal and fetal complications later [10, 11, 15–17]. Our results agree with other studies since repeated CS creates yet another scar that further weakens the lower segment of the uterus, thus increasing the risk of dehiscence.

In 22.2% of women in the study group, compared with 1.9% of the control group, augmentation of labor was required with a statistically significant difference. Overall, seven women from both groups needed augmentation by nipple stimulation. In our department, it is not customary to implement pharmacological augmentation with oxytocin in women who have undergone a cesarean section in the past. Nipple stimulation increased the risk of dehiscence. The reason for this seems to be related to increased uterine contractions due to oxytocin release, but it is also possible that the need for the augmentation itself was due to the inability of the uterus to contract due to early dehiscence. Since our study is retrospective, it is not possible to determine what occurred first. A retrospective research study found that stimulation of the nipples in women with previous births and previous caesarean section is a safe and effective method [18]. In our study, augmentation increased the risk of dehiscence, thus evaluation of the scar is mandatory before any type of augmentation is implemented, and close surveillance is crucial if augmentation is given. The issue of labor induction in previous cesarean section is controversial, as was found in the survey conducted by Udayasankar et al. [19] (2008) among obstetricians in Wales. Although most obstetricians would consider induction of labor in postdate even in the event of previous cesarean section, about 88% of the obstetricians stated that these women should be counseled regarding the increased risk of uterine rupture.

There was no statistically significant difference between the study group and the control group concerning complaints of pain in the surgical scar, 18.5% of the women in the study group reported scar pain vs 14.8% in the control group. This result is also in agreement with previous studies [12–14]. Madaan et al. [2] (2011) concluded that scar tenderness and pain are poor indicators of scar dehiscence: in their series, 10 women were operated due to scar tenderness and dehiscence was not found in any of them.

There was no statistically significant difference between the closing methods of uterine incisions in previous cesarean section (either one or two layers). In our study uterine-incision closure by one layer was the most common method in the two groups. In a retrospective study of 292 women who underwent TOLAC, there was no difference in the percentage of uterine rupture comparing the two closure methods [20]. However, a large cohort study of 3.000 women showed a 4-fold chance of uterine rupture in the one layer vs two-layer closing method [21]. Our results support the first study that shows that the one-layer closure method does not increase the risk of scar rupture, however, a large randomized prospective study is needed to clarify this issue.

We examined the medical records for fever in the previous CS since fever might indicate an infection of the scar, and subsequent impairment of the healing ability of the scar, thereby increasing the chance for dehiscence. However, the results of our study showed no difference between the two groups. One woman from the study group suffered from fever vs three women from the control group, 34.6% of the study group vs 13% of the control group were hospitalized for more than five days (statistically significant), and most hospital stays were for six days (12 women from both groups). One woman from the study group was hospitalized for 18 days due to septic shock, while two women in the control group were hospitalized more than five days suffering from fever. The other reasons for long hospitalization were not related to fever but to other causes such as proteinuria, shortness of breath, suspected pulmonary embolism and back pain.

In the control group, 44.4% were in their active stage of labor during the previous cesarean section compared to 18.5% of the study group, with statistical significance. We attribute this difference to the fact that in women operated during active stage, the lower segment is already formed and the incision in these cases is done mostly without cutting the uterus muscle itself. In women not in active labor, the incision is often done in the muscle itself, even if performed in the lower segment. This hypothesis should also be confirmed in a prospective study with histological proof of the lower segment composition during elective cesarean section vs cesarean section in women in active labor. Irrespective of the reason for this finding, it is particularly important to clarify at what stage of labor the CS was performed in each patient choosing TOLAC.

The weakness of this study, in addition to its being a retrospective analysis, is that some women with dehiscence of scar were not diagnosed because our study included only women with definitive diagnosis of dehiscence during a repeated cesarean. Women with asymptomatic dehiscence were probably discharged undiagnosed.

In conclusion, the need for augmentation of labor (breast stimulation), cesarean section on nonactive stage of labor and more than one cesarean section in the past are parameters that can increase the risk of dehiscence. There was no association between dehiscence and scar pain, time elapsed since the previous cesarean section, method of closure of the scar or fever. It is important to evaluate the scar's status in women with risk factors and especially if they choose a trial of labor.

Conflict of interest

All authors declare no conflict of interest.

Compliance with ethical standards

This study was approved by our Medical Center's Institution Review Board (Helsinki committee). It was not necessary to obtain informed consent due to the retrospective nature of the study.

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The concentration of insulin-like growth factor-1 in pregnancies complicated by pregnancy-induced hypertension and/or intrauterine hypotrophy

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ABSTRACT

Objectives: The aim of the study was to compare Insulin-like Growth Factor-1 (IGF-1)concentration in pregnancies complicated by pregnancy-induced hypertension and/or intrauterine hypotrophy, and its correlation with maternal pressure and umbilical artery pulsatility and resistance indices.

Material and methods: 65 pairs pregnant-newborn were included to four groups: I — control, II — PIH, III — Hypotrophy, IV — PIH and Hypotrophy. In the study we analyzed cord blood IGF-1 concentration, newborns antropometry, umbilical artery pulsatility and resistance indices and maternal pressure before delivery.

Results: The concentration of IGF-1 was the lowest in IV group of hypotrophic newborns from pregnancies complicated by pregnancy-induced hypertension. In this group of patients there was strong negative correlation between IGF-1 concentration and maternal systolic and diastolic pressure.

Conclusions: There is a strong negative correlation between IGF-1 concentration and maternal systolic pressure in group of hypotrophic newborns from pregnancies complicated by pregnancy-induced hypertension.

Key words: intrauterine growth restriction; pregnancy-induced hypertension; insulin-like growth factor-1

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INTRODUCTION

Pregnancy induced hypertension (PIH) is one of the most serious complication of pregnancy significantly increasing the risk of stillbirth, prematurity, and perinatal death, both maternal and neonatal. Coexistence of pregnancy induced hypertension with intrauterine fetal hypotrophy increases these risks [1], but also has long-term negative impact on the further development of the children [2].

It is estimated that PIH occurs with varying intensity in 5–8% of all pregnancies [3], and their etiology is multifactorial and still unclear. It is believed that the basis for the development of this pathology are abnormal differentiation and invasion of extravillous trophoblast cells [4]. In healthy developing placentas, the extravillous trophoblast cells infiltrate spiral arteries, undergoing transformation from an epithelial to an endothelial phenotype. This leads to remodeling of their walls, conversion of muscles and elastic tissue localized in tunica media to fibrinous tissue. This process consists of two phases and enables transformation of these high resistance arteries into high capacitance, easily responsive to increased blood flow utero-placental vessels. The first stage includes the migration of trophoblast cells into the decidua. In the second stage, lasting from the 15th to 20th week of gestation, trophoblast cells infiltrate the spiral arteries. Abnormalities of the second phase was histologicaly observed in placentas from pregnancies complicated by PIH, especially incomplete transformation of spiral arteries. This pathology can result in high resistance in placental vessels, significantly reducing placental blood flow. Impaired placental perfusion leads to fetal growth retardation, activation of inflammatory response cascade and damaging of endothelium [5, 6].

Intrauterine growth restriction occuring before the 32nd week of gestation (early onset IUGR) is more often associ-

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ated with serious structural and functional abnormalities of the placenta, increased resistance in the placental vessels, haemodynamic changes with circulatory redistribution, and in the most severe cases centralization of fetal circulation. This form of IUGR is in 35% associated with pregnancy--induced hypertension [7].

Umbilical arteries pulsatility and resistance indices are good parameters of fetal well-being status. Studies have also shown that screening doppler velocimetry between 20–24 weeks of gestation are good prognostic factors in assessing the risk of developing pregnancy-induced hypertension and intrauterine growth retardation [8].

Objectives

The aim of this study was a comparative analysis of IGF-1 concentrations in cord blood in three clinical situations : 1. In pregnancies complicated by pregnancy-induced hypertension (PIH) and intrauterine fetal hypotrophy. 2. In pregnancies complicated by PIH, with normal intrauterine growth of the fetus. 3. In pregnancies complicated by intrauterine fetal hypotrophy, without pregnancy-induced hypertension. An attempt was also made to assess the correlation between values of the pulsatility and resistance indices in umbilical arteries and maternal blood pressure on the concentration of insulin-like growth factor-1 in cord blood.

MATERIAL AND METHODS

In this study 65 pairs, pregnant-newborn, hospitalized in the Department of Gynecology and Obstetrics of the Specialist Hospital No. 2 in Bytom between 2015–2018 were assessed.

The pairs were included to one of four groups depending on the clinical situation:

- Control — eutrophic newborns from uncomplicated pregnancies;
- PIH — eutrophic newborns from pregnancies complicated by Pregnancy-Induced Hypertension;
- Hypo — Hypotrophic newborns from uncomplicated pregnancies;
- PIH+Hypo — hypotrophic newborns from pregnancies complicated by Pregnancy-Induced Hypertension.

Exclusion criteria:

- prematurity < 32. weeks of gestation;
- multiple pregnancy;
- maternal diabetes;
- congenital diseases (including congenital defects, infections, persistent pulmonary hypertension);
- chronic maternal hypertension.

In all groups, the concentration of Insulin-like growth factor-1 (IGF-1) in the umbilical cord blood was tested by an immunoenzymatic method — ELISA with the use of Human IGF-1 Elisa Kit. The obtained results were analyzed in relation to mean values of systolic and diastolic blood pressure evaluated in the last day before pregnancy termination, pulsation index value and resistance index in umbilical arteries, based on routine pre-natal ultrasound examination with Color Doppler option. Neonatal anthropometric parameters (body length, birth weight, head circumference, chest circumference) were also analyzed.

Statistical analysis of the collected research material was based on the STATISTIKA statistical package, version 9.4. Distribution of quantitative variables was characterized by the mean and median values, standard deviation and standard error. The conformity assessment of the distribution of quantitative variables with normal distribution was performed using the Shapiro-Wilk test. Statistical significance of differences in distributions of quantitative variables between research groups was carried out using analysis of variance (ANOVA), with the assumption of homogeneity variations, whereas in the case where the variable distribution deviate from the normal, or in case of failure assumption of homogeneity of variance, Kruskal-Wallis and non-parametric U Mann-Whitney test was applied. Simple analysis was summarized multivariate regression analyzes. Statistical inference is based on the criterion of statistical significance of $\alpha = 0.05$.

RESULTS

Based on the analysis, it was observed that the concentration of insulin-like growth factor-1 was the lowest in the group of hypotrophic newborns from pregnancies complicated by pregnancy-induced hypertension and was significantly lower compared to eutrophic newborns from normal pregnancies and pregnancies complicated by pregnancy-induced hypertension. In the groups with isolated hypotrophy or pregnancy-induced hypertension, IGF-1 concentrations were also lower than in healthy newborns, but without statistical significance (Fig. 1).

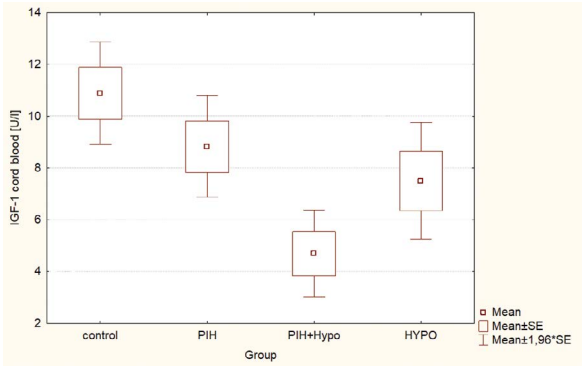

Figure 1. Comparative analysis of mean concentration of IGF-1 in cord blood in study groups with standard error and the 95% confidence interval

Table 1. Patients characteristics — median values with the lowest and the highest values and standard deviations in parentheses

	Control	PIH	Hypo	PIH + Hypo
N	25	14	16	10
Maternal age	30 (6)	34 (5)	34 (6)	33 (4)
Maternal BMI [kg/m^2]	28.8 (4.4)	35.3 (4.6)	29.5 (4.5)	30.3 (6.47)
Maternal Weight [kg]	78.5 (12.9)	95.2 (14.7)	77.4 (13.2)	86.7 (16)
Hbd	37 (34–41)	36 (32–41)	37 (34–40)	**34** $^{CTR, H}$ (32–41)
B.W. [g]	3190 (2170–4480; 492.5)	2870 (1960–3800; 580)	**2257** $^{CTR, P}$ (1600–2900; 352)	**1550** ^{CTR,P} (840–2920; 562)
Lenght [cm]	53.1 (47–60; 2.5)	52.5 (47–60; 4.0)	**49** ^{CTR} (43–54; 3)	**43.0** ^{CTR,P} (35–55; 5.2)
Head circ. [cm]	35 (31–38; 2)	34 (30–37; 2)	**32.0** ^{CTR} (30–34; 1)	**29.0** $^{CTR, P}$ (24–32; 2.2)
Chest circ.[cm]	33 (29–37; 2)	31 (27–34; 2)	**29.0** $^{CTR, P}$ (26–33; 2)	**25.0** $^{CTR, P}$ (20–32; 3.4)
PI in UA	0.86 (0.52–1.26; 0.17)	0.86 (0.55–1.18; 0.20)	0.90 (0.63–1.44; 0.24)	1.1 (0.81–1.9; 0.32)
RI in UA	0.57 (0.39–0.75; 0.08)	0.56 (0.40–0.68; 0.08)	0.59 (0.46–0.79; 0.09)	**0.70** ^{CTR} (0.55–1.00; 0.14)
Sys. Pressure	109 (82–132; 12)	139 (109–178; 24)	124 (105–145; 14)	**160** $^{CTR, P, H}$ (118–249; 36)
Dia. Pressure	68 (50–90; 9)	88 (72–103; 11)	76 (49–92; 11)	**98** $^{CTR, P, H}$ (70–149; 21)
IGF-1 (IU/l)	10.9 (2.02–38.3; 6.4)	**7.50** $^{P+H}$ (3.28–16.81; 3.74)	5,9 (2.44–19.96; 4.62)	**3,4** $^{CTR, P}$ (2.28–11.21; 2.70)

$^{P, H, P+H, CTR}$ — statistical significance comparing to group (respectively): PIH, Hypo, PIH + Hypo and Control (p value < 0.05)

The groups also differed in the values of anthropometric measurements, birth weight, body length, head circumference and chest circumference. These parameters were, of course, significantly lower in hypotrophic neonatal groups with or without gestational-induced hypertension. There was also a tendency to lower values of these parameters in newborns from the PIH group relative to the control group but no statistical significance was found. It may indicate some risks in this group of patients in which pre-natally no intrauterine growth inhibition was observed. Regression analysis showed a strong negative correlation between systolic blood pressure and the concentration of insulin-like growth factor-1 in the hypotrophic neonatal population from pregnancies complicated by pregnancy-induced hypertension (Tab. 1). A similar tendency was observed in the group of eutrophic neonates from pregnancies with PIH, although without statistical significance. Interestingly, there seem to be differences between groups of newborns with pregnancies complicated by pregnancy-induced hypertension and isolated hypotrophy. In the group of isolated hypotrophy we observed possitive correlation between values of pulsatility and resistance indices and concentrations of IGF-1. We also observed surprisingly positive correlation between antropometric parameters and systolic and diastolic maternal pressure, but there was no correlation between the head circumference and body length and the concentration of insulin-like growth factor-1 in cord blood in this group of patients. In the remaining groups, lower concentrations of IGF-1 were observed with the increase of the pulsatility and resistance indices. In the PIH and PIH + Hypo groups, higher values of birth weight and body length, head and chest circumference were observed with an increase in IGF-1 concentration (Tab. 2).

Table 2. Correlation between the concentration of insulin-like growth factor-1 in cord blood and selected parameters in the tested and control groups

	Control	PIH	Hypo	PIH + HYPO
HBD	0	0.28	0	0.24
B.W [g]	0	0.44	–0.31	0.30
Lenght	–0.21	0.39	0	0.41
Head circ. [cm]	–0.21	0.16	0	0.18
Chest circ. [cm]	0	0.37	–0.43	0.35
Sys. Pressure [mm Hg]	0	–0.24	0	–0.81#
Dia. Pressure [mm Hg]	0	–0.20	0	–0.63
Mean UA PI	–0.27	–0.42	0.20	–0.47
Mean UA RI	–0.24	–0.32	0.27	–0.64

means statistical significance (p < 0.05)

DISCUSSION

Insulin-like growth factors (IGF-1 and IGF-2) are peptides with structural similarity to proinsulin. The biological role of insulin-like growth factor-1 is omni-directional, with both aut-, para- and endocrine regulation. Its presence is already observed in the early stages of embryonic development and is one of the most important factors of growth, differentiation and maturation of tissues, affecting fetal growth processes. It also seems to be an extremely important factor, in addition to vascular endothelial growth factor (VEGF), Progesterone induced blocking factor (PIBF) and insulin-like

growth factor-2, which determines normal course of maturation, differentiation and invasion of extravillous trophoblast in spiral arteries, which process, if it goes wrong, is considered the main cause of pregnancy-induced hypertension [9]. The studies also demonstrated the regulatory effect of IGF-1 on endothelial function. It regulates and stimulates the migration of endometrial cells, stimulates neovascularization processes. There are also reports of IGF-1 vasodilatation function in in vivo studies [10]. In animal model studies, low concentrations of IGF-1 were found to be associated with higher blood pressure values due to impaired endothelium-dependent vascular relaxation via nitric oxide and increased expression of vasoconstrictor endothelin [11]. In vitro studies on endothelial cells of human umbilical veins showed a strong effect of insulin and IGF-1 on the synthesis of nitric oxide [12]. It is believed that one of the mechanisms of pregnancy-induced hypertension is the disturbed balance between vasodilators and vasoconstrictors. It is also known that insulin resistance is one of the important risk factor for pregnancy-induced hypertension.

In pregnancies complicated by preeclampsia, a lower IGF-1 concentration was observed as compared to pregnancies with normal maternal blood pressure [13, 14], and the severity of preeclampsia was inversely correlated with IGF-1 expression in the placenta and was in direct proportion to expression of Insulin-like growth factors binding protein-3 [15].

In the present study, similar results were also observed with lower IGF-1 concentrations in pregnancies complicated by pregnancy-induced hypotension and/or hypotrophy compared to the control group. Only in groups with pregnancy-induced hypertension concentration of IGF-1 was negatively correlated with maternal arterial pressure and resistance and pulsatility indices in umbilical arteries.

The limitation of this study was lower mean gestational age in groups of isolated PIH and PIH with hypotrophy compared with the other groups. It resulted from the necessity to terminate such a complicated pregnancy earlier, both due to the mother's condition (preeclampsia, placental abruption) and threatening fetal asphyxia, which was the most common cause.

However, in the statistical analysis, there was no significant relationship between IGF-1 concentration and gestational age. Similar results were published by S. Sifakis et al. [16]. They did not show statistically significant correlations between gestational age, pulsatility index values in the umbilical arteries and IGF-1 concentration.

The other limitation of this study was the lack of information about biochemical profiles and uterine artery flow in the first trimester. Other limitations included the presence of PIH and/or IUGR in previous pregnancies and the usage of acetylsalicylic acid as a prophylaxis of preeclampsia.

Incorrect placental formation leads to its insufficiency, inadequate supply of nutrients to the fetus and, as a result, to fetal growth restriction. These unfavorable intrauterine conditions result in limitations of cell divisions, changes in metabolic and signaling pathways, and even lead to epigenetic changes. This phenomenon was discribed as Barker's hypothesis [17].

An extremely important role in these processes plays reduced insulin secretion, as well as disturbances in the Growth Hormone/IGF-1 axis. The animal model studies have shown a reduction of pancreatic B-cells mass in growth restricted fetuses [18–20]. An animal model presented by Jones et al. in 1984 showed significant endocrine differences between eutrophic and hypotrophic fetuses. In smaller gestational age fetuses (SGA) they observed hypoglycaemia, hypoinsulinaemia, hypocortyzolemia and hypothyreosis, and they were negatively correlated with severity of growth restriction. In the SGA group there was also higher secretion of androstenedione and glucagon [21]. The studies also showed significantly lower insulin-like growth factor-1 secretion and increased insulin-like growth factor-2 secretion compared to normal growing fetuses [22].

SUMMARY

Pregnancy-induced hypertension is strongly associated with the risk of IUGR, and insulin-like growth factor-1 seems to be a common denominator in the pathomechanism of these pregnancy complications, both independently and coexisting. An extremely interesting issue is the increased risk of hypotrophy in the normotensive pregnancy of mothers with history of pregnancy-induced hypertension in previous pregnancies. This phenomenon has not been explained yet, but it has been confirmed in a large cohort study on the Swedish population, which may indicate a common molecular mechanism of both complications of pregnancy [23]. Based on this study it seems that in the case of pregnancy-induced hypertension, the concentrations of insulin-like growth factor-1 are lower with the higher values of maternal systolic and diastolic pressures and pulsatility and resistance indices in umbilical arteries. However, in the case of isolated hypotrophy, despite lower cord IGF-1 concentrations, there was no correlation between maternal blood pressure and IGF-1 concentration. Interestingly, during the study we observed that in the hypotrophy group the IGF-1 concentration was discretely higher at higher values of the umbilical artery resistance and pulsatility indices, which may suggest a completely different pathomechanism. Therefore, it is necessary to broaden the research in this field with the comparison of this two serious complications of pregnancy.

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Intrapartum PCR assay is a fast and efficient screening method for Group B Streptococcus detection in pregnancy

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ABSTRACT

Objectives: The aim of the study is to verify the usefulness of a real-time polymerase chain reaction versus the culture for ante- and intrapartum group B Streptococcus maternal colonization (GBS) and prevalence of discordance during the period between an antepartum screening and delivery.

Material and methods: The study involved 106 pregnant women aged 18 to 39 years. Rectovaginal samples were collected according to CDC guidelines at 35–37 weeks of gestation as well as in the first stage of labour, during physical examination and were analyzed using two independent diagnostic methods: microbiological culture with standard culture and polymerase chain reaction with real-time assay.

Results: The discordance between antenatal and intrapartum GBS prevalence has been demonstrated as well as differences associated with diagnostic strategies, culture and PCR.

Conclusions: Intrapartum detection of GBS colonization using culture or Real-Time PCR assay as well, regardless of antenatal screening test for GBS, is very useful in identifying women who require implementation or withdrawal from prophylactic intrapartum antibiotic therapy. Real-Time PCR is a quick efficient method for GBS screening in pregnant women, which can be even applied during labor due to its short time of analyzing and high sensitivity and specificity. The above fact may indicate the need to perform the GBS test in the intrapartum period in all pregnant GBS negative women using PCR assay as a more adequate diagnostic method as the procedure could reduce the risk of a neonatal GBS infection subsequently to a prophylactic antibiotic therapy in women with an intrapartum positive GBS.

Key words: group B Streptococcus; vaginal colonization; real-time polymerase chain reaction; pregnancy

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INTRODUCTION

Group B Streptococcus (GBS) are β-hemolytic, Gram-positive bacteria that are a leading cause of neonatal infections and maternal GBS colonization during pregnancy is associated with a higher incidence of ascending infection or GBS transfer to the newborn during labor [1]. An independent risk factor for GBS colonization in pregnant women is gestational diabetes [2]. The GBS direct contact with amniotic cavity or placenta may lead to inflammation of the membranes which is associated with premature birth or stillbirth [3–6]. In addition, group B Streptococcus is the leading cause of sepsis and meningitis in the first 3 months of life [7, 8] and in 1 to 3% of children it may cause a severe early-onset group B Streptococcus infection within seven days after birth, with a neonatal mortality rate of around 12% [9]. It is estimated that the incidence of women with vaginal/rectal GBS colonization is 30% and the infection is mostly asymptomatic [10]. The GBS culture identification using microbiological methods with swabbing from the vaginal and rectal area remains the gold standard [11, 12]. The results are obtained after 48–72 hours and have low predictive value of the positive result [13]. As a GBS colonization in pregnancy remains the most important risk factor for newborn disease due its vertical transmission during delivery, the time of GBS detection with immediate antibiotic prophylaxis implementation plays a crucial role. Many assays focus therefore on GBS screening in attempt to validate the fastest and the most effective method. Real-Time PCR screening during labor has

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Table 1. Maternal and neonatal demographic characteristics	
Age (years)	29.1 ± 4.9
Nullipara n (%)	66 (62.3)
Maternal weight at delivery (kg)	79.5 ± 12.9
Height (cm)	166.2 ± 5.8
BMI (kg/m^2)	28.7 ± 4.1
Mean duration of gestation (weeks)	39 ± 1.2
Rupture of membranes > 18 hours n (%)	4 (3.8)
GDM n (%)	22 (20.7)
Cesarean section n (%)	26 (24.5)
Newborns birth weight (g)	3368.7 ± 421.3
Early-onset infection n (%)	2 (1.9)
Late-onset infection n (%)	0 (0)

Data are means ± SD

the advantage of providing results within 1–2 hours with high specificity and sensitivity.

Recently the Cepheid Xpert GBS kit in the GeneXpert® Dx system is found to have been used as a GBS screening method. An automated PCR-assay with vaginal/rectal swab testing for GBS during labor may reduce the incidence of unnecessarily given intrapartum antibiotic prophylaxis (IAP) as well as can be useful for the selection of women to whom IAP should be offered, due to its high sensitivity of 85.71–89% and specificity of 90–95.9% [14–16].

Objectives

The aim of the study is to verify the usefulness of a real-time polymerase chain reaction versus the culture for ante- and intrapartum group B Streptococcus maternal colonization (GBS) and prevalence of discordance during the period between an antepartum screening and a delivery.

MATERIAL AND METHODS

The study involved 106 women aged from 18 to 39 years (mean 29.1 ± 4.9) giving birth at the Department of Perinatology, Obstetrics and Gynecology in Szczecin, Poland in the period 01.10–30.10.2018 and their children (Tab. 1). The inclusion criterion was current term pregnancy (from 37 weeks to 42 weeks), preliminary qualification for vaginal route delivery and the presentation of microbial culture for GBS, taken from vagina/rectal area as a part of routine screening test performed between 35 and 37 weeks of gestation. This investigation did an analysis to establish the prevalence and discordance of GBS colonization among Caucasian women.

Rectovaginal samples were collected from all pregnant women being in the intrapartum period according to CDC guidelines, during a physical examination and were analyzed using two independent diagnostic methods: microbiological culture with standard culture and Real-Time PCR analysis. The material was taken from the vaginal vestibule (without using vaginal speculum) and anus (above the external sphincter) using sterile swabs, appropriate for the given method of determination. After delivery, newborns swabs were taken from the ear and anus and standard culture was performed for bacterial strains identification.

A specimen collection and microbiological culture.

Swabs were collected on a transport medium, not containing activated carbon. In the laboratory, the swabs were used to inoculate two culture tubes and transferred into the tamped medium with addition of gentamicin Todd-Hewitt (8 µg/mL) and nalidixic acid (15 µg/mL). Secondary cultures were incubated for 24–48 h at 35–37°C on air or in a 5% enriched atmosphere (5% CO_2). After an incubation operation, a small number of cultures were streaked on an improved solid medium (5% sheep blood agar plates). The plates were re-incubated for 18–24 hours at 35–37°C on air or in a 5% enriched atmosphere (5% CO_2). After gaining the GBS-corresponding colony grow (characteristic narrow zone of beta-hemolysis), the Gram-positive, catalase-negative grains were identified. In doubtful cases, the CAMP (Christi, N. E. Atkins i E. Munch-Peterson) test were used for presumptive identification of Group B beta-hemolytic streptococci, Streptococcus agalactiae.

For the rapid identification of GBS colonization, in vitro diagnostic tests were used to detect GBS DNA from enriched vaginal and rectal specimens. The Xpert GBS tests of the Cepheid Xpert GBS system were used for this purpose based on a fully automated Real-Time PCR polymerase chain reaction with fluorogenic detection of the amplified DNA. After collecting the swab, it was placed in Lim broth for the enrichment and a secondary clean swab (Cepheid) dipped into the enrichment broth specimen was put in the designated chamber of the cartridge. Once the DNA solution is obtained, mixing with dry PCR reagents is performed. After the transfer into the integrated reaction tube for real-time PCR, the GBS detection takes place. On the base of fluorescent signals measurement and embedded calculation algorithms, the GeneXpert Instrument Systems interpolates the results. A fully automated GBS detection procedure using GeneXpert Instrument Systems takes about 75 minutes and the results are presented in the tables and graphically.

The sensitivity, specificity, positive predictive value and negative predictive value of the Real-Time PCR assay were separately counted with the antepartum or intrapartum culture as the reference. A Fisher's exact test was used for statistical significance ($p > 0.05$) and to analyze the differences among group means in a sample, the analysis of variance (ANOVA) has been used. Statistical analyses were performed with the use of MacStats version 1.01.

Table 2. Group B Streptococcus status of 106 pregnant women recruited for the study

n (%)	Antepartum screening 106 (100)			
	A-CS (+) 29 (27.3)		A-CS (-) 77(72.7)	
Intrapartum screening 106 (100)	I-CS (+) 12 (41.4)	I-CS (-) 17 (58.6)	I-CS (+) 8 (10.4)	I-CS (-) 69 (89.6)
	I-PCR (+) 20 (69)	I-PCR (-) 9 (31)	I-PCR (+) 9 (11.7)	I-PCR (-) 68 (88.3)
	I-CS (+) I-PCR (+) 10 (34.5)	I-CS (-) I-PCR (-) 7 (24.1)	I-CS (+) I-PCR (+) *8 (10.4)	I-CS (-) I-PCR (-) *68 (88.3)
	I-CS (-) I-PCR (+) 10 (34.5)	I-CS (+) I-PCR (-) 2 (6.9)	I-CS (-) I-PCR (+) 1 (1.3)	I-CS (+) I-PCR (-) 0
GBS infection of newborn	None		*1 (1.3)	*1 (1.3)

A-CS — Antepartum culture screening; I-CS — Intrapartum culture screening; I-PCR — Intrapartum PCR assay

Table 3. Sensitivity, specificity, and predictive values for the PCR assay and culture screening for the detection of GBS in the cohort of 106 pregnant women [%]

Performance	Antpartum culture	Intrapartum PCR assay
Sensitivity	64.7	81.8
Specificity	90.3	86.9
Positive predictive value (PPV)	75.9	62.1
Negative predictive value (NPV)	84.4	94.8

RESULTS

Of the 106 pregnant women included in the analysis, in 29 (27.3%) cases microbiological cultures for GBS collected from the vagina/rectum in the antepartum period were positive, while a negative result was found in 77 (72.7%) pregnant women. In the group of antenatal GBS positive women (n = 29), a positive result of intrapartum microbiological culture from vagina/rectum was obtained in 12 (41.4%) women, while the positive result has been confirmed by PCR in 20 (69%) women. In the group of antenatal GBS positive women, a negative result in intrapartum culture was obtained in 17 (58.6%) women, while the PCR showed a negative result only in 9 (31%) women (Fig. 1). The positive result in an ante- and intrapartum test confirmed by two methods (culture, PCR) was demonstrated in 10 (34.5%) of 29 women. On the other hand, the conversion from GBS positive test to negative GBS test in the intrapartum period confirmed by two methods (culture, PCR) was demonstrated in 7 (24.1%) of 29 women. In 10 (34.5%) cases of 29 GBS positive women in the antepartum period, the intrapartum GBS test was negative in culture and positive in PCR, while in 2 (6.9%) cases the intrapartum GBS test was positive in culture and negative in PCR (Tab. 1). In all women with a positive result of antepartum GBS, no GBS infection was found in newborns regardless of maternal intrapartum GBS status. In the group of GBS negative women in antepartum screening (n = 77), a positive result of intrapartum culture was obtained in 8 (10.4%) women, while the PCR test was positive in 9 (11.7%) women. In one case, where the conversion of antepartum negative to positive result confirmed in both the culture and PCR was demonstrated, the newborn was born with GBS infection but did not develop sepsis in the further course of the disease. In the group of women with negative GBS test in the antepartum period, the negative result in intrapartum culture was obtained in 69 (89.6%) women whereas the PCR showed a negative result in 68 (88.3%) women (Fig. 1). Paradoxically, in one case, in which ante- and intrapartum tests (culture and PCR) were negative for GBS, the newborn was born with a GBS infection but did not develop sepsis in the further course of the disease. Also, in one case with maternal negative ante- and intrapartum GBS culture and negative intrapartum PCR but positive intrapartum culture, a GBS infection was found in the newborn which did not result in sepsis. The assessment of the diagnostic reliability of used methods is presented in Table 2. The study shows that the diagnosis of GBS colonization using PCR method indicates a high sensitivity in those women in whom GBS was found to be present in intrapartum culture screening. The lack of colonization of GBS detected by PCR assay equally applies to women in whom the presence of GBS in the culture has not been demonstrated before delivery as well as in an intrapartum test, showing a high specificity in this respect. The analysis of variance did not show any relationship between GBS positive tests (antenatal and intrapartum culture and PCR assay) and the occurrence of gestational diabetes mellitus. The correlation between GBS colonization and the time of premature rupture of membranes has not been proved as well. Regardless of the time that passed from premature rupture of membranes (PROM) and the way of delivery (a vaginal vs cesarean section), the tendency to colonize with GBS was similar.

DISCUSSION

Based on a cohort study conducted in the period 2003–2015 in 60029 pregnant women, colonization with GBS streptococcus was found in 21.6% of women, GBS negative was demonstrated in 78.3% and invasive symptomatic GBS infection was detected in 0.1% of pregnant women [17]. In our material, the percentage of colonized pregnant women was similar and amounted to 27.3%, while the negative result was found in 72.7% of cases. There was no case of symptomatic GBS infection in women. Regardless of the presence of

GBS colonization in the rectovaginal area (chronic, transient or intermittent) in both pregnant and non-pregnant women, its incidence is within 15–35% [18]. Young BC et al. demonstrated differences in the occurrence of GBS colonization, depending on the time when samples were collected for culture. When a swab was taken in the antenatal period, GBS positive results were found in 19.5% of women, compared with 23.8% of women who had performed the intrapartum GBS screening test. It has been demonstrated that there is a discordance of GBS cultures collected in the antenatal period and those collected in the intrapartum period, amounting to as much as 10.4% [18–20]. We obtained similar results in our investigation where among 77 women GBS negative in the antenatal period, the positive culture test was found in 8 (10.39%) and positive PCR assay in 9 (11.69%) individuals. It is reported that the antenatal microbiological screening test has the sensitivity of about 60% and a relatively low specificity [16, 19–21]. Our studies confirmed a high sensitivity of intrapartum PCR assay when compared to antenatal culture, which is consistent with other studies [22–25]. This result is important because as it has been shown in Young et al. report, majority of newborns with sepsis was born from women with GBS negative antenatal screening test and subsequently without the IAP [18]. In our material, among 3 newborns born with GBS infection, 2 newborns came from GBS negative mothers in the antenatal period who did not receive IAP. The above fact may indicate the need to perform the GBS test in the intrapartum period in all pregnant GBS negative but also positive women. The above procedure could reduce the risk of neonatal infection in women with conversion to positive GBS in which IAP has been implemented. In turn, in the group of women with antenatal false-positive GBS result, GBS intrapartum assay would be conclusive, especially when performed using PCR assay and could reduce the incidence of unnecessary IAP. When taking the time of procedure for consideration, the use of Cepheid Xpert GBS is a more adequate method for rapid GBS colonization detection as well as facilitating qualification for antibiotic prophylaxis during labor [1]. In the Plaivert et al. [22] studies, out of 565 women with antenatal positive GBS, only 335 (59.3%) confirmed GBS presence in the intrapartum test regardless of a diagnostic method which proves the unnecessary use of prophylactic antibiotics in almost 40% of women. Our results turned out to be even more unsatisfactory. Among antenatally colonized women only 41.4% confirmed the GBS presence in intrapartum PCR assay. According to the literature, a GBS identification using PCR is a sensitive and specific method acceptable for an intrapartum GBS screening [22, 23]. The meta-analysis of intrapartum GBS colonization conducted among 6368 pregnant women in 15 studies showed high sensitivity (93.7%) and specificity (97.6%) of the PCR assay in relation to the microbiological methods which due to rapid result determines the PCR superiority [26]. Also, in our investigation, despite the relatively small analyzed group, the PCR method proved to be a useful diagnostic tool during delivery. According to Helmig et al. an intrapartum PCR assay is an adequate diagnostic tool to qualify patients for prophylactic antibiotic therapy [23]. In our study, the relationship between two variables: time elapsed from PROM to delivery and incidence of GBS colonization has not been confirmed regardless of the diagnostic method used (PCR, culture), which is consistent with studies of other investigators [27]. In a study conducted by Victoria Parente et al. [28], an early fetal rupture of membranes over 18 hours was associated with a slightly higher chance of occurrence of GBS colonization (OR 1.38).

CONCLUSIONS

We conclude that intrapartum detection of GBS colonization using culture or Real-Time PCR assay as well, regardless of antenatal screening test for GBS, is very useful in identifying women who require implementation or withdrawal from prophylactic intrapartum antibiotic therapy. Real-Time PCR is a quick efficient method for GBS screening in pregnant women, which can be even applied during labor due to its short time of analyzing and high sensitivity and specificity.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical approval

This study was approved by the ethics committee of the Pomeranian Medical University in Szczecin (N^0 KB-0012/23/15). All patients provided written consent before the inclusion in the study.

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Supplementation of dehydroepiandrosterone (DHEA) in pre- and postmenopausal women — position statement of expert panel of Polish Menopause and Andropause Society

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ABSTRACT

Dehydroepiandrosterone (DHEA) concentration decreases with age, therefore, DHEA has been considered a hormone that reduces the symptoms associated with aging, so the usefulness of DHEA in premenopausal and postmenopausal women, and the options of hormone therapy have received a large amount of attention. The effectiveness of DHEA in the premenopausal women remains unclear, while in postmenopausal women with coexisting estrogens deficiency is controversial. Despite many years of study, the use of DHEA is still controversial, especially regarding its effectiveness. The aim of present article was to evaluate DHEA specific effects on metabolic parameters, bone mineral density, insulin resistance as well as the therapeutic potential of DHEA in pre- and postmenopausal women using measures of sexual activity, cognition and well-being. The summary of this article is the position statement of expert group of the Polish Menopause and Andropause Society regarding the efficacy and safety of DHEA supplementation in women. We concluded, that currently available clinical trials and meta-analyses indicate that DHEA supplementation is effective in women with adrenal insufficiency and chronically treated with exogenous glucocorticoids, postmenopausal women with low bone mineral density and/or osteoporosis, premenopausal women with sexual disorders and low libido, and in women with vulvovaginal atrophy due to menopause or genitourinary syndrome of menopause. Currently available clinical trials also suggest that DHEA supplementation is probably effective in postmenopausal women with hypoactive sexual disorders, infertile women with diminished ovarian reserve, women suffering from depression and anxiety, and women with obesity and insulin resistance. No serious adverse effects have been reported.

Key words: dehydroepiandrosterone; perimenopause; menopause

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INTRODUCTION

Dehydroepiandrosterone (DHEA) represents the most abundant steroid hormone in humans. DHEA concentration gradually decreases with age reaching the lowest levels about the time when the incidence of many diseases increases. Therefore, DHEA has been considered a hormone that slows down or prevents aging, or at least reduces the symptoms associated with aging, therefore the usefulness of DHEA in perimenopausal and menopausal women, and the options to hormone therapy have received a large amount of attention.

However, not all aspects of physiology, pathophysiology, mechanism of actions, clinical relevance and safety of DHEA supplementation in women have been known to date. The effectiveness of DHEA in the premenopausal women remains unclear, while in postmenopausal women many symptoms are caused by both estrogens and DHEA deficiency. Therefore, it is difficult to clearly distinguish between menopause and adrenopause. Despite many years of study, the use of DHEA is still controversial, especially regarding its effectiveness.

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The aim of present article was to evaluate DHEA specific effects on metabolic parameters, bone mineral density, insulin resistance as well as the therapeutic potential of DHEA in pre- and postmenopausal females using measures of sexuality, cognition and well-being. The summary of this article is the position statement of expert group of the Polish Menopause and Andropause Society regarding the efficacy and safety of DHEA supplementation in women.

DHEA IN HUMAN PHYSIOLOGY

Dehydroepiandrosterone (DHEA) is an endogenous androgen produced in zona reticularis of adrenal cortex (30%), thecal cells in ovary (20%), and from peripheral conversion of DHEAS (30%).

In peripheral tissues DHEA is converted to more active androgens and estrogens: estrone and testosterone and later to estradiol and dihydrotestosterone (DHT) respectively. The contribution is remarkable, since in postmenopausal women 40–75% of testosterone and 90% of estrogens are derived from adrenal androgens [1]. DHEA-S serves as a circulating reservoir of DHEA. DHEA can be reversely transformed in many tissues by sulphatases from its sulphate (DHEA-S). Together with its sulphate, DHEA is the most concentrated hormone and the most abundant steroid in peripheral blood, thus it became clear that it is more than just an intermediate in steroid hormone synthesis. Serum DHEA concentrations range from 0.2 to 0.9 mcg/dL (7 to 31 nmol/L). Due to its remarkable and gradual decrease that occurs with aging, DHEA was considered as an anti-aging elixir and concept of dietary supplementation of DHEA was promoted and administration was introduced in 1980s [2]. As primary effects improvement in sexual function, well-being, metabolic parameters, immune response, and cognition were suggested. Noteworthy many initial studies regarding anti-aging properties and mechanisms of DHEA were conducted on rodents that naturally do not secrete DHEA from adrenal glands, thus may be irrelevant [3]. DHEA also plays an important role in reproductive endocrinology. As other androgens, DHEA is important in follicular steroidogenesis and oogenesis in ovary. Described effects on ovarian folliculogenesis includes upregulation of insulin-like growth factor-1 (IGF-1) [4], sensitization to gonadotropins and reduction of follicular arrest [5].

Secretion of DHEA is stimulated by ACTH from pituitary gland; however, levels of DHEA-S are not linked directly to ACTH due to long plasma half-life time and high concentration of the sulphate. The hydrophilic DHEA-S is secreted mainly by adrenal glands and is a major circulating and most stable form of DHEA that can easily be interconverted in target tissues. Metabolism of DHEA is modulated by binding to plasma proteins, since SHBG have low affinity to DHEA and no affinity to DHEA-S. Albumins weakly binds DHEA but have high affinity to DHEA-S and increases its half-life time [6].

Physiologically, their production clearly increases in children between 6–8 years of age, which is called adrenarche. These hormones in the child's target tissues are converted to steroids with more activity. The place of conversion is, inter alia, the skin. Pubic hair appears apocrine sweat glands begin to function, and sebaceous gland secretion increases. At this time, slight acceleration is also observed in bone growth and maturation. DHEA and DHEA-S levels have been shown to depend on nutritional status. Obese children have higher levels of DHEA-S and earlier achieve adrenarche than lean children. Some research suggests that adrenal androgens directly or after peripheral conversion to estrogen modulate hypothalamic activity influencing the gonadarche. DHEA and DHEA-S concentrations increase gradually to the third decades of life. Peak secretion of these hormones falls for ages between 20 and 30 years old. Levels since then DHEA and DHEA-S in the blood gradually decrease to reach 20% of its maximum value in its 70s, up to a fall to 5% at the age of 85–90 [7]. Humans and primates are unique in their capacity to produce large amounts of adrenal steroids.

In postmenopausal women ovarian production of DHEA is ceased in contrast to testosterone that is still produced by postmenopausal ovaries for many years [8]. Diverse conditions influence the DHEA(S) activity. DHEA and DHEA-S can be suppressed in many pathological conditions including acute stress, severe chronic systemic diseases, anorexia nervosa, ACTH-independent Cushing's syndrome and chronic medication with anti-inflammatory doses of glucocorticoids (GCS). Higher levels were described in hyperprolactinemia [3].

DHEA exerts its primary effects through its estrogenic and androgenic derivatives since a unique DHEA receptor is not known. However, DHEA exhibits a weak antagonist effect on the androgen receptor (AR) [9]. It was described that DHEA plays a role as a low-affinity ligand for hepatic nuclear receptors, such as the pregnant X receptor, the constitutive androstane receptor, and estrogen receptors α/β (ERα/ERβ) as well as G protein-coupled ER (GPER1) [1]. Moreover, DHEA may act in brain as an stimulatory neuromodulator by blocking the action of the gamma-aminobutyric acid type A (GABA) receptor, as well as, activating the N-methyl-D-aspartate (NMDA) receptor and the σ-subtype 1 receptor, thus having anti-depressive potential. Furthermore, DHEA can play a role in endothelial proliferation and angiogenesis through membrane-bound G-coupled receptor that has been described on vascular endothelial cells [3].

SUPPLEMENTATION OF DHEA AS A HORMONE REPLACEMENT THERAPY IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN

Postmenopausal women present sharp drop in estradiol level and rapid rise of FSH. In elderly males slow but continuous, age-dependent decline of testosterone level is observed. The decrease is more pronounced for free testosterone as a result of the age-associated increase of the levels of sex hormone binding globulin (SHBG). Moreover, the circadian rhythm of testosterone secretion is lost in elderly men. The age-associated decrease of DHEA is the most important and ubiquitous decrease of all hormones in all men and women [10]. In contrast to estradiol breakdown, androgens are secreted for many years after menopause. Interestingly some menopausal women, e.g., after bilateral oophorectomy or premature ovarian failure, experience concomitant deficiency of androgens, thus could clearly benefit from DHEA supplementation [11].

Osteoporosis, metabolic health, and muscular strength

DHEA is the precursor for at least 70% of androgens in elderly women, and a major origin of estrogens in males and postmenopausal females. Therefore, it has been suggested that the drop in DHEA level with aging leads to physiological changes that are dependent on steroid hormones, such as the loss of bone density and muscular mass.

A small number of randomized controlled trials have assessed changes in bone mineral density (BMD) in older females on DHEA replacement therapy at doses of 25–100 mg/d. In a randomized controlled trial, the effect of 12-month supplementation with DHEA on BMD in 70 women, aged 60-88 years with low serum DHEAS concentration levels at baseline was investigated [12]. The intervention compared oral DHEA 50 mg/d with placebo for 12 months. Intent-to-treat analyses showed trends towards increase of BMD with DHEA versus placebo at the total hip (1.0%), trochanter (1.2%), shaft (1.2%), and lumbar spine (2.2%). Also, modest effects of DHEA supplementation on bone density in postmenopausal women was observed when treatment time exceed 52 weeks [3].

A recent systematic review with meta-analysis of randomized placebo-controlled studies of DHEA supplementation of BMD in healthy women revealed, that hip and trochanter BMD increased remarkably above control group in individuals, who took DHEA supplementation [13]. During DHEA therapy, serum osteocalcin also increases (from 1.16 to 2.44 µg/L), which is a marker of bone formation [14].

The aging process in women is associated with changes in muscle, that can lead to sarcopenia, and about 20% of patients with sarcopenia are functionally disabled. An important reason for this phenomenon is a marked decline in serum sex steroids – estradiol and DHEA. In postmenopausal women, DHEA becomes the predominant sex hormone, however the relationship among, DHEA, and mass of muscles or strength in women after the menopause has not been documented.

Therefore, the relation between the level of intramuscular steroid hormones and muscle characteristics in menstruating women was investigated [15]. The authors measured isometric skeletal muscle strength, knee extension strength, and explosive lower body muscle power. They concluded that intramuscular estradiol, testosterone, and DHEA are proved significant, independent predictor factors of strength and power that explains 59–64% of the difference in knee extension strength and 80–83% of the difference of vertical jumping height in women. These results suggests that intramuscular sex hormones are related with strength and power level in female muscles.

In most recent study [16] the relationship between sex steroids (DHEA, testosterone and estradiol) muscle mass, and muscle strength in postmenopausal women were investigated. Women classified within the lowest DHEA and free testosterone tertile had lower muscle strength in comparison to those placed in the highest tertile (adjusted OR for DHEA 1.42; 95% CI 1.02–1.99), however, estradiol was not related to muscle strength.

The accumulation of abdominal fat increases with age, and is associated with insulin resistance, diabetes, and atherosclerosis. Hormonal changes (low DHEA levels) that occur with aging may contribute to the increase in abdominal fat. Administration of DHEA to reduce visceral fat accumulation in rats, and has a protective effect against insulin resistance and the decrease in insulin responsiveness. A possible explanation for these findings is that DHEA is an alpha activator of peroxisome proliferator activated receptor α (PPARα), what is associated with favor increasing of fat oxidation and reducing fat deposition.

The influence of DHEA supplementation of insulin metabolism was also investigated in women. In randomized, double-blind, placebo-controlled trial conducted in 28 women with age-related decrease in DHEA level, participants received 50 mg/d for 6 months [17]. DHEA therapy results in significant decreasing in visceral and fat and subcutaneous fat area. Also, the insulin levels decreased, and insulin sensitivity increased during the OGTT after DHEA therapy. So, authors concluded, that DHEA replacement therapy could play a role in prevention and management of the metabolic syndrome associated with abdominal obesity in women. Despite these results, there are no sufficient data in terms of influence of DHEA supplementation on insulin sensitivity as well as lipid profiles in women [3].

Depression, anxiety, cognitive function and mood improvement

Currently, many data indicate, that DHEA modulate many neurobiological actions and there are evidences showing that DHEA concentrations are inversely correlated with ratings of depressed mood [18]. DHEA as well as glucocorticoids take a part in regulation of many physiological mechanisms and play an important role in regulation of affect and its dysregulation. DHEA levels remarkably decrease throughout adulthood but an increase in circulating cortisol level with advanced age has been proved in human. Therefore, it can be interesting to consider the fraction of both hormones in addition to their absolute levels of concentration. Lower DHEA to cortisol ratio may impact many physiological functions, including learning, memory, and is associated with greater cognitive impairment. It has been recently revealed that DHEA treatments improve cognitive deficits and depressive like behaviors in animals by promoting neurogenesis in the hippocampus [19].

In patients with posttraumatic stress disorder (PTSD), which is classified as the anxiety disorder, elevated DHEA concentration was identified, and researchers have suggested, that DHEA may have a role in resilience and in proper adaptation to stressors [20].

Majority of studies have reported lowered serum concentration levels of DHEA in subjects with poor life quality, satisfaction and psychosocial stress. Higher plasma and serum concentrations of DHEAS have also been associated with greater amount, frequency, and enjoyment of leisure activities and psychological profiles associated with health [21].

Clinical trials of DHEA treatment for depression constantly suggest beneficial consequences. Randomized control trials showed that application of DHEA used alone or as an antidepressant adjunct in unresponsive patients with depression, has significant antidepressant effects (as shown by improvements in Hamilton Depression Ratings and Symptom Checklist) in some of them. In most studies, authors stated that baseline serum DHEA concentrations did not predict antidepressant response, responders to DHEA reached higher serum DHEA concentrations following treatment and antidepressant effects were directly correlated with variation in DHEA levels [22, 23]. However, recent data suggest that higher circulating DHEA(S) levels can predict SSRI-associated remission in major depression [24]. It is obvious that, more trials will be necessary to establish the place of DHEA supplementation in therapy of patients with depression, and to compare DHEA to standard antidepressants.

Libido and sexual satisfaction

Sexual desire and libido in women are affected by endocrine factors. Adrenal androgens are necessary for normal sexual function, while disorders characterized by androgen deficiency are associated with a low frequency of sexual activity.

Although testosterone plasma levels do not correlate directly with sexual function in cross-sectional and longitudinal studies, they have the main role in sexual desire [25], On the other hand, it is suggested, that a poor DHEA level negatively correlates with sexual function in women before and after menopause [26].

DHEA supplementation has been recognized for its beneficial effect on sexual function but still the effects of DHEA therapy on sexuality in postmenopausal women are controversial [27], what is mainly associated with dose of DHEA and treatment time.

In most recent study, authors reported the influence of DHEA supplementation in dose of 10 mg daily on sexual function, frequency of sexual intercourse, and quality of relationship early after menopause [28]. Efficacy was evaluated using the McCoy Female Sexuality Questionnaire during 12 months of treatment. DHEA supplementation related to improvement in sexual function, and significant growth in the numbers of sexual intercourses.

The results of DHEA administration on sexual function was also investigated in premenopausal women. This observational study [29] investigated the effect of DHEA in dose 25 mg three times a day on the Female Sexual Function Index (FSFI) in women with mean age 41 years. The FSFI score for the treated group raised by 7%, domain scores for desire raised by 17% and by 12% for arousal, while no difference in domain scores for orgasm or satisfaction were proved. Women starting in the lowest quartile of FSFI score, experienced 34% growth in total FSFI score during treatment with DHEA. Among participants of a trial, improvements in domain categories were shown for desire (40%), arousal (46%), lubrication (33%), orgasm (54%), and sexual satisfaction (24%). This study implies that administration of DHEA improved sexual function in premenopausal women from low baseline FSFI scores group.

Androgen replacement in unselected postmenopausal women has been administrated for decades as an off-label treatment. Recently a global consensus on testosterone replacement for women was formulated [30]. The statement does not support the systemic and local use of DHEA for hypoactive sexual desire disorder (HSDD) in postmenopausal women with proper adrenal function. While, testosterone treatment, in doses that are in normal range of the physiological testosterone concentrations for premenopausal women, results in a beneficial effect on sexual function. Study included increases, above the effects of placebo/comparator therapy, of an average of one satisfying sexual event per month, and increases in the subdomains of sexual desire, arousal, orgasmic function, pleasure, and sexual responsive-

ness. On the other hand, a reduction in sexual concerns including sexual distress was observed.

More adequately powered, double-blind RCTs, with no selection bias and with consistent reporting and analysis of standardized results, are required to comprehensively document the benefits and risks of DHEA and testosterone supplementation in women.

Cosmetic dermatology

DHEA can also be used in cosmetic dermatology. It has been shown to act on the sebaceous glands, increases sebum production in postmenopausal women, and thus prevents the skin from drying out. This effect is due to presence in these cells, the steroidogenesis enzymes necessary for catalyzing the transformation reaction of DHEA to DHT, which is main stimulator of sebaceous gland activity [31]. In addition, DHEA could improve skin hydration reduction of its pigmentation [32].

SUPPLEMENTATION OF DHEA IN WOMEN WITH ADRENAL INSUFFICIENCY AND CHRONICALLY TREATED WITH EXOGENOUS GLUCOCORTICOIDS

Highest beneficial effect of DHEA supplementation was reported in individuals with virtually complete DHEA deficiency that occurs in adrenal insufficiency [11]. Primary or iatrogenic adrenal insufficiency, despite supplementation of glucocorticoids (GKS), leads to decreased quality of life (QoL) when compared to healthy population [33]. DHEA supplementation has been studied as an accessory treatment to conventional adrenal replacement therapy with glucocorticoids and mineralocorticoids. The exact physiological roles of DHEA still remains not fully elucidated and the routine therapy in individuals with adrenal insufficiency is still controversial. Some papers reported significant improvements of mood, well-being, sexual thoughts, libido, interest and satisfaction following DHEA replacement particularly in females [11, 34]. Other analysis of DHEA administration in women with primary and secondary type of adrenal insufficiency have resulted in inconsistent and unreproducible data [35]. According to recent recommendations, supplementation of DHEA is indicated in hypo-adrenal women with low libido, depressive symptoms, low energy levels or impaired sense of well-being despite optimized glucocorticoid and mineralocorticoid replacement [36].

In women with systemic lupus erythematosus, treated with high, that is anti-inflammatory doses of corticoids DHEA reduced disease activity and had an antiosteoporotic effect. One study suggested also benefits for cognitive function in such patients [37].

SUPPLEMENTATION OF DHEA IN WOMEN WITH VULVOVAGINAL ATROPHY

Efficacy and safety of DHEA were also investigated in women with vulvovaginal atrophy due to menopause, also called genitourinary syndrome of menopause (GSM), especially in women suffering from moderate to severe dyspareunia or pain at sexual activity.

In a prospective, double-blind, and placebo-controlled clinical trials with randomization, the effect of intravaginal 0.50% DHEA (6.5 mg per day) was assessed on four coprimary objectives, i.e., percentage of parabasal cells, percentage or superficial cells, level of vaginal pH, and pain at sexual activity (moderate and severe dyspareunia) identified by the women as most troublesome vulvovaginal atrophy symptom. After intravaginal administration of 0.50% DHEA for a period of 12 weeks, the fraction of parabasal cells was decreased by 27.7%, whereas the percentage of superficial cells were increased by 8.44%, vaginal pH was reduced by 0.66 pH, and the pain at sexual intercourse decreased by 1.42 severity score unit from the baseline. On the other hand, moderate or severe vaginal dryness, that was present in 84.0% of women, have improved at 12 weeks by 1.44 severity score unit in comparison to baseline. At gynecological examination vaginal secretions, epithelial integrity, epithelial surface thickness, as well as, color were all improved [38].

The clinical benefits of DHEA therapy demonstrated in 12-week studies, were also confirmed in 52-week study [39]. After 52 weeks of treatment, a significant betterment was seen in comparison to the baseline in parameters such as a decrease in the fraction of parabasal cells, a growth in the fraction of superficial cells, and a decrease in vaginal pH, as well as the volume of vaginal discharge, and vaginal epithelial integrity, thickness and color. In women who suffer from moderate to severe dyspareunia as the most bothersome symptom of GSM, and who met the inclusion criteria of postmenopausal vaginal atrophy, the pain severity score was decreased by 46.7% during 12 weeks period, and further by 19.4% between 12 and 52 weeks, finale reaching the level of 33.9% of the baseline at week 52. In women that suffer from moderate to severe dyspareunia and considered vaginal dryness or vaginal irritation/itching as the most bothersome symptom of atrophy, the pain severity score reduced from 2.42 ± 0.07 at baseline to 0.77 ± 0.12 at week 52. Moreover, in females with moderate and severe vaginal dryness or vaginal irritation/itching, the severity of each of the symptoms after 52 weeks of administrating prasterone decreased also significantly.

SUPPLEMENTATION OF DHEA FOR FERTILITY IMPROVEMENT

Diminished ovarian reserve (DOR) is a common cause of otherwise unexplained infertility, as well as, early ovarian hor-

monal insufficiency and related endocrinopathies. Recently published meta-analysis that included 14 studies shows that individuals with premature ovarian insufficiency (POI) have lower concentration levels of androgens in comparison with healthy controls: DHEAS, testosterone and androstendione [40]. Others estimated that suboptimal DHEAS concentrations are quite common and were found in 65% of women with diminished ovarian reserve DOR [41]. Androgen levels are lower in POI than in women with regular cycles [42]. Autoimmunological disorders can have a role in deregulated function of DHEA on DOR. Occurrence of the anti-thyroid antibodies in women with POI correlated with deeper drop of DHEAS. Treatment with DHEA caused decrease in thyroid peroxidase autoantibodies levels [43]. In a small group consisting of 25 patients with POF high proportion (44% vs. 4%) of autoantibodies was found. Moreover, occurrence of autoimmunity was associated with higher DHEAS level decrease [44].

Supplementation of DHEA in IVF cycles

The DOR is a leading challenge for artificial reproductive technologies (ART), since growing incidence and significant decrease in the effectiveness of stimulation and outcome of *in-vitro* fertilization (IVF). It can be explained by decreased ovarian reserve and reduced oocyte quality. Although, ESHRE recommendations do not support any therapeutic modality in premature ovarian insufficiency promoting oocyte donation in such cases. Moreover, it is indicated that androgen administration has poor evidence in infertility treatment [45]. Up to date, many attempts were done to improve the effectivity of therapy in infertile DOR women. Supplementation with DHEA is one of the solutions to the problem. A recent meta-analysis proved that the rates of clinical pregnancy (CPR) were improved significantly when DHEA pre-treatment was implemented (OR = 1.47, 95% CI: 1.09–1.99). No differences in the number of oocytes retrieved, the cancellation rate, and the miscarriage rate was reported [46]. Other meta-analysis reported supplementation with DHEA as a therapy that improve IVF results in patients with DOR. However, randomized controlled trials did not give a prove for the performance of DHEA therapy, one prospective randomized trial revealed that DHEA may increase potential for fertility in women without DOR [47]. Since females with normal ovarian reserve supplemented with DHEA had a significantly better live birth rate and a lower miscarriage rate [48], it has been even indicated that all women aged above 35 years may be treated with DHEA to diminish the abortion rate and increase treatment effectiveness [49].

The physiological processes underlying advantageous activity of DHEA occurred to be still not fully determined. Supplementation with DHEA rise the number of top-quality embryos, transfers and fertilization rate compared with placebo group. Moreover, supplementation was shown to decrease DNA damage and rate of apoptosis, increase the mitochondrial fraction, and dehydrogenase activity in mitochondria of cumulus oophorus [50]. DHEA administration caused that higher embryo score is associated by changes in follicular fluid constitution that includes variation in bone morphogenic protein-15 (BMP-15) [51]. Other authors reported that DHEA advantageous effect on gonad is caused by reduced concentration of senescence marker: senescence-associated β-galactosidase (SA-β-gal) in cumulus oophorus and in granulosa cells [52]. Administration of DHEA increases the level of androgen receptor (AR) and follicle-stimulating hormone receptor (FSHR) in granulosa cells. Moreover, higher increase was associated with improved results of stimulation [53]. It is known that DHEA, as well as, its downstream metabolites can act by modulation of immune response. The supplementation could also increase the Th1 immune response and change the balance of the Th1/Th2 ratio. Studies on animal model also indicated some mechanisms. DHEA administration may increase T lymphocyte infiltration on murine model, causing a decrease in the CD4+ T lymphocyte population, an increase of the CD8+ T lymphocyte number. Therefore, change in of balance between CD4+/CD8+ T cells take a place [54].

Supplementation of DHEA in natural cycles

Treatment with DHEA has been applied in infertile females with low ovarian reserve who try to conceive spontaneously. One of first case series by Mamas and Mamas described five females with POI supplemented with DHEA. The treatment caused decrease in level of FSH and spontaneous pregnancy in all reported patients that occurred in 1-6 months from start of treatment [55]. DHEA was evidenced to significantly upregulates AMH in patients with DOR. Advantageous outcomes were reported for antral follicle count (AFC), estradiol, inhibin B and FSH concentrations [56–58]. Trials on patients pre-treated with DHEA, because of low ovarian response, reported remarkably high number of spontaneous pregnancies and ongoing pregnancies (21.05% and 13.15% respectively). Recent paper show that 2 in 20 supplemented patients conceived spontaneously in DHEA group [59]. Many small series of cases advocating for implementation of DHEA supplementation in infertile DOR patients that refuses oocyte donation in IVF treatment have been published so far [60].

In the animal model supplementation of DHEA reversed the DOR phenotype and reduced atresia rate of the follicles in rats. After administration animals had increased number of primordial, primary, and growing follicles in comparison to untreated group. However, androgen supplementation did not reverse the phenotype completely and the number

of follicles was still reduced in comparison to control rats without DOR [61].

Studies on rat model of DOR showed that there is a window for DHEA dose. To high dosage of DHEA did not improve the ovarian reserve and pregnancy outcome, but rather induce PCOs-like morphology of gonads and impair fertility. This fact indicates the necessity of personalization of the treatment. Tailoring the dose of DHEA and proper selection of patients that will have improvement thanks to the therapy would increase the effectiveness [62]. Therefore, assessment of DHEA-S level and supplementation of DHEA selectively in low-DHEAS group seems to be reasonable and proper treatment strategy [60].

SIDE EFFECTS OF DHEA SUPPLEMENTATION AND CONTRAINDICATIONS

Common androgenic side effects of DHEA replacement have been reported so far: hirsutism, acne, greasy skin, itching of the skin scalp, abundant vaginal discharge, increased apocrine secretion of sweat and related odor. Some studies reported no such side effects. The side effects were infrequent, mild and well-tolerated. No serious adverse effects have been ever reported [35, 63, 64].

Prior to initiating supplementation with DHEA a complete family and personal medical record should be obtained. Careful gynecological and breast examination should be done periodically. Contraindications and possible benefit-risk profile should be considered before treatment and during follow-up. Temporal and persistent contraindications for DHEA supplementation that should be consider [63] includes:

- hypersensitivity to the substances contained in the formulation,
- non-diagnosed vaginal bleeding, untreated endometrial hyperplasia
- diagnosed breast cancer, history or suspicion of breast cancer
- estrogen-dependent malignancy (diagnosed or suspected)
- pregnancy or breastfeeding
- acute liver disease, elevated liver enzymes, kidney failure
- previously or currently diagnosed venous thromboembolism, known susceptibility to venous thrombosis
- active or recent arterial thromboembolic disease
- prostatic cancer or benign prostatic hyperplasia
- porphyria.

CONCLUSIONS

Currently available clinical trials and meta-analyses indicate that DHEA supplementation **is effective** in the following cases:

- Adrenal insufficiency and chronically treated with exogenous glucocorticoids
- In postmenopausal women with low bone mineral density and/or osteoporosis
- In premenopausal women with sexual disorders and low libido
- Vaginally in women with vulvovaginal atrophy of menopause or genitourinary syndrome of menopause (GSM)

Currently available clinical trials suggest that DHEA supplementation is **probably effective in the some of the following cases**:

- Postmenopausal women with hypoactive sexual disorders
- Infertile women with diminished ovarian reserve (DOR)
- Women suffer from depression and anxiety
- Women with obesity and insulin resistance

Usual daily doses of DHEA that are administrated in clinical trials and regular off-label use are summarized in Table 1. In majority of conditions oral dose of 25 mg of DHEA given two or three times a day is often implemented.

Commonly used dosage range of DHEA supplementation in therapy of diverse medical conditions. Most prevalent dose was bolded. Daily doses above 25 mg are usually split into

Table 1. Usual daily doses of DHEA that are recommended, administrated in clinical trials and regular off-label use

Indication with confirmed effectiveness	Daily dose titration range	Reference
Adrenal insufficiency and chronically treated with exogenous glucocorticoids	25–**50 mg** p.o.	[33–35]
Postmenopausal women with low bone mineral density and/or osteoporosis	25 mg–**50 mg**–100 mg p.o.	[3, 12]
Premenopausal women with sexual disorders and low libido	**75 mg** p.o.	[29]
Vulvovaginal atrophy/genitourinary syndrome of menopause (GSM)	3.25–23.4 mg p. vag. **6.5 mg** p. vag. — on market	[38–39, 63]
Indication with possible effectiveness	**Daily dose titration range**	**Reference**
Postmenopausal women with hypoactive sexual disorders	10 mg–50 mg–90 mg–300 mg–450 mg p.o.	[3, 27–28]
Infertile women with diminished ovarian reserve (DOR)	**75 mg** p.o.	[46–60]
Women with depression and anxiety	30 mg–60 mg–**90 mg**–450 mg p.o.	[22–23]
Women with obesity/insulin resistance	25 mg–**50 mg**–100 mg p.o.	[3]

2–3 parts. Please note that table summarizes example of doses administered in clinical practice that may differ from recommended by manufacturer or are utilized in off-label treatment. Administration and dose of every drug should rely on current medical knowledge and individual clinical assessment

Recent statement of experts of the Polish Menopause and Andropause Society and the Polish Society of Aesthetic and Reconstructive Gynecology provides a comprehensive literature review that supports the use of intravaginal DHEA supplementation. Clinical studies with high level of evidence proves that topical treatment is effective, safe and well tolerated long-term therapy for vulvovaginal atrophy [63].

In a Cochrane Systemic Review [64] regarding the supplementation of DHEA in peri- and postmenopausal women, the authors questioned the effectiveness of DHEA in women, but the overall quality of the studies analyzed in this review was moderate to low. It was unclear if supplementation of DHEA decrease symptoms of menopause since the study outcomes were inconsistent and could not be pooled to obtain an overall effect due to versatile types of measurement. Insufficient results were available to estimate quality of life and menopausal symptoms during DHEA supplementation as well as, there were inadequate reports accessible to compare the effects of DHEA replacement to hormone therapy (HT) for quality of life, menopausal symptoms, and adverse effects.

Conflict of interest

The authors report no conflict of interest.

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Impact of COVID-19 on pregnancy and delivery — current knowledge

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ABSTRACT

The World Health Organization announced on 12 March 2020 a global pandemic of the new SARS-CoV-2 coronavirus causing COVID-19 disease associated with pneumonia and acute respiratory failure. SARS-CoV-2 has caused so far over 6.66 million recorded cases, of which 393,000 ended in death (as of June 1, 2020). Despite the demographic statistics of incidence, there is no current recording of cases in the group of pregnant or perinatal women. Changes occurring in the female body system during pregnancy also affect and alter the immune system, and as studies based on other viral respiratory infections have shown, the population of pregnant women is at risk of having a severe course of the disease. The aim of the study is to summarize current reports on the course of COVID-19 disease in a group of pregnant women and the possible impact of SARS-CoV-2 on the foetus and vertical transmission, taking into account changes occurring in the woman's immune system during pregnancy. Available advice and recommendations for antenatal and perinatal care of pregnant women during the pandemic period are also included.

Key words: COVID-19; SARS-CoV-2; coronavirus; pregnancy; delivery; breastfeeding

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INTRODUCTION

The global pandemic of the contagious COVID-19 disease, caused by the SARS-CoV-2 coronavirus, was announced by the World Health Organization (WHO) on March 12, 2020 as a result of the dynamic development of the epidemic in many countries and continents [1]. SARS-CoV-2 has caused so far over 6.66 million recorded cases, of which 393,000 have ended in death (as of 1.06.2020) [2]. The SARS-CoV-2 virus is structurally and functionally like the SARS virus and other pathogens from the Coronaviridae family [3–4].

Susceptibility to SARS-CoV-2 infection is observed in patients of all ages. However, according to the available studies, the full-blown COVID-19 disease develops much more frequently in elderly adults with associated comorbidities [5]. Despite the demographic statistics of incidence, there is no current recording of cases in the group of pregnant or perinatal women. Pregnancy is a special type of physiology during which several changes occur not only in the hormonal management, but also in the immune system. According to the available studies, the population of pregnant women is exposed to a severe course of infectious diseases affecting the upper respiratory tract [6–7]. The current state of knowledge is insufficient to clearly indicate and describe the above-mentioned clinical issue.

The aim of the study is to summarize current reports on the course of COVID-19 disease in a group of pregnant women and the possible impact of SARS-CoV-2 on the foetus and vertical transmission, taking into account changes occurring in the immune system of women during pregnancy. The available recommendations and suggestions for antenatal and perinatal care of pregnant women during the pandemic period are also included.

THE ROLE OF THE IMMUNE SYSTEM IN THE PREGNANCY

The immune system undergoes a change during pregnancy, outside the implantation site where suppression occurs. The response to microorganisms varies depending on the type of pathogen and stage of pregnancy. The

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trophoblast and mother's immune system reach a state of agreement where the immune response during pregnancy is a combination of the mother's systemic response and the local foetal-placental response. Therefore, the signals from the placenta are the ones that alter mother's immune system, and the immune system during pregnancy is focused on integrating the actions of placenta, foetus and pregnant woman [8].

Caring for the immune system is one of the elements of pre-conception, prenatal and perinatal education. Therefore, important are protective vaccinations before getting pregnant as well as proper diet and supplementation during pregnancy. Vitamin D3 facilitates the immune adaptation necessary to maintain pregnancy. It regulates the concentration of calcium and phosphate in the plasma, affects the state of mineralization of mother and foetal bones and alters the work of the system, among others immune system [9]. Vitamin D3 deficiency may affect up to 50% of pregnant women in the first trimester. The new guidelines assume that women planning pregnancy and during pregnancy should take vitamin D3 supplements, preferably in doses calculated based on blood tests. If it is not possible to determine D3 in the blood — the use 2000 IU/d is recommended [10]. During lactation, D3 supplementation does not differ from that taken during pregnancy - the dose is selected based on the vitamin D3 levels in the blood or 2000 IU/d [11–12].

Iron, as an essential microelement in the process of cellular respiration, is also responsible for the synthesis of red blood cells, DNA synthesis and regulation of the immune system function. Anaemia during pregnancy also affects the increased risk of preterm delivery and lower foetal birth weight. The demand of a woman's body during pregnancy is 26–27 mg/day, and during the lactation period 20 mg/day [13–14].

VIRAL RESPIRATORY DISEASES AND PREGNANCY

Pneumonia resulting from any infectious aetiology is an important cause of morbidity and mortality in pregnant women. This is the most common non-pregnancy condition that occurs during pregnancy. Twenty-five percent of pregnant women who develop pneumonia require hospitalization in intensive care units and require ventilation support. Viral pneumonia has an even higher level of morbidity and mortality during pregnancy than bacterial. Maternal physiological changes associated with pregnancy — including altered cellular immunity — may increase the susceptibility of pregnant women to illnesses [15].

Analysis based on data from SARS and MERS infections shows that both these coronaviruses can cause maternal death in a small number of cases (risk factors for death during pregnancy have not been clarified). Coronaviruses may also result in adverse effects on the foetus and infant, including IUGR, preterm delivery, miscarriage and perinatal death. Unlike some viral infections, especially Ebola and Zika virus, the probability of coronavirus intrauterine transmission from mother to foetus is low — no cases of SARS or MERS have been documented. The novel coronavirus may influence obstetric diseases and outcomes depending on gestational age, drug effects or other treatment regimens, differences in host resistance reactions, occurrence of comorbidities and obstetric diseases and other accompanying variables [15].

CLINICAL MANIFESTATIONS AND THE COURSE OF COVID-19 DISEASE

According to one recent meta-analysis of the most common symptoms of COVID-19 disease which can be identified in the general population are: fever (87%), cough (58%), shortness of breath (38%), myalgia and fatigue (36%). Headache, excessive sputum production, or smell and taste disorders are less common symptoms. Some patients require admission to the Intensive Care Unit, of which, according to the authors, the risk of death was estimated at 7% [16]. Zaigham et al. performed systematic review based on 18 articles, covering 108 cases of pregnant women infected with SARS-CoV-2. Among the symptoms analysed, high fever (68%) and cough (34%) were the most common ones, while fatigue (13%) and shortness of breath (12%) were less common. Three percent of patients required admission to the Intensive Care Unit. There were no deaths in pregnant women, however one case of neonatal and one case of intrauterine death were recorded [17].

The study, which included the largest group of pregnant women infected with SARS-CoV-2 at one time, was published by Breslin et al. and included 43 women from the United States. Only 7% of women were hospitalized for COVID-19 symptoms while the others were admitted for obstetric reasons. In this group of patients, dry cough (66%) was described as the most common symptom followed by fever (48%) and muscle pain (39%) [18].

Based on the analysis of the available studies and literature, it can be concluded that no significant differences in clinical symptoms have been described in the pregnant women group infected with SARS-CoV-2 in relation to the general population [7]. Although, in most of the cases mentioned above the course of the disease was mild or even asymptomatic, some of them required intensive care and mechanical ventilation.

EFFECT OF SARS-COV-2 ON PREGNANCY AND DELIVERY

A meta-analysis conducted by Di Mascio et al. [19] on six studies which involved 41 pregnant women illustrates registered pathologies during pregnancy and delivery in patients infected with SARS-CoV-2. Premature delivery occurred in

Table 1. Occurrence of selectes pathologies during pregnancy and delivery in patients infected with SARS-CoV-2 [19]

Pathologies	n = 41
Premature birth	41%
PROM (premature rupture of the membranes)	19%
Pre-eclampsia	14%
Foetal intrauterine asphyxia	43%

41% of patients, and premature rupture of the membranes (PROM) was found in 19% of the patients. A significant proportion of patients (*i.e.* 43%) were at risk of foetal intrauterine asphyxia (Tab. 1). However, due to the small group of respondents and the fact that retrospective case studies have been included in the description, certain bias of the results obtained should be taken into account.

The available studies show large differences in the prevalence of preterm deliveries

Metaanalia Zagiham et al. [17] reports that 92% of COVID-19 gave birth via C-section. Studies carried out by Breslin et al. indicate that 44% of deliveries carried out by surgery [18]. Despite different percentages, it can be stated that the infection of the patient with SARS-CoV-2 is associated with an increased rate of giving birth via C-section in comparison to the pregnancies of healthy women population.

In regard to SARS reports, there is an increased risk of miscarriage concerning infected pregnant women, especially at the beginning of pregnancy. Unfortunately, the available publications provide residual information about complications in maintaining pregnancy in the first trimester. Therefore, it cannot be explicitly stated whether SARS-CoV-19 infection increases the risk of miscarriage or not. However, it should be emphasized that one of the most common symptoms of COVID-19 is fever. According to Yin et al. hyperthermia may be a teratogenic factor, especially at the stage of embryogenesis i.e. early pregnancy [20].

In the publication of Zheijang University School of Medicine, the drugs recommended for pregnant women are lopinavir and ritonavir. Chloroquine and favipiravir were forbidden. The Table 2 shows the most important drug interactions [21].

Regarding the vertical transmission of SARS-CoV-2 from mother to foetus, current studies do not provide reliable reports on the eventual possibility of infection. One of the first studies conducted on nine neonates shows that there is no evidence of intrauterine coronavirus infection (Tab. 3). Nine women with laboratory-confirmed COVID-19 (*i.e.*, positive throat swab samples) participated in the study. Evidence of vertical transmission was evaluated by the presence of SARS-CoV-2 in samples of amniotic fluid, umbilical cord blood and neonatal throat swabs. Samples of breast milk were also taken from patients after the first lactation and tested. Nine live births were recorded — in the 1st minute Apgar score 8–9 and 5th minute Apgar score 9–10. Amniotic fluid, umbilical cord blood, neonatal throat swab and milk samples were negative [22]. To date, no SARS-CoV-2 virus has been found in umbilical cord blood, amniotic fluid or placental tissue [23].

PERINATAL CARE AND BREASTFEEDING

Most authors of the publication agree that the mere fact of contracting COVID-19 is not an indication of the urgent delivery of pregnancy. It is recommended, if the obstetric situation permits, to delay the planned intervention, induction of delivery and adopt a waiting attitude until the woman poses an epidemic threat [24–26]. However, increased CTG monitoring is recommended due to reported cases of foetal well-being disorders [26]. According to the recommendations of the Polish Society of Gynecology and Obstetrics, each hospital with a Maternity Ward should have

Table 2. The most important drug interactions [21]

Drug name	Potential interactions	Contraindication in combined medication
Lopinavir/ritonavir	When combined with drugs associated with CYP3A metabolism (*e.g.*, statins, immunosuppressors such as tacrolimus, voriconazole), the plasma concentration of the combined drug may increase; leading to 153%, 5.9 folds, 13 folds increase of the AUC of rivaroxaban, atrovastatin, midazolam, respectively. Pay attention to clinical symptoms and apply the TDM	Combined use with amiodarone (fatal arrhythmia), quetiapine (severe coma), simvastati (rhabdomyolysis) is prohibited
Darunavir/cobicistat	When combined with drugs associated with CYP3A and/or CYP2D6 metabolism, the plasma concentration of the combined drugs may increase. See lopinavir/ ritonavir	See lopinavir/ritonavir
Arbidol	It interacts with CYP3A4, UGT1A9 substrates, inhibitors, and inducers	–
Fapilavir	① Theophyllinum increases the bioavailability of fapilavir ② It increases the bioavailability of acetaminophen by 1.79 folds ③ Its combination with pyrazinamide increases the plasma uric acid level ④ Its combination with repaglinide increases the plasma repaglinide level	–
Chloroquine phosphate	–	Prohibit to combine with the drugs that may lead to the prolonged Q-T interval (such as moxifloxacin, azithromycin, amiodarone, etc.)

Table 3. Maternal clinical characteristics [22]

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	n (%)
Age [years]	33	27	40	26	26	26	28	29	34	–
Gestational age on admission	37 weeks, 2 days	38 weeks, 2 day	36 weeks	36 weeks, 2 days	38 weeks, 1 day	36 weeks, 3 days	36 weeks, 2 days	38 weeks	39 weeks, 4 days	–
Epidemiological history	Yes (exposure to relevant environment)	Yes (contact with infected person)	Yes (contact with infected person)	Yes (exposure to relevant environment)	Yes (exposure to relevant environment)	Yes (contact with infected person)	Yes (contact with infected person)	Yes (contact with infected person)	Yes (exposure to relevant environment)	9 (100%)
Other family members affected	No	Yes	Yes	No	No	Yes	No	Yes	No	4 (44%)
Complications	Influenza	None	Gestational hypertension	Pre-eclampsia	Fetal distress	None	PROM	Fetal distress	PROM	--
Typical signs of viral infection	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	8 (89%)
Method of delivery	C-section	C-section	C-section	C-section	C-section	C-section	C-section	C-section	C-section	–
Indication for C-section	Severely elevated ALT or AST; COVID-19 pneumonia	Mature; COVID-19 pneumonia	History of C-section (× 2); COVID-19 pneumonia	Pre-eclampsia; COVID-19 pneumonia	Fetal distress; COVID-19 pneumoni	History of stillbirth (× 2); COVID-19 pneumonia	PROM; COVID-19 pneumonia	Fetal distress; COVID-19 pneumonia	PROM; COVID-19 pneumonia	–

a separated so-called „Epidemiological emergency room". It is proposed that it should be created on the model of existing "Departments of rapid prenatal diagnosis". Such rooms have the conditions to perform ultrasound, CTG and examination of the patient maintaining an epidemiological regime whether patient's condition requires hospitalization. If pregnancy is endangered, or there are symptoms that may threaten pregnancy, it's necessary to inform the main doctor, or if there is no such possibility, go to the closest open hospital with obstetrics facilities. In the hospital, after TRIAG, if there is no suspicion of infection, every pregnant woman will be examined and further treatment will be established. If there are infectious symptoms, patient will be transferred to the„epidemiological" admission room, where, if the pregnancy threat symptoms requiring hospitalization are confirmed, the medical team will first have to rule out COVID19 infection [27]. Furthermore, according to recommendations to suspend visits to hospitals, an analogous procedure should apply to the family births. It is dictated by the overarching goal, i.e. ensuring epidemiological safety of patients, pregnant and giving birth hospitalized in maternity hospitals. All actions should be aimed at minimizing the possibility of spreading the above mentioned infection and minimizing virus transmission in the environment [28].

Limited studies in women suffering from COVID-19 or infected with other Coronaviridae (SARS-CoV) did not confirm the presence of the virus in human milk. However, it is not known for certain whether infection is transmitted through breast milk, but given the low rate of respiratory viral transmission through breast milk, the World Health Organization (WHO) states that mothers with COVID-19 can breastfeed [29]. It is not recommended to routinely isolate healthy new-borns from their suspected or COVID-19 mothers. Isolation can only be recommended if the mother's and/or new-born's health deteriorates and prevents them from being in the rooming-in system. Childcare is best entrusted to a healthy family member. These rules should be used to suppress symptoms in the mother (generally 5–7 days). So far, there are no reported cases regarding new-borns contracting Coronavirus through the milk of infected biological mothers. Furthermore, there is no evidence in favour of early clamping and cutting of the umbilical cord or early washing of the new-born [26, 31–33].

Recommendations regarding the participation of the neonatal team and clinical factors indicating the need for resuscitation remain unchanged, regardless of the status of the pregnant woman regarding COVID-19. These guidelines were issued on April 24, 2020, they will change as knowledge and experience in the field of COVID-19 treatment develops. Due to the varied level of pandemic severity, there may be differences in clinical practice in individual countries. The decision to separate the COVID-19 mother

from her child should be based on local guidelines. As a rule, the child should stay with the mother if her condition allows. If observation is required, it may be conducted by midwives. Skin-to-skin contact and breastfeeding is possible if proper precautions are taken, including strict hand hygiene and the use of masks [30].

SUMMARY

The current clinical knowledge does not present significant differences in clinical symptoms or the course of the disease in the pregnant women's group in relation to the general women's population and to the similar age. It can be assumed that the routine examination of pregnant women before planned hospitalization seems to be justified, due to the high percentage of asymptomaticity of pregnant women with SARS-CoV-2 infection. Based on the current research, patients with confirmed COVID-19 may be included in the risk group of preterm delivery, PROM or intrauterine infection during pregnancy. The dynamic development of the global pandemic emphasizes the need to constantly update knowledge and follow the latest recommendations to ensure the highest quality of health services and care for patients infected with SRARS-CoV-2.

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VIA MEDICA

CLINICAL VIGNETTE

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A triplet's ectopic pregnancy in a non-communicating rudimentary horn and spontaneous rupture

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ABSTRACT

Our aim is to feature the management of ectopic pregnancy in a non-communicating rudimentary horn. It has a remarkable life-threatening potential, being rare and difficult to be considered during differential diagnosis of acute abdomen or sudden maternal collapse in early pregnancy. Therefore, this is a report of mid-trimester triplet's ectopic pregnancy which presented with sudden repeated syncopal attacks and hemodynamic instability that necessitated emergency laparotomy to treat ruptured non-communicating rudimentary horn. The rarity of this clinical condition can lead to multiple challenges. When a diagnosis is confirmed, the intervention plans should be independently tailored based on the patient's age, obstetric history, fertility wishes, as well as, surgeon's experience. Moreover, most cases passed unnoticed till complications took place. Thus, early diagnosis of Mullerian anomalies preconceptionally or even during the initial antenatal visits is crucial step regarding the avoidance of such catastrophic maternal outcomes.

Key words: second trimester; ectopic pregnancy; mullerian anomalies; rudimentary horn; unicornuate uterus

Ginekologia Polska 2020; 91, 9: 569–570

CASE REPORT

A 25 years old woman G2P1 + 0 presented gestation to emergency unit of Benha University Hospital at 20 weeks complaining of sudden onset of acute abdominal pain and repeated syncopal attacks. By history, she had no unusual medical or surgical history. She delivered uneventfully three years prior. After a period of secondary infertility, she was prescribed ovulation induction in the form of clomiphene citrate three months before the current pregnancy. Cervical cerclage by the end of first trimester after being diagnosed as triplet pregnancy.

By general examination, she was drowsy with extreme pallor, blood pressure 90/60 mmHg, pulse 120–130 beat per minute, respiratory rate 29 per minute and no fever. Upon abdominal examination, generalized tenderness and rebound tenderness all over the abdomen with shifting dullness on percussion. A pelviabdominal ultrasound revealed marked free intraperitoneal fluid collection, three dead fetuses 20 weeks gestation by biometric measurements with an empty uterus. Her immediate laboratory results showed hemoglobin level of 5 gm/dL with satisfactory kidney and liver function tests without evidence of coagulopathy.

It was explained precisely to the couple that she was most likely suffering from uterine rupture which needed immediate intervention after cross-matching of packed RBCs and other blood products required accordingly. They signed a clearly informed, high-risk consent form after full counselling about the management modalities, as well as, the risks and the complications.

AneEmergency laparotomy was performed through pfannensteil incision. The findings were marked hemoperitoneum with three dead fetuses, ruptured non-communicating rudimentary horn and intact empty unicornuate uterus. An excision of the rudimentary horn was done at the line of contact with the unicornuate uterus. Multiple U-shaped sutures with vicryl-1 were held to ensure hemostasis. (Fig. 1) Two drains were inserted intraperitoneally and subrectally, then a mass closure of abdominal wall was adequately completed.

The patient received three units of packed RBCs, six units of plasma and NovoSeven ® RT (recombinant coagulation Factor VIIa) intra-operatively, then she was transferred to ICU for close monitoring. She received 12 units of platelets and cryoprecipitate. After two days, she became fully conscious with improvement of her laboratory results, so she was discharged from ICU to the ward. She was finally discharged home three days later in good condition with an appointment for follow up in the outpatient clinic. She was offered combined oral contraceptive pills as birth control for at least 18 months before planning subsequent pregnancy.

The infrequent detection of unicornuate uterus (nearly 1:100,000 women), which is a result of Mullerian duct fusion defect, makes its diagnosis challenging as the presentation varies from being asymptomatic to sever obstetric hemorrhage.

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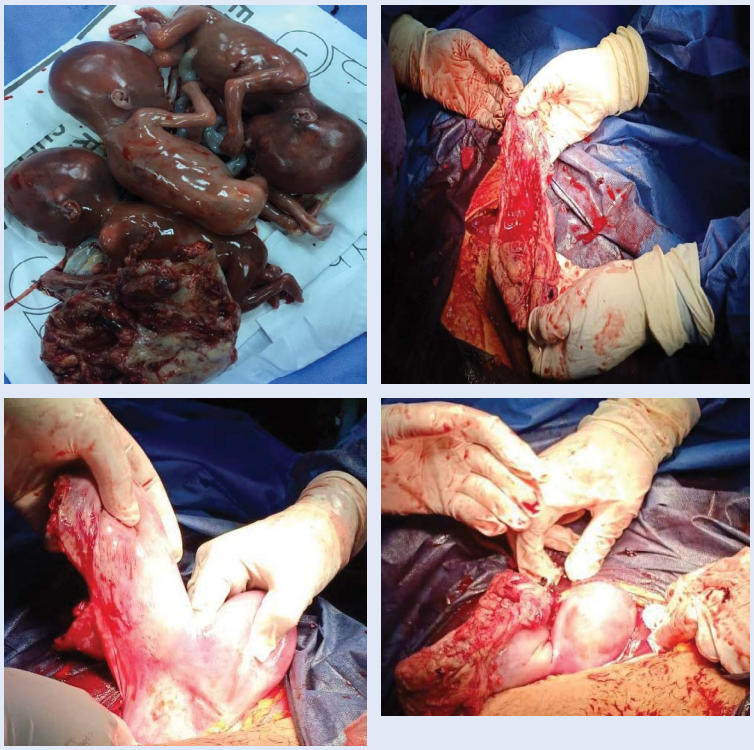

Figure 1. Showing three dead fetuses, ruptured non-communicating rudimentary horn, unicornuate uterus and treatment by excision

Moreover, when a pregnancy takes place within non-communicating rudimentary horn, as rare instance (1:76,000 pregnant women), the condition might worsen. It was explained hypothetically by relocation of the sperm or the fertilized oocyte trans-peritoneally from the contralateral tube or through a minute tract within the unicornuate uterus's walls.

Early diagnosis of unicornuate uterus is difficult due to the fact it is mostly asymptomatic and its discovery is mainly incidental during work-up of infertility, pelvic pain or recurrent miscarriage or unfortunately during the second trimester when uterine rupture is most likely to complicate pregnancy. Thus, the condition is rare and mostly diagnosed throughout complication. It must be kept in mind during preconception evaluation and early antenatal booking visit to avoid unexpected outcomes [1–3].

Conflict of interest

The authors report no conflicts of interest.

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Primary non-Hodgkin's lymphoma masquerading as cervical cancer

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ABSTRACT

Genital tract lymphomas are rare entities that can be diagnosed at advanced stages. The uterine cervix is not generally infiltrated by lymphoma. Nevertheless it can be seen as a consequence of either a systemic disease or primary disease. The infrequency of primary cervical lymphoma makes the diagnosis challenging.

Key words: uterin cerviks; Non Hodgin's Lymphoma; CIN 3

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Herein we report a case of 46-year-old woman, referred to our clinic because of vaginal bleeding. Physical examination revealed a cervical mass of approximately 10 cm in diameter that filled the vagina entirely. There was bilateral parametrial involvement, and this examination resembled cervical cancer. Colposcopy-directed punch biopsies resulted in a diagnosis of cervical intraepithelial neoplasia (CIN) 3. Subsequently, a cone biopsy was performed and histopathological examination revealed the diagnosis of diffuse B cell non-Hodgkin's lymphoma along with CIN 3. Tumor cells showed diffuse CD20, PAX5, CD79a positivity (Fig. 1, 2). Positron Emission Tomography (PET CT) showed a bulky cervical mass and with probable involvement of the parametrium. There was no evidence of extension to other sites.

The surgical margins of the cone biopsy were free of tumors. The patient was referred to the Medical Oncology Clinic for further evaluation. Bone marrow examination was normal. Depending upon the findings, a diagnosis of the primary cervical Non-Hodgkin's Lymphoma with diffuse large B cell type was made. The patient received three cycles of CHOP (Cyclophosphamide, adriamycin, vincristine, and prednisone) chemotherapy. After three cycles of chemotherapy, the tumor completely disappeared. An additional three cycles of chemotherapy were administered. The patient was referred to the Radiation Oncology Department and treated with external beam radiotherapy (46 Gy). Radiographic and clinical findings of the disease resolved completely. For CIN 3 surveillance, the patient was observed with colposcopy and cytology in 6-months intervals.

The prognosis and treatment of primary cervical lymphoma is entirely different from the squamous cell carcinoma of the cervix. Although being rare, this diagnosis should be kept in mind in the differential diagnosis of cervical masses. The coexistence of CIN 3 and non-Hodgkin's lymphoma could be purely coincidental and an accompanying, cervical lymphoma can be overlooked, if biopsies were not adequately taken.

Based on the described clinical vignettes, cervical punch biopsy, especially cone biopsy and detailed immunohistochemical studies, are essential for cervical lymphoma diagnoses. In the treatment of cervical non-Hodgkin's lymphoma, combination radiotherapy with chemotherapy has a good response rate.

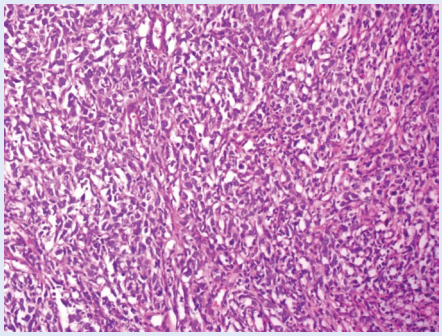

Figure 1. Diffuse large B-cell lymphoma infiltration in the cervix (H&E, x400)

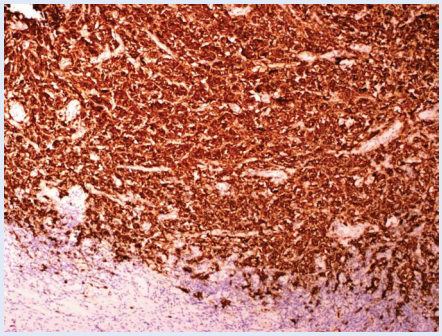

Figure 2. Immunohistochemically, tumor cells showed diffuse CD20 staining (DAP, x200)

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