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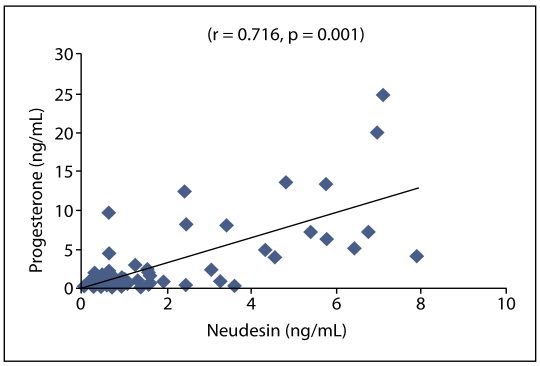

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EDITORIAL

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Perfect cesarean section — the Holy Grail of obstetricians

Michal Pomorski, Mariusz Zimmer

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Caesarean section (CS), as the most frequently performed major surgical procedure worldwide (21.1% women give birth by CS — almost 30 million CSs annually), has several advantages [1]. The obvious one is it's a safe option for delivery when due to maternal or fetal indications the vaginal delivery is contraindicated. Moreover, there are long-term advantages like decreased rates of pelvic floor dysfunction (especially stress urinary incontinence and pelvic organ prolapse) when compared to women who deliver vaginally [2, 3].

Major progress has been achieved in the reduction of early CS complications, such as wound infection (*i.e.*, antibiotic prophylaxis), postpartum haemorrhage (*i.e.*, carbetocin infusion), pain control (*i.e.*, transversus abdominis plane block) and thromboembolic complications (*i.e.*, low molecular weight heparin, early mobilization) [4, 5].

However, there are no roses without thorns. Above all, there are ongoing concerns about increased maternal mortality related to CS [6]. Also, an urgent problem is maternal morbidity expressed in skyrocketing frequency of long-term CS complications. These complications are mostly related to the incomplete healing of the uterine CS scar. In pregnant women they include potentially life-threatening complications like cesarean scar dehiscence or rupture, CS scar pregnancy and its direct consequence — placenta previa accreta [7]. In nonpregnant women the long-term CS complications include abnormal uterine bleeding, subfertility and pelvic pain syndrome [8–10]. Also, CS related intra-abdominal adhesions, mainly between the uterus and abdominal wall, negatively affect the safety of subsequent surgical procedures and increase the risk of incomplete healing of the uterine CS scar [11].

As the incompletely healed uterine CS scar seems to play crucial role in the etiology of long-term CS complications the current research should be focused on the improvement of surgical techniques that allow better healing of the uterine CS scar and decrease adhesion formation.

Making a long story short — the cornerstone on the way to modern CS was change from vertical uterine incision to low transverse incision introduced by John Martin Munro Kerr in 1926, which was then combined with transverse "Pfannenstiel" abdominal entry [12]. The next step was the introduction of blunt dissection techniques for abdominal entry by Joel-Cohen and uterine entry – the Misgav-Ladach method. These novel techniques allowed decreased blood loss, shortening of the operation time and recovery period [12]. However, after millions of CSs and dozens of studies the uterine closure technique that allows complete healing of the CS scar is still missing [13, 14]. There are also no general guidelines on CS technique from skin incision to skin closure. The American College of Obstetricians and Gynecologists advises autonomy of obstetricians in choosing their preferred CS technique, considering their safety regarding short-term complications [12]. However, the impact of this techniques on the above listed long-term CS complications is still under evaluation, with no final conclusions [12, 15].

One of the steps forward on the way to the „perfect" CS might be the "novel technique uterine suturing" (NTUS) described by Ugur Erkayiran and Tufan Arslanca in the current issue of *Ginekologia Polska* in a study entitled: "Comparative analysis of classical primary continuous and novel technique uterine suturing methods on uterine scar formation after caesarian section: a prospective clinical study" [16]. In this study the CS scar niche incidence did not differ between the group with NUTS closure and the group with classical primary continuous suturing, however the residual myometrial thickness (RMT) was significantly thicker in the NUTS group.

According to the current knowledge the RMT has crucial value in terms of risk for scar related complications in subsequent pregnancies. Randomized studies revealed that low RMT values measured in the non-pregnant uterus using transvaginal ultrasound (TVUS) predict the occurrence of CS scar dehiscence and rupture in the next pregnancy [17, 18].

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Moreover, in women with cesarean scar pregnancy (CSP) the RMT value of ≤ 2 mm measured at the first prenatal ultrasound predicts in all cases the development of placenta previa accreta when the pregnancy is continued [19].

In our opinion the key to success in improvement of CS technique is the evaluation of own results. In our centre — the 2nd Department of Gynecology and Obstetrics, Medical University Wroclaw, Poland (Head: Prof. Mariusz Zimmer) in 2005 we introduced the first standardized ultrasonographic assessment of the CS scar in the non-pregnant uterus. The scar was assessed six weeks after CS. The results published in *Ginekologia Polska* in 2007 revealed that in 94.5% of women the scar niche was detected [20]. After analysis of the results a mandatory full thickness single layer uterine closure without the inclusion of decidua was implemented. Further studies on women after CS performed in our department revealed significant decrease in niche detection [18]. Among 204 women included in the latest publication from our center only five patients (2.4%) had a RMT < 2.2 mm [21].

The last word in the topic on the improvement of the CS technique is not said. We should be aware that even small progress in the most common major surgery worldwide may have positive impact on wellbeing of millions of women and thus on public health. The key to the success is the evaluation of own surgical results both in the settings of big clinical trials and in the micro scale — by the surgeons themselves. The tool for CS scar assessment – TVUS is widely available and uterine CS scar assessment techniques are easy to implement and use [22]. Dear Readers, we encourage all of you to assess the CS scars after the cesareans you have performed — maybe your technique will turn out to be a milestone on the way to a perfect CS.

Conflict of interest

The authors have no conflicts of interest to declare.

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Serum neudesin levels in patients with polycystic ovary syndrome

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ABSTRACT

Objectives: We aimed to investigate serum neudesin levels that has neural, metabolic functions in patients with polycystic ovary syndrome (PCOS).

Material and methods: The study included 180 women (age range, 18–44 years) with a diagnosis of PCOS and a control group that included 100 healthy females (age range, 18–46 years). Body mass index (BMI), waist circumference, Ferriman-Gallwey score, was evaluated and plasma glucose, lipid profile, estradiol, progesterone, total testosterone, prolactin, insulin, dehydroepiandrosterone sulfate (DHEA-S), FSH, LH, free T3, free T4, thyroid stymulating hormone (TSH), anti-thyroperoxidase (anti-TPO) antibody and neudesin levels were evaluated in all participants.

Results: BMI and waist circumference were similar between two groups. Ferriman-Gallwey score was significantly higher in the patient group. Fasting blood glucose, HbA1C, lipid parameters except triglyceride levels, free T3, free T4, TSH, anti-TPO were similar between the two groups. Triglyceride, insulin and HOMA values were significantly higher in PCOS patients. While follicle-stimulating hormone (FSH), estradiol, progesterone, prolactin and DHEAS levels were similar, LH was significantly higher in patients with PCOS. Serum neudesin level was significantly lower in PCOS patients with respect to controls ($p = 0.015$). Neudesin was positively correlated with insulin ($r = 0.224$, $p = 0.037$), and progesterone ($r = 0.716$, $p = 0.001$). Multiple regression analysis revealed that neudesin correlated with only progesterone (beta = 0.308, $p = 0.001$).

Conclusions: Due to the association of decreased levels of neudesin with PCOS and correlation of neudesin with progesterone, neudesin may be related with one of patophysiologic pathways of PCOS. Still, it is not certain that decreased neudesin is involved in the pathogenesis of PCOS or is the result of the disorder.

Key words: neudesin; membrabe-associated progesteron receptors; pathogenesis of polycystic ovary syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most frequent reason of chronic anovulation with hyperandrogenemia affecting 5–10% of women of reproductive age [1, 2]. This syndrome is characterized by hyperandrogenism (clinical and/or biochemical), oligo-amenorrhea and polycystic ovaries detected by pelvic ultrasound. Two out of these three criteria are sufficient for diagnosis according to the most widely used Rotterdam criteria (2003) [1]. The Androgen Excess Society (2006) defined PCOS by the presence of hyperandrogenism (clinical and/or biochemical), ovarian dysfunction (oligo-anovulation and/or polycystic ovaries) and the exclusion of related disorders such as Cushing's syndrome, congenital adrenal hyperplasia and/or androgen-secreting tumors [1, 3]. PCOS not only leads to menstrual irregularities, infertility and miscarriage, but also increases the risk of endometrial cancer due to the long-term undefied estrogen effect [3]. PCOS is also associated with metabolic abnormalities and cardiovascular risk factors such as type 2 diabetes, dyslipidemia and obesity related to insulin resistance [1, 4–6].

The definite etiology of PCOS has not been determined; it is considered a heterogenous disorder with multifactorial causes and the genetic contributions remain incompletely

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described. It has been suggested that the underlying causes of PCOS include the increased pulse frequency of gonadotrophin-releasing hormone (GnRH), leading to increased amplitude and frequency of luteinizing hormone (LH) secretion and stimulation of theca cells to produce androgens; decresed levels of follicle-stimulating hormone (FSH) relative to LH, insulin resistance in adipose tissue and skeletal muscles via a post-receptor defect (abnormal phosphorylation of thyrosine kinase), pancreatic beta-cell dysfunciton and obesity [3, 7, 8].

Neudesin, one of the membrane-associated progesterone receptors, is a secreted protein with cytochrome 5-like heme/steroid-binding domain, has neural functions, participates in energy metabolism and tumorigenesis [9]. The distribution pattern of neudesin gene expression is very similar to the progesterone receptor in the rat forebrain, especially regions including anteroventral, periventricular, arcuate and ventromedial nuclei [10]. Therefore, it has been suggested that neudesin might be involved in regulation of neuroendocrine functions via progesterone receptors [10].

Objectives

As neuroendocrine abnormalities contribute to the pathogenesis of PCOS, we aimed to investigate serum neudesin levels (with neural and metabolic functions) in patients with PCOS. To date, there has only been one study about neudesin levels in patients with PCOS [11]. Therefore due to the scarcity of data, we aimed to investigate serum neudesin levels in patients with PCOS.

MATERIAL AND METHODS

Patients study design

This cross-sectional, case-control study included 180 women (aged 18–44 years) with a diagnosis of PCOS defined in accordance with the Rotterdam criteria [8]. The control group consisted of 100 healthy females (aged 18–46 years). The study was carried out between January 2017 and October 2018 at the Department of Endocrinology at Tepecik Research and Training Hospital. Women with chronic diseases such as overt hypothyroidism or hyperthyroidism, kidney or liver failure, hyperprolactinemia, late-onset adrenal hyperplasia, diabetes, hypertension or Cushing's syndrome as well as women taking thyroid hormones or anti-thyroid medication were excluded from the study. Additionally, women who had been receiving hormonal therapy, including oral contraceptive pills or steroids (glucocorticoids) within six months were excluded. The study was approved by the medical ethics committee of the Tepecik Research and Training Hospital and written informed consent was obtained from all the study subjects.

Body mass index (BMI) and waist circumference were measured in all study subjects. Hirsutism was evaluated based on the Ferriman-Gallwey scoring index over nine body areas [12].

Fasting venous blood was obtained from all study subjects to evaluate biochemical parameters including plasma glucose and lipid profile (total cholesterol, HDL cholesterol, LDL [low density lipoprotein] cholesterol and triglycerides) as well as hormones including estradiol, progesterone, total testosterone, prolactin, insulin, DHEA-S, FSH, LH, free T3, free T4, TSH and anti-thyroperoxidase (anti-TPO) antibodies. Serum samples were aliquoted, frozen and stored at — 80°C for neudesin analysis. The blood samples were obtained during the third to ninth days of the menstrual cycle or 60 days after the last menstrual period.

Pelvic ultrasonography was perfomed for all participants.

Laboratory assessments

Glucose, triglycerides, total cholesterol and high density lipoprotein (HDL) cholesterol levels were measured by enzymatic methods using an AU5800 autoanalyzer (Beckman Coulter Inc., CA, USA). LDL cholesterol was calculated by the Friedewald 's equation method. Insulin, FSH, LH, total testosterone, estradiol, progesterone, prolactin, DHEAS levels, were analysed by chemiluminescence assay method using a DxI immunoanalyser (Beckman Coulter Inc., CA, USA). Free T3, free T4, TSH, anti-TPO, levels were measured by chemiluminescent method by Immulite 2000 otoanalyzer (Immulite XPi, Siemens, Germany). Glycated hemoglobin (HbA1c) was measured using boronate affinity high-performance liquid chromatography method (Trinity Biotech, Kansas City, MO, USA). Serum neudesin levels were measured using sandwich- enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Cat. No: E-EL-H3302, Elabscience Biotechnology Co.,Ltd, China). The intra-assay and inter-assay coefficients of variations were 7.4 % and 6% for neudesin (ng/mL) respectively.

Homeostasis model assessment (HOMA) was calculated to measure insulin sensitivity by using the equation = fasting insulin (mU/L) × glucose (mmol/L)/22.5. Insulin resistance is determined by having a HOMA value > 2.7 [13].

Pelvic ultrasonography

Transabdominal pelvic ultrasonography was performed by using a Logiq 5 Pro unit (GE Medical Systems, WI, USA) and C1-5-RS (2–5 MHz) transducer. The ovaries were imaged in the sagittal and transverse planes. The presence of polycystic ovaries were defined as existence of 12 or more follicles throughout the ovary measuring 2–9 mm in diameter [1]. All ultrasonographic evaluations were performed by the same radiologist.

Statistical analysis

Results are expressed as means ± SD. The Chi-Square test was used for the comparison of non-parametric variables in both groups. The patient and control groups were compared by using Student-t test and Mann-Whitney U test. Correlation between serum neudesin levels and other parameters were assessed by Pearson's correlation analysis. Multiple regression analysis was used to assess the contribution of correlated parameters to neudesin. P < 0.05 was considered statistically significant. Statistical analysis was performed with SPSS 20 statistical software.

RESULTS

Clinical characteristics of the patient and control groups are described in Table 1. The groups were similar according to age, BMI and waist circumference. Ferriman-Gallwey score was significantly higher in patient group –8.95 ± 2.94 — compared to control group 7.10 ± 4.11 (p = 0.002).

Fasting blood glucose and HbA1C values, lipid parameters except triglyceride levels, free T3, free T4, TSH, anti-TPO were similar between the two groups (Tab. 2). Triglyceride levels were significantly higher in patients with PCOS com-

Table 1. Clinical characteristics of the study population

	Group 1 (Patients with PCOS) (n = 180)	Group 2 (Control Group) (n = 100)	p value
Age [years]	25.94 ± 6.18	28.12 ± 7.27	0.088
BMI [kg/m^2]	29.77 ± 6.65	28.04 ± 6.16	0.173
Waist circumference [cm]	89.72 ± 15.36	84.33 ± 12.21	0.072
Ferriman-Gallway index	8.95 ± 2,94	7.10 ± 4.11	0.002*

*Statistically significant, p value < 0.05; BMI — body mass index; PCOS — polycystic ovary syndrome

Table 2. Laboratory parameters of the study population

	Group 1 (Patients with PCOS) (n = 180)	Group 2 (Control Group) (n = 100)	p value
Fasting glucose [mg/dL]	89.14 ± 8.71	86.92 ± 7.12	0.170
Insulin [µU/mL]	17.31 ± 9.72	10.23 ± 7.76	0.004*
HOMA	3.85 ± 2.22	2.01 ± 1.18	0.004*
HbA1C [%]	5.29 ± 0.38	5.21 ± 0.24	0.231
LDL-cholesterol [mg/dL]	117.31 ± 37.02	116.35 ± 29.22	0.889
HDL-cholesterol [mg/dL]	49.36 ± 12.42	50.35 ± 9.41	0.655
Total cholesterol [mg/dL]	191.78 ± 44.12	186.21 ± 35.25	0.497
Triglyceride [mg/dL]	126.72 ± 72.59	97.35 ± 42.18	0.023*
FSH [mIU/mL]	5.67 ± 2.15	5.76 ± 2.14	0.834
LH [mIU/mL]	5.97 ± 2.11	2.53 ± 2.91	0.024*
Estradiol [pg/mL]	71.24 ± 68.07	69.38 ± 53.21	0.884
Total testosterone [ng/dL]	68.50 ± 24.79	46.71 ± 13.31	0.001*
Progesterone [ng/mL]	1.81 ± 1.32	1.96 ± 0.48	0.225
Prolactin [ng/mL]	11.44 ± 5.02	12.10 ± 6.25	0.548
DHEA-S [µg/dL]	287.48 ± 127.82	252.56 ± 111.08	0.150
fT3 [pg/mL]	3.64 ± 0.38	3.51 ± 0.31	0.100
fT4 [ng/mL]	0.84 ± 0.15	0.85 ± 0.11	0.816
TSH [uIU/mL]	2.12 ± 1.07	2.15 ± 0.64	0.882
Anti-TPO [IU/mL]	58.92 ± 35.87	36.29 ± 28.54	0.453
Neudesin [ng/mL]	1.19 ± 1.08	2.12 ± 1.04	0.015*

*Statistically significant, p value < 0.05; anti-TPO — anti-thyroperoxidase; DHEA-S — Dehydroepiandrosterone sulfate; FSH — follicle-stimulating hormone; HDL — high density lipoprotein; HOMA — homeostasis model assessment; LDL — low density lipoprotein; LH — luteinizing hormone; PCOS — polycystic ovary syndrome; TSH — thyroid stymulating hormone

pared to individuals in control group (p = 0.023). Likewise, fasting insulin levels and HOMA values were significantly higher in PCOS patients. Insulin level was 17.31 ± 9.72 µU/mL in patient group, and 10.23 ± 7.76 µU/mL in control group (p = 0.004). HOMA values in PCOS and control groups were 3.85 ± 2.22 and 2.01 ± 1.18, respectively (p = 0.004). While FSH, estradiol, progesterone, prolactin and DHEAS levels were similar, LH and total testosterone were significantly higher in patients with PCOS. LH level was 5.97 ± 2.11 mIU/mL in patient group and 2.53 ± 2.91 mIU/mL in control group (p = 0.024). Total testosterone level was 68.50 ± 24.79 ng/dL in patient group, and 46.71 ± 13.31 ng/dL in control group (p = 0.001). Progesterone levels were 1.81 ± 1.32 ng/mL and 1.96 ± 0.48 ng/mL in patient and control group, separately (p = 0.225). Serum neudesin level was significantly lower in PCOS patients (1.19 ± 1.08 ng/mL) with respect to controls (2.12 ± 1.04 ng/mL) (p = 0.015).

The percentage of individuals with polycystic ovarian apperance on pelvic ultrasonography was higher in patients with PCOS compared to control group (70% vs 18%, p = 0.001).

Correlation analysis were performed between neudesin and all the other parameters. Neudesin was positively correlated with insulin (r = 0.224, p = 0.037), HOMA (r = 0.234, p = 0.029) and progesterone (r = 0.716, p = 0.001) (Fig. 1). No correlation was observed between neudesin level and other parameters. Multiple regression analysis showed that progesteron levels significantly contributed to neudesin levels (beta = 0.308, p = 0.001), however the contribution of insulin to neudesin was not statistically significant (beta = –0.155, p = 0.140) (Tab. 3).

DISCUSSION

PCOS is the most common endocrine disorder in reproductive-aged women and is associated with signs and symptoms of hyperandrogenemia as well as increased metabolic and cardiovascular risk factors. However, the exact etiology remains unrevealed. Increased GnRH pulse frequency and LH pulsatility and relatively decreased FSH levels contribute to the pathogenesis [3, 14]. Increased LH pulsatility leads to increased androgen production from theca cells, and relatively decreased FSH levels cause impaired aromatization to estrogens, follicle maturation and ovulation. The increased GnRH pulse frequency is attributed to loss of negative feedback inhibition by progesterone which may be due to decreased progesterone levels or decreased effects because of hyperandrogenemia [14, 15]. In addition, PCOS-related insulin resistance due to abnormal phosphorylation of the insulin receptor by intracellular serine kinases in adipose tissue and skeletal muscle contributes to increased 17, 20-lyase activity of P450c17 in steroidogenic tissue and up-regulation of testosterone formation via increased *HSD17B5* gene expression in adipose tissue [16, 17]. Hyperinsulinemia augments LH stimulation of ovarian androgen production by up-regulating LH-binding sites and enhancing androgen production at the level of cytochrome P450c17 [16, 18].

Membrane associated progesterone receptors are a group of four proteins with a similar heme-binding domain related to cytochrome b5 (a membrane-bound hemoprotein that functions as an electron carrier for microsomal cytochrome P450 monooxygenase systems) [19]. These four proteins progesterone receptor membrane component 1 (PGRMC1), PGRMC2, neudesin and neuferricine, have diverse functions associated with cholesterol/steroid biosynthesis, drug metabolism and response. They interact with Cytochromes P450 (CYPs) and modulate their functions [19, 20]. Only PGRMC1 has been reported to bind progesterone [10, 21, 22] and it is suggested that PGRMC1 might mediate

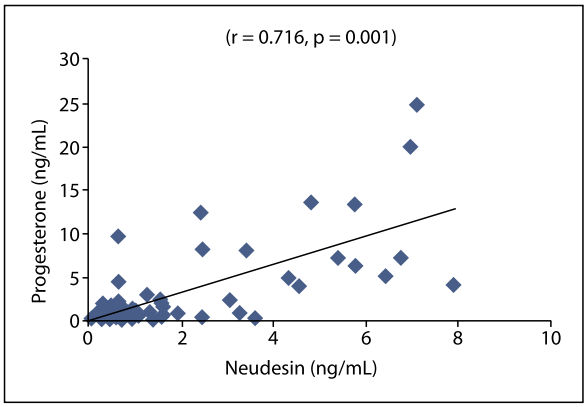

Figure 1. Correlation between neudesin and progesterone

Table 3. Evaluation of the effect of insulin, HOMA and progesterone on neudesin using the multiple regression analysis ($R^2 = 0.577$)

Variables	β	95% CI Min Max	p value
Insulin	-0.155	-0.363 0.052	0.140
HOMA	0.843	-0.069 1.755	0.07
Progesterone	0.308	0.244 0.373	0.001*

Multiple regression analysis was used. β: Unstandardized regression coefficient; *p value of < 0.05 was considered as significant; CI — confidence interval; HOMA — homeostasis model assessment

the rapid effects of progesterone [10]. PGRMC1 was found in immortalized GnRH neurons (GT1-7cells) [23] and rapidly inhibited the fluctuations of intracellular calcium levels in GnRH neurons [10, 24], leading to inhibition of GnRH and LH release. Neudesin is expressed in numerous tissues such as, brain, adipose tissue, heart and lungs, preferentially in neurons. It promotes differentiation of neurons through protein kinase and phosphotidylinositol-3 kinase pathways by using its' (cytochrome b5-like) heme/steroid-binding domain [10, 25]. It is also strongly expressed in hypothalamic nuclei that regulate food intake and recombinant neudesin administration into the cerebral ventricle, resulting in decreased appetite and body weight with increased expression of pro-opiomelanocortin and melanocortin 4 receptors in the hypothalamus [12, 25–27]. In contrast to this finding, in another study, increased sympathetic activity was found in neudesin KO mice fed with high fat diet leading to increased energy consumption, fatty acid oxidation in brown adipose tissue and enhanced lipolysis in white adipose tissue [12, 28, 29]. It was suggested that the discrepancy between the two sets of data might be caused because the physiologic effect of neudesin was analyzed in neudesin KO mice and the pharmacological effect of neudesin was analyzed by the administration of recombinant neudesin [9]. Neudesin expression was also identified in various cancers and it was involved in tumorigenesis. Although the mRNA distribution pattern of neudesin and progesterone receptors is similar, and there is a structural similarity between PGRMC1 (which binds to progesterone) and neudesin, there is no data about the interaction between neudesin and progesterone [24, 28]. It is proposed that neudesin may act as a binding protein for lipophilic progesterone and hold it on the cell sufrace in the extracellular enviroment and this complex may act on the unknown cell surface progesterone receptor to exert a rapid effect [28].

The data are limited on the evaluation of membrane-associated progesterone receptors in patients with PCOS. PGRMC1 expression was reported in ovary tissues of PCOS rat models [29] and PGRMC1 expression in peripheral leukocytes was decreased in a study of patients with PCOS [30]. In the latter study, serum PGRMC1 levels were evaluated twice weekly, in addition to estradiol and progesterone levels, over four-week period. PGRMC1 levels were stable during the study period, and there was no correlation between PGRMC1 and estradiol and progesterone levels. Markedly reduced levels of PGRMC1 protein in PCOS patients were reported compared to healthy cycling women in the early follicular phase and it was suggested that PGRMC1 levels were strongly associated with ovulatory function [30]. However, to date, there is only one recently published study about neudesin as related to PCOS. In that study, researchers found decreased levels of neudesin in patients with PCOS and a negative correlation between neudesin and progesterone. Likewise, in our study, we observed significantly decreased neudesin levels despite of similar progesterone levels between the two groups and a positive correlation between progesterone and neudesin levels. Although this cross-sectional study that does not imply causality, it might lead to a discovery of a relation between neudesin and PCOS. It may support the hypothesis that progesterone cannot exert a negative feedback effect on GnRH in combination with a relative deficiency of neudesin, and this may contribute to the pathogenesis of PCOS. Still, it is not certain that decreased neudesin is involved in the pathogenesis of PCOS or is the result of the disorder.

CONCLUSIONS

Due to the association of decreased levels of neudesin with PCOS and the correlation of neudesin with progesterone, neudesin may be hypothesized as an inter-relater in one of the pathophysiologic pathways of PCOS. Still, it is not certain that decreased neudesin is involved in the pathogenesis of PCOS or is the result of the disorder. However, further studies should be carried out to elucidate the neuroendocrine functions of neudesin and novel mechanisms regulating the progesterone effect in patients with PCOS.

Author contributions

All the authors contribute to conception of the work, acquisition, analysis and interpretation of data, drafting the work or revising it for intellectual content, final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Statement of ethics

All procedures performed in this study were in accordance with the ethical standarts of the medical ethics commitee of the Tepecik Research and Training Hospital with a decision file number: 2018/3-19 and with the declaration of Helsinki. All the participants of the study have given their informed consent.

Conflict of interest

The authors have no conflicts of interest to declare.

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Male factor infertility in the Comprehensive Procreational Health Protection Program at the University Hospital in Cracow

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ABSTRACT

Objectives: Quality of semen is one of the most important factors contributing to couples' chance of natural conception. There are many confirmed or potential factors that influence semen analysis results.
To estimate the incidence and analyze male factor infertility.

Material and methods: The retrospective observational study was in the Clinical Department of Gynecological Endocrinology and Gynecology, University Hospital in Cracow. The study included men from subfertile population, aged ≥ 18 years, without prior diagnosis and obvious cause of infertility, whose initial seminograms were used to characterize the population. Seminograms of men remaining in the follow-up were used to analyze the variability of sperm parameters in relation to lifestyle modification and the use of fertility supplements containing antioxidants. Control semen tests were performed at 1-3-month intervals.

Results: The study included 870 men. In 68.5% of men, at least one abnormal sperm parameter was found and 40.7% had complex sperm abnormalities. Averaged values of sperm parameters of men from subfertile couples were within the WHO reference ranges, except for the normal morphology, whose median was 3.8%. No significant differences in the selected sperm parameters after the implementation of conservative management were observed. The percentage of pregnancies not resulting from IVF in the follow-up population was 7.7%.

Conclusions: One semen sample is representative of an individual in the diagnostics of male infertility. Expectant management and lifestyle modification should not be proposed as first-line treatment when more effective procedures are available.

Key words: male infertility; semen parameters; natural conception

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INTRODUCTION

Reference Center of Infertility Treatment at the University Hospital in Cracow was the implementer of the National Program for the Comprehensive Protection of Procreational Health for the years 2016–2020. This program was aimed at not only diagnostics, but also treatment of infertility and was dedicated to couples in cohabitation, who for at least 12 months of regular unprotected intercourse, were unable to achieve pregnancy [1] and had no previous medically established diagnosis of infertility. The main assumption of the program was to reduce the number of couples affected by infertility.

Among the factors contributing to couples' chance of natural conception, the most important are woman's age, length of time-to-pregnancy period and quality of semen [2]. The inability of a man to elicit a pregnancy in a healthy female partner defines male infertility [3], thus the evaluation of man's reproductive potential is an inseparable element of assessing couple's fertility and the result of semen analysis is believed to correlate with the chance of conceiving [4]. There are a number of conditions leading to impaired spermatogenesis. Abnormal semen parameters may be the result of hypergonadotropic hypogonadism (primary testicular failure), where there is no possibility of improving fertility, hypogonadotropic hypogonadism (secondary testicular failure), where hormonal treatment is used and normogonadotropic hypogonadism (mostly abnormal sperm parameters of unknown cause), where hormonal

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treatment is usually ineffective. In any case of an abnormal seminogram, the patient should be examined by a urologist to exclude pathologies of reproductive functions and to implement treatment of detected disorders, if possible [5].

In addition to several well-documented causes, there are numerous environmental, occupational and lifestyle factors that through a negative effect on spermatogenesis lead to subfertility, limiting reproductive capacity of a couple and contributing to the diagnosis of idiopathic male infertility.

Data from literature indicate an oxidative stress as the cause of reduced male fertility in the above-mentioned conditions, a significant part of which are partially modifiable lifestyle elements [6]. Reactive oxygen species had been shown to disrupt sperm function and motility, damage cell membranes and DNA. Scientific studies had shown that in some situations, antioxidant treatment can improve sperm parameters and increase the chance of pregnancy, however, there is no consensus on dose, duration of treatment, nor qualitative composition of combined oral antioxidants [7]. An important element in the management of idiopathic male infertility is therefore counselling on modifiable risk factors that have a negative long-term effect on overall health [8].

Other factor with potential impact on semen parameters is the alteration of sperm parameters over time [9] due to lifestyle, environmental and genetic factors [10].

Considering that the main goal of couples seeking medical help in Reference Center of Infertility Treatment was not only to determine the cause of reduced fertility, but primarily to achieve pregnancy in a situation where *in vitro* fertilization (IVF) was not financed by public funds, it was deemed justified to conduct a retrospective study and its objectives were defined.

Objectives

The main aim of the study was to estimate the incidence of the male factor and its cause in the population of men from infertile couples examined in the Comprehensive Procreational Health Protection Program. Other specific research objectives were:

1. Analysis of the variability of sperm parameters over time and the relationship between the alteration of sperm parameters and lifestyle modification.
2. Estimation of the effectiveness of therapy with oral antioxidants in terms of improving sperm parameters in men with idiopathic subfertility.
3. Calculation of the percentage of clinical pregnancies (not resulting from the use of IVF procedures).

MATERIAL AND METHODS

The retrospective observational study was conducted based on the data of the Clinical Department of Gynecological Endocrinology and Gynecology over a period of time from March 1, 2017 to March 1, 2020. The positive opinion of the Bioethical Committee of the Jagiellonian University no. 1072.6120.94.2020 was obtained. The study group consisted of men living in subfertile couples seeking medical help at the University Hospital's Infertility Treatment Center. The following inclusion criteria were used: i) age at least 18 years old, ii) living in a relationship in which pregnancy has not been achieved despite regular unprotected intercourse for at least 12 months, iii) no prior diagnosis and treatment of infertility, iv) no obvious cause of infertility. No exclusion criteria were applied.

The study population was characterized in relation to age and the following semen parameters: volume (mL), liquefaction time (min.), pH, abstinence (days), viscosity, sperm count in 1 ml of ejaculate (million/ml), total sperm count in ejaculate (million), motility (%), morphology (%), teratozoospermia index (TZI) [3], multiple anomaly index (MAI), defined as the average number of abnormalities per abnormal sperm [3, 11], head defects (%), midpiece defects (%), tail defects (%), cytoplasmic droplets (%), vitality (%).

In order to maintain the repeatability of the evaluated parameters, more advanced computer-assisted sperm analysis (CASA) parameters and results of additional sperm tests were not considered.

In the next step, medical documentation of men who underwent extended diagnostics were analyzed. During follow-up visits it was recommended to use one of common antioxidant supplements, to quit smoking, reduce body weight, increase physical activity and modify dietary and working habits, if applicable. Control semen analyses were performed at 1–3-month intervals. By analyzing the change in semen parameters, the effectiveness of the management was assessed. Finally, the percentage of clinical pregnancies not attributable to IVF techniques was calculated.

Semen analysis

Sperm samples were processed in accordance with WHO guidelines [3] and assessed by means of Sperm Class Analyzer® CASA System. In cases of very low semen parameters manual seminograms were performed. All semen analyses were performed by the person with the statutorily required qualifications [12], trained to work in an embryological laboratory in accordance with the standards of the Polish Society of Reproductive Medicine and Embryology [13] and the European Society of Human Reproduction and Embryology [14].

Qualification

Couples seeking help because of presumed subfertility were consulted by a supervising obstetrician-gynecologist.

All men with an incorrect seminogram were referred for urological consultation. Complementary tests including examination of male genitals, rectal examination, transrectal and scrotal ultrasound were performed by a urologist, who managed further treatment, and ordered hormonal and genetic testing, if necessary.

Statistical analysis

Descriptive statistics methods were used to characterize the male population. Categorical variables were summarized as the number of cases (n) and percentage (%). Continuous variables were presented using means and standard deviations (SD) in the case of normal distribution and medians, lower (LQ) and upper (UQ) quartiles in the other cases. The maximum (max.) and minimum (min.) values of the variables were also given. Kolmogorov-Smirnov test, skewness test, histogram, boxplot and Q-Q plot were used to assess normality.

Partitioning of the variance attributable to intra-individual variability and inter-individual variability was estimated using ANOVA estimation methodology. Significance of inter-individual to intra-individual variability ratio was estimated using F test. Assessment of differences in sperm count, progressive motility and normal forms between three or more measurements, without inclusion of lifestyle factors, was done using General Linear Model for Repeated Measurements (GLM-RM) for normally distributed variables and Friedman's Two-way Analysis of Variance by Ranks in other cases, with the Bonferroni correction when appropriate. GLM-RM was used to assess factors affecting average level of above-mentioned parameters when dependent variable had normal distribution in all measurements and Generalized Estimating Equations (GEE) was used in other cases. IBM SPSS Statistics 25 for Windows was used for the calculations.

RESULTS

Characteristics of the studied population

Using inclusion and exclusion criteria a database of seminograms was created, representative of 870 men who had done semen analysis at least once.

Population characteristics were based on the results of the initial semen analysis (Tab. 1A–C). Considering the parameters of sperm motility, morphology and viability, the population was classified according to the diagnoses presented in Table 2. During the extended diagnostics, a few cases of urological disorders were found (Tab. 3). Among men diagnosed with azoospermia and cryptozoospermia, only 3 out of 18 patients came for hormone level testing and in all cases FSH concentrations exceeded the reference range. Patients with azoospermia and cryptozoospermia were also referred for genetic testing and, among patients who performed it (3/18), not a single case of abnormal karyotype or deletion in the AZF region of the Y chromosome was found.

Seventy-two (72/870; 8.28 %) out of 870 men remained in the follow-up including at least three semen tests, performed at 1–3-month intervals. For 65 out of them, some extra data concerning lifestyle factors and pregnancy rate were collected in Table 4. The mean body weight was 78.7 kg (SD = 9.8), and the average frequency of physical activity was 2.0 days a week (SD = 1.6). In addition to the data included in the table, one man quit smoking and one man took clomiphene acetate.

In the next step, the variability of sperm concentration, morphology and motility over time in patients who had at least three semen analyses were assessed.

In terms of all three analyzed parameters, inter-individual variability dominated over intra-individual, and for sperm concentration and morphology these ratios were statistically significant (Tab. 5).

There was no significant difference in sperm concentration between three consecutive samples. Similarly, no significant difference was observed regarding progressive motility. There was significant difference in terms of sperm morphology ($p = 0.031$), however pairwise comparison did not reveal significant differences in particular pairs of measurements (Tab. 6).

Multivariate analysis of factors related to lifestyle revealed that some of them influenced the value of sperm concentration, i.e., measurement time point ($p = 0.014$), change in dietary habits ($p = 0.018$), weight loss ($p < 0.001$), weight at first measurement ($p = 0.012$), physical activity ($p < 0.001$) and interaction between measurement time point and weight at first measurement ($p = 0.018$), as well as between measurement time point and physical activity ($p < 0.001$). Change in dietary habits was related to lower sperm concentration, whereas weight loss was related to higher sperm concentration, both differences were stable throughout the measurements. Sperm concentration increased with an increase in physical activity and decreased along with increasing body weight at all measurement points. (Tab. 7).

Multivariate analysis revealed that mean progressive motility was associated with the change in dietary habits ($p = 0.047$), whereas impact of body weight was close to significant ($p = 0.073$). Table 8 presents the differences related to change in dietary habits and body weight difference of 1 kilogram at particular measurement points (Tab. 8).

Multivariate analysis revealed that only a few of lifestyle factors influenced sperm morphology. According to the multivariate model mean percentage of morphologically normal forms was higher in those who smoked ($p = 0.019$), throughout all measurement points. The percentage of mor-

Table 1A. Population characteristics in relation to the averaged values of the most important parameters

Variable	Mean	SD	Median	1st quartile	3rd quartile	Min.	Max.	n
Age [years]	34.6	5.6				22	62	870
Semen volume [mL]	3.49	1.66				0.40	9.93	870
pH	8.0	0.2				6.8	8.9	870
Abstinence [days]			3	3	5	0	30.0	870
Sperm concentration [mln/mL]			27.3	10.0	55.4	0	381.0	870
Total sperm number [mln]			88.9	30.0	177.0	0	1817.0	870
Progressive motility [%]	32.7	17.0				0	76.3	870
Non-progressive motility [%]	11.0	6.2				0	42.6	870
Total motility [%]	43.7	18.2				0	81.6	870
Immobile sperm [%]	56.2	18.2				18.4	100	870
Rapid progressive motility [%]	18.7	12.1				0	64.9	777
Slow progressive motility [%]	15.8	8.3				0	49.5	777
Normal morphology [%]			3.8	2.0	5.4	0	18.0	870
TZI			1.63	1.48	1.82	0	14.3	776
MAI			3.13	2.48	4.37	0	67	815
Head defects [%]			93.8	90.4	96.2	0	100	787
Inlet defects [%]			45.0	35.0	54.5	0	100	819
Tail defects [%]			4.6	2.0	9.0	0	60	776
Cytoplasmic droplets [%]			4.1	2.0	7.0	0	100	776
Vitals forms [%]	63.8	15.4				4.3	92.0	851

SD — standard deviation; TZI — teratozoospermia index; MAI — multiple anomaly index

Table 1B. Population characteristics in relation to the cut-off points for the studied sperm parameters

Variable	Value	n	Percentage [n/870 %]
Semen volume	< 1.5	62	7.1
	≥ 1.5	808	92.9
Semen pH	< 7.2	2	0.2
	≥ 7.2	868	99.8
Sperm concentration [mln/mL]	<1	39	4.5
	≥ 1 & < 5	83	9.5
	≥ 5 & < 15	175	20.1
	≥ 15	573	65.9
Total sperm number [mln]	<39	267	30.7
	≥ 39	603	69.3
Progressive motility [%]	< 32%	423	48.6
	≥ 32%	447	51.4
Total motility [%]	< 40%	349	40.1
	≥ 40%	521	59.9
Normal morphology [%]	< 4%	449	51.6
	≥ 4%	421	48.4
Vital forms [%]	< 58%	226	26.0
	≥ 58%	626	72.0

Table 1C. Population characteristics in terms of semen viscosity, aggregation and agglutination

Variable	Value	n	Percentage (n/870 %)
Semen viscosity	Normal	813	93.4
	+	34	3.9
	++	23	2.6
Sperm aggregation	None or typical	778	89.4
	+	83	9.5
	++	9	1.0
Sperm agglutination	None	612	70.3
	1	3	0.3
	1A	47	5.4
	1B	28	3.2
	1C	2	0.2
	1D	93	10.7
	2A	28	3.2
	2B	3	0.3
	2D	49	5.6
	3A	5	0.6

Table 2. Diagnosis by seminogram results in the studied population

Diagnosis	n	n/N %
Azoospermia	13	1.5
Cryptozoospermia	5	0.6
Oligoasthenoteratozoospermia	182	20.9
Oligoasthenozoospermia	22	2.5
Oligoteratozoospermia	36	4.1
Oligozoospermia	39	4.5
Asthenoteratozoospermia	115	13.2
Asthenozoospermia	86	9.9
Teratozoospermia	98	11.3
Normozoospermia	274	31.5
N	870	100.0

Table 3. Diagnosed urological disorders

Diagnosis	n	n/N* %
Varicocele eligible for surgery	4	0.5
Phimosis	6	0.7
Testicular tumor	2	0.2
Retrograde ejaculation	1	0.1

*N = 870

Table 4. Characteristics of the follow-up subpopulation in relation to selected parameters studied

Variable	Value	n	Percentage (n/65 %)
Smoking	no	60	92.3
	yes	5	7.7
Diet modification	no	24	36.9
	yes	41	63.1
Body weight loss > 3 kg	no	47	72.3
	yes	18	27.7
Work style habits modification	no	61	93.8
	yes	4	6.2
Fertility supplements	no	1	1.6
	yes	64	98.4
Other medications	no	60	92.3
	yes	5	7.7
Pregnancy not resulting from IVF	no	60	92.3
	yes	5	7.7

IVF — *in vitro* fertilization

phologically normal sperm decreased at the first measurement point with an increase in physical activity, although non-significantly (p = 0.55). In the second measurement the percentage of normal sperm slightly increased with an increase of days of physical activity, whereas in the third measurement the percentage of normal sperm was actually independent from men's physical activity (Tab. 9).

Finally, the difference between the value of given parameter (sperm concentration, progressive motility, morphology) from the first semen analysis and the average value of this parameter from subsequent tests was assessed. No significant difference was observed between the value of the first measurement and the average value drawn from subsequent measurements for any parameter (Tab. 10).

Complications

There were no complications from any of the routine procedures used.

DISCUSSION

Diagnosis of male infertility due to sperm dysfunction is based on semen analysis. Precise determination of the cause of male infertility proves impossible in 30–40% of cases [5]. However, the definition of infertility is based on duration of the problem, not on identifying specific disorders or causative agent. The time limit of 12 months in which pregnancy cannot be achieved indicates that there is less than a 5% chance that the failure is due to random factors [15].

The fifth edition of the WHO Semen Manual gave reference ranges for the most important sperm parameters, determined based on seminograms obtained from a large population of men who conceived within 12 months [3]. The set reference values identify the lower 5% threshold of one-sided confidence interval in the population of fertile men and should not be interpreted as absolute norms [16]. This means that 5% of fertile men from reference population who achieved pregnancy within 12 months had values below the cut-off points and therefore outside the "fertile norms".

The first finding of the study was that the semen parameters of the studied subfertile male population were largely within the WHO reference ranges [17]. The detected abnormalities principally concerned sperm morphology, for which population median value of normal forms was 3.8%. However, not all morphological indices were beyond the desired values. TZI index was equal to 1.63, that was below the WHO maximum value of 1.72, while median MAI index was above the WHO maximum values of 2.55 [3]. The median percentage value (4.1 %) of spermatozoa with cytoplasmic droplets (excess residual cytoplasm) [18] was lower than WHO maximum value of 7% [3].

Among men with no apparent cause of reduced fertility, there are those with abnormal sperm parameters (idiopathic infertility), and those with normal sperm parameters (unexplained infertility) [19]. There are currently no criteria to distinguish between men with disturbed sperm parameters and a high chance for pregnancy from those with poor prognosis.

Table 5. Inter-individual and intra-individual variability of sperm parameters

Variable	Intra-individual	Inter-individual	Inter-individual/ /Total	Inter-individual/ /Intra-individual	p value for Inter-individual/ /Intra-individual
Sperm concentration	298.617	417.634	58.3%	139.9%	0.046
Progressive motility	70.860	129.653	64.7%	183.0%	0.223
Morphology	1.223	2.461	66.8%	201.2%	< 0.001

Table 6. Sperm concentration (mln/mL), progressive motility (%) and sperm morphology (%) in 3 consecutive samples

Parameter	Measurement	n	median	Lower quartile	Upper quartile	p
Sperm concentration [mln/mL]	1	72	9.40	3.92	22.87	0.350
	2	72	9.09	5.52	22.13	
	3	72	9.58	5.26	17.49	
Parameter	**Measurement**	**n**	**mean**	**Lower 95% CI**	**Upper 95% CI**	**p**
Progressive motility [%]	1	72	23.315	19.922	26.708	0.677
	2	72	24.609	21.233	27.985	
	3	72	24.216	21.004	27.427	
Parameter	**Measurement**	**n**	**median**	**Lower quartile**	**Upper quartile**	**p**
Sperm morphology [%]	1	72	2.00	1.00	3.00	0.031*
	2	72	2.00	1.00	4.00	
	3	72	2.13	1.27	3.94	

* no significant differences in pairs of measurements 1–2, 2–3, 1–3; CI — confidence interval

Table 7. Sperm concentration (mln/ml) in multivariate model

Parameter	B	95% Wald CI		p
		Lower limit	Upper limit	
constant	25.126	16.855	33.398	0.000
[Measurement = 1]	0	–	–	–
[Measurement = 2]	8.852	0.978	16.727	0.028
[Measurement = 3]	14.880	4.811	24.949	0.004
[Diet modification = No]	0	–	–	–
[Diet modification = Yes]	–9.227	–16.874	–1.580	0.018
[Body weight loss = No]	0	–	–	–
[Body weight loss = Yes]	0.856	0.512	1.200	0.000
Body weight	–0.279	–0.463	–0.096	0.003
Physical activity	2.290	–0.199	4.778	0.071
[Measurement = 2] * Body weight	0.026	–0.320	0.372	0.883
[Measurement = 3] * Body weight	–0.237	–0.572	0.098	0.166
[Measurement = 2] * Physical activity	7.066	2.492	11.640	0.002
[Measurement = 3] * Physical activity	10.065	5.258	14.872	0.000

CI — confidence interval

The value of a single parameter correlates poorly with a chance of natural conception, but taking into account three parameters (motility, morphology, concentration) probably increases the prognostic value of seminogram [16, 20]. There are also situations where the seminogram result in an infertile couple may be normal, but the biological potential of the sperm may be impaired due to intracellular abnormalities [21]. For this reason, the prognostic value of basic semen parameters is limited by the influence of sperm characteristics not included in the routine seminogram,

Table 8. Sperm progressive motility (%) in multivariate model

Measurement	Parameter	B	95% CI		p
			Lower limit	Upper limit	
1	Constant	48.133	20.170	76.095	0.001
	[Diet modification = 0]	0	–	–	–
	[Diet modification = 1]	–6.371	–13.488	0.746	0.078
	Body weight	–0.349	–0.702	0.004	0.053
2	Constant	46.545	18.031	75.059	0.002
	[Diet modification = 0]	0	–	–	–
	[Diet modification = 1]	–3.581	–10.838	3.677	0.328
	Body weight	–0.296	–0.656	0.065	0.106
3	Constant	43.860	17.123	70.596	0.002
	[Diet modification = 0]	0	–	–	–
	[Diet modification = 1]	6.729	–13.534	0.077	0.053
	Body weight	–0.275	–0.613	0.063	0.109

CI — confidence interval

Table 9. Sperm morphology (%) in multivariate model

Parameter	B	95% Wald CI		p
		Lower limit	Upper limit	
(Constant)	2.748	2.024	3.471	0.000
[Measurement = 1]	0	–	–	–
[Measurement = 2]	–0.412	–1.028	0.203	0189
[Measurement = 3]	–0.176	–0.768	0.416	0.561
[Smoking = 0]	0	–	–	–
[Smoking = 1]	1.972	0.319	3.624	0.019
Physical activity [days/week]	–0.190	–0.416	0.037	0.101
[Measurement = 2] * Physical activity	0.275	0.036	0.514	0.024
[Measurement = 3] * Physical activity	0.175	–0.011	0.361	0.065

CI — confidence interval

Table 10. The first measurement and the average value of subsequent measurements for selected parameters

p = 0.110	Median	1st quartile	3rd quartile	n
Sperm concentration [mln/mL] Measurement 1	9.40	3.92	22.87	72
Sperm concentration [mln/mL] (Mean of measurements 2+)	9.43	6.03	22.64	72
p = 0.222	Mean	SD		N
Progressive motility [%] Measurement 1	23.32	14.44		72
Progressive motility [%] (Mean of measurements 2+)	24.89	12.99		72
p = 0.233	Median	1st quartile	3rd quartile	N
Morphology [%] Measurement 1	2.00	1.00	3.00	72
Morphology [%] (Mean of measurements 2+)	2.27	1.36	3.67	72

SD — standard deviation

e.g., chromatin maturity or DNA fragmentation [22]. Assessment of additional parameters, *e.g.*, DNA, RNA, centrioles is not routinely performed because treatment methods are limited [23]. Nevertheless, despite existing limitations, sperm analysis remains the main test in routine fertility assessment until better diagnostic tools are invented [16]. What can improve the

prognostic value of sperm analysis by eliminating temporal variability of parameters is to perform it more than once. The above-mentioned variability is caused by physiological processes (intra-individual variability), biological factors (inter-individual variability) or laboratory technique and implies the limited value of a single semen analysis [15]. Two or three semen samples should probably be evaluated prior to diagnosing reduced fertility [22], however, no consensus on this issue was made [24–26]. In this research, the calculations were based on the analysis of a single initial sample [17], but after evaluating semen parameters variability over time (concentration, progressive motility, morphology), no significant intra-population differences were observed. The presented data would indicate that just one semen sample is sufficient for diagnostic purposes.

Literature data indicate that 30 to 80% of men with idiopathic infertility have an increased concentration of free oxygen radicals [7], therefore during follow-up visits, empiric treatment with fertility supplements and lifestyle modification were recommended. Of the lifestyle factors, only weight loss, diet modification, physical activity and smoking were suitable for calculations, due to the fact that virtually no one changed working environment and that virtually everyone took supplements. It was shown that weight loss and increased physical activity improve sperm concentration and progressive motility, which coincide with the results of some other studies [27, 28]. Interpretation difficulties were caused by the correlation between diet modification and decrease in sperm concentration, as well as smoking and greater percentage of normal forms. While the first may be because it was an unhealthy diet before its change that was the reason for worsened parameters, the second is rather the result of a bias (a small subgroup of smokers, 7/65), making it impossible to extrapolate the data to the general population. Lifestyle factors were measured only once during the study, although value may vary at particular points of measurements, making the estimations imprecise.

An attempt was also made to evaluate the effectiveness of the conducted management by comparing the sperm parameters from the first sperm analysis with the mean of analogous values from subsequent measurements, but no significant difference was observed for any parameter. Therefore, the results presented suggest that proposing conservative management with an eventual change in lifestyle and antioxidants supplementation does not bring measurable benefits in the aspect of improving sperm parameters, not to mention improving fertility.

No consensus was established so far on how to treat male subfertility. In the case of male or idiopathic infertility, the essential available therapy affects a woman and usually involves one of the ART modalities [23]. The therapeutic success of IVF-ICSI technique led to the fact that since 1992 [29], virtually no progress had been made in investigating the underlying etiology of male infertility and, consequently, in methods of treatment. The IVF-ICSI allows to overpass the problem of *e.g.*, reduced concentration or motility but may not be as effective in the case of intracellular defects. Moreover, treatment using IVF-ICSI can significantly burden the household budget, which is an important issue when treatment is not financed from public funds. On the other hand, the expectant procedure provided by public health in situations of complex sperm abnormalities seems to be hardly justified given the existence of more effective treatment methods. Couples with idiopathic infertility or a mild male factor obviously still have a chance of getting pregnant by natural fertilization, but no recognized method has been developed to determine this chance.

CONCLUSIONS

Limited conclusions can be drawn that the proposed conservative treatment was not effective in improving sperm parameters or that the population of men under observation was too small to draw conclusions. It is therefore reasonable not to propose conservative management as a possible therapeutic approach for couples with a male infertility factor, at least until the development of a technique that allows improving the fertilization capacity of sperm or invention of efficient model defining the chance of natural conception.

Conflict of interest

The study received no financial support. The authors have nothing to disclose.

Author's role

I.G.: study conception and design; data collection, analysis and interpretation; article drafting and revision; final approval of the version to be published. R.B.: data collection, analysis and interpretation; article drafting and revision; final approval of the version to be published. J.D.: data collection, analysis and interpretation; article drafting and revision; final approval of the version to be published. M.S.: data collection, analysis and interpretation; article drafting and revision; final approval of the version to be published. E.P.: data collection, analysis and interpretation; article drafting and revision; final approval of the version to be published. R. J.: study conception and design; article drafting and revision; final approval of the version to be published.

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Overall survival (OS) in patients after chemotherapy for cervical cancer in Poland in years 2008–2015

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ABSTRACT

Objectives: To analyze cervical cancer prevalence as well as treatment methods, and its effects and assessment of overall survival of patients after chemotherapy for cervical cancer in Poland.

Material and methods: Data were collected from the registry of the National Health Fund (the only public payer in Poland). The data of patients treated in 2008–2015, who were shown to the payer with the diagnosis of malignant neoplasm of cervix (C53 according to the ICD-10 classification), were included in the analysis. The annual and eight-year prevalence rates were calculated. The overall survival was calculated for patients treated with chemotherapy.

Results: In the analyzed period (2008–2015), 83,100 women were diagnosed with C53, of which 33,300 (40%) were reported in the group of hospital treatment. The median age of patients was 59 years (58.8 ± 12.87). The highest prevalence rate was observed in 2008 (16.94 patients/100,000 inhabitants). The highest annual and period (2008–2015) prevalence rates patients per 100,000 inhabitants were observed in the Podlaskie (17.03 and 115.53 respectively) and Pomorskie (14.19 and 101.43 respectively) voivodeships and the lowest in Dolnośląskie voivodeship (10.47 and 78.87 respectively) and Podkarpackie voivodeship (10.79 and 71.29 respectively). Mean survival time was 55.12 months and its median 45.46 months. Annual survival time was observed in 76.79% of patients, 2-year in 60.61%; 3-year in 53.08% and 5-year in 46.65%.

Conclusions: In the years 2008–2015 in Poland, the incidence of cervical cancer was even 4 times higher than the EU average, and the mortality was as much as 70% higher than the average for EU countries.

Key words: cervical cancer; epidemiology; survival rate; Poland

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INTRODUCTION

Cervical cancer (cancer of cervix uteri) belongs to the group of the most common neoplasms in the world population, it is the fourth most commonly occurring cancer in women and the eighth most commonly occurring cancer overall [1–4]. According to the World Health Organization (WHO) data, in 2008 there were 530,000 new cases in the world and 275,000 deaths recorded due to cervical cancer [1, 2]. More than 85% of new cases affected women living in developing countries, but the highest incidence rates were recorded in African countries (35/100,000), South America (23.9/100,000) and Central-South Asia (24.6/100,000). The lowest incidence rates were observed in Western Asia, Australia and New Zealand (6.0/100,000) [1, 4].

The global burden of cervical cancer is increasing — in 2018, 570,000 new cases and 311,000 deaths were reported worldwide [3]. In 2020 the number of new cases of cervical cancer exceeded 600,000 [3]. It is estimated, that in 2020, cervical cancer was the most diagnosed cancer in 23 countries and was the leading cause of cancer death in 36 countries [3].

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Although cervical cancer is relatively rare in high-income countries, cervical cancer continues to be an important public health problem in Europe [4]. In 2009, over 54,000 new cases of cervical cancer were registered in Europe which at that time was the fifth most common cancer in women in Europe in terms of incidence [5]. In the countries belonging to the European Union (EU), the number of new diagnoses was 31,000 and 13,000 women died from this neoplasm. In 2020 the burden of cervical cancer in the EU remained at a similar level — in 2020, 30,447 new cases were reported in the EU countries [5]. However, there is a markable disparity between the European populations in morbidity and mortality due to the cervical cancer, primarily due to differences in the prevalence of human papilloma virus (HPV) infection, cancer control strategies as well as an access to cervical cancer screening programs [3, 4]. In 2020, the highest incidence of cervical cancer (age standardized rate per 100,000) was observed in Romania (32.3), Estonia (27.4) and Lithuania (26.8) and the lowest in Malta (5.7) and Finland (6.7) [5]. The highest mortality rates were recorded in Romania (16.9), Bulgaria (12.4) and Latvia (11.7) and the lowest in Finland (2.1) and Malta (2.2) [5].

Poland is one of the countries with an average incidence rate of this type of cancer, however, it has one of the highest incidence and mortality rates in Europe. In 2020, Poland was eight in the EU in terms of the incidence of cervical cancer and fifth in terms of deaths from this cancer in the EU [5]. In Poland, the peak incidence of cervical cancer falls on the sixth decade of life. Recent years indicate an increase in the number of cases in the population of younger women (from 35 to 44 years of age). In addition, Poland has also one of the lowest five-year survival rates in Europe noted, which is a measure of cure rate of this cancer. It was only 48.3% with the European average of 62.1%. The discussed parameter depends primarily on the stage of the tumor at the time of diagnosis, histopathological type with the assessment of the degree of maturity, depth of cervical tissues involvement and presence of metastatic lymph nodes [6]. High incidence and mortality from cervical cancer in some regions of the world are mainly related to socioeconomic conditions, education, low health awareness and lack of primary and secondary prevention in women living in those areas. Nevertheless, over the past 20 years, there has been a gradual decrease in both incidence and mortality from cervical cancer including Poland. This tendency is probably related to the improvement of the quality of cancer prevention (access to cytology tests) as well as systematically growing expenditures on health care, health promotion and access to newer drugs.

Objectives

The aim of the study was to analyze (1) cervical cancer prevalence as well as (2) treatment methods, and its effects and assessment of overall survival (OS) of patients after chemotherapy for cervical cancer in Poland based on the data contained in the reports from the National Health Fund.

MATERIAL AND METHODS

Data were collected from the registry of the National Health Fund — dataset of the only public payer in Poland, where all data on healthcare services provided under public healthcare services are recorded. From the reporting reports of the National Health Fund, the data of patients treated in 2008–2015, who were shown to the payer with the diagnosis C53% (the "%" sign replaces any number) — malignant neoplasm of cervix according to ICD-10 classification, were collected for analysis. Based on the patient's ID and the data from the Statistics Poland, a retrospective analysis was performed to calculate the annual and eight-year prevalence rates. All subsegments of the health market operating in Poland (types of services — Tab. 1), which were then limited to hospital treatment, were analyzed. The prevalence rates were calculated based on the number of pairs "patient's ID + ICD-10 diagnosis = C53% *". The annual index was calculated separately for each year (unique patient ID in a given year reported with the analyzed ICD-10) and the period index for the years 2008–2015 (unique patient ID in the entire analyzed period shown with the described diagnosis). Data were presented for the whole country as well as individual administrative regions (16 voivodeships). Patients treated with chemotherapy were selected based on the data from hospitals and for which the probability of overall survival (OS) was calculated. The beginning of the observation was the date of their first given chemotherapy, the cut-off date was set for 30th June 2016. Analyzes were performed using software SAS Enterprise E.G.5.1. This study was carried out in accordance with the principles of the Declaration of Helsinki. As we were working on secondary, anonymous data collected at the voivodship level, written informed consent to participate in the study was not required.

RESULTS

In the analyzed period (2008-2015), medical entities in the entire health care system in Poland showed 83,100 women diagnosed with malignant neoplasm of cervix (C53), of which 33,300 were reported in the group of hospital treatment (approximately 40%). The average annual number of patients showed high stability at the level of about 24,100 patients (SD = ± 704). The median age of patients was 59 years (mean 58.8; SD = ± 12.87).

Annual and period (8-year) prevalence rate per 100,000 inhabitants for a given voivodeship was calculated for each region and year separately, with detailed information included in Table 2. These parameters in the analyzed period are characterized by negative dynamics. The high-

Table 1. Types of healthcare services included offered to patients with cervical cancer included in the analysis

Number according to the National Health Fund	Type of healthcare service
1	Primary care
2	Ambulatory specialized services
3	Hospital treatment
4	Psychiatric care and treatment of addictions
5	Medical rehabilitation
6	Long-term care
7	Dental treatment
9	Emergency assistance and sanitary transport
10	Preventive health programs
11	Separately contracted services
12	Supplied of orthopedic equipment, auxiliaries and medical technical measures
14	Nursing and care services
15	Palliative and hospice care
16	Emergency Medical services
17	Emergency assistance and sanitary transport from 2009

Table 2. Incidence rate per 100,000 inhabitants for a given voivodeship in 2008–2015 and eight-year period morbidity (2008–2015)

Voivodeship	2008	2009	2010	2011	2012	2013	2014	2015	Avarage	8 year indicator
Dolnośląskie	14.13	10.04	11.07	9.46	7.84	9.87	8.60	12.76	10.47	78.87
Kujawsko-Pomorskie	17.75	15.17	14.98	14.98	14.93	14.26	13.59	15.45	15.14	97.37
Lubelskie	15.50	12.80	10.94	11.13	11.64	11.59	11.78	11.31	12.09	85.02
Lubuskie	15.19	14.70	11.76	12.94	12.45	13.72	12.35	10.00	12.89	90.36
Łódzkie	17.45	14.62	13.26	12.86	11.46	11.78	11.82	11.14	13.05	91.65
Małopolskie	14.55	14.78	13.60	12.29	10.00	9.68	10.48	11.16	12.07	82.44
Mazowieckie	16.91	14.45	14.51	10.57	10.70	11.53	12.20	12.09	12.87	89.46
Opolskie	18.08	16.49	12.89	13.19	10.09	10.59	10.99	10.49	12.85	89.62
Podkarpackie	12.12	11.98	10.90	10.05	10.99	10.38	10.99	8.92	10.79	71.29
Podlaskie	24.83	20.97	16.11	15.94	16.36	12.84	14.01	15.19	17.03	115.53
Pomorskie	17.07	17.64	15.46	14.33	12.21	14.29	11.38	11.12	14.19	101.43
Śląskie	18.67	16.46	15.13	14.04	12.04	11.97	10.99	12.02	13.91	94.96
Świętokrzyskie	16.94	17.02	15.12	14.80	9.26	9.10	7.28	11.87	12.68	86.21
Warmińsko-Mazurskie	15.72	13.57	12.53	11.91	9.14	9.90	10.39	10.39	11.70	85.39
Wielkopolskie	17.25	14.77	13.68	14.31	11.06	11.37	11.26	12.41	13.26	90.88
Zachodniopomorskie	18.95	13.35	13.87	11.66	10.84	10.55	10.49	12.07	12.72	91.06

est value of the prevalence rate for Poland was observed in 2008 (16.94 patients/100,000 inhabitants), in the following years the value gradually decreased to the level of 11.77 patients/100,000 inhabitants in 2015. In the eight-year follow-up period, the incidence rate of cervical cancer in Poland was 90.09 patients/100,000 inhabitants.

The highest annual and period prevalence rates were observed in the following voivodeships: Podlaskie (115.53 patients per 100,000 inhabitants in the period 2008–2015 and 17.03 patients per 100,000 inhabitants on average annual) and Pomorskie (101.43 patients per 100,000 inhabitants in the period 2008–2015 and 14.19 patients per 100,000 inhab-

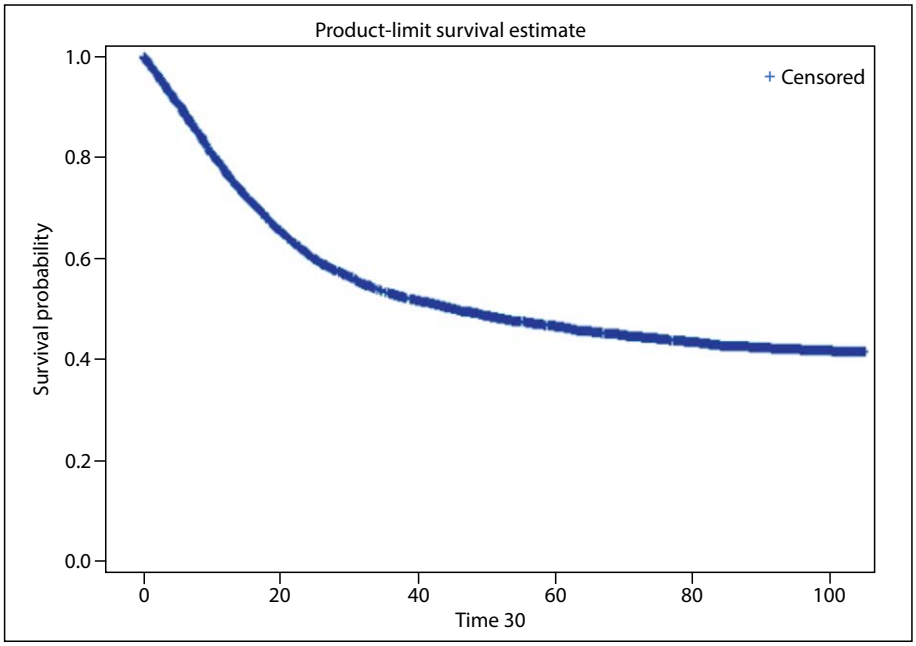

Figure 1. Kaplan-Meier estimation of overall survival in patients with cervical cancer undergoing chemotherapy

itants on average annual). High average annual value of this indicator was also found in Kujawsko-Pomorskie voivodeship (15.14) and Śląskie voivodeship (13.91), but the value of the eight-year prevalence for those voivodeships was lower than the value of that parameter in Podlaskie voivodeship and Pomorskie voivodeship. The lowest values of annual prevalence rate were observed in Dolnośląskie voivodeship (10.47) and Podkarpackie voivodeship (10.79) as well as the lowest value of the eight-year indicator was also observed in those voivodships, (78.87) and (71.29), respectively.

The median follow-up was 77.33 months. The survival curve was generated only for patients who received systemic therapy in the analyzed period. The number of analyzed observations was 5952, of which 49.06% were censored. Mean survival time was 55.12 months and its median 45.46 months. Annual survival time was observed in 76.79% of patients, two-year in 60.61%; three-year in 53.08% and five-year in 46.65% (Fig. 1).

DISCUSSION

To the best of our knowledge, this is the most comprehensive epidemiological analysis of cervical cancer incidence and overall survival in patients after chemotherapy for cervical cancer. Within the collected material, numerical data of diagnoses according to ICD-10 classification and the type of hospital treatment in the form of chemotherapy were analyzed. Based on the collected data and comparison with the data on the population according to the Statistics Poland, the parameters describing overall survival (OS) were calculated and presented. The actual picture of prognoses for patients with cervical cancer in Poland was also obtained in the context of effectiveness of chemotherapy used, quality of treatment and degree of survival. The probability of overall survival is a determinant of the quality and effectiveness of cancer therapy in relation to that cancer in Poland.

In Europe, cervical cancer is responsible for about 13% of neoplasms in women and 4% of those cases are malignant neoplasms. Cervical cancer accounts for about 4% of female deaths in Poland [7].

Depending on the stage of cervical cancer according to FIGO classification and the patient's reproductive plans, there are several methods of its treatment which include: radical or fertility-sparing surgery, various surgical techniques, radiotherapy (brachytherapy and/or teletherapy), chemotherapy or a combination of those methods [7].

The analyzed data show that in the period 2008–2015, the Polish health care system recorded 83,100 women diagnosed with cervical cancer, which on average annual amounted to about 24,100 patients aged about 59 years. General data indicate a decrease in the number of cases in the analyzed period as the highest percentage of the incidence per 100,000 inhabitants in individual voivodships was recorded in 2008, which systematically decreased by about 30% in the analyzed eight-year observation period. In the scale of the entire period under observation, it can be observed that the highest percentage of cases was recorded in Podlaskie voivodship and Pomorskie voivodship as well as in Kujawsko-Pomorskie voivodeship. On the other hand, the

lowest incidence rate for this type of neoplasm was recorded in Dolnośląskie voivodship and Podkarpackie voivodship.

In addition to the data describing the incidence of cervical cancer in Poland, the information on treatment with chemotherapy was also analyzed. According to the materials provided by the treating centres, about 40% of the analyzed total number of cases (33,300) received treatment as part of hospital therapy. The observation period was about 78 months and the results of treatment of patients who received systemic chemotherapy were analyzed. In the group of 5,952 observed subjects, the median survival time was almost 4.5 years (55.1 months), and its median was 3.8 years. In the analyzed period, almost 77% of patients survived more than one year, 61% — two years, while only slightly more than half survived a three-year period and less than half (46.65%) lived more than five years. This means that the median survival time after systemic chemotherapy does not reflect the actual survival rate and the data are very scattered. Compared to the data from the 1970s, the death rate from cervical cancer decreased by over 45%. Nevertheless, mortality from this cancer in Poland is 70% higher than the average for the European Union countries [1, 5]. Thus, the survival rates in Poland in comparison with the European Union countries as well as other developed countries indicate still insufficient quality of therapy, prevention or reporting errors. As indicated in the introduction, high incidence and mortality from cervical cancer in some regions of the world are mainly related to socioeconomic conditions, education, low health awareness and lack of primary and secondary prevention in women living in those areas. The data from WHO and the European Commission show that the number of cases of cervical cancer in Poland among women aged 55–59 years is still exceeding four times the number of cases in this group of women in Great Britain and over two times in Slovenia. However, in the same age group mortality in Poland is over five times higher than in Italy and over four times higher than in the Netherlands and Great Britain [7]. The above differences between the data on patients from Poland and other European countries indicate that the strategies of prophylaxis and chemotherapy used in Poland are still ineffective compared to the therapies used in other countries.

Almost all cervical cancer cases are linked to infection with high-risk human papilloma viruses (HPV) [2–4]. A common introduction of HPV vaccinations gives a real opportunity to reduce the number of cervical cancer cases. In 2020, < 30% of low- and middle-income countries had implemented national HPV vaccination programs compared with > 80% of high-income countries [8]. Prophylaxis in the form of vaccine preventing HPV infection is the most effective method of reducing the incidence of cervical cancer. We can see examples of the effectiveness of such a program based on data from, for example, Australia [9]. HPV vaccinations have been available in Poland for almost 20 years, but it was not until January 2021 that HPV vaccination refunds for adolescent girls were introduced based on National Oncology Strategy. This creates the possibility of increasing the percentage of the population vaccinated against HPV, which will directly translate into a reduction in the incidence of cervical cancer.

Secondary prevention measures such as screening programs (the Pap tests) also lead to the drop in the cervical cancer death rate [10–12]. Whereas in Poland, since 2005, programs of early diagnosis of cervical cancer in the form of cytological tests are characterized by an unsatisfactory response from women to invitations - about 20%, which results in presently high percentage of cases and diagnoses of clinically advanced forms of cervical cancer [12]. It is also believed that most of advanced-phase research on modern treatments of cervical cancer is carried out in the United Kingdom and the United States. Unfortunately, in those countries there is a lack of adequate research material in the form of advanced stage cases of cervical cancer that are recorded in Poland, which results in the lack of new drugs available in the case of late cancer diagnosis and initiation of cervical cancer treatment in Polish patients [5].

This study has several limitations. Firstly, our study is based on the data collected from the registry of the National Health Fund. The quality of reported data may influence the obtained results. However, the National Health Fund is the only institution in Poland collecting data on healthcare services provided to patients with cervical cancer throughout Poland, with a uniform method of reporting data. Secondly, our analysis is limited to an eight-year period and further analysis are needed to assess the impact of HPV vaccinations on the incidence and mortality related to cervical cancer. Nevertheless, our study also has practical implications. Epidemiological analysis of the incidence of cervical cancer in individual voivodeships can be used in planning the national oncological strategy and screening programs at the regional level. Moreover, data on overall survival may be the basis for further comparisons of the effectiveness of treatment between centers and the appointment of dedicated "cancer units" in the future.

CONCLUSIONS

1. In the years 2008–2015 in Poland, the level of cervical cancer incidence still exceeded the level recorded in other European Union countries (sometimes more than 4 times), while mortality in relation to other European countries was as much as 70% higher than an average for other countries.
2. The level of survival in the group of analyzed in 2008––2015 patients, who received systemic chemotherapy,

was different depending on the survival period and very dispersed. Over a five-year survival period was observed only in less than half of the patients referred for treatment, which was more than four times less than the European average.

3. Relatively low survival rate of Polish women diagnosed with cervical cancer and receiving systemic chemotherapy may result from still insufficient prevention strategy, information and promotion of preventive examinations and underfunding of therapy in Poland.
4. Only systemic, coordinated and appropriately funded activities can contribute to lowering of the incidence rate (due to mandatory HPV vaccinations) and increasing the survival rate of Polish patients (due to early diagnosis and treatment) compared to other European countries.

Conflict of interest

All authors declare no conflict of interest.

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Genotyping of human papillomavirus DNA in Wielkopolska region

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ABSTRACT

Objectives: Human papillomavirus infection (HPV) is one of the most common sexually transmitted diseases. Long-term exposure to the HPV leads to development of high-grade squamous intraepithelial lesions that can eventually transform into cervical cancer.
The aim of the study was to assess the HPV genotype distribution in patients with abnormal pap smear and provide prospective study.

Material and methods: We obtained material from 674 women who registered to Specialist Medical Practice in the years 2008–2020. The sample for the molecular test was collected using combi brush and forwarded to the independent, standardized laboratory. HPV detection was done using PCR followed by DNA enzyme immunoassay and reverse hybridization line probe assay for virus genotyping. Sequence analysis was performed to characterize virus genotypes in HPV — positive samples.

Results: We found that 53% of patients tested positive for HPV. The percentage decreased with age. The following HPV types were the most common: HPV — 16 (24.5%), HPV — 53 (13.1%), HPV — 31 (10.3%), HPV — 51 (9.7%), HPV — 56 (9.5%).

Conclusions: Our results suggest that type-specific, high–risk HPV DNA — based screening should focus on HPV types 16, 31, 51, 56.

Key words: HPV; HPV genotypes; HPV screening; cervical cancer

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INTRODUCTION

Cervical cancer (Cc) remains the fourth most frequent cancer in women worldwide causing about 275,000 deaths annually [1, 2]. There are many factors affecting the development of this life-threatening disease, such as the socio-economic status, the age of first sexual intercourse, alcohol consumption or smoking, as well as genetic load, immunosuppression and a large number of pregnancies and births (especially for young women) [3]. However, the most important factor in developing cervical cancer is primarily persistent infection with high-risk HPV (HR HPV). It can lead to an uncontrolled course of infection and is the direct cause of the vast majority of cervical intraepithelial neoplasia and invasive cervical cancers. The oncogenic potential of particular HPV genotypes has been acknowledged since the discovery of the definitive association of HPV as the indubitable etiological agent for development of SIL and cervical cancer. The role of human papillomavirus in cervical cancer was established over 40 years ago [4, 5].

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Genotypes 16 and 18 are assumed to be responsible for about 70% of cc cases [6, 7].

A growing number of countries are replacing Pap-smears with molecular HPV testing as the primary screening modality. Both the American Cancer Society (ACS) and the European Society for Medical Oncology (ESCO) recommend a new pattern of cervical cancer screening [8, 9]. ACS recommends testing patients between 25 and 65 years of age every five years. Pap-smear has been the standard method for cervical cancer screening for over half of the century. It has reduced the incidence by 60–90% and the death rate by 90%. However, the limitation of Pap-smear is sensitivity (~50%) and a significant proportion of inadequate specimens. A pooled analysis of four randomized controlled trials of HPV-based cervical screening versus Pap-smear showed 60–70% greater protection against invasive cancer in favor of HPV-test [10]. Thirteen HPV genotypes are recognized to be oncogenes with high-risk potential by the International Agency for Research on Cancer [11].

On a global scale, HPV infections cause more than half of infection-linked cancers among women and barely 5% in males. Vaccines against the high-risk HPV types 16 and 18 represent the first prophylactic vaccines developed directly to prevent a major human cancer (cc). A significant decrease in the incidence of cervical cancer has been observed over the past several decades due to preventive measures and screening.

Objectives

This paper summarizes the results of HPV DNA genotyping in the Wielkopolska region. So far, we do not have reliable data on the contribution of selected oncogenic HPV types in the formation of cervical pathology in the Polish population. Our aim is to provide distribution of particular HPV genotypes in specific age groups. This knowledge might enable estimating the potential effectiveness of HPV vaccines as primary prevention.

MATERIAL AND METHODS

This study included 674 patients who registered to Specialist Medical Practice in the years 2008–2020 for regular cervical screening. Parallel to the Pap-smear, the women were tested for the presence of HPV which genotypes were later determined. The sample for a molecular test (Linear Array HPV Genotyping-Roche Diagnostics) was collected from the external os of the cervix and vaginal wall with a use of combi brush. The obtained specimen was placed into a liquid-based medium Solution. An HPV test is a quality test that serves to identify high- risk HPV DNA of the following genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 68a, 68b, 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, CP6108, 90 *in vitro*. A positive result in molecular tests confirms the presence of DNA of at least one of the mentioned above oncogenic types of human papillomavirus in the collected specimens.

If needed, a following colposcopy and biopsy were performed. Specialist in gynecologic oncology with 10-year experience examined colposcopy with SmartOPTIC colposcope. Trial with a 5% aqueous solution of acetic acid as well as Schiller's test with Lugol's iodine were performed in all cases. The colposcopic images were evaluated according to Reid's Colposcopic Index which assesses the color, lesion boundaries and surface, blood vessels, and iodine test. All colposcopic images were archived. We used classification created by The International Federation of Cervical Pathology and Colposcopy and recommended by the Polish Society of Colposcopy and Cervical Pathophysiology.

Calculations were performed using the statistical package Statistica (ver. 13.3). Graphs were created with the help of Excel. Statistical hypotheses were verified at the level of significance of 0.05. The Shapiro-Wilk test was used to assess whether the data distribution is normal and Spearman's rho coefficient was used in order to analyze its correlation. The correlation between individual genotypes and age groups was analyzed with a Chi-square test.

RESULTS

The mean age of the entire population was 34. A total of 359 patients (53.3%) tested positive for HPV DNA. The quantitative and percentage distribution of individual genotypes is presented in Table 1. Figure 1 shows the percentage distribution of HPV-positive women in each age group. The HPV genotype 16 and 53 were the most common amongst HPV-positive women. They accounted for 24.5% and 13.4%, respectively. As far as both genotypes are concerned, the correlation between them and particular age groups was not found ($p > 0.05$). A detailed analysis is presented in Table 2 and 3.

The individual HPV genotypes have been allocated to three groups:

- Group A — carcinogenic to humans: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 64, 67, 68a, 68b, 73, 82;
- Group B — either probably or possibly carcinogenic to humans: 26, 53, 66, 69;
- Group C — unclassifiable as carcinogenic to humans: 6, 11, 40, 42, 44, 54, 55, 61, 62, 70, 71, 72, 81, 83, 84, 87, 90, CP6108.

Table 4 presents the basic descriptive statistics and the result of the normality distribution of the Shapiro-Wilk test (W). The result is statistically significant for all variables; therefore the distribution of the examined variables is highly deviating from normal ($p < 0.001$). The correlation of the

Table 1. The quantitative and percentage distribution of individual genotypes

HPV genotype	Presence n	Presence %	Deficiency n	Deficiency %
16	88	13.1	586	86.9
53	48	7.1	626	92.9
31	37	5.5	637	94.5
51	35	5.2	639	94.8
56	34	5.0	640	95.0
54	32	4.7	642	95.3
52	27	4.0	647	96.0
59	27	4.0	647	96.0
66	27	4.0	647	96.0
18	26	3.9	648	96.1
73	24	3.6	650	96.4
6	23	3.4	651	96.6
61	21	3.1	653	96.9
42	20	3.0	654	97.0
39	19	2.8	655	97.2
45	19	2.8	655	97.2
62	17	2.5	657	97.5
CP6108	15	2.2	659	97.8
33	14	2.1	660	97.9
84	14	2.1	660	97.9
67	11	1.6	663	98.4
68	11	1.6	663	98.4
90	11	1.6	663	98.4
35	10	1.5	664	98.5
58	10	1.5	664	98.5
82	8	1.2	666	98.8
81	8	1.2	666	98.8
83	7	1.0	667	99.0
11	5	0.7	669	99.3
40	5	0.7	669	99.3
55	5	0.7	669	99.3
70	5	0.7	669	99.3
87	3	0.4	671	99.6
44	2	0.3	672	99.7
68a	1	0.1	673	99.9
68b	1	0.1	673	99.9
72	1	0.1	673	99.9
64	0	0	674	100
26	0	0	674	100
69	0	0	674	100
71	0	0	674	100

n — number, HPV — human papillomavirus

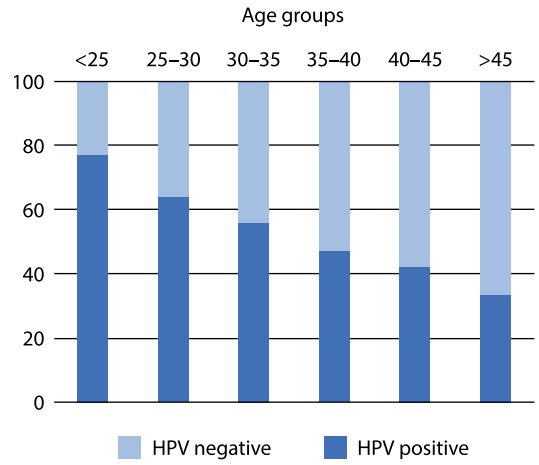

Figure 1. Distribution of HPV positive patients in specific age groups; HPV — human papillomavirus

occurrence of particular genotypes in specific age groups is statistically significant. This correlation is negative, so the frequency of occurrence of particular groups of HPV genotypes decreases with age (Fig. 1 and Tab. 4). The relationship calculated using Spearman's rho coefficient, however, is weak (Tab. 5).

For individual genotypes, the following relationships were found:

- genotype 51 (carcinogenic): significantly more frequent in patients under 25 years of age in comparison to all other age groups ($p = 0.001$), significantly more frequent in group 25–30 in comparison to group 30–35 ($p < 0.001$);
- genotype 56 (carcinogenic): significantly more frequent in groups 25–30 and 30–35 in comparison to group 40–45 ($p = 0.005$ and $p = 0.024$ respectively);
- genotype 59 (carcinogenic): significantly more frequent in patients under 25 years of age in comparison to groups 30–35, 35–40 and over 45 years ($p < 0.001$, $p = 0.0015$ and $p = 0.009$, respectively) and statistically significantly more frequent in patients in group 25–30 in comparison to groups 30–35, 35–40 and over 45 years of age ($p = 0.006$, $p = 0.015$ and $p = 0.049$, respectively);
- genotype 67 (carcinogenic): significantly more frequent in patients in group 25–30 in comparison to groups 30–35 and 35–40 ($p < 0.001$ and $p = 0.004$, respectively), significantly more frequent in group 25–30 in comparison to groups 30–35 and 35–40 ($p = 0.014$ and $p = 0.037$, respectively) and significantly more frequent in group 30–35 in comparison to group over 45 years of age ($p = 0.027$);
- genotype 73 (carcinogenic): significantly more frequent in patients in group under 25 years of age in comparison to groups 30–35 and 40–45 ($p = 0.016$ and $p = 0.008$, respectively) and significantly more frequent in group 25–30 in comparison to groups 30–35 and 40–45 ($p = 0.01$ and $p = 0.01$, respectively);
- genotype 66 (possibly carcinogenic): significantly more frequent in patients under 25 years of age in comparison to all other age groups ($p = 0.0035$).

There were also some significant interactions between other genotypes, such as 6, 52, 54 but because of their non-carcinogenic character, these were not mentioned.

In case of a positive HPV result, abnormal Pap-smear or a clinically suspicious cervix image, colposcopy with biopsy was performed. As a result, a biopsy was examined in 321 patients. In over half of the cases no pathology was found (NILM was diagnosed in 50% of patients). LSIL was present in 87 (27%) whereas HSIL in 71 (22%) samples. No squamous cervical cancer was histologically confirmed. However, what is noteworthy, two cases of adenocarcinomas were detected.

Table 2. Correlation between studied age groups and the presence of HPV genotype 16

Genotype 16	Group < 25	Group 25–30	Group 30–35	Group 35–40	Group 40–45	Group > 45	Line all	X2	p
Deficiency	43	129	156	120	70	68	586		
% Column	89.58	83.23	85.25	90.91	86.42	90.67			
% Line	7.34	22.01	26.62	20.48	11.95	11.60			
% All	6.38	19.14	23.15	17.80	10.39	10.09	86.94		
Presence	5	26	27	12	11	7	88		
% Column	10.42	16.77	14.75	9.09	13.58	9.33		5.41	0.368
% Line	5.68	29.55	30.68	13.64	12.50	7.93			
% All	0.74	3.86	4.01	1.78	1.63	1.04	13.06		
All	48	155	183	132	81	75	674		
% All	7.12	23.00	27.15	19.58	12.02	11.13	100		

HPV — human papillomavirus; p — p value

Table 3. Correlation between studied age groups and the presence of HPV genotype 53

Genotype 53	Group < 25	Group 25–30	Group 30–35	Group 35–40	Group 40–45	Group > 45	Line all	X2	p
Deficiency	42	142	171	122	77	72	626		
% Column	87.50	91.61	93.44	92.42	95.06	96.00			
% Line	6.71	22.68	27.32	19.49	12.30	11.50			
% All	6.23	21.07	25.37	18.10	11.42	10.68	92.88		
Presence	6	13	12	10	4	3	48		
% Column	12.50	8.39	6.56	7.58	4.94	4.00		4.29	0.508
% Line	12.50	27.08	25.00	20.83	8.33	6.25			
% All	0.89	1.93	1.78	1.48	0.59	0.45	7.12		
All	48	155	183	132	81	75	674		
% All	7.12	23.00	27.15	19.58	12.02	11.13	100		

HPV — human papillomavirus; p — p value

Table 4. Correlation between age groups and the oncogenic potential of the studied HPV genotypes

HPV type	M	SD	LMod.	Min.	Max.	Skew.	W	p
Group A	0.60	0.84	394	0	5	1.53	0.713	p < 0.001
Group B	0.11	0.35	606	0	2	3.20	0.348	p < 0.001
Group C	0.29	0.63	529	0	4	2.70	0.511	p < 0.001

HPV — human papillomavirus; M — mean; SD — standard deviation; p — p value

DISCUSSION

This study provides comprehensive information on the HPV prevalence and genotype distribution among a cohort of Polish women who were referred to a single center for HPV genotyping following either a diagnosis of abnormal cytology or for screening. We have not found such a database of one roof patients.

In comparison to another recent study conducted in Poland, we have noticed some discrepancies. As expected, the most frequent HPV genotype was 16. It was present in 26% of all HPV-positive patients compared to 20% in mentioned study. On the other hand, negative patients constituted 46.7%, and in the cited study 32.1%. According to Smolarz et al. HPV genotype 18 was found in about 14% of women, while in our observation, it was in 10th place and occurred twice less often (7.2%) [12]. Contrary to the literature, we did not observe genotype 18 occurring frequently. That, however, could the result of our focus on a heterogeneous group, where neither SIL nor cervical cancer was the criterion. In line with previous studies, HPV 16, 31, and 45 genotypes were most often detected in patients diagnosed with ASC-US or LSIL, whereas in patients with HSIL, genotypes 16, 33, 18, 31, 56 were the most common [13, 14]. We also provide data for the HPV types that are phylogenetically classified as oncogenic, such as HPV types 26, 67, 69, and 82, but seldomly described in epidemiological studies [15]. Little is known about the exact mechanism of HPV-associated carcinogenesis of these rare types due

Table 5. Correlation between age groups and HPV genotypes divided into three groups

	rho Spearman	p
Age group & Group A	–0.23	0.000
Age group & Group B	–0.08	0.033
Age group & Group C	–0.17	0.000

HPV — human papillomavirus; p — p value

to insufficient epidemiological evidences. The biological properties of the rare high-risk HPV types have only been investigated in a few studies, which included mostly cervical intraepithelial neoplasms lesions and a few cases of invasive cervical cancer [16].

As far as prevention is concerned, it is both important to detect lesions in the early stage and to identify risk-factors of carcinogenesis. Early diagnosed HPV-positive patients will be eligible for a high risk of cancer development. As a consequence, they will be subjected to tighter inspection and follow-up visits. The prevalence of HPV infection among women with subclinical or latent disease leads to different results. It depends on the studied population and used method of HPV detection. The highest percentage of infections is diagnosed using a PCR method which is recognized to have the highest sensitivity among all molecular biology techniques. It allows to detect the presence of one copy of HPV in 105–106 cells. PCR is now becoming a common diagnostic technique that is used in numerous laboratories. The results obtained from PCR are comparable and allow to avoid their false interpretation. The introduction of DNA testing has increased the effectiveness of screening programs in women over 30 years of age with the NILM (negative for intraepithelial lesion or malignancy) and reduced the number of unnecessary colposcopies and treatment in younger patients [17–20].

What is noticeable, the correlation of the appearance of particular genotypes in specific age groups is statistically significant — the frequency of occurrence of particular groups of HPV genotypes decreases with age. Over the past four to five decades the assessment of the distribution of HPV types in cervical cancer has been crucial for determining the cause of age-related differences. If the reason is the cohort effect, that could allow us to predict changes in the distribution of HPV types in the upcoming years, resulting in improvement of implementing preventive HPV-vaccination.

Originally, risk stratification in cervical screening based on the underlying HPV genotype was suggested in 2003 when the primary clinical HPV assays for screening indicated the detection of high-risk HPV genotype was performed either in a research setting or as an in-house test. Clifford et al. [21], suggested that HPV genotypes 16, 18, and 45 would merit closer surveillance than infection with other high-risk HPV genotypes. Subsequently, large-scale studies of cervical cancers displayed the contribution of different HPV genotypes to squamous cell carcinoma and adenocarcinoma. It served as a foundation to determine the hierarchy of high-risk HPV genotypes [22]. Throughout the next decade, studies showed that genotypes 31, 33, 52, and 58 confer risks similar to HPV 18 and 45, thereby establishing impetus for contemplating more complex screening algorithms using genotype-specific risk stratification. That resulted in forming more precise colposcopy referral recommendations and allowed to reduce [23–26] overtreatment. Thus, today's application of HPV diagnostics in screening distinguishes between a partial genotyping result for reporting of HPV 16 and 18, with the remaining high-risk HPV genotypes as a pooled result. A recent expert review by Xu et al., [27] assessing the accuracy of HPV 16/18 genotyping to triage LSIL cytology, points out that although the partial genotyping strategy increases the positive predictive value, the specificity declines compared with cytology. A more complete differentiation between genotypes may improve this strategy.

This work provides estimates of the important contribution of HPV types 16, 31, 51, 56, 52, 59, and 18. These types might be considered while developing new vaccines with a wider efficacy range. The early detection of cancers associated with HPV types 16, 31, and 51 could be considered in screening programs aimed at clinical management based on the HPV genotype. Our results indicate which HPV types should be emphasized on when the cross-protective effects of current vaccines are assessed. What is more, they could come as applicable while preparing recommendations for HPV vaccines usage. According to our findings those type-specific, high-risk, HPV-DNA-based screening tests and protocols should be focused on HPV types 16, 18, 31, 51, 52, 56, and 59.

CONCLUSIONS

Cervical cancer screening is recommended by clinical practice guidelines for being effective cancer preventive method. HPV 16 and 18 partial genotyping is implemented in several clinical screening guidelines. Evidence, that have been accumulated for over a decade, suggests that the definition should be expanded to include risk stratification on the full spectrum of high-risk HPV genotypes of women undergoing screening.

In the future, follow-up and vaccination status of patients may indicate a trend related to the extinction of some HPV genotypes in the vaccinated population. The advantage of our research is the long duration of the study. Close follow-up should last two years as up to 25% of relapses are observed within that period of time. During follow-up, both LSIL and HSIL were detected in 158 patients. It is a proof of

necessity of supervision over the patients. Two cases of adenocarcinoma furtherly confirm that statement. That is why it is essential to build trust in the doctor-patient relationship, conduct social campaigns reminding about regular check-ups and expand diagnostics beyond the exclusive cytology.

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Conflicts of interest

The authors declare no conflict of interest.

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Comparative analysis of classical primary continuous and novel technique uterine suturing methods on uterine scar formation after caesarian section: a prospective clinical study

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ABSTRACT

Objectives: The study investigated isthmocele rate, residual myometrium thickness, blood loss, and closure lengths through comparing the classical primary continuous suturing (CPCS) and novel technique uterine suturing (NTUS) after caesarian section.

Material and methods: A total of 402 C/S patients were included in this single-center prospective clinical study. All patients were divided into two groups according to suture technique. Classical primary continuous suturing (CPCS) was applied to the patients in Group 1, while the novel technique uterine suturing (NTUS) was applied in Group 2 as Z suture on both corners and 8 sutures in the remaining middle part incision closure.

Results: Patients in the NTUS group bled less than in the CPCS groups ($p < 0.0001$). Incision length after closure was longer in the CPCS than in the NTUS ($p < 0.0001$). Similarly, the number of sutures we applied was higher in the CPCS ($p < 0.0001$). In comparison of residual myometrium thickness, the mean values measured 197 ± 50 mm in the NTUS and 146 ± 39 mm in the CPCS ($p < 0.0001$). Residual myometrium thickness showed a negative strong correlation with incision length after closure ($r = –0.436$; $p < 0.0001$), how many times the needles have been passed ($r = –0.423$; $p < 0.0001$) and time for suturing ($r = –0.237$; $p < 0.0001$). NTUS and CPCS groups were similar in comparison to isthmocele.

Conclusions: The NTUS, termed as Erkayiran's suture, showed a successful reflection in our surgical cesarean section application compared to the classical suture. Although the occurrence of isthmocele in patients was similar, results were quite successful operationally in terms of both minimal blood loss and increased residual myometrium thickness.

Key words: caesarian section; uterine scar; isthmocele; suturing method; residual myometrium

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INTRODUCTION

In general, women have their first child at the beginning of their fertility years, and nowadays, the rate of preferring to deliver by the cesarean method has increased [1]. In cesarean operations, the lower regions of the uterus are opened in women, the baby is removed and then sutures are applied [2]. Normally this suture can be sewn in a single layer, double layer, or by locking the threads together, but there is no standard for the suture approach in practice [3]. When the cesarean section does not blend well, that area may remain open for any reason in women or create tissue in the open part there [4].

The isthmocele, which has a fluid, sac-like defect at the cesarean incision site, is detected in the anterior uterus in the previous cesarean area [5]. There is a prevalence that varies country by country, concerning the increasing number of cesarean sections [6]. Although patients with isthmocele are commonly asymptomatic, most of the symptoms are infertility, bleeding, and pain [7]. In particular, monthly menstrual blood can accumulate in this sac and cause continuous bleeding in the form of stains for 15–20 days [7]. Uterine rupture, ectopic pregnancy, and miscarriage implantation are among the pregnancy complications arising from this

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condition [8]. Although the surgical approach seems to be a controversial issue today, surgery should be recommended to women with future pregnancy expectations [9, 10].

Surgical sutures allow the tissues to be held together and have crucial importance in a cesarean [11]. Correctly applied sutures contribute to accelerating the healing process, thereby reducing scarring in the affected areas and reducing the recovery period and postoperative complications [6]. Although, new studies have reported possible risks [12], especially related to scar defect of cesarean section, there is no suture method comparison for isthmocele, which is one of the cesarean scar defects [13, 14]. The surgical method used during the closure of the incision to be made to the uterus increases and decreases the probability of occurrence of complications such as isthmocele, rupture, location, and invasion anomalies [15 16]. These complications cause difficulties in the next pregnancies. In cases such as isthmocele formation, the incidence of invasion anomalies such as scar pregnancy and percreata increata increases [17]. Besides, it is known that there is a relationship between residual myometrium tissue and rupture in subsequent pregnancies [18, 19].

In the present study, we wanted to examine the isthmocele rate, residual myometrium thickness, the difference in blood loss, duration of the closure, pre- and post-closure lengths compared to the classical primary continuous suturing (CPCS) of the operations performed with the novel technique uterine suturing (NTUS).

MATERIAL AND METHODS

Study design

A total of 402 C/S patients enrolled in this single-center prospective clinical study. We performed the current research at the Faculty of Medicine in the University. It was carried out after being approved by our institution's scientific research approval center and the Clinical Research Ethics Committee of the University (Approval Date: 2020/13-04; Protocol: 252). All individuals who participated in the study gave informed written consent before enrollment. The current study followed the guidelines of the Consolidated Standards of Reporting Trials and the Declaration of Helsinki [20].

Sample size

Before beginning the study, the participation of 176 individuals for each group in total was considered sufficient as a result of the power analysis performed at alpha: 0.05 and beta: 0.20 levels and the power of 0.80 test. We planned to include at least 200 patients for each group in the study to have higher participation or power in evaluation. As a result, 446 women were analzyed, with 412 eligible for inclusion, of whom 402 were enrolled (Fig. 1).

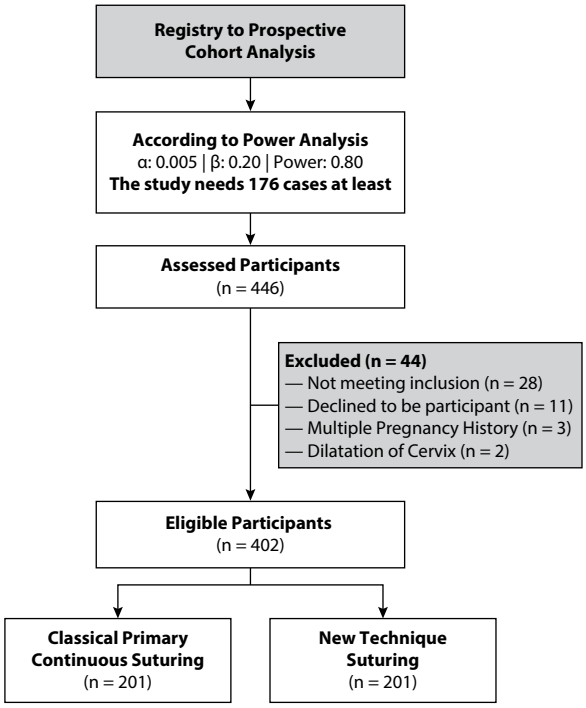

Figure 1. Flow chart for the selection and enrollment of study participants

Criteria for participants

The criteria for inclusion in the study were determined as follows: healthy patients between 18 and 40 years of age, who did not have a chronic disease, who did not take any anticoagulant drugs recently, who did not have a systemic disease such as blood pressure, and who had no previous uterine incision. Our exclusion criteria from the study were as follows: anticoagulant use, history of one or more cesarean section, uterine incision, hypertensive disease such as preeclampsia in pregnancy.

Surgical technique

Patients with primary cesarean section decided for cesarean section due to maternal or baby-related indications, regardless of the gestational age, were included in the study. Ages, cesarean indications, and weeks of gestation of the patients were recorded. All surgical procedures were applied to each patient as standard following working standards. The surgical process was performed by two surgeons (U.E. and T.A) experienced in obstetric surgery with the same suture materials and method. In patients with normal body mass index, 1g cefazolin sodium was administered one hour before the incision, while 2 g cefazolin sodium was used in patients with high BMI. The patients underwent cesarean delivery by making a lower segment uterine incision [21]. Classical primary continuous suturing (CPCS) was applied to the patients in Group 1, while the novel technique

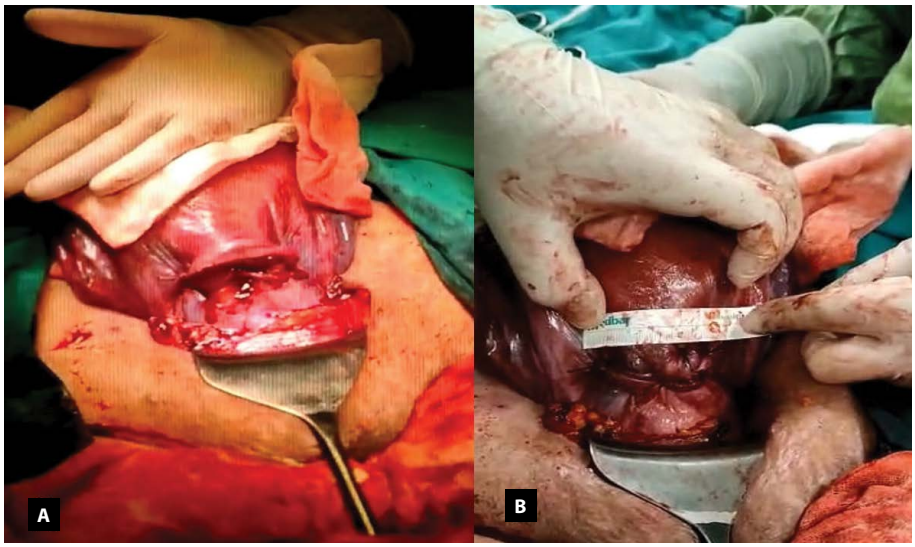

Figure 2. The pictures of the lower uterine segment before and after the NTUS; **A.** Closure of both corners of the uterine incision; **B.** Measurement of the uterine incision after closure

uterine suturing (NTUS) was applied in Group 2 as Z suture on both corners and eight sutures in the remaining middle part incision closure. The lengths were measured before and after the incision was closed (Fig. 2). During the closing of the incision, how many needles were passed through both uterine lips and how long this period was recorded, in addition to preoperative and postoperative hemogram values of the patients. Patients were called for the control at the postoperative 12th week and examined by transvaginal USG when they came. Uterine scar line thickness, size, and presence of isthmocele were evaluated.

Statistical technique

Data including continuous variables were expressed as mean ± standard deviation and categorical data as percentage. While the Chi-square test was used for categorical variables, the Mann-Whitney U test was used to compare the means of both groups in non-normal distributions. Student T-test was used to compare continuous variables between groups for normally distributed data. The paired analysis was performed to compare the preoperative and postoperative results of the patients. Chi-square analysis was applied for the categorical variables when the indications for the cesarean section were compared with the cross table. Data were analyzed using SPSS version 24 (SPSS) and a p-value less than 0.05 value was considered significant.

RESULTS

The patients' groups dividing according to the suture technique did not differ in terms of body mass, age, gestational week, uterine position, gravity-parity, cervical dilatation, and surgery indication, including demographic data ($p > 0.05$). Figure 1 provides a study flow chart for all the patients, including those enrolled in the present study and those who did not meet the criteria.

Paired analysis

The preoperative hemoglobin value measured for the comparison of the pre-and postoperative bleeding amount for all patients was 11.2 ± 1.2 mg/dL, while the postoperative value was found to be 10.1 ± 1.2 mg/dL ($p < 0.01$). The difference between preoperative and postoperative hemoglobin values in the CPCS group was 1.19 ± 0.83 mg/dL ($p < 0.0001$), while the difference in hemoglobin value between preoperative and postoperative in the NTUS group was 0.82 ± 0.65 mg/dL ($p < 0.0001$). It was observed that there was less bleeding in the NTUS group between the two groups.

Group comparison

In comparison to the suturing groups, pregnancy age and uterine incision length were similar between the groups. Incision length after closure was significantly longer in the CPCS than in the NTUS ($p < 0.0001$). Similarly, the number of sutures we applied was higher in the CPCS group ($p < 0.0001$). In the comparison of residual myometrium thickness, the mean values measured 197 ± 50 mm in the NTUS and 146 ± 39 mm in the CPCS ($p < 0.0001$). All the details of the group comparisons were given in Table 1.

Correlation analysis

There was a significant positive correlation between gestational age and the suture related parameters including uterine incision length ($r = 0.601$; $p < 0.0001$), incision length

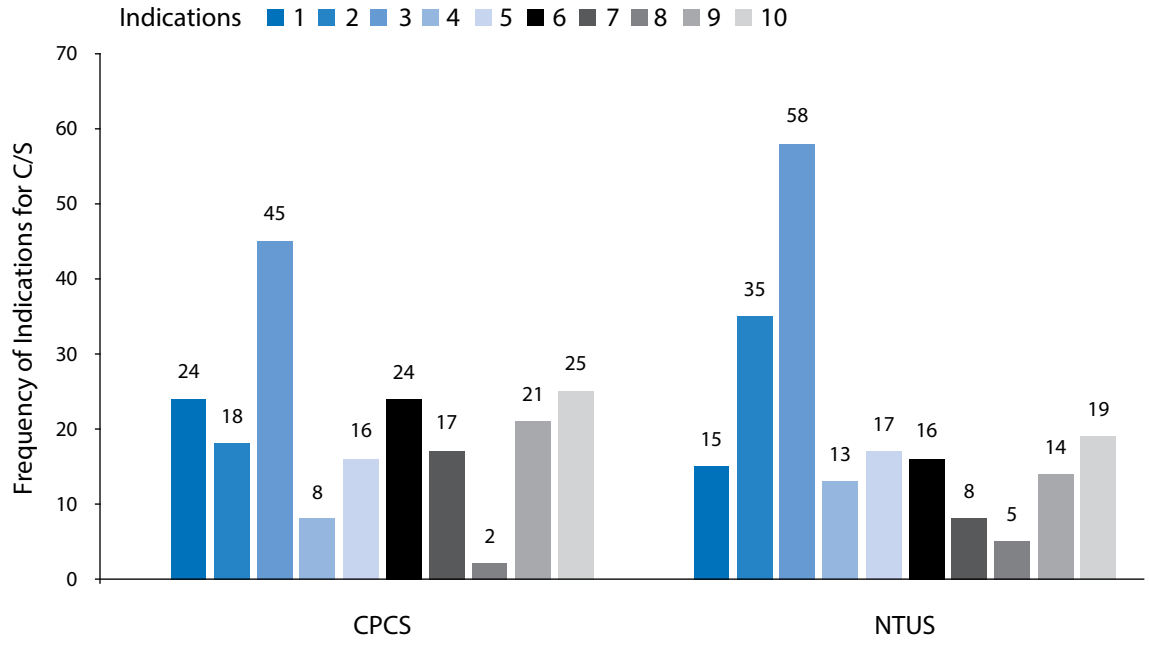

Figure 3. Frequency of cesarean section indications by the suture groups

after closure (r = 0.195; p = 0.009), how many times the needles have been passed (r = 0.253; p < 0.0001) and time for suturing (r = 0.307; p < 0.0001). Residual myometrium thickness showed a negative strong correlation with incision length after closure (r = –0.436; p < 0.0001), how many times the needles have been passed (r = –0.423; p < 0.0001) and time for suturing (r = –0.237; p < 0.0001).

Indications and isthmocele prevalence

A comparison graph of the patients' indications which we included in the present study was given in Figure 3. According to the chi-square analysis, the frequency of indications including cephalopelvic disproportion, fetal distress, multiple pregnancies, and maternal heart disease was found higher in the NTUS group than in CPCS (p = 0.025). NTUS and CPCS groups were similar in comparison to isthmocele. It was found in only one patient in both suture groups.

DISCUSSION

The NTUS, termed as Erkayiran's suture technique, which we examined in the present prospective study, showed a successful reflection in the results in our surgical cesarean section application compared to the classical suture approach. Although the occurrence of isthmocele in patients was similar, we showed that the results were quite successful operationally in terms of both minimal blood loss and increased residual myometrium thickness.

Most cesarean-related studies held several mechanisms that were associated with scar formation responsible, attributing to the surgical procedure. As one of these, Vervoort et al. directly tried to explain scar formation as the use of lower segment transverse incision [22]. Their results showed that the surgical incisions they applied due to the difficulty of separating the cervix from the uterus brought it closer to the cervix. Although this situation slows wound healing due to the accumulation of secretion in the wound area, these hypotheses are insufficient to explain scar formation after previous cesarean section. In another study conducted on scar formation, Yasmin et al. stated that scar formation is directly related to uterine wall closure techniques [23]. According to them, the inclusion/removal of the decidua, how many times the uterine wall was closed, the suture technique were the most effective parameters on scar development, such as the isthmocele. Tulandi et al. supported the results of Yasmin et al. that closing the uterus with a single layer reduced the risk of scars [24].

As an indisputable result, it is inevitable that the number of wounds in the uterus increases as the preference of cesarean section increases in both patients and surgeons. Bamber et al. conducted a study to investigate the outcome of uterine closure and evaluate uterine scar thickness, and ultimately compared the single/double layer suture. According to the results of their studies, double-layer closure was associated with a thicker myometrial scar in the primary or elective cesarean section [13]. While the focus of current studies is on suture material or surgical approaches in isthmocele formation, these analyzes do not address the importance of wound healing in the development of the suture type or technique. In this sense, it would be appropriate to work on different techniques to reduce the risks according

to each cesarean operation as a forward return risk for the patient. In the caesarean section surgical techniques study, researchers found no difference in postpartum blood loss when comparing catgut, a monofilament suture, and Vicryl, a multifilament suture [25]. The more sutures, lower tension, and longer operative time with monofilament sutures seemed less beneficial for hemostasis.

Başbuğ et al. [26] reported the effectiveness of suture material on the cesarean scar in a clinical analysis in single pregnancies. They performed closure using absorbable sutures and measured myometrial thicknesses remaining in the scar area, as measured by a transvaginal USG. Secondary results covered alterations in blood loss, operation time, and postoperative outputs. Although they could not find a significant alteration between the compared subgroups in terms of gynecological sequelae, according to their results, scar closure showed a positive difference on the increased myometrial thickness and healing. Unlike their study, we focused on the suture technique, not the suture material, and investigated similar clinical values in the same patient group. Besides, we included not only single pregnancies but also patients with different indications.

In the present study, according to the comparison of preoperative and postoperative hemoglobin values to assess the bleeding amounts, we observed fewer amounts of blood loss in the NTUS than in the CPCS. In comparison to the suturing groups, pregnancy age showed a similarity. Incision length after closure was significantly longer in the CPCS than in the NTUS. Similarly, the number of sutures we applied was higher in the CPCS as expected due to the longer incision length in this group. In the comparison of residual myometrium thickness, NTUS had a higher thickness than classical suturing. Our acknowledgment supports that the increase in endometrial thickness may lead to a decrease in the rupture risk. Gestational age showed a positive relationship with all the suture-related parameters including uterine incision length, incision length after closure, how many times the needles have been passed, and time for suturing. Residual myometrium thickness showed a negative strong correlation with incision length after closure, how many times the needles have been passed, and time for suturing. NTUS and CPCS groups were similar in comparison to isthmocele. It was found in only one patient in both suture groups.

There are some limitations in this clinical study. We included all patients with cesarean section without separating any indications according to the admission and exclusion criteria, and therefore we could not rule out the possibility of bias due to indication differences. Although we found the isthmocele occurrence rate similar in both groups, we could reach stronger data if we could also perform ROC analysis with a higher number of cases. The strengths of the study were enough participants according to the power analysis, having a prospective design, high follow-up rates, and successful results.

CONCLUSIONS

While recent clinical studies have focused directly on the closure methods and suture material of the uterine tissue, the current study focused on different, specifically, the suture technique and the importance of its factors affecting uterine scar healing. Our results showed that the 'Erkayiran' technique decreased the number of sutures and suture time, by the way, reduced the operation time and the rate of intraoperative complications with intraoperative less bleeding. Besides all these benefits of the 'Erkayiran' technique, we noted our surgical expectation for the possibility of a decreased intra-abdominal adhesion due to the less incision length after suturing and the possibility of a decrease in the rupture risk due to the increased endometrial thickness that needs to be investigated with future planned studies.

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Availability of study data

The data has not been publicly available due the institution policy but it has been available for the corresponding author on a reasonable demand.

Ethics approval

The present study was carried out after being approved by our institution's scientific research approval center and the Clinical Study/Research Ethics Committee of the University(Approval Details: 2020/13-04; Protocol: 252).

Consent to participate

All individuals who participated in the study gave informed written consent before enrollment.

Conflict of interest

The authors declared no conflicts of interest relevant to the present article.

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The role of individual blood flow parameters through ductus venosus in the first and second trimesters of pregnancy in predicting the condition of the fetus and newborn

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ABSTRACT

Objectives: To predict fetal and neonatal outcome during pregnancy based on detailed analysis of ductus venosus blood flow velocities in first and second-trimester fetuses.

Material and methods: A retrospective analysis was made in 680 patients with single pregnancies in years 2015 and 2016. The following ductus venosus blood flow velocities in first and second-trimester were analyzed: S-wave velocity, D-wave velocity, a-wave velocity, Tmax velocity, PIV. Results were divided into sub-groups with reduced value, normal value and increased value and compared with fetal and neonatal condition.

Results: The relationship between the increased PIV value in the first trimester of pregnancy and an increased risk of chromosomal aberrations was observed, whereas the increased DV PI value in the second trimester of pregnancy with reduced A -wave were associated with a higher incidence of FGR. No correlation between the remaining DV blood flow velocities in the first and second trimester of pregnancy and the more frequent occurrence of fetal and neonatal complications has been confirmed.

Conclusions: The increased DV PIV is a good prognostic tool for the detection of chromosomal aberrations in first trimester of pregnancy. In the second trimester, the increased DV PIV and the reduced A- wave velocity correlate with the fetal growth restriction. Ductus venosus seems to be an indirect indicator of intrauterine hypoxia with moderate prognostic value for adverse obstetric outcomes.

Key words: ductus venosus; Doppler examination; FGR; neonatal outcome

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INTRODUCTION

Ductus venosus is a vessel unique to the fetal circulation, whose task is to supply well-oxygenated blood from the umbilical vein to the coronary circulation and the central nervous system, thanks to the privileged blood stream directed through the foramen ovale to the left atrium. In the first half of pregnancy, ductus venosus carries 60% of the volume of oxygenated blood from the umbilical vein. In the second half of pregnancy, this flow is reduced to 20–30%, but in certain situations of hypoxia, changes in pressure in the umbilical vein or changes in blood viscosity, it may increase [1]. In ductus venosus, a characteristic three-phase

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flow diagram is observed with high velocities during contraction and relaxation of the ventricles and preserved flow towards the heart during atrial contraction [2]. An important role in prenatal diagnosis is played by Doppler examination of blood flow through ductus venosus. Its abnormal waveform and pulsation index for the veins are described in the case of chromosomal aberrations, cardiac defects and arrhythmias, intrauterine restriction of fetal growth, blood transfusion syndrome in unicellular twin pregnancy, edema fetus and intrauterine infections [3–5]. The aim of the study was to assess the usefulness of the analysis of individual parameters of blood flow velocity through ductus venosus in the prognosis of the condition of the fetus and newborn.

MATERIAL AND METHODS

Blood flow through ductus venosus velocity tests and ultrasound assessment of the fetal anatomy were performed in the first trimester between 11w0d and 13w6d and in the second trimester of pregnancy between 18 and 22 weeks of pregnancy in patients who were referred in the first trimester to the Prenatal Research Clinic. The total number of patients who underwent prenatal screening and subsequent pregnancy analysis was 680. Data on the clinical condition of the newborn and the analysis of the course of pregnancy in terms of specific complications were obtained based on hospitalization of the studied patients. Clinical evaluation of the condition of the newborn was performed in 643 cases. In the ultrasound examination carried out between 11w0d and 13w6d, all fetuses were measured with blood flow through ductus venosus velocity. In the study, between 18 and 22 weeks of gestation, DV PIV was measured in 161 fetuses as an extension of routine diagnostics. In 38 cases, the values of other blood flow velocities through ductus venosus were also recorded. In this study, the results of blood flow through ductus venosus, fetal anatomy and NT measurement obtained during the ultrasound examination were analyzed. The NT value above the 95th percentile for normal fetuses was considered an abnormal value. In the case of venous flow measurement, the rules of the Fetal Medicine Foundation (FMF) [4] test were used. For the purposes of this study, in addition to the qualitative assessment of the flow wave, the analysis of individual components of the flow through ductus venosus obtained during the Doppler examination was performed.

The following parameters were assessed: pulsation index for veins (PIV); maximum ventricular systolic velocity (Vs); maximum velocity in the early diastolic phase of the ventricles (Vd); maximum velocity during atrial contraction (Va); time-averaged maximum velocity (T max) — the average maximum velocity of blood flow.

The obtained results of blood flow through ductus venosus in the first trimester of pregnancy were compared with the reference values obtained in the group of uncomplicated pregnancies based on the following studies:„Ozhan M. Turana „Reference Ranges for Ductus Venosus Velocity Ratios in Pregnancies with Normal Outcome" [6] as well as R. Axt-Fliedner „Reference values of ductus venosus blood flow velocities and waveform indices from 10 to 20 weeks of gestation" [7].

In the second trimester of pregnancy, ultrasound examinations were performed in accordance with the recommendations of PTU PTGiP [8].

The following pregnancy complications were analyzed based on the course of hospitalization: gestational diabetes, gestational arterial hypertension, fetal growth restriction, ODFD, assessment of the birth weight of the newborn, assessment of the newborn's acid-base balance immediately after birth, the need to stay in the Neonatal Intensive Care Unit (NICU) , the occurrence of genetic and structural defects and neonatal deaths.

The following statistical tests were used in the study: Kruskal Wallis ANOVA, Mann-Whitney U-Test, frequency analysis using Fisher's exact test, supplemented with the analysis of standardized residuals and calculation of the odds ratio with confidence interval, statistical regression analysis for the parameter DV PIV I trimester and DV PIV II trimester. When verifying the hypotheses, the significance level was adopted for $p = 0.05$. The calculated p-values are presented. Statistical analyses were performed using the TIBCO Statistica 13.3 and R 3.5.3 software.

RESULTS

A statistically significant difference was found for the following parameters: DV PIV in the first trimester of pregnancy, Va in the first trimester of pregnancy, DV PIV in the second trimester of pregnancy, and Va in the second trimester of pregnancy. In the case of DV PIV in the first trimester of pregnancy, a statistically significant difference was found for the birth weight of the newborn between the increased and normal value ($p = 0.03$) and the increased and decreased value ($p = 0.04$). It has been shown that an increase in the DV PIV parameter by one unit increases the chance of the occurrence of a chromosomal aberration almost twice (OR 1.98) (Tab. 1). For Va in the first trimester of pregnancy, the exact Fisher test turned out to be statistically significant for the ODFD variable ($p = 0.005$) (Tab. 2). The analysis of standardized residuals showed that in the group with the increased value, a thirteen times higher chance of ODFD was observed. There was a statistically significant difference between the reduced and normal velocity in relation to the birth weight of the newborn ($p = 0.032$). For the DV PIV II trimester, a statistically significant difference was shown in the following complications: suspicion of heart disease ($p = 0.037$), fetal growth restriction ($p = 0.002$) and the need to stay in the

Table 1. The relationship between the individual values of the pulsation index for the veins of ductus venosus DV PIV (N, O, P) in the first trimester of pregnancy and the occurrence of complications in the further course of pregnancy and perinatal complications

DV PIV (n = 680)										
Complication	**Normal value (n = 440)**			**Decreased value (n = 48)**			**Increased value (n = 192)**			**p (exact Fisher test)**
	Yes	**No**	**No data**	**Yes**	**No**	**No data**	**Yes**	**No**	**No data**	
Suspicion of a heart disease	7	421	12	0	46	2	4	177	11	0.323
Death of the newborn	4	407	29	0	47	1	4	178	10	0.505
Gestational hypertension	14	399	27	2	45	1	4	179	9	0.672
FGR	29	384	27	2	45	1	10	173	9	0.761
Genetic aberration of the fetus	5	433	2	0	48	0	6	186	0	0.353
ODFD	35	378	27	4	43	1	9	173	10	0.47
GDM	92	321	27	12	35	1	37	146	9	0.73
NICU	3	44	1	43	368	29	18	164	10	0.737

FGR — fetal growth restriction; ODFD — operative delivery for fetal distress; GDM — gestational diabetes mellitus; NICU — Neonatal Intensive Care Unit

Table 2. The relationship between individual a-wave velocity values in ductus venosus DV Va (N, O, P) in the first trimester of pregnancy and the occurrence of complications in the further course of pregnancy and perinatal complications

DV PIV (n = 680)										
Complication	**Normal value (n = 635)**			**Decreased value (n = 36)**			**Increased value (n = 9)**			**p (exact Fisher test)**
	Yes	**No**	**No data**	**Yes**	**No**	**No data**	**Yes**	**No**	**No data**	
Suspicion of a heart disease	11	603	21	0	34	2	0	7	2	0.102
Death of the newborn	8	590	37	0	34	2	0	8	1	0.757
Gestational hypertension	17	584	34	3	31	2	0	8	1	0.207
FGR	40	561	34	0	34	2	1	7	1	0.256
Genetic aberration of the fetus	11	623	1	0	35	1	0	9	0	0.197
ODFD	43	557	35	1	33	2	4	4	1	0.005
GDM	135	466	34	5	29	2	1	7	1	0.623
NICU	61	537	37	2	32	2	1	7	1	0.697

FGR — fetal growth restriction; ODFD — operative delivery for fetal distress; GDM — gestational diabetes mellitus; NICU — Neonatal Intensive Care Unit

Neonatal Intensive Care Unit (p = 0.008) (Tab. 3). It was calculated that an increase in the pulsation index for the veins of ductus venosus in the second trimester of pregnancy by one unit causes a 38-time greater risk of fetal growth restriction. For the DV Va II trimester, the Fisher exact test showed a statistically significant difference in relation to the FGR (p = 0.025) (Tab. 4). It was found that the reduced value of a-wave velocity causes an 11-time greater risk of fetal growth restriction than for the normal values. Other parameters of blood flow through the venous duct showed no statistical significance for any of the complications.

DISCUSSION

Blood Doppler flow in ductus venosus is an important parameter used in prenatal diagnostics [8, 9]. When performing Doppler examination of venous flow, its flow waveform is assessed using quantitative indicators such as DV PIV, DV Vs, DV Vd, DV Va and DV Tmax, and qualitative indicators - presence, absence or inversion of the "a wave". In the first trimester of pregnancy, the relationship between abnormal parameters of blood flow through the venous duct and an increased risk of heart defects and chromosomal aberrations has been known for a long time [10]. The relationship between abnormal values of blood flow through ductus venosus and an increased risk of chromosomal aberration may be explained by the more frequent coexistence of heart defects in genetic syndromes or developing circulatory failure in the fetus [11]. It is noteworthy that in the case of a fetus without an accompanying heart defect, the parameters of blood flow through ductus venosus may be completely normal. In the study of Czuba B. et al. [12] it was found that the inclusion of DV in the study increases the

Table 3. Relationship between individual DV PIV (N, O, P) pulsation index values in the second trimester of pregnancy and the occurrence of complications in the further course of pregnancy and perinatal complications

DV PIV (n = 161)										
Complication	Normal value (n = 152)			Decreased value (n = 4)			Increased value (n = 5)			p (exact Fisher test)
	No	Yes	No data	No	Yes	No data	No	Yes	No data	
Suspicion of a heart disease	141	3	8	3	0	1	3	0	2	0.037
Death of the newborn	132	0	20	4	0	0	5	0	0	0.999
Gestational hypertension	128	4	20	4	0	0	5	0	0	0.999
FGR	127	5	20	3	1	0	2	3	0	0.002
Genetic aberration of the fetus	149	3	0	4	0	0	4	1	0	0.207
ODFD	124	8	20	3	1	0	5	0	0	0.474
GDM	95	37	20	4	0	0	3	2	0	0.714
NICU	117	15	20	1	3	0	3	2	0	0.008

FGR — fetal growth restriction; ODFD — operative delivery for fetal distress; GDM — gestational diabetes mellitus; NICU — Neonatal Intensive Care Unit

Table 4. Relationship between individual a-wave velocity values in ductus venosus DV Va in the second trimester of pregnancy and the occurrence of complications in the further course of pregnancy and perinatal complications

DV PIV (n = 38)										
Complication	Normal value (n = 22)			Decreased value (n = 16)			Increased value (n = 0)			p (exact Fisher test)
	No	Yes	No data	No	Yes	No data	No	Yes	No data	
Suspicion of a heart disease	21	0	1	14	0	2	0	0	0	0.562
Death of the newborn	21	0	1	14	0	2	0	0	0	0.562
Gestational hypertension	21	0	1	14	0	2	0	0	0	0.562
FGR	20	1	1	9	5	2	0	0	0	0.025
Genetic aberration of the fetus	22	0	0	15	1	0	0	0	0	0.421
ODFD	19	2	1	14	0	2	0	0	0	0.485
GDM	15	6	1	13	1	2	0	0	0	0.18
NICU	14	7	1	7	7	2	0	0	0	0.53

FGR — fetal growth restriction; ODFD — operative delivery for fetal distress; GDM — gestational diabetes mellitus; NICU — Neonatal Intensive Care Unit

detection rate and lowers the false-positive rate for trisomy 21. Wagner et al. [13] performed a retrospective analysis comparing the predictive value of a-wave alone, DV PIV alone, and both parameters together for the detection of trisomy 21. Reversed wave occurred in 2.3% of fetuses with normal karyotype and 66.1% of fetuses with trisomy 21. DV PIV above the 95th percentile occurred in 8.3% of fetuses with normal karyotype and 77.2% of fetuses with trisomy 21 [13]. Abnormalities in blood Doppler flow through the DV also occur in fetuses with normal karyotype who have been diagnosed with a heart defect. Timmermann [14] in his study pointed to the relationship between thickened NT, heart defect and increased DV PIV parameter in fetuses with normal karyotype. Martinez et al. [15] showed that information on venous flow increased the detection rate of heart defects by 11% in relation to the NT measurement alone. Also, Oh C. et al. [16] proved the correlation between the incidence of DV RAV and an increased risk of the presence of a heart defect, chromosomal aberrations and perinatal death. The analyzed material confirmed the usefulness of the assessment of blood flow through ductus venosus, especially the values of the pulsation index for the veins for ductus venosus, in detecting chromosomal aberrations.

Contrary to the first trimester of pregnancy, Doppler ultrasonography of the venous flow in the second trimester of pregnancy is not a mandatory component of the examination. The most common application of the Doppler examination of ductus venosus is the assessment of the cardiovascular capacity of the fetus in the case of identified abnormalities, *e.g.*, intrauterine growth restriction. Węgrzyn P. et al. [17] compared the values of individual DV indices: PVIV, PIV, PLI and the S/A ratio in 89 fetuses diagnosed with fetal growth restriction and 119 healthy ones between 22–42 weeks of gestation. Their study showed

that in healthy fetuses these parameters gradually decrease with the duration of pregnancy, however, compared to the groups of fetuses with fetal growth restriction, they remain higher [17]. Analysis of the duration of blood flow abnormalities through ductus venosus in the form of the absence or inverted a-wave was performed by Turan et al. [18]. Based on the conducted research, he concluded that the persistence of the absence or the reverse a-wave for more than seven days is associated with an almost certain risk of intrauterine fetal death [18]. Alfirevic in his analysis of blood Doppler flows in high-risk pregnancies showed that the assessment of early and late changes in ductus venosus in pregnancies complicated with early FGR did not significantly correlate with the difference in perinatal death. The benefit of evaluating DV as a parameter influencing the decision to induce labor in the case of late births has been observed in the form of better long-term neurological outcomes in children [19]. In the study by Dahlbäck et al. [20] on changes in the velocity of ductus venosus in 358 high-risk pregnancies between 20–42 weeks of gestation, the DV PI and the following coefficients of its velocity were analyzed: S/Es, S/a and Es/a [systolic (S), final systolic (Es), atrial contraction (a)] and compared these results with the flows in umbilical vessels. These results were compared with specific complications: preterm labor (< 37 Hbd and < 34 Hbd), low birth weight (< 10 percentile and < 3 percentile), Apgar score < 7 points, arterial < 7.1 and venous < 7.2 blood pH, treatment within the Neonatal Intensive Care Unit and perinatal mortality. It was observed that S/Es > 2 SD, DV-PIV > 2 SD and all types of pulsations are associated with poorer blood pH results: umbilical artery < 7.1 and umbilical vein pH < 7.2, S/Es > 2 SD is also related to more frequent occurrence of SGA. It has been shown that the systolic coefficients were less abnormal than the diastolic coefficients, while changes in the end-diastolic velocity of ductus venosus may give false positive results towards the deteriorating condition of the fetus. It was found that umbilical vein pulsation better predicts unfavorable obstetric outcomes than specific indicators of ductus venosus [20]. On the other hand, the Fetal Medicine Foundation recommends the evaluation of the DV PIV in the case of fetal growth restriction, as a significant increase in the pulsation index in ductus venosus along with other abnormalities may be associated with an increased risk of intrauterine death before 34 Hbd. It is also one of the abnormalities suggesting induction of labor after 34 weeks of pregnancy [21]. The analyzed own material showed a relationship between increased values of DV PIV and a decreased value of wave velocity and an increased risk of fetal growth restriction. This is consistent with the pathophysiology of fetal growth restriction, in which a reduced blood exchange area in the placental villi leads first to an increase in vascular resistance in the umbilical artery and its reduction in the middle cerebral artery, and then to an increase in vascular resistance in DV, absence of reverse wave or the presence of reverse wave during atrial contraction due to an increase in venous pressure in the right atrium and right ventricle [22, 23]. No statistically significant results were obtained for the remaining parameters and complications.

CONCLUSIONS

Ductus venosus is an indirect indicator of intrauterine hypoxia, it does not show a high prognostic value for unfavorable obstetric outcomes. Doppler examination of blood flow through the venous duct plays a significant role in the prenatal diagnosis of the first trimester. Especially DV PIV has a prognostic value for the detection of chromosomal aberrations. In the second trimester of pregnancy, the assessment of Doppler blood flow parameters through ductus venosus, especially DV PIV, and the a-wave assessment is helpful in the detection of fetuses with an increased risk of intrauterine growth restriction.

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Conflict of interest

All authors declare no conflict of interest.

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Effects of dietary structure on the incidence of gestational diabetes mellitus and macrosomia

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ABSTRACT

Objectives: To explore the relationship between dietary structure and the incidence of gestational diabetes mellitus and macrosomia.

Material and methods: In this retrospective study, the diet records of pregnant women admitted to the Shanghai Jiao Tong University Affiliated Sixth People's Hospital between August 2017 and August 2018 were collected with the approval of the local ethics committee. Corresponding medical and clinical information of pregnant women were obtained from the medical system. The relationship between diet structure and the incidence of gestational diabetes and macrosomia was analyzed.

Results: A total of 93 pregnant women with elevated blood sugar (including new gestational diabetes mellitus and diabetes mellitus with pregnancy) were enrolled. There were 21 newborns with macrosomia. The consumption of tofu was negatively correlated with the occurrence of macrophages. The consumption of pork eaten was negatively correlated with blood sugar levels two hours after eating. The consumption of vegetables was positively correlated with the blood glucose level one hour after eating. Eggs may increase triglycerides and blood sugar, which is an important inducer of pregnancy complicated with diabetes and macrosomia.

Conclusions: The diet structure of pregnant women is correlated with the occurrence of diabetes mellitus and macrosomia in pregnancy. It is recommended to eat more potatoes and not fried noodles with edible oil and to eat more high-quality protein, such as vegetable protein and lean pork.

Key words: dietary structure

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INTRODUCTION

Gestational diabetes is a common complication during pregnancy, including pregestational diabetes mellitus (PGDM) and gestational diabetes mellitus (GDM), with an incidence of 6–9% [1]. The glucose metabolism of most patients returns to normal after delivery, but the risk for developing type 2 diabetes in the future increases [1]. Gestational diabetes is harmful to both mothers and children and one of the most important complications is macrosomia [2]. During pregnancy, due to estrogen, progesterone, and placental lactogen, B cell proliferation increases, hypertrophy and hypersecretion of islets occurs, insulin secretion increases, leading to slightly increased levels of blood sugar in pregnant woman than the non-pregnant woman [2]. The increase of insulin content in blood was higher in pregnant women compared with non-pregnant women after intravenous glucose injection, and the decrease in blood glucose levels after insulin injection was not as effective as that in non-pregnant women, indicating that the islet B cells are active and secreted [3]. There are many hypotheses for the pathogenesis of gestational diabetes mellitus, including genetic factors, insulin resistance, abnormal fat factors and inflammatory factors [3]. It is well known that improper eating habits may lead to obesity, which is a high-risk factor for diabetes [4]. Diet control has become an effective control method for pregnancy complicated with diabetes [4]. Therefore, we suspected that dietary structure may also affect the incidence of gestational diabetes mellitus. Previous literature suggested that red meat, eggs, and sea fish are the main sources of methylamine in the diet [5]. Methylamine produces trimethylamine oxide, while circulating trimethylamine oxide increases the risks of type 2 diabetes

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and cardiovascular disease [5]. In addition, some studies have shown that diet can affect the occurrence of diabetes by changing intestinal flora. Ferrocino et al. [6] analyzed the dietary structure and intestinal flora of 41 GDM pregnant women who were under the guidance of dietitians and found that patients following dietary recommendations showed better metabolic and nutritional structure, and decreased the number of bacteria associated with high-fat diet [7]. In this retrospective study, we collected the diet records of pregnant women who were admitted to the Shanghai Jiao Tong University Affiliated Sixth People's Hospital between August 2017 and August 2018 and aimed to explore the relationship between diet structure and the incidence of pregnancy with diabetes.

MATERIAL AND METHODS

With the approval of the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital, the diet records of pregnant women who were admitted to the Shanghai Jiao Tong University Affiliated Sixth People's Hospital between August 2017 and August 2018 were retrospectively reviewed. All pregnant women who were admitted between this period and diagnosed with gestational diabetes were enrolled, except for those with other severe acute or chronic diseases. Corresponding medical and clinical information of pregnant women were obtained from the medical system. The relationship between diet structure and the incidence of pregnancy with diabetes and macrosomia was explored using statistical analysis.

The SPSS software (version 22.0) was used for data analysis. The t-test was used to analyze the difference between two groups, while rank test was performed to analyze the difference among multiple groups. Chi-square test was used to analyze the correlation between two factors.

RESULTS

A total of 93 pregnant women diagnosed with gestational diabetes (including GDM and PGDM) were enrolled. There were 21 newborns diagnosed as macrosomia (9 cases with diabetes-related macrosomia and 12 cases with macrosomia unrelated to diabetes). As shown in Table 1, there was no significant difference in delivery, neonatal score, and postpartum hemorrhage in these women. Obesity is a high-risk factor for pregnancy with diabetes and macrosomia. Low family income is also a high-risk factor for fetal macrosomia.

The relationship between diet and gestational diabetes mellitus and macrosomia, as well as the levels of blood glucose during pregnancy was analyzed (Tab. 2 and 3).

Staple food: potato food reduced blood sugar for two hours. Fried pasta significantly increased the levels of low--density lipoprotein and cholesterol. When the intake of edible oil fried noodles more than 42 per week, low density lipoprotein level increased 0.12 mmol/L and cholesterol increased 0.12 mmol/L. Proteins: Fatty fish raised blood sugar in pregnant women. Tofu reduced the levels of triglycerides and was negatively correlated with the occurrence of macrosomia. The consumption of pork was negatively correlated with the levels of blood sugar two hours after meal. Vegetables: the amount of vegetables consumed was positively correlated with the levels of blood sugar one hour after meal, indicating that vegetables should be eaten properly. Drinks: Juice, yogurt lowered blood sugar. Other drinks raised the levels of LDL and cholesterol, Average absolute value of LDL increased by 0.21 mmol/L in pregnant women who drank other beverages. Oil: soybean oil and animal oil are better choices. Soy oil reduced the incidence of pregnancy and diabetes. Animal oil reduced the incidence of macrosomia. Eggs raised triglyceride and blood sugar levels. When the consumption of eggs was more than 30 per month (1 per day), an increase of 0.33 mmol/L in absolute value of triglyceride, an increase of 0.13 mmol/L in fasting blood glucose, and an increase of 0.38 mmol/L in Blood glucose one hour after meal were observed. Excessive consumption of eggs is the cause of pregnancy with diabetes and macrosomia diet. Social factors: obesity and old age were important risk factors for pregnancy with diabetes. Low-income and low education level were risk factors for giant children.

DISCUSSION

Obesity and old age are high risk factors for pregnancy with diabetes, both PGDM and GDM [7]. In this study, we also found that obesity and old age are important risk factors for pregnancy with diabetes and macrosomia. Pregnant women with diabetes have an increased risk for later hypertension in addition to the risk of macrosomia [8]. Pregnancy with diabetes may also be accompanied by fetal malformation, hyperbilirubinemia, hypocalcemia and neonatal respiratory distress, and may cause amniotic fluid excess and premature delivery [8].

According to the results of this study, pregnant women who ate much staple food should add coarse grain reasonably. Although potato food is a main source of starch, it contains more cellulose and can increase satiety [9]. Fried pasta can increase blood lipid levels; therefore, should be avoided during pregnancy [9]. The fat content in the fat fish is relatively high, which can raise the blood sugar levels, increase the risk of diabetes [10]. Thus, fat fish is not a high-quality protein choice for pregnant women. Lean red meat and bean products can reduce blood sugar and blood lipids; therefore, they are a high-quality protein source during pregnancy. Juice and yogurt can reduce blood sugar levels, but other drinks (such as cola) can increase blood lipid levels [10].

Our data showed that soybean oil and animal oil were better choices for pregnant women. Soybean oil can reduce

Table 1. Baseline information of pregnant women

	Gestational diabetes mellitus		p value	Fetal macrosomia		p value
	Yes	No		Yes	No	
Age			0.000			0.653
≥ 35 years old	16	12		2	26	
< 35 years old	77	291		19	349	
BMI	27.70	26.49	0.002	28.22	26.69	0.041
Educational level			0.234			0.315
Primary school and below	1	0		0	1	
Junior high school	4	30		2	32	
Senior high school, technical secondary school	14	48		7	55	
Junior college	19	67		4	82	
undergraduate	44	122		7	159	
Master and above	11	36		1	46	
Per capita household income			0.365			0.000
1K below	0	2		1	1	
1K–3K	1	3		0	4	
3K–5K	6	41		3	44	
5K–8K	21	72		6	87	
8K–10K	28	69		6	91	
More than 10K	35	99		5	129	
Delivery mode			0.481			0.144
Eutocia	45	172		8	209	
Forceps Delivery	3	10		1	12	
Elective cesarean section	22	64		4	82	
Emergency cesarean section	21	47		8	60	
Neonatal weight	3257	3178	0.283	4126	3142	0.000
Neonatal score			0.311			1.000
10 points	88	272		20	340	
< 10 points	3	19		1	21	
Postpartum blood loss	352	358	0.781	387	352	0.353

the incidence of pregnancy with diabetes, while animal oil can reduce the incidence of macrosomia. We also found that consumption of vegetables was positively correlated with the levels of blood sugar at one hour after meal, indicating that too much consumption of vegetables may increase. Excessive consumption of eggs (more than 1 per day) significantly increased blood lipid levels, and hyperlipidemia caused a series of complications, such as acute fatty liver and coagulation disorders [11].

Intestinal microecology, which can be affected by many factors (e.g., age, heredity, dietary structure, and body mass index) play a key role in diabetes during pregnancy [12]. Pedersen et al. have shown that the intestinal flora of pregnant women with gestational diabetes mellitus is abnormal at many levels, including phylum and genus, compared with pregnant women with normal blood sugar [12]. Zheng et al. [13] evaluated the dynamic changes of intestinal microbiota in 141 pregnant women from the first three months to the middle three months, and they found significant differences in intestinal microecology of pregnant women with diabetes and normal control pregnant women as gestational weeks progressed, as evidenced by a continuous downward trend fecal coccus and streptococcus in pregnant women.

The potential mechanisms by which intestinal microecology affects metabolism are as follows: (1) changes in intestinal flora lead to changes in the levels of hormones, such as insulin, gastric inhibitory peptides and adipokines, results in metabolic disorders [14]; (2) disruption of homeostasis between intestinal microbes and the immune system leads to intestinal bacterial endotoxin entering the systemic circulation, causing "metabolic endotoxemia", systemic inflammatory response, and insulin resistance [14]; (3) changes

Table 2. Pregnancy diet for pregnant women with diabetes mellitus and fetal macrosomia

	Gestational diabetes mellitus		p value	Fetal macrosomia		p value
	Yes	No		Yes	No	
Rice (twice/week)	13.33 ± 7.75	13.18 ± 9.52	0.894	9.60 ± 6.19	13.42 ± 9.34	0.068
Flour (twice/week)	5.96 ± 5.64	6.06 ± 5.64	0.880	6.62 ± 5.42	6.00 ± 5.65	0.626
Crops (twice/week)	2.49 ± 3.26	3.19 ± 4.28	0.149	4.04 ± 4.81	2.84 ± 3.59	0.141
Potato (twice/week)	2.62 ± 2.89	3.24 ± 3.94	0.163	2.07 ± 1.82	3.15 ± 3.80	0.199
Fried dough foods (twice/week)	3.06 ± 6.47	2.84 ± 8.45	0.814	1.86 ± 2.39	2.95 ± 8.23	0.544
Pork (twice/week)	5.19 ± 4.82	6.15 ± 4.95	0.099	3.43 ± 3.21	6.07 ± 4.98	0.017
Beef and mutton (twice/week)	8.21±13.355	6.29±9.70	0.13	6.62 ± 6.09	6.75 ± 10.89	0.957
Poultry (twice/week)	7.97 ± 9.57	8.75 ± 11.0	0.543	7.76 ± 5.00	8.61 ± 10.91	0.724
Viscera (twice/week)	1.17 ± 1.75	1.70 ± 4.67	0.278	1.71 ± 2.26	1.57 ± 4.26	0.878
Fatty fish(twice/week)	6.38 ± 10.58	4.10 ± 7.93	0.026	3.14 ± 3.95	4.72 ± 8.85	0.418
Other fish (twice/week)	6.87 ± 17.61	4.11 ± 7.93	0.034	2.90 ± 4.55	4.86 ± 11.28	0.430
Alga (twice/week)	2.17 ± 2.95	2.41 ± 3.86	0.575	1.40 ± 1.54	2.41 ± 3.74	0.222
Other aquatic product (twice/week)	8.22 ± 16.60	7.42 ± 13.68	0.639	6.09 ± 6.68	7.69 ± 14.72	0.622
Milk (twice/week)	84.98 ± 65.10	72.51 ± 73.02	0.141	92.76 ± 68.04	74.47 ± 71.50	0.254
Milk powder (twice/week)	9.68 ± 25.16	15.86 ± 33.24	0.099	20.38 ± 45.41	14.07 ± 30.70	0.374
yogurt (twice/week)	23.20 ± 26.30	28.27 ± 31.39	0.159	16.76 ± 20.59	27.66 ± 30.69	0.109
Egg (twice/week)	26.11 ± 18.04	22.08 ± 15.83	0.039	31.71 ± 29.98	22.54 ± 15.26	0.013
Tofu (twice/week)	14.25 ± 14.35	13.30 ± 12.86	0.547	7.43 ± 5.66	13.86 ± 13.43	0.030
Soybean milk (twice/week)	28.27 ± 36.16	31.57 ± 40.76	0.484	28.81 ± 34.62	30.91 ± 40.01	0.814
Dried bean (twice/week)	2.68 ± 7.20	3.06 ± 7.75	0.679	2.00 ± 4.97	3.02 ± 7.74	0.550
Vegetables (twice/week)	125.8 ± 75.68	115.7 ± 88.31	0.377	98.86 ± 104.1	118.9 ± 84.39	0.295
Pickles (twice/week)	4.37 ± 14.08	3.57 ± 9.94	0.537	3.00 ± 6.75	3.89 ± 11.23	0.747
Cake (twice/week)	6.54 ± 9.58	8.92 ± 17.44	0.208	3.76 ± 4.96	8.62 ± 16.33	0.175
Fruit (twice/week)	161.0 ± 113.4	162.9 ± 124.6	0.899	136.2 ± 107.5	163.9 ± 122.7	0.312
Nut (twice/week)	7.58 ± 16.07	5.71 ± 10.04	0.179	7.56 ± 14.78	6.07 ± 11.57	0.672
Fruit juice (twice/week)	2.09 ± 3.90	3.42 ± 7.45	0.100	2.43±6.64	3.14 ± 6.82	0.639
Other drinks (twice/week)	1.09 ± 2.39	2.24 ± 12.52	0.377	0.62 ± 2.62	2.04 ± 11.31	0.565
Peanut oil	55/93	163/302	0.406	8/21	210/374	0.119
Soya-bean oil	37/93	164/302	0.018	11/21	190/374	1.000
Colza oil	50/93	187/302	0.183	13/21	224/374	1.000
Salad oil	26/93	101/302	0.375	5/21	122/374	0.479
Sesame oil	49/93	184/302	0.185	12/21	221/374	1.000
Animal oil	18/93	62/301	0.883	1/21	79/373	0.050
Quantity of oil						
1-more	7/87	17/259	0.336	0	24	0.650
2-middle	57/87	180/259		14	223	
3-less	22/87	62/259		4	80	
Quantity of salt						
1-more	2/90	15/284	0.203	1	16	0.934
2-middle	52/90	159/284		10	201	
3-less	35/90	110/284		9	136	
Quantity of sugar						
1-more	8/89	17/287	0.725	2	23	0.775
2-middle	31/89	110/287		6	135	
3-less	45/89	141/287		10	176	

Table 3. The effect of some foods on blood lipid and glucose levels.

	Low density lipoprotein	Cholesterol	Triglyceride	Blood glucose (0 min)	Blood glucose (postprandial 1 h)	Blood glucose (postprandial 2 h)
Potato	/	/	/	/	/	–
Fried dough foods	+	+	/	/	/	/
Cake	/	/	/	–	/	/
Fatty fish	+	/	/	/	/	/
Pork	/	/	/	/	/	–
Tofu	/	/	–	/	/	/
Eggs	/	/	+	/	+	/
Vegetables	/	/	/	/	+	/
Milk	/	/	/	/	+	/
Soybean milk	/	+	/	/	/	/
Yogurt	/	/	/	–	/	/
Fruit juice	/	/	/	–	/	–
Other drinks	+	+	/	/	/	/

This table is measured by chi-square test, "+" represents a positive correlation and a statistically significant difference; "–" represents negative correlation and statistically significant difference; "/" means no statistical difference

in short-chain fatty acids caused by intestinal dysbacteriosis, followed by a series of signal transduction pathways, lead to low-grade intestinal inflammatory response, dyskinesia, and increased intestinal mucosal permeability, which ultimately affect maternal and fetal energy metabolism [15].

Some social factors also affect the occurrence of pregnancy with diabetes and macrosomia. Low income and low education level are risk factors for macrosomia. It has been long believed that nutrition should be strengthened during pregnancy, which reduces the intrauterine growth restriction caused by malnutrition [15]. However, many pregnant women overeat during pregnancy and gain significant weight, resulting in obesity and diabetes [15]. The risk of macrosomia also increases. The results of our study showed that pregnant women with higher education level and better medical compliance had the concept of weight control. The weight increased reasonably during pregnancy, and the size of fetus was controlled within a reasonable range. In the low-educated and low-income population, due to the neglect of pregnancy care and conservative concept, a considerable proportion of pregnant women still do not pay attention to the control of calorie intake during pregnancy, resulting in obesity, diabetes, macrosomia and other complications. Diabetes is no longer a "rich disease". It is pay attention to pregnant women with low income and low education level.

CONCLUSIONS

The occurrence of gestational diabetes mellitus and macrosomia was related to the diet structure of pregnant women. It is recommended to eat more potato and high-quality proteins, such as vegetable protein and lean red meat. Polyfat fish are rich in protein and easy to digest but have a high fat content and a small amount of consumption. vegetables should be consumed moderately, and excessive consumption may increase blood sugar levels. Juice and yogurt, but not other beverages, are recommended. Soybean oil and animal oil are better choices. The intake of eggs cannot be more than one per day. There are also important social factors in the occurrence of gestational diabetes mellitus and macrosomia. Obesity, old age, low income, and low education level are risk factors.

Ethics approval and consent to participate

The ethic approval was obtained from the Ethic Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Consent to publish

All the authors have Consented to publish this research.

Availability of data and materials

The data are free access to available upon request.

Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

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Conflict of interests

All authors declare no conflict of interest.

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Prenatal diagnosis and molecular cytogenetic characterization of hereditary complex chromosomal rearrangements in a Chinese family

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ABSTRACT

Objectives: To report a family with an extremely rare and previously undescribed complex chromosomal rearrangement (CCR). To explore the molecular cytogenetic mechanism of 'octaradial chromosome'.

Material and methods: G-banding karyotype analysis was performed on all the members of the family. Chromosomal microarray analysis(CMA) was performed on the five members of the family.

Results: This case presented with a karyotypically balanced CCR (46,XX,t(2;4;11;5)(p21;q34;q21;p15)). The familial CCR was stably transmitted across three generations.

Conclusions: We report an extremely rare and previously undescribed complex chromosomal arrangement that is transmitted across three generations. The clinical outcome of this CCR is complex. Careful characterization of all the breakpoint regions is required for prenatal diagnosis and genetic counseling.

Key words: Chromosome karyotype; chromosomal microarray analysis (CMA); complex chromosomal rearrangements; octaradial chromosome; prenatal diagnosis

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INTRODUCTION

Complex chromosomal rearrangements (CCRs) are rearrangements involving more than two chromosomes or having more than two breakpoints [1]. The occurrence of constitutional CCRs is rare with approximately 250 cases reported so far [2, 3]. The CCRs detection rate increases with the application of molecular cytogenetic technology. Karyotypically detected complex rearrangements are further analyzed using methods such as chromosomal microarray analysis (CMA), fluorescence *in situ* hybridization (FISH) and whole-genome sequencing (WGS) [4]. Here we report an extremely rare and previously undescribed CCR that stably is transmitted across three generations.

Objectives

To report a family with an extremely rare and previously undescribed complex chromosomal rearrangement (CCR). To explore the molecular cytogenetic mechanism of 'octaradial chromosome'.

MATERIAL AND METHODS

In 2011, a 24-year-old primigravid woman underwent amniocentesis at 20 weeks of gestation because her adjusted Down syndrome risk was 1/50.

G-banding karyotype analysis was performed on all the members of the family.

CMA has been introduced in clinical diagnosis to rapidly detect genome-wide gains and losses with higher resolution. CMA was performed on the five members of the family.

RESULTS

Conventional karyotyping using cultured amniocytes revealed a karyotype of 46,XY,t(2;4;11;5)(p21;q34;q21;p15) (Fig. 1). Conventional karyotyping using peripheral blood

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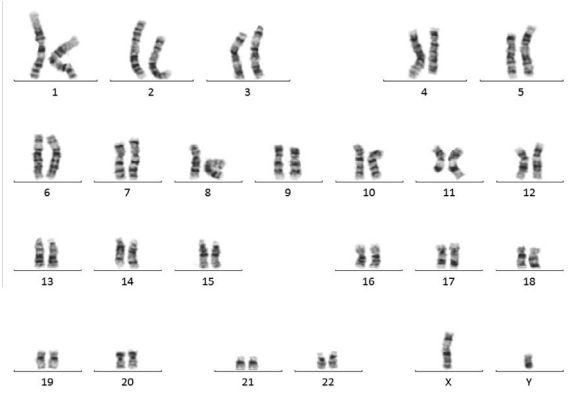

Figure 1. Karyotype of III-1, 46,XY,t(2;4;11;5)(p21;q34;q21;p15)mat

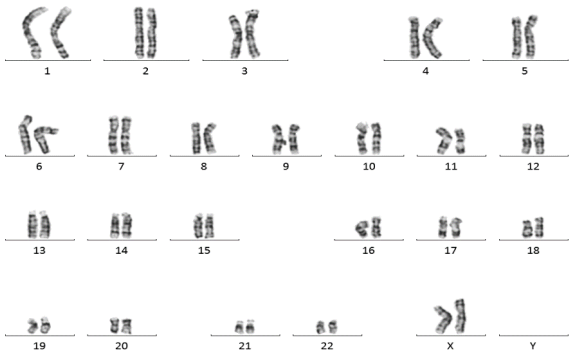

Figure 3. Karyotype of I-2, 46,XX,t(2;4;11;5)(p21;q34;q21;p15)

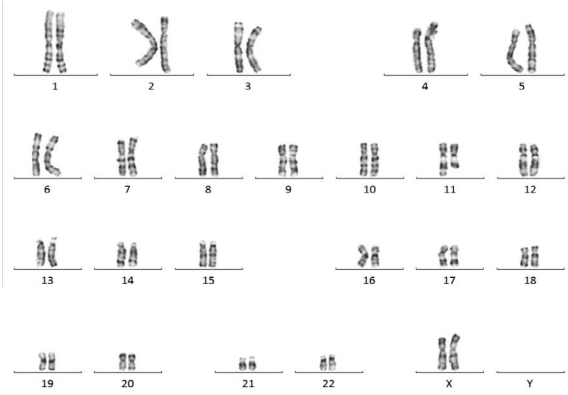

Figure 2. Karyotype of II-2, 46,XX,t(2;4;11;5)(p21;q34;q21;p15)mat

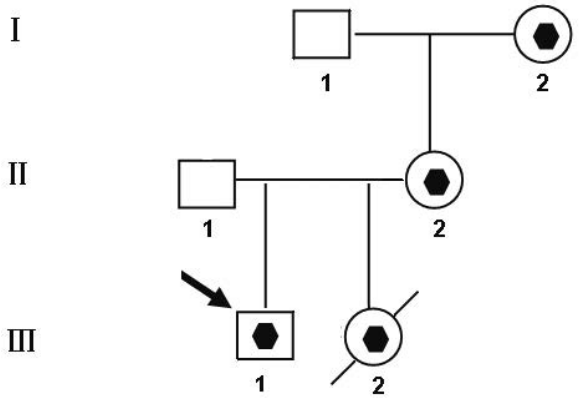

Figure 4. Pedigree of the family. The proband, III-1 (arrow), karyotype was 46,XY,t(2;4;11;5)(p21;q34;q21;p15)mat. III-2, karyotype was 46,XX,der(2;4;5)t(2;4;11;5)(p21;q34;q21;p15)mat. II-2, karyotype was 46,XX,t(2;4;11;5)(p21;q34;q21;p15)mat. I-2, karyotype was 46,XX,t(2;4;11;5)(p21;q34;q21;p15.

revealed the same chromosome rearrangement in the mother and the maternal grandmother (Fig. 2, 3) and a normal karyotype in the father and the maternal grandfather. The pedigree of the family showed that this chromosome rearrangement was stably transmitted across three generations (Fig. 4).

CMA analysis on uncultured amniocytes detected no pathogenic variants. Parental CMA didn't detect any pathogenic variants. Ultrasound examination showed no dysmorphism and intrauterine growth restriction (IUGR) [5] in the fetus. Because both the mother and the grandmother carry the same translocation and have a normal phenotype, we conclude this is a balanced translocation. After genetic counseling, the couple decided to continue the pregnancy. At 38 weeks of gestation, a 3100 g physically normal male baby was delivered naturally. The infant was phenotypically normal at birth. He had normal growth and psychomotor development by age 9.

In 2020, this woman was pregnant again and underwent amniocentesis at 20 weeks of gestation. Conventional karyotyping revealed a karyotype of 46,XX,der(2;4;5)t(2;4;11;5)(p21;q34;q21;p15)mat (Fig. 5). CMA analysis on uncultured amniocytes detected two pathogenic variants: arr[hg19] 4q34.2q35.2(176,442,864–190,957,460)x1 and arr[hg19] 11q21q25(92,897,617–134,937,416) x3 (Fig. 6, 7). Ultrasound examination showed cleft lip and palate in the fetus. After genetic counseling, the couple decided to terminate the pregnancy.

DISCUSSION

About one-third of all CCRs are familial. Familial CCRs tend to have fewer breakpoints and are mainly maternally transmitted via oogenesis, as in the case reported by Binsbergen et al. [1]. Here we present an extremely rare and previously undescribed CCR involving chromosomes 2, 4, 5 and 11. Surprisingly, the CCR was stably transmitted across three generations. The woman inherited this CCR

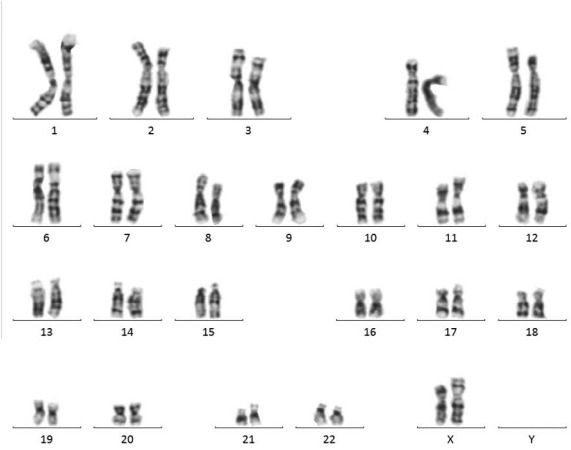

Figure 5. Karyotype of III-2, 46,XX,der(2;4;5)t(2;4;11;5)(p21;q34;q21;p15)mat

from her healthy mother and transmitted it to her son, who is phenotypically normal suggesting this is a balanced CCR. However, when the woman was pregnant with her second child, the fetus carries two pathogenic variants and presents with cleft lip and palate (Fig. 6, 7). Our case provides further evidence that balanced CCR carriers can produce abnormal offspring. Therefore, once a CCR is identified, it is necessary to narrow down all the breakpoint regions to determine whether any copy number variants (CNVs) are associated with it. The mechanisms underlying the formation of CCRs remain unknown. There are several events that could lead to complex rearrangements, including replication-based mechanisms and chromothripsis [4]. In our case, the second baby of the CCR carrier has two CNVs, both are close to the breaking region (Fig. 6, 7). Further characterization of these breakpoint junctions in our patient will help understand

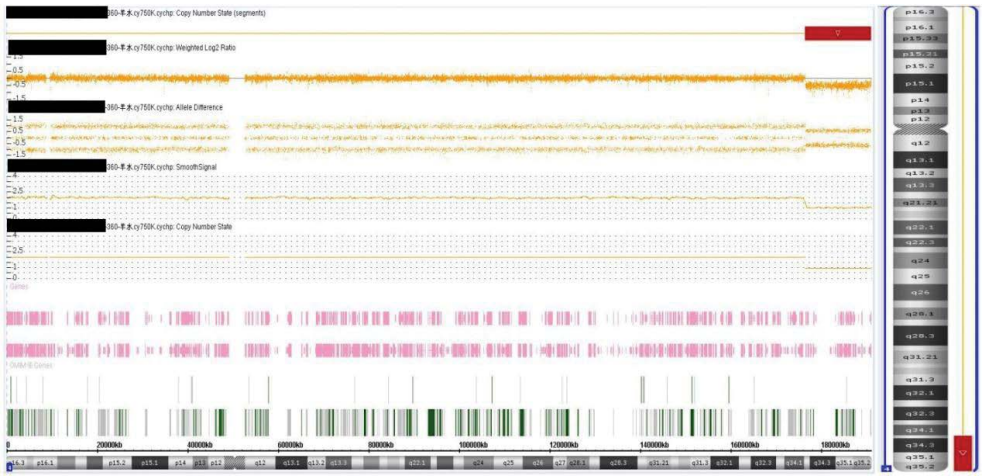

Figure 6. CMA result. 4q34.2q35.2(176,442,864-190,957,460)x1

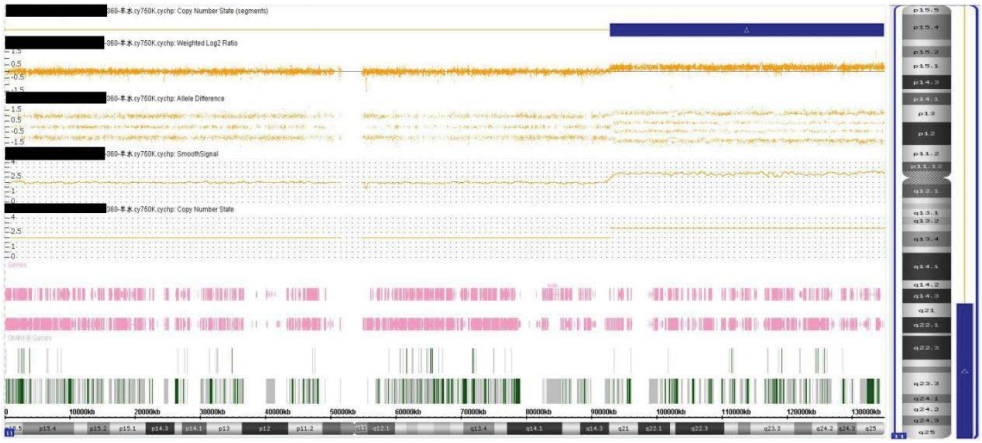

Figure 7. CMA result. 11q21q25(92,897,617-134,937,416)x3

the molecular mechanisms responsible for the process of this complex CCR.

The outcomes of the CCR carriers are more complex than previously appreciated [6]. Even balanced chromosomal translocations may cause damage or alteration of the functional genes at the breakpoints of the defective chromosomes resulting in the disease phenotype [7]. On the other hand, the possibility of producing normal gametes is theoretically low in CCR carriers. For example, carriers of a balanced chromosomal reciprocal translocation could form a quadriradial chromosome during the pachytene stage of meiosis I. According to the classical gamete formation theory, they can form 18 kinds of gametes and only one is the same balanced chromosomal reciprocal translocation. In our case, the CCR carriers (I-2, II-2 and III-1) could form a 'octaradial chromosome' (Fig. 8) during the pachytene stage of meiosis I, which will result in $18^4 = 104976$ kinds of gametes. Therefore, there are only one out of 104976 chance that the offspring will carry the same balanced CCR, suggesting there are must be some unknown mechanisms underlying gamete formation.

CONCLUSIONS

We report an extremely rare and previously undescribed complex chromosomal arrangement that is transmitted across three generations. The clinical outcome of this CCR is complex. Careful characterization of all the breakpoint regions is required for prenatal diagnosis and genetic counseling.

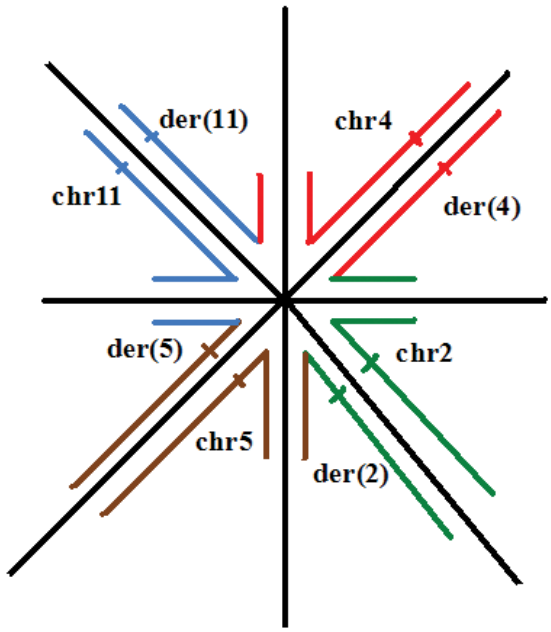

Figure 8. The octaradial chromosome of CCRs (I-2, II-2 and III-1)

Ethics approval and consent to participate

The research was approved by the Ethics Committee of Renmin Hospital of Wuhan University. All patient guardians gave informed consent to the study.

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Consent for publication

All patient guardians gave informed consent to the publication of this study.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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Prediction of preterm birth using PAMG-1 test: a single centre experience — preliminary report

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ABSTRACT

Objectives: Placental alpha microglobulin-1 (PAMG-1) is a novel biomarker detected in cervicovaginal discharge in patients threatened with preterm birth (PTB). This study aimed to show a single centre experience of assessment of imminent spontaneous PTB risk in patients with symptoms suggesting preterm labour (PTL).

Material and methods: The study group consisted of 46 women with singleton pregnancies between 24 + 0/7 and 33 + 6/7 weeks of gestation who presented with symptoms of threatened PTL, with cervical dilatation of < 3 cm, cervical length (CL) of < 30 mm and clinically intact fetal membranes. CL was measured via transvaginal ultrasound and the PAMG-1 test was performed in all of the objectives.

Results: Sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of prediction of PTB within seven days for CL were 100%, 11.11%, 5.88% and 100%, respectively. The PAMG-1 test SN, SP, PPV and NPV of the same endpoint were 50%, 80.56%, 12.5% and 96.67%, respectively.

Conclusions: PAMG-1 is a more accurate predictor of PTB when compared to CL. Routine use of both mentioned tests could allow identification of low-risk patients and reduction of rate of unnecessary hospitalizations and treatments.

Key words: preterm birth, preterm labou; placental alpha microglobulin-1 (PAMG-1); PartoSure; cervical length (CL)

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INTRODUCTION

Preterm birth (PTB) is a delivery between 22 + 0/7 and 36 + 6/7 weeks of gestation and is one of the most frequent complications occurring during pregnancy. Preterm labour (PTL) is manifested via regular uterine contractions leading to the cervical shortening or effacement. It affects approximately 5–18% of pregnancies worldwide [1] and is a leading reason of neonatal mortality and morbidity [2, 3]. Predisposing factors of PTB are: multiple pregnancy, uterus anomaly, previous PTB, cervical insufficiency, ethnicity, low socio-economic status, maternal weight, smoking, periodontal status [4]. Accurate prediction of PTB remains a challenge, whereas more than 2/3 of symptomatic patients do not go on to deliver within seven days from the onset of symptoms [5, 6]. It leads to partly unnecessary admissions to hospital and administrations of tocolytics and antenatal corticosteroids [7], which are not devoid of its adverse consequences [8, 9].

The most widely used but also not the most accurate tool to assess likelihood of PTB is cervical length (CL) measurement via transvaginal ultrasound examination. There are several novel biomarker tests commercially available, based on fetal fibronectin (fFN), phosphorylated insulin-like growth factor-binding protein-1 (phIGFBP-1) and placental alpha microglobulin-1 (PAMG-1) concentrations in cervicovaginal discharge. The most promising test out of the mentioned three seems to be PAMG-1, with reported relatively highest values of sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) [10–15].

Objectives

This study aimed to show a single centre experience of the Clinical Department of Perinatology, Gynaecology and Obstetrics in Ruda Śląska, Poland of using PAMG-1

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(the PartoSure Test) in assessment of imminent spontaneous PTB risk in patients with symptoms suggesting preterm labour.

MATERIAL AND METHODS

The prospective study was performed in the period between June 2018 and July 2019 at the Clinical Department of Perinatology, Gynaecology and Obstetrics in Ruda Śląska, Medical University of Silesia, Poland. Forty-six patients, who presented with uterine contractions, were recruited into the study. Inclusion criteria were age of 18 years and above, gestational age between 24 + 0/7 and 33 + 6/7 weeks, CL of < 30 mm in transvaginal ultrasound examination, cervical dilatation of < 3 cm, clinically intact fetal membranes and absence of vaginal bleeding at presentation. As exclusion criteria were used: previous tocolytic therapy, intercourse within the past 24 hours before examination, cervical cerclage or pessary, placenta praevia, multiple pregnancy and patient's disapproval of participation in the study.

A patient who presented to the Department with symptoms of threatened PTL was firstly screened for exclusion criteria, asked for informed consent and then recruited to the study group. A sample for the PAMG-1 test was collected accordingly to the manufacturer's instructions. Then, clinical examination and transvaginal ultrasound with CL measurement were performed as well as the patient's medical history was collected. The objectives of this study were admitted for a minimum 7-day clinical observation and possible inpatient treatment. A test-to-spontaneous-delivery interval was calculated for all patients.

RESULTS

There were 46 patients included into the study group initially. Seven were excluded from the final analysis due to lack of follow-up data on the delivery and one due to equivocal PAMG-1 test result, thus 38 objectives represented the study group. The mean age was 30.08 ± 5.37 years, the mean gestational age at presentation was 28.61 ± 2.73 weeks. A total of 15.79% (4) of the patients had a history of PTB, 28.95% (10) of miscarriage (before 22+0/7 weeks of gestation), 34.21% (13) were taking vaginal progesterone treatment.

Table 1. Summary of CL measurements and deliveries within 7 and 14 days of presentation

	Delivery ≤ 7 days		Delivery ≤ 14 days	
CL < 25 mm	+	–	+	–
+	2 (5.26%)	32 (84.21%)	3 (7.89%)	31 (81.58%)
–	0	4 (10.53%)	0	4 (10.53%)

Table 2. Summary of the PAMG-1 test results and deliveries within 7 and 14 days of presentation

	Delivery ≤ 7 days		Delivery ≤ 14 days	
PAMG-1	+	–	+	–
+	1 (2.63%)	7 (18.42%)	1 (2.63%)	7 (18.42%)
–	1 (2.63%)	29 (76.32%)	2 (5.26%)	28 (73.68%)

In total 5.26% (2) of the patients delivered within seven days of presentation and 7.89% (3) within 14 days. The mean value of CL was 16.39 ± 6.44 mm. 92.11% (34) of the objectives were evaluated as those with a short cervix (CL < 25 mm), while only two delivered within 7 days and three delivered within 14 days of presentation (Tab. 1). In total, 21.05% (8) of the study group had a positive result of the PAMG-1 test, out of which one delivered within 7 days of presentation (Tab. 2).

Prediction of delivery within 7 days of testing using CL measurement had sensitivity (SN) of 100%, specificity (SP) of 11.11%, positive predictive value (PPV) of 5.88% and negative predictive value (NPV) of 100%. SN, SP, PPV and NPV of CL measurement for prediction of delivery within 14 days of testing were 100%, 11.43%, 8.82% and 100%, respectively. The PAMG-1 test had SN of 50%, SP of 80.56%, PPV of 12.5% and NPV of 96.67% of prediction of delivery within 7 days of testing. Its SN, SP, PPV and NPV of prediction of delivery within 14 days of testing were 33.33%, 80%, 12.5% and 93.33%, respectively (Tab. 3).

A test-to-spontaneous-delivery interval ranged from 0 to 109 days, with a mean value of 58.79 ± 28.32 days.

Table 3. Performance of CL measurement and the PAMG-1 test of prediction of spontaneous preterm delivery within 7 and 14 days

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Delivery ≤ 7 days				
CL < 25 mm (95% CI)	100% (15.81–100%)	11.11% (3.11–26.06%)	5.88% (5.28–6.56%)	100%
PAMG-1 (95% CI)	50% (1.26–98.74%)	80.56% (63.98–91.81%)	12.5% (2.98–39.92%)	96.67% (87.78–99.15%)
Delivery ≤ 14 days				
CL < 25 mm (95% CI)	100% (29.24–100%)	11.43% (3.2–26.74%)	8.82% (7.91–9.83%)	100%
PAMG-1 (95% CI)	33.33% (0.84–90.57%)	80% (63.06–91.56%)	12.5% (2,47–44.68%)	93.33% (86.08–96.94%)

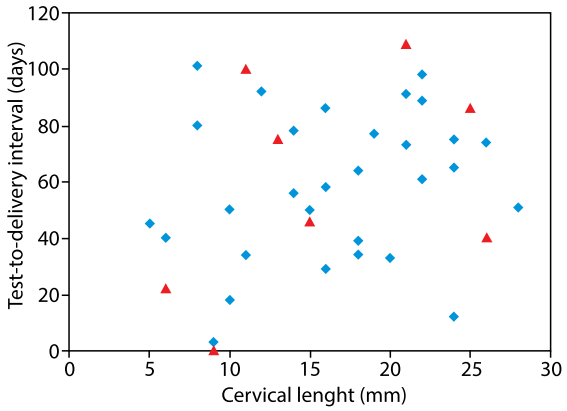

Figure 1. Cervical length (mm) and test-to-spontaneous-delivery interval in the study group. ▲ — positive result of PAMG-1 test; ◆ — negative result of PAMG-1 test

Additionally, there was found no statistically significant correlation between CL and the test-to-spontaneous-delivery interval (p = 0,1253) (Fig. 1).

DISCUSSION

In the study group only 5.26% of patients presenting with symptoms suggesting PTL went on to deliver within 7 days. According to the literature, rate of PTB within 7 days of presentation in symptomatic patients ranges from 1.8 to 29.7% [5, 6]. It shows that screening for patients truly endangered with PTB is still very poor and leads to partially unnecessary admissions to hospital and treatments [16]. Empana et al. [7], revealed that 85% of European centres administer multiple courses of antenatal corticosteroids. Although it is proven to be a treatment that promotes neonatal lung maturation, uncontrolled use of corticosteroids may lead to several neonatal adverse consequences, such as respiratory distress syndrome [8] or delay in myelination in the central nervous system [9].

Assessment of likelihood of PTB within 7 days with CL measurement via transvaginal ultrasound in the study group is characterized by high SN and NPV but also very low SP and PPV. Performance of CL measurement in the literature is also unsatisfactory, with SN, SP, PPV, and NPV values of 57%, 73%, 89% and 30%, respectively [11] and proves CL to be an inaccurate tool in prediction of imminent PTB.

As expected, the PAMG-1 test in the study group obtained a much higher value of SP and similar value of NPV of PTB prediction within 7 days when compared to CL. SN and PPV values of the PAMG-1 test in the study group were not consistent with the literature [17], most likely due to a small size of the group. Nevertheless, obtained data show that PAMG-1 is a superior predictor of imminent PTB than CL.

Since the prediction of PTB in symptomatic patients is still a challenge for clinicians, it needs to provide focus on more efficient screening for patients truly threatened with PTL. Combination of routine CL measurement and the PAMG-1 test could allow identification of patients at low risk of imminent PTL and reduction of rate of unnecessary hospitalizations and antenatal corticosteroids administrations. It would be beneficial not only for patients but also for the healthcare system budget.

CONCLUSIONS

Our centre experience of predicting imminent spontaneous PTB using the PAMG-1 test is consistent with currently available literature. It shows that the PAMG-1 test is more accurate in assessment of likelihood of PTB than CL measurement. Therefore, routine use of this test should be recommended in order to identify low-risk patients.

Conflict of interest

All authors declare no conflict of interest.

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Intrahepatic cholestasis of pregnancy — prevalence and ethnic distribution in northern Israel

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ABSTRACT

Objectives: Intrahepatic cholestasis of pregnancy (ICP) is charachterized by pruritis and elevated serum bile acids (BA) and is associated with adverse obstetrical outcomes. ICP etiology is poorly understood and its incidence varies with ethnicity and geographical distribution.

Explore the prevalence and characteristics of ICP in the different Northern Israeli ethnic groups and compare maternal and perinatal outcomes according to disease severity.

Material and methods: Single-center retrospective study. Women who were diagnosed with ICP based on clinical presentation and elevated fasting BA (≥ 10 µmol/L) were included. Disease incidence, maternal and neonatal complications were explored according to ethnic subgroups analysis and obstetrical complications were examined according to disease severity.

Results: The incidence of ICP in the study population was 0.58%. Higher ICP incidence was found in our cohort compared with other reports arising from Central Israel ($p < 0.001$). The Christian patients had a higher incidence of ICP (1.1%) and preeclampsia (23.1%). A higher rate of neonatal intensive care unit (NICU) admissions was found in the Arab Muslim and Christian groups compared with the Jewish and Druze groups ($p = 0.007$).

A higher rate of preeclampsia was found in the severe (BA ≥ 40 µmol/L) ICP group ($p < 0.001$). Patients in the severe ICP group had earlier gestational age at delivery (37 versus 38.14 weeks, $p < 0.001$). Birth weight was significantly lower in the severe ICP group ($p = 0.018$).

Conclusions: The incidence of ICP at our institution was 0.58%, which is higher compared with previous reported Israeli incidence. Higher ICP and preeclampsia incidence were found among Arab Christian patients.

Keywords: intrapartum cholestasis of pregnancy; ethnicity; retrospective cohort review; maternal outcome; perinatal outcome

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a cholestatic disorder characterized by pruritus with increased serum bile acids (BA) and elevated serum aminotransferases. ICP manifests in the second half of pregnancy and rapidly resolves after delivery. It is associated with increased rates of fetal morbidity and mortality. It increases the risk of preterm delivery, meconium-stained amniotic fluid and fetal distress [1, 2]. Higher total serum bile acid levels are associated with higher rates of fetal complications, and severe cholestasis is defined as bile acids over 40 µmol/L [3].

There is significant geographical and ethnical variation in the incidence of ICP which suggest a genetic predisposition. For instance, in certain areas of Chile, rates have been reported up to 22% [4], while the rates from France and the United Kingdom are less than 1% [5]. Recent studies emerging from Central Israel have reported an incidence of 0.1–0.36% for ICP, but ethnical charataristics have not been studied before [6, 7].

Compared with Central Israel, Northern Israel consists of a widely diverse population which includes several ethnic groups with unique genetic makeup. This study was

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conducted in the Galilee Medical Center in Nahariya, located in Northern Israel, which serves a population of over 600,000 comprising of Jews, as well as Christian and Muslim Arabs and Druze. Our study sought to investigate the prevalence and characteristics of ICP in the different Northern ethnic populations and to compare its incidence with Central Israel. Additionally, we aimed to compare maternal and perinatal outcomes between mild and severe disease.

MATERIAL AND METHODS

Study design

We conducted a historical cohort study of women diagnosed with ICP.

Study population

Women hospitalized between April 2013 and December 2017 in the Galilee Medical Center (Nahariya, Northern Israel) with a diagnosis of ICP were included in the study group.

We excluded women without a definitive diagnosis of ICP or missing information on pregnancy outcome.

Data collection

Demographic, clinical, obstetrical and laboratory data were collected from computerized medical records and the hospital's laboratory database.

Maternal data included: age, gravidity, parity, mode of conception, multiple pregnancy, ethnicity (Jews, Arab Christian, Arab Muslim, Druze), gestational age at diagnosis and at delivery, medications, comorbidities including pre-gestational diabetes mellitus, chronic hypertension, thrombophilia and any other renal, liver or cardiac disease. Data concerning current pregnancy complications included: rates of preeclampsia (PET), oligohydramnios, polyhydramnios, premature uterine contractions and gestational diabetes. Laboratory parameters included serum levels of alanine aminotransferase (ALT) and fasting total BA.

Data concerning the course of labor was collected: onset of labor (spontaneous/iatrogenic), gestational age at delivery, mode of delivery, rate of preterm delivery (spontaneous or iatrogenic), postpartum hemorrhage (PPH), meconium-stained amniotic fluid, non-reassuring fetal monitoring.

Neonatal data included: gender, birthweight and birthweight percentile — which was calculated according to Dolberg fetal growth calculator [8], Apgar score at 1 and 5 min, arterial umbilical cord pH, neonatal intensive care (NICU) admission, respiratory complications (respiratory distress syndrome, transient tachypnea of newborn, ventilation, respiratory support), hypoglycemia, hyperbilirubinemia, anemia and perinatal mortality. In addition, pediatric emergency department (ED) visits during the neonatal period were examined

Definitions

ICP was defined as a combination of pruritus and an increased fasting serum BA ≥ 10 μmol/L, while other causes of itching and liver dysfunction were excluded. Severe ICP was defined by fasting serum BA ≥ 40 μmol/L.

PET was diagnosed by the combination of new-onset hypertension and proteinuria after 20 weeks of gestation. Elevated blood pressure was defined as systolic blood pressure of 140 mmHg or more or diastolic blood pressure of 90 mm Hg or more on two occasions at least four hours apart and proteinuria as 300 mg protein or more per 24-hour urine collection.

Postpartum hemorrhage was defined as estimated blood loss of ≥ 500 mL in the 24 h after vaginal delivery or ≥ 1000 ml after cesarean delivery.

Spontaneous delivery versus iatrogenic: according to our local management protocol of ICP and in line with accepted guidelines, iatrogenic induction of labor was performed at 37 weeks of gestation if spontaneous delivery has not occurred and ICP was diagnosed before term.

Preterm delivery was defined as delivery < 37 gestational weeks (either spontaneous or iatrogenic).

Three types of cesarean deliveries (CD) were defined: elective CD was done for maternal or neonatal indications, without evidence for compromise of either one of them, (e.g., repeated cesarean sections) and was performed at its scheduled time. Non-elective CD was carried out for maternal or fetal indications (e.g., ruptured membranes and breech presentation) and was not performed immediately. Emergency CD was performed immediately in cases of fetal or maternal compromise.

Small for gestational age (SGA) newborn was defined as birthweight below the 10th percentile according to local growth curves (Dolberg fetal growth calculator) [8].

Ethics

Approval for this retrospective cohort study was given by the local Helsinki Committee prior to the initiation of data collection, reference no. 0042-18-NHR. All procedures were in accordance with the requirements of the Declaration of Helsinki.

Statistics

Statistical analysis was conducted by the statistical department at Galilee Medical Center using SPSS software (IBM SPSS Statistics version 25.0). Continuous variables are presented as the mean ± standard deviation or as median and range. Qualitative variables are presented as Frequencies and percentages. Comparisons of continuous variables between the groups were performed with an independent sample t-test or a Mann-Whitney test (according to the sample size of the groups and the variables'

distribution shape). Categorical variables were analyzed using a Pearson's chi-square test or Fisher's exact test (if expectancy < 5). The prevalence of ICP was compared with findings of previous Israeli studies using Binomial Test. A two tailed p value < 0.05 was considered statistically significant.

RESULTS

Out of a total of 25,379 deliveries during the study period, there were 148 diagnosed cases of ICP. Another 128 cases were excluded due to non-definitive diagnosis of ICP or missing information regarding pregnancy outcome.

Of the 148 ICP cases, there were 20 (13.5%) twin pregnancies. Mean maternal age was 29.5 and mean gestational age at diagnosis was 35.5 weeks.

The incidence of ICP in our study population was 0.58% (148 cases out of 25,379 deliveries).

We found a higher ICP incidence in our cohort compared with other reports arising from Central Israel (6) (0.58% compared with 0.36%, p < 0.001).

The clinical characteristics of our study population are presented in Table 1.

ICP incidence and outcomes according to ethnical groups

The incidence of ICP among Jewish and Druze patients was similar (0.69% and 0.62%, respectively). We found a lower ICP incidence among Muslim patients (0.4%). Compared with the other ethnic groups, the Christian patients had a higher incidence of ICP (1.1%, p = 0.005) (Tab. 2).

Maternal characteristics and outcomes of the different ethnical groups are shown in Table 3.

The mean maternal age at the time of diagnosis was similar in the Jewish, Christian and Muslim groups (30.37, 30.15 and 29.56 years, respectively). On the other hand, a lower maternal age of 26 years was found in the Druze group (p = 0.012). No difference was found in gravidity, parity, mode of conception or multiple pregnancy rates between the group. Mean fasting BA levels and ALT levels were comparable.

Table 1. Clinical characteristics of the general study population

ICP cases (n = 148)			
Pregnancy complications, n (%)	Gestational diabetes		21 (14.1)
	Polyhydramnios		9 (6.1)
	Oligohydramnios		3 (2)
	Preeclampsia		6 (4.1)
Gestational age at delivery, weeks, mean (± SD)			37.5 ± 2.1
Mode of delivery, n (%)	Vaginal delivery (n= 84)	Spontaneous	16 (10.8)
		Induced	66 (44.6)
		Instrumental	2 (1.4)
	Cesarean delivery (n = 64)	Elective	11 (7.4)
		None-elective	15 (10.1)
		Emergency	38 (25.7)
Birthweight, grams, mean (± SD)			2873.7 ± 630.8
Labor related complications, n (%)	Iatrogenic preterm delivery		27 (18.2)
	Spontaneous preterm delivery		9 (6.0)
	Non-reassuring fetal monitoring		20 (1)
	Meconium-stained amniotic fluid		15 (10.1)
	PPH		8 (5.4)
Newborn related complications, n (%)	5-min Apgar < 7		0 (0)
	Cord around the neck		8 (4.8)
	True knot of cord		2 (1.2)
	Respiratory morbidity		16 (10.1)
	SGA		2 (1.2)
	NICU admission		42 (26.6)
	Hypoglycemia		1 (0.6)
	Hyperbilirubinemia		99 (62.7)
	Anemia		12 (7.6)

ICP — intrahepatic cholestasis of pregnancy; PPH — postpartum hemorrhage; SGA — small for gestational age; NICU — neonatal intensive care unit

Table 2. ICP incidence according to ethnical groups

	Pregnancies diagnosed with ICP	Total no. of deliveries in the Galilee Medical Center	Incidence of ICP (%)
Jew, n (%)	62 (41.9)	8890 (35.0)	0.69
Arab Muslims, n (%)	41 (27.7)	10,095 (39.7)	0.4
Arab Christians, n (%)	26 (17.6)	4175 (16.4)	1.11
Druze, n (%)	13 (8.8)	1167 (4.5)	0.62
Missing data on ethnicity	6 (4.0)	1052 (4.1)	0.57
Total	148	25,379	0.58

Table 3. Maternal characteristics, obstetrical and neonatal outcomes of the different ethnical groups

	Jews (n = 62)	Arab Muslims (n = 41)	Druze (n = 26)	Arab Christians (n = 13)	p value
Maternal age, years, mean (± SD)	30.3 ± 5.6	29.5 ± 6.5	26 ± 4.2	30.1 ± 5.5	0.01
Bile acids, mean (range)	16.3 (10.2–149.2)	17.6 (10–160.5)	21.8 (10.1–114.1)	23.2 (11–184.8)	0.54
ALT, mean (range)	24 (6–619)	48 (6–339)	34.5 (6–200.4)	121 (6–330)	0.16
GA at diagnosis, weeks, mean (range)	37 (27.8–40.2)	36.4 (25.2–40.2)	35.2 (25–41.2)	33.8 (30.2–40.1)	0.18
Preeclampsia	3.2 (2)	0 (0)	3.8 (1)	3 (23.1)	0.013
GA at delivery, weeks, mean (± SD)	37.7 (28.2–41.2)	37.7 (29.4–40.5)	38.4 (32.1–41.4)	37 (32.5–40.4)	0.17
Mode of delivery					
Vaginal delivery, % (n)	50 (31)	63.4 (26)	69.2 (18)	53.8 (7)	0.68
Spontaneous	19.4 (6)	15.4 (4)	27.8 (5)	14.3 (1)	
Induced	80.7 (25)	76.9 (20)	72.2 (13)	85.7 (6)	
Instrumental	0 (0)	7.7 (2)	0 (0)	0 (0)	
Cesarean delivery, % (n)	50 (31)	36.6 (15)	30.7 (8)	46.1 (6)	0.97
Elective	19.4 (6)	20 (3)	12.5 (1)	0 (0)	
Non-elective	25.8 (8)	20 (3)	25 (2)	33.3 (2)	
Emergency	54.8 (17)	60 (9)	62.5 (5)	66.7 (4)	
Birthweight, grams, mean (±SD)	2974.9 ± 576.6	2805.2 ± 727.3	2864.1 ± 637.0	2554.2 ± 476.7	0.08
Labor-related complications, % (n)					
Iatrogenic preterm delivery	16.1 (10)	14.6 (6)	19.2 (5)	38.5 (5)	0.27
Spontaneous preterm delivery	32. (2)	7.3 (3)	11.5 (3)	7.7 (1)	0.37
PPH	6.5 (4)	7.3 (3)	0 (0)	15.4 (2)	0.54
Meconium-stained amniotic fluid	4.8 (3)	14.6 (6)	19.2 (5)	5.9 (1)	0.11
Non-reassuring fetal monitoring	13.4 (9)	15.2 (7)	10 (3)	5.9 (1)	0.82
Newborn related complications, % (n)					
Respiratory morbidity	9.5 (6)	11.6 (5)	10.7 (3)	11.8 (2)	0.96
SGA	0 (0)	2.1 (1)	3.3 (1)	0 (0)	0.42
NICU admission	17.5 (11)	39.5 (17)	14.3 (4)	47.1 (8)	0.007
5-min Apgar < 7	0 (0)	0 (0)	0 (0)	0 (0)	
Perinatal death, % (n)	0 (0)	0 (0)	0 (0)	0 (0)	

ALT — alanine aminotransferase; GA — gestational age; PPH — postpartum hemorrhage; SGA — small for gestational age; NICU — neonatal intensive care unit

No difference in mean maternal age at diagnosis or at delivery was observed between the groups.

Higher incidence of PET (23.1%) was found in the Christian group compared with the other groups (3.2% Jews, 0% Arab Muslims, 3.8% Druze) (p = 0.013). No difference was observed in rates of oligohydramnios, polyhydramnios or gestational diabetes between the groups.

Mode of delivery and fetal gender were similar between the groups. Fetal weight was lower in the Christian group (2554.2 grams) (p = 0.088), but no difference was found

in the SGA rates. No cases of 5-minutes Apgar less than 7 or perinatal death were found.

Higher rate of NICU admissions was found in the Arab Muslim and Christian groups (39.5% and 47.1%, respectively) compared with the Jewish and Druze groups (17.5% and 14.3%, respectively) (p = 0.007).

Preterm delivery (whether iatrogenic or spontaneous), PPH, meconium-stained amniotic fluid and non-reassuring fetal monitoring rates were not different between the four ethnic groups.

Obstetrical outcome according to the ICP severity

According to fasting BA levels (mild, fasting BA < 40 μmol/L and severe, BA ≥ 40 μmol/L) a subgroup analysis was performed. One hundred seven patients consisted the mild group, while 41 patients had severe ICP (Tab. 4).

Median ALT was significantly higher in the severe group compared with the mild group (79 versus 22 U/L, p < 0.001). A higher rate of PET was found in the severe group (14.6%

Table 4. Maternal characteristics, obstetrical and neonatal outcomes of the mild and severe ICP groups

	Mild ICP (n = 107)	Severe ICP (n = 41)	p value
Maternal age, years, mean (± SD)	29.4 ± 5.6	29.8 ± 6.3	0.69
Ethnicity, % (n)			
Jews	79 (49)	21 (13)	0.21
Arab Muslims	63.4 (26)	36.6 (15)	
Druze	73.1 (19)	26.9 (7)	
Arab Christians	61.5 (8)	38.5 (5)	
Bile acids, mean (range)	14.8 (10–36)	71.9 (41–184.8)	–
ALT, mean (range)	22 (6–313)	79 (6–619)	< 0.001
GA at diagnosis, weeks, mean (range)	36.8 (25–41.2)	35.8 (29.4–39.7)	0.09
Preeclampsia	0 (0)	14.6 (6)	< 0.001
GA at delivery, weeks, mean (± SD)	38.1 (28.2–41.4)	37 (32.1–40.1)	< 0.001
Mode of delivery			
Vaginal delivery, % (n)	55.1 (59)	60.9 (25)	0.01
Spontaneous	13.6 (8)	32 (8)	
Induced	84.8 (50)	64 (16)	
Instrumental	1.7 (1)	4 (1)	
Cesarean delivery, % (n)	44.8 (48)	39.0 (16)	0.71
Elective	18.8 (9)	12.5 (2)	
Non-elective	20.8 (10)	31.3 (5)	
Emergency	60.4 (29)	56.3 (9)	
Birthweight, grams, mean (± SD)	2945.2 ± 657	2689 ± 520.4	0.018
Labor-related complications, % (n)			
Iatrogenic preterm delivery	15.9 (17)	24.4 (10)	0.24
Spontaneous preterm delivery	3.7 (4)	12.2 (5)	0.11
PPH	4.7 (5)	7.3 (3)	0.68
Meconium-stained amniotic fluid	7.5 (8)	19.5 (8)	0.07
Non-reassuring fetal monitoring	11.7 (14)	12.8 (6)	1.0
Newborn related complications, % (n)			
Respiratory morbidity	12.5 (14)	4.3 (2)	0.15
SGA	0.8 (1)	2.1 (1)	0.48
NICU admission	24.1 (27)	32.6 (15)	0.32

ALT — alanine aminotransferase; GA — gestational age; PPH — postpartum hemorrhage; SGA — small for gestational age; NICU — neonatal intensive care unit

versus 0%, p < 0.001). No difference was observed in rates of oligohydramnios, polyhydramnios or gestational diabetes between the groups.

Although not statistically significant, the median age at diagnosis was earlier in the severe ICP group (35.85 versus 36.85 weeks, p = 0.095). In addition, earlier gestational age at delivery was found in the severe group compared with the mild group (37 versus 38.14 weeks, p < 0.001). Although the mode of delivery was comparable between the two groups, a higher rate of induced vaginal delivery was found in the mild group (84.8% versus 64%, p = 0.01). Higher rates of iatrogenic preterm deliveries and meconium-stained amniotic fluid were found in the severe ICP group.

Birth weight was significantly lower in the severe ICP group (2689.72 versus 2945.2 grams, p = 0.018).

No correlation was found between ICP severity and neonatal emergency department (ED) visits during the neonatal period.

DISCUSSION

We found a higher ICP incidence in our cohort compared with other reports arising from the center of Israel (0.58% compared with 0.36%, p < 0.001) [6]. Furthermore, among our ethnically heterogenous study population, a higher ICP incidence was found in the Arab Christian group (1.11%) and a lower incidence in the Arab Muslim group (0.4%) compared with the Jewish (0.69%) and Druze (0.62%) groups.

The exact pathogenesis of ICP is not known, but likely involves genetic, hormonal, immunological and environmental factors. Previous Israeli studies on ICP were conducted in Rabin Medical Center in Petach-Tikva [6] and Sourasky Medical Center in Tel Aviv [7], both located in Central Israel and ethnical differences were not explored. We believe that unlike our cohort (which consisted of over 50% non-Jewish patients), and according to the demographics of Central Israel, the vast majority of Raz and Mor cohorts [6, 7] consisted of Jews. Our findings could be explained by the diverse genetic backgrounds of the different ethnic groups, although other factors such lifestyle factors may play a role and cannot be ruled out.

Inter-group comparison reveals a significantly lower maternal age at ICP diagnosis in the Druze group (mean age of 26 years) compared with the other groups. The Druze maternal average age at birth according to the Israeli Central Bureau of statistics is 28.5–29.5 years during the years 2013–2018 [9]. This finding raises the questions of whether Druze patients might suffer from ICP at an earlier maternal age and whether patient age affect ICP incidence. Others have reported that ICP is more prevalent in older women [10].

We found a significantly higher PET rate in the Arab Christian group (23.11%) compared with others, and a trend toward a lower gestational age at diagnosis and a lower birthweight. Previous studies have reported that ICP is associated with an increased risk for PET, and earlier diagnosis of ICP was associated with higher incidence of PET [6]. Recent study found that early-onset ICP is associated with a lower birth weight than late-onset ICP [11].

In line with earlier research [12], we found a higher GDM prevalence in our study cohort (14.9%) compared with a recent Israeli report of 3.9% [13]. In animal modality studies BA was demonstrated to take part in glucose and lipid metabolism [14]. FXR, a primary BA receptor, has a regulatory role in both the glucose and cholesterol homeostasis [15].

More neonates in the Arab Christian and Muslim groups have been admitted to NICU which may be explained in part by the lower birth weight in the Arab Christian group and higher GDM rate in the Arab Muslim group.

In addition, a comparison was made between severe and mild ICP based on BA levels as mentioned earlier in the methods section. In the severe group, ICP diagnosis was made at an earlier gestational age. Previous study reported a trend for less severe BA levels when the ICP diagnosis was made closer to term [16]. Although it has been speculated that pruritis is a result of bile acids accumulation in the interstitial fluid of the skin and higher BA levels may lead to an earlier diagnosis, former studies have not found a correlation between pruritis and BA levels. Furthermore, some studies have reported that pruritus might precede the onset of biochemical abnormalities [17].

We found an elevated PET rate and meconium-stained amniotic fluid in the severe ICP group. In addition, the lower birthweight in this group might be attributed to the earlier gestational age at delivery; those findings are consistent with previous studies [6].

Strengths and limitations

To our knowledge, this is the first Israeli study exploring ICP incidence and characteristics in the different Israeli ethnical groups. Another strength of our study lies in it being a single-center study, with a uniform practice, treatment and clinical care. Furthermore, cases which were included followed strict criteria for ICP diagnosis, as only cases with pruritis and elevated fasting BA levels. Other etiologies had been excluded through carefully taken history and comprehensive physical examination while upper abdomen sonography was performed if needed. Despite the retrospective study nature, maternal and neonatal outcomes are well documented.

Our study is not without its limitations, foremost, due to its retrospective design and small cohort size, while confounding bias is impossible to eliminate. In addition, data on the Jewish ethnical subgroups (e.g., Ashkenazi, Northern Africa origins) was unavailable.

CONCLUSIONS

In conclusion, we found a higher ICP prevalence in our Northern Israeli cohort compared with previous reports from Central Israel, a higher ICP incidence among Arab Christian patients and a lower incidence in the Arab Muslim group. In line with previous studies, severe ICP is associated with certain pregnancy complications.

Further research is required to investigate reasons for the increased incidence of ICP in certain ethnical groups and to explore mechanisms responsible for the association between ICP, GDM and PET.

Conflict of interest

All authors declare no conflict of interest.

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Ureteric injury after laparoscopic hysterectomy: a report of 3 cases and brief literature review

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ABSTRACT

Objectives: The application of minimally invasive laparoscopic techniques in gynecologic surgery gained popularity due to quicker recovery, shorter hospital stays as well as lower risk of complications. Ureteric injuries at laparoscopic hysterectomies are incidental and occur in less than 1% of cases. They can be identified intra-operatively but most of them are undetected. In most cases, the symptoms of an injury are non-specifically manifested after several days or even months following surgery.

Case reports: We described different clinical symptoms suggesting ureteric injury based on 3 laparoscopic hysterectomies. Methods of diagnosis and repair techniques were also presented.

Conclusions: All complications following laparoscopic hysterectomy should be analyzed meticulously and ureteral injury must be considered as one of the possible causes of abnormal patient recovery.

Key words: ureteric injury; laparoscopic hysterectomy; gynecologic surgery; ureteric damage

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INTRODUCTION

The use of minimally invasive laparoscopic procedures in gynecologic surgery gained popularity due to quicker recovery, shorter hospital stays as well as lower risk of peri- and post-operative complications. While there is an increasing number of procedures including laparoscopic hysterectomy (LH) [1], there is still a risk of complications related to the technique applied. One of the most intriguing is ureteric injury with a reported incidence of less than 1% worldwide [2]. From 2018 until 2020, we performed 487 laparoscopic hysterectomies (total and supracervical) and ureteral injuries were diagnosed in three cases reported herein. The complication rate was 0.61%, which is in the middle average in relation to the data reported worldwide. The risk diminishes significantly with increased surgeon experience, and it is estimated that 30 LHs is a safety threshold for this procedure [3, 4]. Indications for LH and anatomic pelvic conditions greatly influence ureteric injury. The highest rates are linked to operations for gynecological malignancies, being also connected with difficult surgical conditions [5, 6]. Ureteral damage in open surgery is usually caused by ligation, crushing by forceps, partial or complete incision, excision of a segment, or even secondary ischemic wall necrosis due to ischemia. Endoscopy with the extensive use of energy-generating instruments could be linked to thermal injury, while all of the above-mentioned damage mechanisms are also possible. Ureteral injuries can be identified intra-operatively but most of them are undetected. In most cases, the symptoms of an injury are non-specifically manifested after several days or even months following surgery [7].

Our study aimed to describe the clinical symptoms suggesting urinary tract injuries based on 3 cases of laparoscopic hysterectomy. Methods of their diagnosis and repair techniques were also presented.

MATERIAL AND METHODS

Case 1

A 54-year-old woman with negative history of previous pelvic surgery was admitted due to metrorrhagia, atypical endometrial hyperplasia, and left ovarian cyst.

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Table 1. Case 1. Results of laboratory tests

WBC [× 10^9/L]	NEU [× 10^9/L]	RBC [× 10^{12}/L]	HGB [g/dL]	HCT [%]	PLT [× 10^9/L]	D-Dimer [ng/dL]	CRP [mg/L]	Creatinine [mg/dL]	PCT [ng/mL]	Blood culture
15.88	**14.85**	4.23	13.2	40.7	314	**1995**	**92.97**	**1.2**	0.15	negative

WBC — white blood cells; NEU — neutrophils; RBC — red blood cells; HGB — hemoglobine; HTC — hematocrit; PLT — thrombocytes; CRP C Reactive Protein, PTC — procalcitonin

Imaging studies did not reveal pelvic abnormalities. Total laparoscopic hysterectomy (TLH) with left adnexectomy and right salpingectomy was performed. At surgery, moderate pelvic peritoneal adhesions were released. Perioperative and early postoperative periods were uneventful. The patient was discharged in a good condition on the third postoperative day. On the 15th postoperative day, the patient was readmitted with significant lower left quadrant abdominal pain, elevated body temperature, and chills. At admission, her body temperature was 37.4°C, systemic blood pressure 135/90, heart rate 95 beats/ per minute and the results of laboratory tests suggested the likelihood of the infection (Tab. 1). The abdominal X-ray in the standing position showed no signs of gut perforation or intestinal obstruction. Transvaginal ultrasound examination revealed a small volume of fluid in the pouch of Douglas. On the next day, the patient reported an outflow of a great amount of clear and odorless fluid from the vagina. Antibiotics were administered (metronidazole, cefuroxime) and intravenous urography was performed (Fig. 1). The examination revealed leakage of the contrast from the left ureter, which has been consistent with the presence of a ureterovaginal fistula. Further management consisted of the placement of a double-J stent into the left ureter with subsequent confirmation of its position by abdominal X-ray. The urinary bladder was catheterized. A day after the procedures, the patient reported complete resolution of the symptoms. She was discharged on the third day with scheduled follow-ups in Week 2 to confirm the right placement of the DJ-catheter and a week later to remove it. The repeated intravenous urography showed no abnormalities in the left ureter and the other segments of the urinary tract.

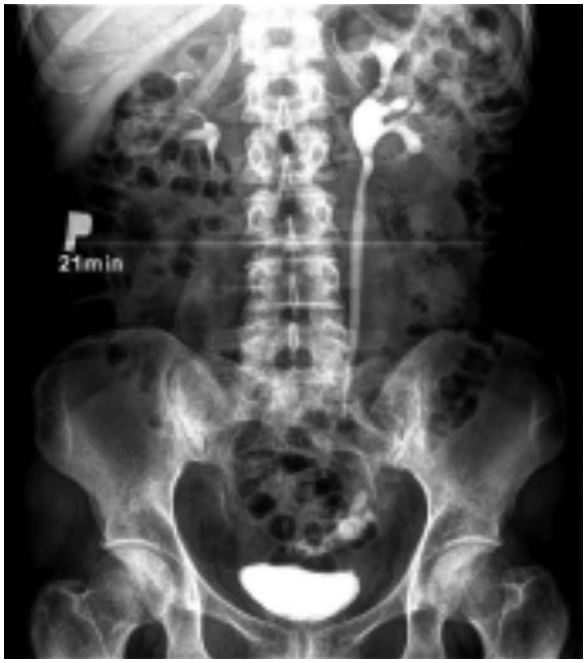

Figure 1. Urography — left-sided ureterovaginal fistula

Case 2

A 50-year-old patient was admitted due to CIN3 and abnormal uterine bleedings. She was scheduled for a total laparoscopic hysterectomy with bilateral salpingectomy. The early postoperative period was uncomplicated, and the patient was discharged on the second postoperative day in a good condition.

On the 19th postoperative day, the patient was readmitted with vaginal bleeding and enlargement of the abdominal girth. Physical examination did not reveal abdominal tenderness, guarding, or abdominal masses. Transvaginal ultrasound examination showed a large volume of fluid in the Douglas pouch and the upper right abdominal quadrant. Laboratory tests showed an increased serum level of CRP with normal leucocyte counts (Tab. 2).

Since the reason for the collection of fluid was unknown, the patient underwent diagnostic laparoscopy. Intraoperative meticulous inspection excluded abdominal bleeding or gastrointestinal damage. A large amount of a clear and yellowish fluid was found. Findings on the laparoscopy suggested injury of the urinary tract. A sample of abdominal fluid was analyzed which enabled us to confirm the presence of the urine. The next step was intravenous urography that demonstrated contrast outflow from the right ureter (Fig. 2).

The uretero-abdominal fistula was diagnosed. Attempt to place a DJ stent into the right ureter was unsuccessful due to its stenosis related likely to thermal injury. The next day, the patient reported leakage of a copious amount of clear fluid from the vagina. Since right-sided mild hydronephrosis developed, there was a plan to establish percutaneous nephrostomy. However, the first attempt on Day 6 failed but another procedure scheduled two days later was successful. During the hospital stay, all parameters of renal function normalized. After another unsuccessful attempt to place the

Table 2. Case 2. Results of laboratory tests

	WBC [$\times 10^9$/L]	NEU [$\times 10^9$/L]	RBC [$\times 10^{12}$/L]	HGB [g/dL]	HCT [%]	PLT [$\times 10^9$/L]	D-Dimer [ng/dL]	CRP [mg/L]	Creatinine [mg/dL]	PCT [ng/mL]
Day 1	7.25	5.44	**3.9**	**11.5**	**34.6**	**444**	—	**227.26**	0.9	—
Day 9	7.54	5.18	**3.83**	**11.1**	**34.5**	**453**	—	—	—	—
Day 11	5.58	3.4	**3.43**	**9.7**	**31.0**	**431**	—	**56.78**	0.8	—
Day 16	—	—	—	—	—	—	—	—	—	0.07

WBC — white blood cells; NEU — neutrophils; RBC — red blood cells; HGB — hemoglobine; HTC — hematocrit; PLT — thrombocytes; CRP C Reactive Protein, PTC — procalcitonin

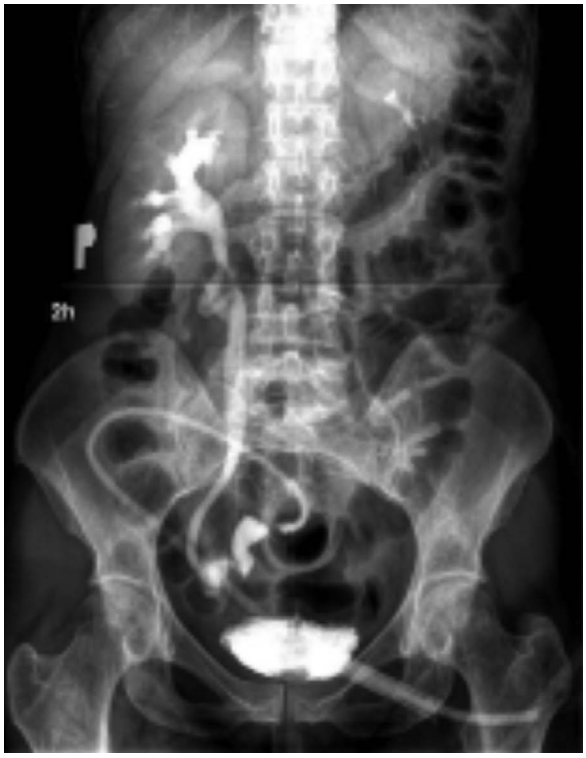

Figure 2. Urography. Right ureteric injury

ureteral stent, the patient was discharged in good clinical condition with a functioning nephrostomy tube. Four weeks later, the DJ stent was inserted into the right ureter, and the nephrostomy was removed. The repeated intravenous urography showed no abnormalities in the right ureter and other segments of the urinary tract.

Case 3

A 40-year-old patient was hospitalized with symptomatic uterine leiomyoma and abnormal uterine bleeding. Laparoscopic supracervical hysterectomy with bilateral salpingectomy was performed without intraoperative complications. The subsequent postoperative course was uneventful, and the patient was discharged on Day 2.

Three weeks later, the patient was readmitted with abdominal pain and flatulence. The woman had occasional vomiting, micturition was normal, body temperature was 37.2°C and peritoneal signs were negative. Results of blood tests suggested intra-abdominal infection (Tab. 3). Transvaginal ultrasound showed a small amount of fluid in the Douglas pouch. The abdominal X-ray in the standing position revealed several air-fluid levels in the lower right abdominal quadrant but with no suspicion of intestinal perforation. Abdominal ultrasonography showed thickened and swollen intestinal walls with decreased motility and a moderate amount of intra-abdominal fluid. CT abdominal scan confirmed the presence of fluid in the abdominal cavity especially around the liver and the Douglas pouch. On the 30th postoperative day (day 8 of re-admission), the patient was scheduled for reoperation. Diagnostic laparoscopy revealed solid adhesions of the fibrin, bowel, and parietal perineum and around 1500 mL of a clear fluid in the cavity. The adhesions were partially released. Due to technical difficulties, the surgeon decided to convert to laparotomy. After complete adhesiolysis, fluid and tissue samples were collected for further analysis. Two drainage tubes were placed in the abdominal cavity.

On the 1st postoperative day, 200 mL of the yellowish fluid was collected from the drainage tubes. A high concentration of urea and creatinine in this fluid raised suspicion of uretero-abdominal fistula. The URO-CT showed right-sided hydronephrosis with contrast leakage from the ureter into the abdominal cavity located on the level of the uterine cervix (Fig. 3). An attempt to insert a DJ catheter into the right ureter failed but percutaneous nephrostomy was successfully performed. The patient was in a stable condition and was discharged with an indication for a follow-up visit after 4 weeks to remove the catheter. On the 39th postoperative day, the nephrostomy tube was removed. The follow-up renal blood test and URO-CT confirmed normal kidney function.

DISCUSSION

The diagnosis of ureteric injury may be made intra- or postoperatively but approximately 70% of ureteric injuries are diagnosed after surgery [8–10]. The most common symptoms of ureteric injury are fever and abdominal flank pain. The patient may present hematuria, oliguria, anuria, and retroperitoneal urinoma with the risk of abdominal

Table 3. Case 3. Results of laboratory tests

	WBC [$\times 10^9$/L]	NEU [$\times 10^9$/L]	RBC [$\times 10^{12}$/L]	HGB [g/dL]	HCT [%]	PLT [$\times 10^9$/L]	D-Dimer [ng/dL]	CRP [mg/L]	Creatinine [mg/dL]	PCT [ng/mL]
Day 1	**10.8**	7.16	**3.9**	**11.8**	**35.9**	**510**	**881**	**71**	0.8	< 0.10
Day 5	**11.91**	7.91	4.32	12.8	39.9	**557**	—	**79**	0,8	—
Day 7		—	—	—	—	—	**1535**	**70**	0.8	—
Day 8	**13,8**	**11,28**	**3.85**	**11.5**	**35.2**	**418**	—		—	—
Day 9	**11.57**	**9.25**	**3.57**	**10.6**	**33.0**	389	—	**289**	**0.4**	0.13
Day 10	9.08	**6.86**	**3.16**	**9.5**	**28.5**	357	—	**273**	**0.4**	—
Day 11	5.96	3.72	**2.96**	**9.1**	**26.4**	376	—	b.d.	**0.4**	< 0.10
Day 13	—	—	—	—	—	—	—	52.96	—	—
Day 14	—	—	—	—	—	—	**2279**	**39.61**	—	—
Day 19	9.47	5.98	**3.83**	**11.3**	**35.9**	**505**	—	**20.92**	—	—
Day 22	8.42	5.48	**3.84**	**11.6**	**35.8**	**487**	—	**12.98**	—	—
Day 28	6.14	3.0	**3.77**	**11.2**	**34.7**	307	—	**9.74**	0.6	—
Day 34	—	—	—	—	—	—	445	2.05	—	—
Day 35	7.28	3.57	4.23	12.6	38.7	278	447	2.38	—	—

WBC — white blood cells; NEU — neutrophils; RBC — red blood cells; HGB — hemoglobine; HTC — hematocrit; PLT — thrombocytes; CRP C Reactive Protein, PTC — procalcitonin

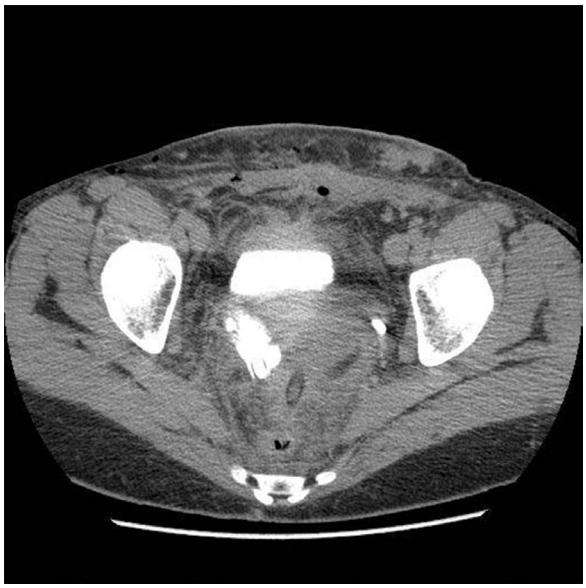

Figure 3. URO-CT showing right ureteric injury

or even retroperitoneal abscess formation. The presence of vaginal or cervical urine leakage is an important clue in the diagnostic procedure. Unfortunately, in approximately 50% of cases, the symptoms are not specific, and the first manifestation of ureteric injury may be hypertension caused by obstructive uropathy.

Postoperative symptoms in the above-mentioned cases were blurred and non-specific. Hysterectomized patient (Case 1) had mainly gastrointestinal symptoms *e.g.*, difficulty to pass a stool that made diagnosis more difficult by mimicking postoperative ileus. Another patient (Case 2) reported vaginal bleeding and distension of the abdomen that raised suspicion of abdominal bleeding. Finally, the main complaint reported by the woman after LASH (Case 3) was abdominal pain. She was also feverish, which initially suggested intra-abdominal organ infection, visceral injury, or even peritonitis.

Diagnostic methods of ureteral damage depend on the time of diagnosis. Perhaps the best strategy is to verify the ureter's course with the reservation that the observed peristalsis does not prove the ureter's viability. Another intraoperative strategy is intravenous injection of dye followed by cystoscopy, which reveals dye-stained urine (negative test) or bubbles, eventually blood-tinged urine (positive test). Unfortunately, intraoperative methods of ureteral damage assessment were not applied thereafter, because the likelihood of urinary injury was deemed by the surgeon as low. Moreover, there have been no definitive guidelines regarding the role of cystoscopy at the time of benign hysterectomy so far. American Association of Gynecologic Laparoscopists (AAGL) recommended that routine cystoscopy should be performed after all laparoscopic hysterectomies, whereas ACOG limited the indication to prolapse and incontinence procedures [11, 12]. The research of Barber et al. [13] highlighted the limitations of cystoscopy in the prevention of delayed lower genitourinary tract injury. They showed a significant 27% increase in the risk of urinary tract infections after they performed cystoscopy. In conclusion, they encourage the use of other strategies, beyond cystoscopy, to improve surgical quality and decrease the rate of delayed urinary tract injury in women undergoing benign hysterectomy [13].

There are few methods of imaging in suspected ureteral damage but the cheapest and most readily available is intravenous urography. This method was applied in two cases and proved sufficient to make the correct diagnosis. However, in patient three, we used URO-CT to assess the urine outflow tract. It allowed to precisely diagnose the ureteral damage.

Laboratory tests (Tab. 1–3) must be evaluated in every case of suspected ureteral injury. All (except case 2) patients had leukocytosis and significantly elevated levels of CRP but both markers are not specific for urinary tract injury. Of note is the lack of increase of creatinine level. It is likely that transient early postoperative elevation of blood creatinine went unnoticed and renal function was well compensated at the time of hospital re-admission.

Once diagnosis is made, medical intervention should be immediate. The first line of treatment is usually stent placement especially in the partial transection or subtotal obstruction of the ureter. Complications such as abscess, urinoma, urinary tract infection, sepsis, or even renal failure must be treated with a combination of surgery, antibiotics, and supportive care.

Intraoperative identification of the ureters could reduce the risk of their injury especially when normal anatomy is severely distorted [14]. Obviously, this crucial surgical step was not taken in the cases described above, but it is worth noting that anatomy was normal except uterine with leiomyomas. The cadaver study highlights a few steps of protecting the ureter during laparoscopic extra-fascial hysterectomy [15]. They recommend combined lateralization and elevation of the uterus, sectioning the ascending branch of the uterine artery, and dissecting it along the uterosacral ligaments. Moreover, Feng et al. [16] reported that ureteral catheterization before surgery should be recommended for complicated gynecological surgical interventions or unskilled surgeons to prevent iatrogenic ureter injuries. However, routine stent placement before gynecological laparoscopy remains controversial and is not recommended. The use of trans-illuminating stents has also been suggested to identify the ureter during laparoscopy; however, this application is limited by costs, as well as by the additional equipment required [16].

Most likely, the mechanisms described above were thermal destruction that highlight the value of a proper application of electro-surgery at laparoscopy. The anatomical proximity of the ureter which passes with an average distance of 2.3 ± 0.8 cm or is as close as 0.5 cm to the cervix makes the area of uterine artery-ureter crossing especially vulnerable [17]. Indeed, all described ureteric injuries were located on that level and lateral to the cervix. The penetration of heat from bipolar forceps used in the sealing of uterine vessels is the most likely underlying cause of this injury. The risk may be reduced by the employment of advanced electro-surgery devices that are able to limit the power settings. However, all described safety managements to prevent laparoscopic electrosurgical ureteric injuries are of utmost importance.

CONCLUSIONS

All complications following laparoscopic uterine surgery should be analyzed meticulously and ureteral injury must to be considered as one of the possible causes of abnormal patient recovery. This is of paramount importance in preventing ureteric damage-related morbidity.

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Conflict of interest

The authors have no conflict of interest relevant to this article.

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Coffee consumption during pregnancy — what the gynecologist should know? Review of the literature and clinical studies

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ABSTRACT

Coffee is one of the most consumed beverages in the world. The impact of coffee consumption on human health has been the subject of many clinical studies and meta-analyses. Taking into account the results of these studies, it can be concluded that coffee has a number of health benefits in terms of the population, including the reduction of the risk of death from any cause. From a clinical point of view, the safety of coffee consumption in a specific subpopulation of pregnant women is important. A large percentage of women continue to consume this drink during pregnancy, while a significant proportion of them exceed the permissible daily dose of caffeine (≤ 200 mg). During pregnancy, the metabolism of caffeine slows down significantly, which prolongs its action and penetrates into the body of the fetus. These biochemical observations have become the driving force behind numerous clinical studies assessing the impact of coffee consumption during pregnancy on its course, complications and the health of the newborn. This review article summarizes the current knowledge of these important issues.

Key words: coffee; caffeine; pregnancy

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COFFEE — AN OVERVIEW OF THE MOST IMPORTANT INFORMATION

Coffee, next to water, is the second most consumed drink in the world [1]. According to the National Coffee Association USA, about 2.25 billion cups of coffee are consumed worldwide every day. The data of the International Coffee Organization indicate that the inhabitants of the Netherlands consume the most coffee — on average around 8.3 kg/*per capita*/year (data from 2020). In Poland, coffee consumption *per capita* is about 3 kg/year, which is on average 1–2 cups of coffee/day. In the last 10 years, coffee consumption in Poland has increased significantly, by over 80% [2].

In the world, from a commercial point of view, the most important are Arabica coffee (*Coffea arabica*), Robusta coffee (*Coffea canephora*) and Liberian coffee (*Coffea liberica*) [3]. The largest amounts of coffee are produced in Brazil [1]. It is estimated that there are over 1000 chemical compounds in coffee, and the most common ones are phenols (chlorogenic acid and diterpenes: kahweol and cafestol) and alkaloids (caffeine and trigonelline). Less abundant compounds found in coffee include: mannose, galactose polysaccharide chains, melanoidins, flavonoids, catechins, anthocyanins, ferulic acid, caffeic acid, p-coumaric acid, and tocopherols [4].

The composition of coffee depends on many factors, including the type of coffee (*e.g.*, *Coffea Arabica*, *Coffea Canephora*, *Coffea Liberica*), the production method (wet, dry, semi-dry/semi-wet, bio-processing) and the method of preparation (*e.g.*, traditionally brewed coffee, espresso) [1, 5]. Factors before harvest (*e.g.*, sunlight) and after harvest (*e.g.*, the way of processing coffee beans) account for approx. 40% and 60% of the organoleptic, physical and chemical properties of coffee, respectively [1, 5]. Interestingly, due to a different capacity of the "coffee cup", the caffeine content varies depending on the geographic region. In Northern Europe and Great Britain, a cup of coffee contains 140 mg of caffeine, in Southern Europe 50 mg, and in the United States 85 mg [6]. Caffeine is also found in drinks such as

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decaffeinated coffee (small amounts, approx. 0.02 mg/mL), cola, green tea, black tea, and energy drinks [7].

Coffee is a very popular drink all over the world. Consuming coffee, especially brewed with a paper filter and without the addition of sugar or milk, in a regular and moderate manner (2–3 cups/day) has a beneficial effect on human health in the general population. It was shown that consumption of coffee according to such a pattern was characterized by antihypertensive properties and had a positive effect on the activity of the nervous, digestive, cardiovascular and kidney systems [3, 8–11]. In a recent study by Simon et al. [12], including 468,629 subjects, drinking 0.5–3 cups of coffee/day was associated with a lower risk of death from any cause [HR (hazard ratio) = 0.88; 95% CI (confidence interval): 0,83–0.92; $p < 0.001$] and death from cardiovascular causes (HR = 0.83; 95% CI: 0.74–0.94; $p = 0.006$), and stroke (HR = 0.79; 95% CI: 0.63–0.99 $p = 0.037$) during 11 years of follow-up. A number of other studies have also shown that coffee consumption reduces the risk of death from any cause [13].

Thus, consumption of coffee is associated with a number of health benefits in the population dimension, which makes it a very popular drink. However, special attention should be paid to the safety and impact of coffee consumption on a very specific population group, that is pregnant women. This issue is the subject of current discussions and numerous clinical studies. The most significant and recent information on this issue is summarized in the further part of the article.

CONSUMING COFFEE DURING PREGNANCY: RECOMMENDATIONS AND REALITY

The European Food Safety Authority (EFSA) concludes that maternal consumption of up to 200 mg of caffeine per day "is of no concern for fetal safety", and the UK National Health Service (NHS) recommends that women in pregnancy "limit" the daily intake to 200 mg [14, 15]. This amount of caffein is contained in approx. two cups of coffee. Recent studies show that a significant proportion of pregnant women consume more caffeine than allowed by EFSA and the NHS. A study by Lehtonen et al. [16] of 2840 Finnish women in the third trimester of pregnancy showed that 31% of them consumed 200–299 mg of caffeine/day and 10% over 300 mg of caffeine/day. The most common source of caffeine was coffee (81%). The highest amounts of caffeine were consumed by elderly, multiparous, overweight or obese women and smokers. Moreover, the amount of caffeine consumed by the mother correlated with the amount of this compound in the hair of the newborns ($p < 0.001$). Thus, in this study, over 40% of women in the third trimester of pregnancy consumed more or much more of the daily allowable dose of caffeine. A study by Lama et al. [17] assessed caffeine consumption among 724 pregnant French women. Coffee consumption was declared by 47.1% of women. Average daily caffeine consumption has been shown to decrease slightly from the first to third trimester of pregnancy: 587 caffeine users with 59.2 ± 61.5 mg/day caffeine intake in the first trimester compared with 577 users (54.3 ± 55.4 mg/day) in the third trimester, respectively. In a study by Mannucci et al. [18], which included 5,405 pregnant Italians, it was shown that 42.3% of them reported consuming coffee during pregnancy (70% consumed one cup/day, 23% two cups/day, and 6% at least three cups/day). Differences in the amount of caffeine consumed between Finnish and French and Italian women are most likely due to the general pattern of coffee consumption in these countries. A study by Peacock et al. [19] involving 1,232 Australian women assessed adherence to recommendation for caffeine consumption during pregnancy. With regard to the first trimester of pregnancy, the prevalence of coffee consumption depended on awareness of this condition. Among women who were not aware of being pregnant, 89% consumed caffeine in the amount of 107 mg/day (60–147 mg), while among those aware of pregnancy, this percentage was lower and amounted to 68% in the amount of 60 mg/day (40–107 mg). The percentage of women in the second and third trimesters of pregnancy who consumed caffeine was 79% (80 mg/day; 40–107 mg) and 80% (80 mg/day; 40–107 mg), respectively. It is worth noting that the percentage of women who consumed ≥ 200 mg/day of caffeine was 22% (first trimester, unaware pregnancy), 7% (first trimester, conscious pregnancy), 11% (second trimester) and 13% (third trimester). A very high frequency of caffeine consumption by pregnant women was also shown in a study by Alamneh et al. conducted among women in Ethiopia. The consumption of caffeine out of 352 women was declared by 98.2% of them, while the average daily consumption of this substance was 170.5 mg. The consumption of ≥ 300 mg of caffeine/day was declared by 17.6% of the surveyed women. Caffeine was most often consumed by the richest pregnant women and those in the first trimester of pregnancy [20]. In the United States, approximately 70% of women still consume caffeine during pregnancy [21, 22]. It was even reported that some women consumed more than 300–500 mg of caffeine per day during pregnancy [23].

To sum up, the available literature shows that pregnant women frequently consume caffeine during pregnancy (mainly in coffee), while a significant percentage of it exceeds the acceptable daily consumption. This is important due to the negative effect of *in utero* exposure to caffeine that has been described in numerous clinical studies. A recent review of the literature by James indicated that the available data support the conclusion that caffeine consumption during pregnancy is associated with a higher risk of: miscarriage, stillbirth, low birth weight and/or low pregnancy weight, premature birth and/or premature pregnancy, childhood

acute leukemia as well as overweight/obesity later in the life of the child. It has been found that there is no safe dose of caffeine to consume during pregnancy [24].

CAFFEINE METABOLISM DURING PREGNANCY

The metabolism of caffeine and its influence on the function of individual organs and systems depends on many factors. It has been shown that the effect of caffeine on the course of pregnancy and fetal development is highly variable between different women [25]. This variability results mainly from: 1) the ability to metabolize caffeine, which is determined by the activity of CYP1A2 (mono-oxygenase and xanthine oxidase enzymes); 2) interaction of caffeine with cells, mainly related to the sensitivity of ADORA1 and ADORA2A adenosine receptors; and 3) the presence of various factors of fetal or maternal origin modulating the effect of caffein [25].

Cytochrome P450 A1 activity and caffeine metabolism

The rate of caffeine metabolism depends primarily on the activity of CYP1A2, because this enzyme is responsible for 95% of the metabolism of this compound that takes place in the liver [26]. It has been shown that in the case of exposure to the same doses of caffeine, women with higher activity of the enzyme CYP1A2 (rapid caffeine metabolism) were characterized by an increased risk of pregnancy disorders compared to women with lower activity of this enzyme [25]. In women who metabolize caffeine quickly (CYP1A2 AA polymorphism) after coffee consumption, a higher concentration of paraxanthine (the main metabolite of caffeine) was found compared to women who metabolize caffeine more slowly (CYP1A2 AC/CC polymorphisms) [27].

Another very important issue is the influence of pregnancy on the activity of CYP1A2. During pregnancy, the half-life of caffeine is significantly extended up to 18 hours, especially in the third trimester. Several weeks after childbirth, the caffeine metabolism rate normalizes to the baseline state. The prolongation of the half-life of caffeine in the body is related to the decreasing activity of CYP1A2 during pregnancy (Fig. 1) [28].

It has been found that the activity of CYP1A2 decreases in the first to third trimester of pregnancy [28]. In a study by Liang et al. [29], the metabolomic profile of 784 weekly blood samples from 30 pregnant women was analyzed. Five metabolites have been shown to belong to the same pathway of caffeine metabolism. All five metabolites were continuously elevated during pregnancy and caffeine reached levels three times higher at the end of pregnancy than at the beginning. The authors of the study indicate that this increase could have been caused by slower metabolism of caffeine in pregnant women, and not by an increase in coffee consumption. The study by Abduljalil et al. [30] summarized the changes in caffeine metabolism occurring during pregnancy (Tab. 1).

It should also be emphasized that apart from polymorphisms and pregnancy itself, the activity of CYP1A2 is also influenced by other factors, such as smoking and larger amounts of coffee consumed, which increase the activity of this enzyme [9, 25]. Moreover, genetic and environmental

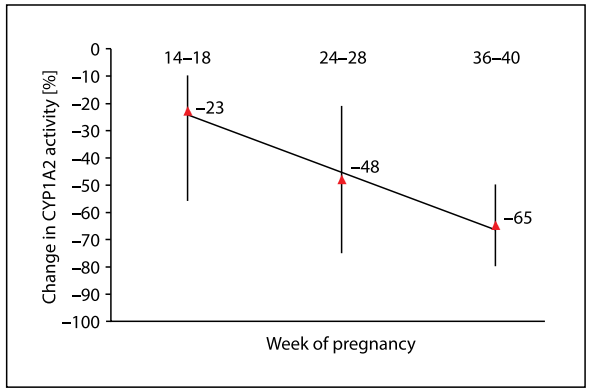

Figure 1. Changes in CYP1A2 activity during pregnancy in humans. Prepared on the basis of [28]

Table 1. Summary of changes in caffeine metabolism during pregnancy

Parameter	Change during pregnancy	Reference
CYP1A2 activity (compared to the level in non-pregnant women)	Progressive reduction throughout pregnancy	[30]
CYP1A2 abundance (µmol CYP1A2/whole liver)	Progressive reduction throughout pregnancy	[30]
Caffeine concentration (mg/L)	Longer lasting increase in blood levels compared with non-pregnant women	[31]
Caffeine half-life (h)	Progressive extension throughout pregnancy	[32, 33]
Caffeine clearance (l/h/kg)	Progressive reduction throughout pregnancy	[33]
Elimination of caffeine from the body (1/h)	Progressive reduction throughout pregnancy	[33]

factors explain only 30–40% of the individual variability in CYP1A2 activity, suggesting the complexity of CYP1A2 regulation, which may involve epigenetic mechanisms [25].

As mentioned, a reduction in the rate of caffeine metabolism leads to a prolonged period of this compound's presence in the mother's body. Caffeine, due to its high lipophilicity, quickly crosses the placental barrier, and since the cytochrome P450 system remains undeveloped until infancy, this compound accumulates in the tissues of the fetus (the placenta is also unable to metabolize caffeine), which is additionally confirmed by the presence of caffeine in its hair (a clear correlation especially in the third trimester of pregnancy) and a high percentage of unchanged caffeine removed (80% in the fetus vs 3–4% in an adult) [33].

Taking into account the significant changes in caffeine metabolism described above, an important question is the safety of coffee consumption in this period for the mother and child. Clinically important are the effects of coffee consumption on fertility, course of pregnancy and the health of the newborn in the short and long term.

POTENTIAL CONSEQUENCES OF MOTHER'S COFFEE CONSUMPTION ON THE COURSE OF PREGNANCY — DATA FROM CLINICAL STUDIES

Fertility

The effects of coffee consumption on fertility have been the subject of numerous clinical studies. It is an important clinical topic as the incidence of fertility disorders in 2017 was 1571.35/100000 women (95% CI: 1115.30–2111.94) and it is increasing year by year [34]. In a meta-analysis of 2 cohort studies by Lyngsø et al. [35], caffeine consumption was not shown to significantly affect time to pregnancy. A more recent meta-analysis of four studies by Bu et al. [36] showed that the consumption of caffeine at a dose of ≤ 100 mg/day, ≥ 200 mg/day, and ≥ 400 mg/day was not significantly associated with the risk of infertility (OR = 0.95; 95% CI: 0.78–1.16, OR = 1.14; 95% CI: 0.69–1.86 and OR = 1.86; 95% CI: 0.28–12.22). Interestingly, a study by Lyngsø et al. [37], involving 1708 Danes undergoing fertility treatment, assessed the effect of coffee consumption on the success of this therapy. It was shown that consumption of coffee by women treated with *in vitro* fertilization and intracytoplasmic sperm injection did not significantly affect the risk of pregnancy and having a live child. However, it was found that consumption of 1–5 cups of coffee/day by women undergoing intrauterine insemination increased the likelihood of becoming pregnant (RR = 1.49; 95% CI: 1.05–2.11) and having a live child (RR = 1.53; 95% CI: 1.06–2.21). No negative influence of coffee consumption on the risk of infertility was also demonstrated by Soylu et al. [38] in a prospective cohort study involving 7,574 Danish women. These investigators concluded, based on a 20-year follow-up, that coffee consumption did not influence the risk of primary infertility (HR = 1.00; 95% CI: 0.97–1.03). In a meta-analysis conducted by Lyngsø et al. no association was found between coffee/caffeine consumption and outcomes of fertility treatment (based on 2 studies). No clear association was found between exposure to coffee/caffeine and natural fertility as measured by fecundability odds ratio (based on 3 studies) [35].

In conclusion, the results of clinical studies and their meta-analyses do not indicate that consumption of coffee increases the risk of infertility.

Pregnancy loss

Bleeding within the first 20 weeks of gestation is one of the most common pregnancy complications (15–20% of ongoing pregnancies) and is an unfavorable prognostic factor, increasing the risk of miscarriage [39, 40]. A cross-sectional analysis by Choi et al. [40], including 3510 Korean women, showed that women up to 35 years of age, consuming ≥ 2 cups of coffee/day, had a significant bleeding risk (adjusted OR = 1.358; 95% CI: 1.050–1.757). Consumption of ≤ 1 cup of coffee/day was not significantly associated with the risk of bleeding (regardless of the age of the pregnant woman).

Spontaneous abortion (SAB), defined as loss of pregnancy naturally before twenty-four weeks of gestation, is a not uncommon clinical problem. In the study by Zhao et al. [41], covering 102,259 pregnant women, it was shown that 14.3% of them experienced SAB. In a prospective cohort study by Gaskins et al. [42], including 15590 pregnant women, it was shown that compared to women who had not consumed coffee before pregnancy, women consuming ≥ 4 cups of coffee/day had 20% (RR = 1.20; 95% CI: 1.06–1.36) higher risk of SAB. Consumption of < 1–3 cups of coffee/day did not significantly affect the risk of SAB. Similar results were obtained by Hahn et al. [43] in a prospective cohort study of 5,132 Danish women planning pregnancy. It has been shown that in the pre-pregnancy period, coffee consumption ≥ 3 cups/day and consumption of up to 2 cups/day in early pregnancy were not significantly associated with SAB risk. A mendelian randomization study by Yuan et al. [44], which included 259,142 women from the UK and Finland, assessed the relationship between coffee consumption and the risk of pregnancy loss taking into account genetic predisposition. It was shown that coffee consumption was not significantly associated with the risk of pregnancy loss (OR = 0.96; 95% CI: 0.87–1.06). In a meta-analysis of 27 studies by Lyngsø et al. [35], compared with no caffeine consumption, the relative risk of SAB was 1.08 (95% CI: 1.03–1.13) for 100 mg caffeine/day, 1.37 (95% CI: 1.19–1.57) for 300 mg caffeine/day and 2.32 (95% CI:

1.62–3.31) for 600 mg caffeine/day. Similar results were obtained in a meta-analysis of 26 studies by Li et al. [45]. The authors showed that caffeine consumption was associated with an increased risk of pregnancy loss (OR = 1.32; 95% CI: 1.24–1.40), as was coffee consumption (OR = 1.11; 95% CI: 1.02–1.21). A dose-response analysis suggested that risk of pregnancy loss rose by 19% for every increase in caffeine intake of 150 mg/day and by 8% for every increase in coffee intake of two cups per day. The effect of coffee consumption on the risk of pregnancy loss was also assessed by Chen et al. [46] in a meta-analysis of 14 prospective clinical studies. It has been shown that women who consumed ≥ 350 mg/day of caffeine had a significant risk of pregnancy loss (350–699 mg/day, RR = 1.40; 95% CI: 1.16–1.68; ≥ 700 mg/day, RR = 1.72; 95% CI: 1.40–2.13). It was found that for every 100 mg/day increment of caffeine consumption, the risk of SAB was increased by 8% (RR = 1.08; 95% CI: 1.04–1.13) and stillbirth by 9% (RR = 1.09; 95% CI: 1.02–1.16). In a meta-analysis of 2 studies by Ng et al. [47] it was shown that the risk of recurrent pregnancy loss in the group of women with higher caffeine consumption (> 99 mg/day) compared to the group with lower caffeine consumption (< 99 mg/day) was higher, but not statistically significant (OR = 1.35; 95% CI: 0.83–2.19).

The potential mechanisms by which coffee consumption may increase the risk of pregnancy loss are not fully understood. It is indicated that caffeine may reduce blood flow through the placenta, and disrupt the hormonal profile during pregnancy. Caffeine and estradiol are both metabolized by the hepatic enzyme CYP1A2 so a possible pathway for caffeine to interfere with estradiol levels. Moreover, besides caffeine, coffee contains numerous other bioactive substances including lignans and isoflavonoids, both belonging to the phytoestrogen family with great affinity for the estrogen receptor. It should also be mentioned that caffeine may cause chromosomal anomalies (structure of caffeine is similar to that of adenine and guanine, so it might be incorporated into the DNA macromolecule during mitosis) [35, 45].

Thus, the results of clinical studies and meta-analysis indicate that caffeine consumption may increase the risk of pregnancy loss.

METABOLIC DISORDERS

The most common metabolic complications of pregnancy include diabetes mellitus (10.9% in Europe; 95% CI: 10.0–11.8) and hypertension (approx. 4.4%) [48, 49].

In a prospective multicenter cohort study by Hinkle et al. [50], involving 2,583 women, the impact of the consumption of caffeinated beverages on the cardiometabolic profile of pregnant women was assessed. Daily total caffeine consumption was estimated over the period: 10 to 13 weeks of gestation and 16 to 22 weeks of gestation. It has been shown that consumption of caffeinated beverages in the first trimester of pregnancy did not affect the risk of gestational diabetes and gestational hypertension, while in the second trimester it reduced the risk of gestational diabetes (only 1–100 mg caffeine/day; RR = 0.53; 95% CI: 0.35–0.80, up to 200 mg/day — no effect), and did not affect the risk of gestational hypertension. Similar results were obtained in a prospective cohort study of 85533 Japanese women, Kawanishi et al. [51]. These authors showed that the consumption of 2–3 cups of coffee/day was associated with a lower risk of hypertension-related pregnancy disorders (OR = 0.79; 95% CI: 0.62–0.99). It is also worth taking note of the results of the study by Bakker et al. [52], which assessed the influence of coffee consumption on the risk of pre-eclampsia among 7890 pregnant women. It was shown that as compared to women with caffeine intake of < 2 cups/day, those using 2–4 cups/day had a lower risk of pre-eclampsia (OR = 0.63; 95% CI: 0.40–0.96). It is worth mentioning that coffee may reduce the risk of pre-eclampsia by reducing the concentration of lipoprotein (a) in plasma [53].

Thus, studies results do not indicate that coffee consumption increases the risk of metabolic disorders during pregnancy.

PRETERM BIRTH

The incidence of preterm labor is not a rare clinical problem. According to the World Health Organization, the global prevalence of preterm birth is around 5–18%. The prevalence of preterm births is a measure of the level of gynecological and obstetric care in a given country.

In a case-control study by Sindiani et al. [54], involving 1110 pregnant women, the relationship between coffee consumption and the risk of preterm birth was assessed. It was shown that after taking into account numerous risk factors, coffee consumption did not affect the risk of preterm labor (OR = 0.72; 95% CI: 0.40–1.29). In a cohort study by Vitti et al. [55], involving 7,607 Brazilian women, no association was found between high caffeine consumption and risk of preterm birth (RR = 1.03; 95% CI: 0.65–1.63). The results of these studies are confirmed by an earlier meta-analysis by Maslova et al. [56], including 15 cohort and 7 case-control studies. This meta-analysis shows no significant association between caffeine intake during pregnancy and the risk of preterm birth.

Thus, the results of the available literature indicate that coffee consumption is unlikely to influence the risk of preterm birth.

NEWBORN HEALTH

The impact of maternal coffee consumption on the health of the newborn is a very important issue. As al-

ready mentioned, the fetus does not have the ability to metabolize caffeine, and therefore it accumulates in the body. Measuring the caffeine content in a newborn's hair is a good method to assess fetal cumulative caffeine exposure [16]. So far, many studies have been conducted to assess the effects of coffee consumption during pregnancy on the health of the newborn.

Low birth weight and childhood obesity

In a cross-sectional study by Mannucci et al. [57], involving 5,405 pregnant Italian women, the effect of coffee consumption during pregnancy on the birth weight of the newborn was assessed. It was shown that newborns of women who consumed ≥ 3 cups of coffee/day during pregnancy were characterized by significantly lower birth weight (OR = 1.566; 95% CI: 1.081–2.267, p = 0.018). A retrospective study by Oh et al. [58] of 1657 pregnant Korean women also found that consumption of ≥ 2 cups of coffee/day during pregnancy was associated with an increased risk of low birth weight (OR = 1.92; 95% CI: 1.22–3.03). Less pessimistic observations came from a study by Lamy et al. [59], involving 724 pregnant French women. It was shown that the influence of caffeine consumption on anthropometric parameters of the newborn, adjusted for other factors, was statistically insignificant. Similar results were obtained by Wierzejska et al. [60] in a study involving 100 pregnant Polish women. No relationships were found between caffeine intake and neonatal weight, length, or head and chest circumference (p > 0.05). The authors of the study indicate, however, that the amount of caffeine consumed by the studied women was low (only 2% consumed > 200 mg of caffeine/day). Nevertheless, the literature predominates data showing that coffee consumption during pregnancy increases the risk of low birth weight. An interesting multicenter cohort study by Gleason et al. [61], involving 2055 pregnant women, has shown that women who consumed about 50 mg of caffeine/day (~ 1/2 cup of coffee) gave birth to lower birth weight newborn with smaller arms, smaller thighs and a smaller anterior flank skin fold. Moreover, these observations did not differ depending on the genotype of fast or slow caffeine metabolism. The adverse effect of maternal coffee consumption on the child's birth weight is also confirmed by a cross-sectional study by Ferreira et al. [62], involving 260 pregnant Brazilian women. It was found that women who consumed, inter alia, coffee during pregnancy more often gave birth to children with low birth weight (PR = 1.27; 95% CI: 1.11–1.45). In the meta-analysis of 8 cohort and 4 case-control studies conducted by Rhee et al. [63], the impact of coffee consumption on birth weight was summarized. It was shown that the risk of low birth weight, comparing the highest versus lowest level of caffeine intake during pregnancy, was 1.38 (95% CI: 1.10-1.73). Every additional 100 mg of caffeine intake (1 cup of coffee) per day during pregnancy was associated with a 3.0% increase in odds ratio for low birth weight. These observations were confirmed in a more recent meta-analysis of 15 cohort studies by Jin and Qiao, which showed that the risk of low birth weight was 1.33 (95% CI: 1.12–1.57) for mothers with the highest compared with the lowest level of caffeine intake during pregnancy. In the dose-response meta-analysis, this risk was found to be 1.07 (95% CI: 1.02–1.11) for each 100 mg/day increase of caffeine intake [64]. Adverse results were also obtained in the meta-analysis of seven studies by Soltani et al. [65]. Was showed a significant positive association between maternal caffeine intake and the risk of low birth weight (RR = 1.70; 95% CI: 1.19–2.43). Moreover, each additional 100 mg per day of maternal caffeine intake was shown to be significantly associated with an increased risk of low birth weight (RR = 1.12; 95% CI: 1.03–1.22). The results consistent with these observations were also obtained in the meta-analysis of 13 prospective studies by Chen et al. [66].

It should also be mentioned here that the previously cited meta-analysis by Jin and Qiao also found that the risk of childhood overweight and obesity was 1.39 (95% CI: 1.15–1.69) for mothers with the highest compared with the lowest level of caffeine intake during pregnancy. In the dose-response analysis, this risk was 1.31 (95% CI: 1.11–1.55) for each 100 mg/day increase of caffeine intake [64].

The exact pathophysiological mechanisms underlying the above observations are not fully understood. It is indicated that caffeine has been shown to inhibit phosphodiesterase and increase concentration of cyclic adenosine monophosphate in cells which in turn may interfere with fetal cell growth and development. Moreover, caffeine consumption during pregnancy is associated with reduced placental blood flow and hypoxia (A1AR receptor antagonism) that result from the blockage of adenosine receptors, as well as from increased epinephrine concentrations in the mother and in the fetus [65]. *In-utero* caffeine exposure has been linked to abnormal fetal growth through impacting normal development of the fetal hippocampus and the hypothalamic — pituitary — adrenal axis, which may lead to abnormal neuroendocrine changes. Children with low birth weight have a tendency to accumulate intra-abdominal fat mass [64]. Caffeine, a neural stimulant, can alter fetal brain development and impact normal neural transmission, which is important to normal brain function and metabolic processes [64].

Thus, the results of the studies and their meta-analyzes indicate a clinically significant influence of coffee consumption on the risk of low birth weight and childhood obesity.

Table 2. Summary of the safety of coffee consumption for the health of mother and child

Conditions	Consumption of coffee in an amount containing ≤ 200 mg of caffeine per day	
Risk of:	**Probably safe**	**Probably unsafe**
Infertility	X	
Bleeding		X
Pregnancy loss		X
Gestational diabetes	X	
Gestational hypertension	X	
Preeclampsia	X	
Preterm birth	X	
Low birth weight		X
Childhood obesity		X
Childhood acute leukemia		X

Childhood acute leukemia

We cannot fail to mention the reports indicating the influence of coffee consumption during pregnancy on the risk of childhood acute leukemia. A meta-analysis of 12 case-control studies by Thomopoulos et al. [67] found that high maternal coffee consumption was positively associated with acute lymphoblastic leukemia (OR = 1.43, 95% CI: 1.22-1.68) and acute myeloid leukemia (OR = 2.52; 95% CI: 1.59-3.57). Similar observations were made in the meta-analysis of 7 studies by Cheng et al. [68]. These authors found that compared with non/lowest drinkers, the combined odds ratio regarding the relationship of maternal coffee consumption during pregnancy and childhood acute leukemia was 1.22 (95% CI: 1.04–1.43) for ever drinkers, 1.16 (95% CI: 1.00–1.34) for low to moderate-level drinkers, and 1.72 (95% CI: 1.37–2.16) for high-level drinkers [68]. The Childhood Leukemia International Consortium (CLIC) analysis by Milne et al. [69], including 2,552 cases and 4,876 controls showed that the risk of childhood acute lymphoblastic leukemia for > 2 cups/day vs none was 1.27 (95% CI: 1.09–1.43, p = 0.005) [69]. Another CLIC study by Karalexi et al. [70], including 318 cases and 971 controls, also showed a positive association between increasing coffee intake and the risk of acute myeloid leukemia (> 1 cup per day; OR = 1.40; 95% CI: 1.03–1.92, increment of one cup per day; OR = 1.18; 95% CI: 1.01–1.39) [70]. However, it should be emphasized that not all studies have confirmed a relationship between coffee consumption during pregnancy and the risk of childhood acute leukemia. In a cohort study of 96 children by Madsen et al. [71], maternal coffee intake of 0.5–3 cups/day during pregnancy was not associated with a higher risk of childhood acute leukemia (RR = 0.89; 95% CI: 0.48–1.65), however, an intake of > 3 cups/day resulted in insignificant increase of this risk (RR = 1.37; 95% CI: 0.56–3.32) [71].

As in other cases, the mechanism underlying the effect of coffee consumption during pregnancy on the risk of childhood acute leukemia observed in some studies is unclear. It is indicated that high levels of caffeine may inhibit the activity of topoisomerase II, which is involved in gene transcription, DNA recombination, and replication. Moreover, caffeine has the capacity to inhibit some genes, such as the tumor suppressor gene *p53* and ataxia telangiectasia mutated gene which were associated with childhood acute leukemia [68].

Thus, the results of clinical trials and their meta-analyzes indicate that coffee consumption during pregnancy may increase the risk of childhood acute leukemia.

There are also reports that coffee consumption during pregnancy may increase the risk of autism spectrum disorder in offspring [72].

CONCLUSIONS AND LIMITATIONS

This literature review shows that coffee consumption during pregnancy is not completely safe (Tab. 2).

It should be emphasized that even consumption of the amount of coffee containing an acceptable daily dose of caffeine in some studies has turned out to be unsafe.

Of course, it should be remembered that most of the studies were carried out using self-reporting of the amount of coffee consumed during pregnancy, which means that the obtained results could be influenced by recall/respondent bias [73]. These are observational studies, the results of which do not allow for the determination of a cause-and-effect relationship [9]. Another factor limiting the strength of the evidence obtained is the capacity of the cup of coffee and the strength of the brew. What is a cup of coffee? 150 mL or 240 mL… What is a mug of coffee? 240 mL or 300 mL or maybe 360 mL. Similar concerns apply to the "strength of the brew", as well as to additions (*e.g.*, sugar, non-nutritive sweeteners, milk, cream) [9, 73]. An important limitation is also the fact that the influence of other risk factors, such as

smoking (smoking while pregnant increases the risk of childhood acute leukemia, and coffee drinkers are more likely to smoke) has not been analyzed [73]. Publication bias may also influence the obtained results. Despite many confounding factors, the results of clinical studies and their meta-analyzes presented in this paper should be taken seriously.

The authors of this study indicate that patients should certainly be asked about the amount of coffee and other caffeine-rich products consumed during pregnancy. You should actively look for women who clearly consume more caffeine than the currently accepted limit of 200 mg/day. This group of women should be particularly intensively educated and encouraged to reduce caffeine consumption. All women who decide to consume coffee during pregnancy should be educated about the possible risks associated with it. Taking into account the research results cited by us, a revision of the current recommendations regarding the safety of caffeine consumption during pregnancy should be expected.

Conflict of interest

None.

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VIA MEDICA

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Macro-TSH — tips and tricks for gynecologists

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ABSTRACT

Thyroid disorders are one of the most common endocrinopathies in women of reproductive age.

Measurement of TSH (thyroid-stimulating hormone) concentration in women planning pregnancy/pregnant is a golden standard of thyroid function assessment. When the laboratory findings do not correspond with the clinical signs, it is reasonable to mark macro-TSH.

Key words: macro-TSH; pregnancy; thyroid dysfunction

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CASE STUDY

Thyroid function disorders are among the most common diseases, and subclinical hypothyroidism affects up to 10% of the adult population [1].

There is evidence that thyroid dysfunction could be one of the reasons for female infertility, as well as obstetric complications [2]. That is the reason why thyroid function should be assessed by TSH concentration in every woman planning pregnancy.

According to the recommendations of the Polish Endocrine Society and American Thyroid Association, TSH concentration should be routinely determined, especially in the first trimester of pregnancy. This should also be the case in women planning pregnancy and in patients undergoing treatment for subfertility with a cut-off point of 2.5 mIU/L [2, 3].

A 27-year-old female after thyroidectomy (due to nodular goiter) in spite of constant levothyroxine supplementation was referred with persistently elevated TSH (> 100 mIU/L) measured by the of method Chemiluminescent Microparticle Immunoassay (Abbott, USA). The patient's complaints were unspecific and she appeared clinically euthyroid. The plasma levels of free thyroxine (T4) and free triiodothyronine (T3) were within the normal range, thyroid autoantibodies were negative, and thyroid ultrasonography did not present any known abnormalities. The gastro-intestinal examination had been previously carried out to eliminate any gastric reason of malabsorption.

In the diagnosis, we initially checked the levothyroxine absorption by measuring the free T4 concentration after a morning dose of 200 µg of levothyroxine which was followed by increasing fT4 levels. Then, we decided to check the sample for possible macro-TSH presence. As we hadn't the possibility of gel-filtration chromatography (GFC), which is the state-of-art method for detection of macro TSH, we checked TSH concentration after polyethylene glycol (PEG). The results are presented in Table 1.

DISCUSSION

Measurement of TSH concentration in women planning pregnancy/pregnant is a golden standard of thyroid function assessment. To prepare the patient for pregnancy we must be sure about the function of this gland. When the laboratory

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Table 1. Hormonal and antibodies data during hospitalization

Reference values for the laboratory	2 July 2021 at 8 am	2 July 2021 at 9 am	5 July 2021
TSH — 0.35–4.94 (mIU/L)	> 100		
fT4 — 0.7–1.48 (ng/dL)	0.81	0.90	
fT3 — 1.7–3.7 (pg/mL)	1.72	2.19	
T4 — 4.87–11.72 (µg/dL)	6.03	8.02	
T3 — 0.58–1–59 (ng/mL)	0.63	0.73	
A-TPO — 0–5.61 (IU/mL)	< 0.3		
A-TG — 0–4.11 (IU/mL)	0.61		
post-PEG TSH recovery (%)			10.7

A-TG — thyroglobulin antibodies; A-TPO — thyroid peroxidase antibodies; PEG — polyethylene glycol

findings (TSH elevated, free hormones in normal) do not correspond with the clinical signs and we are sure about the patient's compliance we must keep in mind possible macro-TSH presence.

As there is, unfortunately, no specific immunoassays which can reveal the presence of macro-TSH, we can adopt measurement of TSH after addition of PEG — as it is used in the diagnosis of macroprolactinoma [4]. This method is based on either the post-PEG recovery level of monomeric prolactin or the use of post-PEG monomeric prolactin reference intervals. Dilution studies might be helpful in identifying assay interference. In literature, the percentage of recovery calculation less than 20 or 25% is used [5]. Considering lower costs and higher accessibility than with GFC, such an approach may be a valid alternative for detection of macro-TSH [5].

Every gynecologist should be aware of its possible presence if considering patient planning pregnancy, undergoing levothyroxine treatment with good compliance and elevated TSH level.

Conflict of interest

All authors declare no conflict of interest.

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