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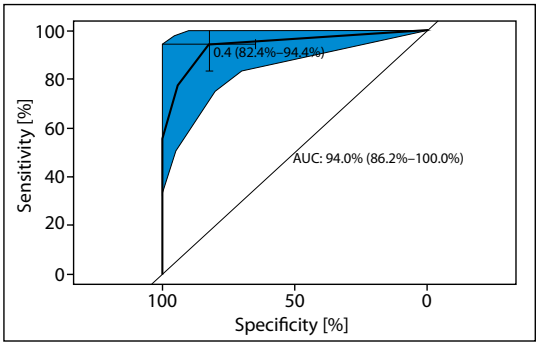

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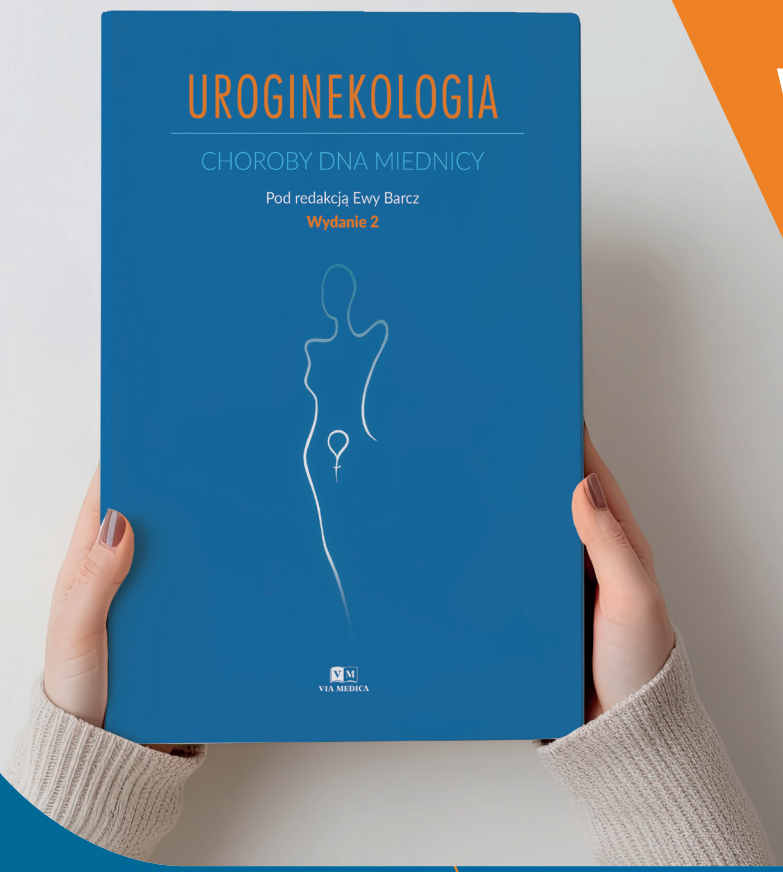

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Reproductive health of women and men with obesity: a global challenge in gynaecological and obstetric care within the KOS-BAR program

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Obesity has been a human health issue for centuries. Historically, it was viewed as a symbol of prosperity, wealth, and high social status. However, as civilization has progressed, obesity has become increasingly visible and dangerous, now considered an epidemic of the 20th and 21st centuries and classified as a "disease of civilization." The body mass index (BMI), calculated as weight in kilograms divided by height in meters squared (kg/m^2), is commonly used to assess body weight status. According to World Health Organization (WHO) guidelines for adults, BMI values are classified as follows: 25.0–29.99 for overweight, 30.0–34.99 for first-degree obesity, 35.0–39.99 for second-degree obesity, and above 40.0 for third-degree (so-called morbid obesity) [1, 2].

The primary cause of obesity is excessive caloric intake, but genetics also play a role, with around 40% of body fat variability attributed to genetic factors. Obesity contributes to approximately 7.1% of deaths globally, is a cause of disability in 1 in 20 patients, and is associated with a range of health complications over 200 have been identified so far. The most common complications include metabolic disorders such as type 2 diabetes, insulin resistance, and lipid imbalances, as well as hypertension, ischemic heart disease, obstructive sleep apnoea, osteoarthritis, depression, and an increased risk of various cancers [3–5]. Data from Poland after 2020 indicate that more than 65% of Poles are overweight or obese, with men comprising a majority of this group. Obesity affects 15.4% of men and 15.2% of women, with 0.5% of men and 0.4% of women classified as morbidly obese (BMI ≥ 40 kg/m^2) [6, 7]. It is estimated that around 5 million people in Poland suffer from obesity, including nearly 1.5 million with second-degree obesity and approximately 290,000 with third-degree (morbid obesity) [8]. According to estimates from the Supreme Audit Office (NIK) in 2022, over 9 million adults in Poland were affected by obesity, with direct healthcare costs for the condition reaching PLN 9 billion [9].

CLINICAL EFFECTS OF OBESITY ON INFERTILITY IN WOMEN

Obesity negatively impacts women's reproductive potential, primarily through functional changes in the hypothalamic-pituitary-ovarian (HPO) axis. Obese women often exhibit elevated blood insulin levels, which stimulate the production of ovarian androgens. These androgens, converted into oestrogen through excess adipose tissue, create a negative feedback loop on the HPO axis, disrupting gonadotropin production. This disruption leads to menstrual irregularities and ovulatory dysfunction. Hyperinsulinemia plays a key role in the pathogenesis of polycystic ovary syndrome (PCOS), which is characterized by oligomenorrhea and hyperandrogenism. Obesity stimulates insulin resistance and appears to worsen PCOS symptoms, with more severe cases frequently observed in obese women. Elevated androgen levels in PCOS contribute to visceral fat accumulation, furthering insulin resistance and hyperinsulinemia, which in turn stimulate additional production of ovarian and adrenal androgens creating a self-reinforcing cycle. The prevalence of PCOS among obese women may be as high as 30%, although a direct causal link between obesity and the development of PCOS has not been established. Obesity has also been associated with a longer time to conception. Two large cohort studies of Danish women attempting to conceive showed a decrease in fertility rates as body mass index (BMI) increased. Notably, obesity may reduce fertility even in the absence of ovulatory dysfunction. A study of over 7,000 U.S. women found reduced fertility among obese women with regular menstrual cycles, and data from a cohort of over 3,000 Dutch women with

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regular cycles indicated that the likelihood of spontaneous conception decreased with increasing BMI above 29 kg/m^2. Obesity also affects the outcomes of assisted reproductive technology (ART), suggesting that its impact on fertility extends beyond ovulatory issues. Obese women undergoing in vitro fertilization (IVF) tend to have smaller oocytes with lower fertilization success rates. Numerous studies have demonstrated a negative effect of obesity on live birth rates (LBR), with an inverse correlation between LBR and BMI. A review of ART outcomes in overweight and obese women found a slight reduction in LBR, with an average odds ratio of 0.90. However, a large study of women with class III obesity (BMI > 40 kg/m^2) observed a 50% reduction in live birth chances [10–19].

CLINICAL EFFECTS OF OBESITY ON INFERTILITY IN MEN

Obesity in men significantly impacts fertility, as demonstrated by numerous clinical and epidemiological studies. Men with excess body weight often have poorer semen parameters, including lower sperm concentration, reduced motility, and abnormal morphology. Adipose tissue produces reactive oxygen species that can damage sperm DNA, diminishing their fertilizing potential. Additionally, obesity disrupts the hormonal system-excess adipose tissue promotes oestrogen production via aromatization of androgens, while reducing testosterone levels. This imbalance leads to hypogonadism, which in turn results in poor semen quality and reduced libido. Increased body weight is also linked to higher scrotal temperatures, which can negatively impact spermatogenesis, as the testes are sensitive to even slight temperature increases. Furthermore, insulin resistance and elevated leptin levels, commonly observed in obese men, may disrupt the hormonal function of the hypothalamic–pituitary–gonadal axis, resulting in decreased testosterone production and impaired spermatogenesis. Research suggests that weight loss, a balanced diet, and regular physical activity can improve fertility in obese men. Studies of patients following bariatric surgery have shown improvements in testosterone levels and semen parameters in some cases, indicating that weight loss may benefit reproductive health. The complex effects of obesity on male fertility highlight the need for further research into effective strategies to enhance reproductive capacity in overweight men [20–23].

REPRODUCTIVE HEALTH AND BARIATRIC OBESITY TREATMENT

According to the WHO, sexual health is an essential component of overall human health. The WHO defines sexual health as "the integration of biological, emotional, intellectual, and social aspects of sexual life necessary for the positive development of personality, communication, and love" [24]. Sexual dysfunctions are common among individuals with overweight and obesity, with studies indicating significantly poorer sexual functioning compared to individuals with normal weight. This includes reduced libido, painful intercourse in women, and erectile and ejaculatory disorders in men. Sexual dysfunction often accompanies obesity-related conditions: hypertension is frequently associated with lowered libido and erectile and ejaculatory issues, while diabetes can lead to painful intercourse, vaginal dryness, and decreased libido. Additionally, excess weight reduces physical fitness and endurance, leading to rapid fatigue, which further contributes to reduced libido and satisfaction with sexual activity [22–25].

In the context of the global obesity epidemic, bariatric surgery has gained recognition as the only treatment method that offers sustainable weight loss while improving metabolic health and overall quality of life. Bariatric surgery is a medical specialty focused on treating severe obesity and includes comprehensive interventions, such as conservative therapy (developing personalized dietary plans, teaching proper nutrition, and self-monitoring), non-surgical methods (*e.g.*, gastric balloon implantation), as well as plastic surgery and gastrointestinal surgery [8].

The KOS-BAR program was established in response to the growing challenge of morbid obesity, which presents significant medical and socioeconomic issues. In Poland, the program was introduced by the Regulation of the Minister of Health on August 12, 2021, establishing a pilot initiative for comprehensive specialist care for patients with morbid obesity, known as KOS-BAR (Journal of Laws 2021, item 1622). The program provides coordinated, comprehensive care before and after surgery at designated centres. It is available to patients aged 18 and older with an ICD-10 diagnosis of E66, indicating obesity due to excessive caloric intake. To join, patients must register a referral to the General Surgery Clinic with this diagnosis. Eligibility is determined by a bariatric surgeon based on the following criteria: (1) a body mass index (BMI) of ≥ 40 kg/m^2, or (2) a BMI of 35–40 kg/m^2 in patients for whom weight loss could improve obesity-related conditions such as type 2 diabetes, hypertension, cardiovascular diseases, sleep apnoea, joint diseases requiring surgery, non-alcoholic steatohepatitis, hyperlipidaemia, or female infertility, including infertility related to polycystic ovary syndrome [26].

SUMMARY

Clinical studies clearly show that obesity increases the risk of subfertility, affecting not only natural fertility but also responses to assisted reproductive technologies (ART). Prevention and treatment of obesity are therefore essential components of reproductive health care and play a crucial

role in developing therapeutic strategies for individuals facing fertility challenges. A comprehensive approach including lifestyle modifications, psychological support, and, when appropriate, surgical interventions such as bariatric surgery can significantly improve reproductive health outcomes in patients with obesity.

Article information and declarations

Author contributions

K. Zborowska — review of the literature on the impact of obesity on reproductive health and text editing; D. Jorg — review of the literature on obesity and the KOS-BAR program; Lukasz Wicherek — manuscript, substantive evaluation

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. World Health Organization (WHO) 2024. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. (7.10.2024)
2. World Health Organization (WHO) Global Database on Body Mass Index. http://apps.who.int/bmi/index.jsp?intropage=intro_3.html. (7.10.2024)
3. Pierzak-Stępień M. Role of surgical treatment for obesity in weight reduction and its effect on comorbidities. Med Og Nauk Zdr. 2024; 30(1): 7–13, doi: 10.26444/monz/183053.
4. Bogdański P, Mamcarz A, Ostrowska L. Otyłość i jej powikłania: praktyczne zalecenia diagnostyczne i terapeutyczne. PZWL Wydawnictwo Lekarskie 2022.
5. GBD Spinal Cord Injuries Collaborators, GBD 2019 Meningitis Antimicrobial Resistance Collaborators, GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020; 396(10258): 1223–1249, doi: 10.1016/S0140-6736(20)30752-2, indexed in Pubmed: 33069327.
6. Profilaktyka i leczenie otyłości u osób dorosłych. Delegatura NIK w Rzeszowie, LRZ.40. 430 3 2023 Nr ewid 99/2023/P/23/080/LRZ Rzeszów; 2023.
7. Bąk-Sosnowska M, Białkowska M, Bogdański P, et al. Zalecenia kliniczne dotyczące postępowania u chorych na otyłość 2022 — stanowisko Polskiego Towarzystwa Leczenia Otyłości. Med Prakt 2022; maj: 1–87.
8. Wyleżoł M. Chirurgia bariatryczna. In: Ostrowska L, Bogdański P, Mamcarz A. ed. Otyłość i jej powikłania. Praktyczne zalecenia diagnostyczne i terapeutyczne. PZWL 2021.
9. NIK (2023). Informacja o wynikach kontroli Profilaktyka i leczenie otyłości u osób dorosłych. NIK Delegatura w Rzeszowie. https://www.nik.gov.pl/plik/id,28874,vp,31705.pdf. (7.10.2024)
10. Bomba-Opon D, Gutaj P, Kedzia M, et al. Guidelines of the Polish Society of Gynecologists and Obstetricians on the obstetric care of women with obesity. Ginekol Pol. 2023; 94(12): 1011–1029, doi: 10.5603/gpl.97361, indexed in Pubmed: 38099668.
11. Fuchs A, Dulska A, Drosdzol-Cop A. Is weight just a number? Relationship between overweight, obesity and domains of sexual functioning among young women. Ginekol Pol. 2020; 91(10): 595–599, doi: 10.5603/GP.2020.0162, indexed in Pubmed: 33184827.
12. Ostrowska L, Lech M, Stefańska E, et al. The use of contraception for patients after bariatric surgery. Ginekol Pol. 2016; 87(8): 591–593, doi: 10.5603/GP.2016.0050, indexed in Pubmed: 27629135.
13. Stupak A, Geca T, Krzyzanowski A, et al. Obesity — a still underestimated risk factor during antenatal corticosteroids therapy. Ginekol Pol. 2022 [Epub ahead of print], doi: 10.5603/GP.a2022.0132, indexed in Pubmed: 36448344.
14. Misan N, Korszun P, Gruca-Stryjak K, et al. Does predelivery body mass index really matter in pregnancy? Ginekol Pol. 2022; 93(11): 922–929, doi: 10.5603/GP.a2022.0005, indexed in Pubmed: 35325456.
15. Radzicka-Mularczyk SA, Pietryga M, Brazert J. How mother's obesity may affect the pregnancy and offspring. Ginekol Pol. 2020; 91(12): 769–772, doi: 10.5603/GP.2020.0116, indexed in Pubmed: 33447997.
16. Fuchs A, Dulska A, Drosdzol-Cop A. Is weight just a number? Relationship between overweight, obesity and domains of sexual functioning among young women. Ginekol Pol. 2020; 91(10): 595–599, doi: 10.5603/GP.2020.0162, indexed in Pubmed: 33184827.
17. Jakubowska-Kowal KM, Skrzynska KJ, Gawlik-Starzyk AM. Prevalence and diagnosis of polycystic ovary syndrome (PCOS) in adolescents — what's new in 2023? Systematic review. Ginekol Pol. 2024; 95(8): 643–649, doi: 10.5603/gpl.98849, indexed in Pubmed: 39140356.
18. Lin M, Xu H, Qiu J. Inflammation in recurrent miscarriage — a comprehensive perspective from uterine microenvironment and immune cell imbalance to therapeutic strategies. Ginekol Pol. 2024; 95(4): 266–275, doi: 10.5603/gpl.97320, indexed in Pubmed: 38334340.
19. Czech I, Partyka M, Drosdzol-Cop A. Does body mass index affect anti-mullerian hormone levels in girls and adolescents? Ginekol Pol. 2023 [Epub ahead of print], doi: 10.5603/GP.a2023.0013, indexed in Pubmed: 36929787.
20. Leisegang K, Sengupta P, Agarwal A, et al. Obesity and male infertility: Mechanisms and management. Andrologia. 2021; 53(1): e13617, doi: 10.1111/and.13617, indexed in Pubmed: 32399992.
21. Carson S, Kallen A. Diagnosis and Management of Infertility. JAMA. 2021; 326(1): 65, doi: 10.1001/jama.2021.4788.
22. Kahn BE, Brannigan RE. Obesity and male infertility. Curr Opin Urol. 2017; 27(5): 441–445, doi: 10.1097/MOU.0000000000000417, indexed in Pubmed: 28661897.
23. Eisenberg ML, Esteves SC, Lamb DJ, et al. Male infertility. Nat Rev Dis Primers. 2023; 9(1): 49, doi: 10.1038/s41572-023-00459-w, indexed in Pubmed: 37709866.
24. WHO: Education and treatment in human sexuality: the training of health professionals. World Health Organization, Geneva.
25. Zborowska K, Skrzypulec-Plinta V. Otyłość a seksualność. In: Olszanecka-Glinianowicz M. ed. Obesitologia kliniczna. Alfa-Medica Press, Bielsko-Biała .
26. Rozporządzenie Ministra Zdrowia z dnia 12 sierpnia 2021 r. w sprawie programu pilotażowego w zakresie kompleksowej opieki specjalistycznej nad świadczeniobiorcami leczonymi z powodu otyłości olbrzymiej KOS-BAR (Dz.U. 2021 poz. 1622 i Dz.U. 2022 poz. 1517).

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Level of IGF1 in follicular fluid associated with *in vitro* fertilization pregnancy outcome in the application of growth hormone

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ABSTRACT

Objectives: The combination of growth hormone (GH) with gonadotropin was a prevalent method to improve clinical reproduction in adjuvant for assisted reproduction treatment (ART). However, the contradictory results from previous studies failed to confirm the benefits. The present study is focused on the mechanism analysis of GH-IGF1-gonadal axis in ART and the changes of IGF1 in follicular fluid among different types of patients.

Material and methods: We recruited 136 patients and divided them into eight groups according to their ages and ovarian reserves. The baseline characteristics of the study population were summarized. The therapeutic outcomes in the study population were observed. In the meantime, concentrations of IGF1 in follicular fluids from different types of patients who underwent GH strategy were measured by Western blot. The functional mechanism of GH-IGF1-gonadal axis in ART was also analyzed.

Results: We analyzed the baseline characteristics of the study population, the therapeutic outcome of GH-IGF-1-gonadal axis, as well as the relative protein level of IGF1 and IGFBP1 in follicular fluid from different groups. The chemical pregnancy rate was significantly increased in different degrees for groups with GH co-treatment compared to groups without GH co-treatment. The IGF1 in follicular fluid of patients under 35 years' old showed an upward trend compared with groups of poor, normal and high ovarian reserves. After GH induction, IGF1 in follicular fluid was significantly increased in patients over 35 years old.

Conclusions: The study suggested that the application of GH might be beneficial to the pregnancy outcome in patients. GH application in patients older than 35 years might have a beneficial effect on pregnancy outcome via promoting the expression of IGF1. Our study indicates a different mechanism from GH application among younger and older patient in ART and provides a new clue for individual clinical treatment in infernity patients.

Keywords: growth hormone, IGF-1, follicular fluid, in vitro fertilization

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INTRODUCTION

In recent years, the incidence of female infertility among adult women has been rising all over the world [1]. The age-related fertility decline in the older women makes more and more families unable to achieve the desire of re-pregnancy and childbirth [2]. At present, assisted reproduction treatment (ART) based on *in vitro* fertilization (IVF) and embryo transfer (ET) has been considered as the most helpful method for female infertility. However, it can only ensure 1/3 of women get pregnant successfully, which cannot meet the urgent needs of the majority [3].

Previous studies have shown that patients with GH deficiency experienced reduced fertility rates based on IVF technology [4], while GH supplementation can successfully complete assisted reproduction [5]. Some researchers have indicated that co-treatment of gonadotropin and GH demonstrated significant improvement of the pregnancy, implantation and live birth rates in the patient with poor ovarian responders [6]. However, there is still great controversy in the application of GH in IVF, as well as the inconsistent meta-analysis in recent years [7, 8]. Further studies are urgently needed to gap the bridge between theoretical

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analysis and clinical application for the co-treatment of ART and GH.

GH-Insulin like growth factor 1 (IGF-1), also called somatomedin C, is a hormone with similar molecular structure to insulin, which plays an important role in pregnancy. It has been well established that IGF-1/gonadal axis exerts essential functions in follicular development, ovarian response and ovulation [9]. In the circulation, IGFs are bound to binding proteins (IGFBPs) that can prolong the half-life and modulate its bioavailability. In the ovary, IGFs are released by ovarian granulosa cells. Local IGFs exert function via IGF receptors to regulate normal follicular growth and development and raise the ovary sensitivity to the follicle-stimulating hormone (FSH) [10]. Thus, the effects of GH on ovarian response may synergistically function as direct regulation and indirect stimulation of IGF1 synthesis.

Based on the potential synergistic effect of GH and IGF1 in IVF clinical output, we speculated that the difference in the clinical output of patients with different ages and ovarian reserves might be related to the concentration of IGF1 in ovaries. The identification of this speculation may provide valuable information for the explanation of the different clinical output of GH co-treatment strategies in ART. In this study, we were focused on the exploration of the role of GH-IGF1-gonadal axis in ART.

MATERIAL AND METHODS

Study population

The current research was performed in a single-blinded clinical trial, including 136 patients for IVF who referred to the Reproductive Center of The Hunan Maternal and Child Health Care Hospital, between June 2013 to June 2014. The patients were evaluated at the beginning and then assigned into eight groups. The study was approved by the Hunan Provincial Maternal and Children Health Hospital Ethics Committee. Written consents were obtained from the patients. The ethics approval number was provided (Identify Number: 2014005) by the ethics committee in 2014. The experiments were carried out according to guidance from Helsinki Declaration [11].

Patients were considered as eligible when they met the following criteria: (1) the causes of infertility are primarily due to fallopian tube malfunction or male sterility; (2) age between 20 and 45 years; (3) normal uterine cavity with regular spontaneous menstrual cycles of 25–30 days; and (4) FSH, luteinizing hormone (LH), and estradiol concentrations in the normal range during the early follicular phase. The exclusion criteria were as follows: (1) malignant tumor; (2) serious pelvic adhesions or hydrosalpinx; (3) endocrine disorder; (4) recurrent spontaneous abortion, (5) considering the side effect of GH, patients who got positive results in OGTT were excluded, (6) patients who required fertilization by ICSI (single sperm microinjection) were excluded. In our study, we firstly recruited 136 patients. However, we found two women had high FSH levels (1 more than 20 IU/L, and 1 more than 15 IU/L), 4 were OGTT positive, and two gave up. They were excluded.

One hundred twenty-eight patients with ages less than 35 were classified into (1) poor, (2) normal and (3) high ovarian reserve groups according to their baseline FSH, FSH/LH ratio, anti-Müllerian hormone (AMH), and antral follicle count (AFC). For those patients, 36 patients who were identified as poor ovarian reserve were further divided into group A and group B randomly. Thirty-two patients who were identified as normal ovarian reserve were divided into group C and D randomly. Thirty patients who were identified as high ovarian reserve were set into groups E and F randomly. Thirty patients with ages over 35 were randomly categorized into groups G and H. All the randomization work was done by a computerized random sampling table, with consideration on patient blindness. Finally, 15, 15, 15, 15, 18, 18, 16, 16 patients were distributed into group A, B, C, D, E, F, G and H, respectively.

Study intervention

The patients in all groups received a one-time gonadotropin-releasing hormone (GnRH) agonist injection of triptorelin acetate (Diphereline, 3.75 mg/bottle, Ipsen Pharma Biotech, France) for long-term pituitary down-regulation on day 15 of the preceding oral contraception pill cycle. GnRH-agonist dose ranged from 1.25 mg to 1.875 mg depending on the patients' body weights. On day 2–5 of the next menstrual cycle, pituitary down-regulation was confirmed by an ultrasound scanned endometrial thickness (less than 5 mm), as well as the serum FSH and LH levels (less than 5 mIU/mL) and E2 level (less than 50 pg/mL).

RFSH was given from the time when the downregulation is successful indicated in the above. The recovery of follicles was monitored. When the diameter of three or more follicles reached 7–9 mm, rFSH was given with doses varies from 150 to 300 IU depending on individual ovarian responses and reserves. The rFSH dose was kept unchanged throughout the ovarian stimulation. If it is very necessary, the dose could be reduced by about 1/3 in the last few days, and the reduction was maintained for 2–3 days each time. In addition to common regimens, groups B, D, F, H received 6 IU daily r-GH (Ansomon, Anke Co. LTD., Anhui, China) subcutaneously from the first day of gonadotropin (Gn) stimulation for 10 days. Group A, C, E, G received 10 days' placebo (normal saline, 0.1 mg/day) from the first day of Gn stimulation for 10 days, subcutaneously. 0.25 mg Ovidrel (Merck Serono, Germany) was injected as the final trigger when dominant follicles reached 18 mm in diameter.

Ultrasound-guided oocyte retrieval was performed at 36 h after the trigger.

Sixty mg per day of progesterone was started intramuscularly from the day of oocyte retrieval until 14 days after embryo transfer, together with 200 mg oral progesterone capsules (Yimaxin, Xianju Pharmaceutical Co. LTD., Zhangjiang, China). Chemical pregnancy was confirmed with serum HCG > 40 IU/L at day 14 after embryo transfer. Clinical pregnancy was confirmed with foteal heart activity that was observed under transvaginal ultrasonography at 4–5 weeks after embryo transfer and positive HCG indication. Progestin support continued up to 10–12 weeks' gestation if the pregnancy was achieved. After embryo transfer, we performed the measurement of total dosage and duration of gonadotropin usage, endometrial thickness, numbers of metaphase II oocyte, numbers of transferred embryos, and rates of early miscarriage, implantation, and clinical pregnancy.

Assisted reproduction technique

Oocytes were retrieved under vaginal ultrasonography guidance at 36 hours after r-HCG administrations and fertilized by traditional IVF procedures. During retrieval, 10 mL follicular fluid was collected, frozen, and sent for analysis. Granulosa cell and corona radiata of cumulus oophorous were taken off. We assessed the maturity of the ova and found that the ova were naturally fertilized. After that, the zygotes were incubated for 18 hours in IVF nutrient solution at 37 Celsius with 5% CO_2. We observed the fertilization status at 24 hours and refreshed the nutrient solution. Embryo's evaluation was made on the 3rd day after retrieval using Peter Score System. 1 or 2 embryos were transferred on the 3rd day at cleavage stage to the uterine cavity.

Evaluations of embryos and zygotes were made by the standards previously reported by Tesarik et al. [12]. The morphology of the cleavage embryos was observed on the 2nd and 3rd day based on the number of fragmentations, equality, mono nuclearity, and early compaction. Patients in 8 groups were assessed in terms of collected oocytes, MII oocytes, fertilized oocytes, the number of transferred oocytes, and chemical or clinical pregnancy.

Follicular fluid testing

We measured the concentration of IGF1 in follicular fluid of different types of patients who underwent GH strategy and analyzed the functional mechanism of GH-IGF1-gonadal axis in ART. Based on the randomized and double-blind principle, we collected the follicular fluid of the above eight groups. During retrieval, 10 mL follicular fluid was collected, frozen, and sent for analysis. Western blot was utilized to measure the levels of IGF1 and IGFBP1 in the follicular fluid of the patients. Image J (NIH software) was utilized to analyze the intensity of the western blot bands. Among the 128 patients, only 70 patients agreed to perform this test. Thus, 70 sets of western blot results were obtained for eight groups, with their number recorded in the experimental record sheet. Finally, there were 15, 13, 14, 12, 7, 2, 3, 4 samples in groups A, B, C, D, E, F, G, and H, respectively.

Statistical analysis

In this study, the data were expressed as mean ± standard deviation (SD). The results were summarized utilizing absolute frequency and percentage for the categorical variable. Data normalization was performed utilizing Kolmogorov-Smirnoff tests. The differences among different groups were compared by one-way ANOVA or Kruskal–Wallis H test. SPSS (16.0) was utilized for statistical analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

One hundred twenty-eight patients were divided into eight groups according to their age and ovarian reserves. Table 1 showed the baseline characteristics of the study population. We investigated the mean age for female and male, BMI, number of IVF, level of AMH, antral follicle counts, and duration of infertility in the groups from A to H. The classifications are in the following: group A represented patients with poor responses (age: < 35 years' old); group B represented patients with poor responses treated with growth hormone (age: < 35 years' old); group C represented patients with normal responses (age: < 35 years); group D represented patients with normal responses treated with growth hormone (age: < 35 years); group E represented patients with high responses (age: < 35 years); group F represented patients with high responses treated with growth hormone (age: < 35 years); group G represented infertile women of advanced age (age: > 35 years); and group H represented infertile women of advanced age treated with growth hormone (age: > 35 years). From Table 1, we could find that the baseline characteristics of each group showed no significant difference ($p > 0.05$), including mean age, body mass index, the number of IVF procedure, the level of AMH, FSH, and LH hormones, antral follicle counts, and duration of infertility.

Then, we investigated the therapeutic outcome in the study population, including the number of collected oocytes, number of MII oocytes, number of fertilized oocytes, number of transferred embryos, chemical pregnancy percentage, IVF rate, cleavage rate, high-quality embryo rate, embryo implantation rate, and pregnancy rate. Table 2 revealed that the chemical pregnancy rate was significantly increased in different degrees for groups with GH co-treatment compared to groups without GH co-treatment. In patients with poor responses (age: < 35 years'

Table 1. Baseline characteristics of the study population

Index	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
Mean age, female	28.83 ± 3.72	29.33 ± 2.19	27.81 ± 3.17	29.00 ± 2.63	28.00 ± 2.83	28.73 ± 3.07	38.67 ± 3.46	37.8 ± 2.48
Mean age, male	32.03 ± 5.28	34.32 ± 5.33	32.01 ± 4.47	33.94 ± 5.10	32.77 ± 4.87	33.39 ± 5.19	41.58 ± 2.97	41.73 ± 2.26
BMI [kg/m^2]	22.66 ± 2.87	22.58 ± 2.73	23.03 ± 3.17	23.30 ± 3.26	25.05 ± 5.34	25.13 ± 4.75	24.83 ± 2.22	25.11 ± 3.09
Number of IVF	2.63 ± 1.81	2.28 ± 1.99	2..48 ± 1.99	2.57 ± 1.91	2.39 ± 1.80	2.56 ± 1.63	2.86 ± 1.97	2.93 ± 2.05
Level of AMH	1.08 ± 0.42	0.97 ± 0.51	2.48 ± 0.55*	2.73 ± 0.64*	5.31 ± 1.22*,#	6.03 ± 1.76*,#	1.52 ± 0.61	1.48 ± 0.62
Antral follicle counts	5.46 ± 4.88	7.37 ± 2.59	16.04 ± 4.21*	15.98 ± 5.13*	18.23 ± 8.76*	14.32 ± 5,16*	5.97 ± 4.82	8.18 ± 6.11
Duration of infertility	4.39 ± 2.69	4.67 ± 2.62	2.88 ± 1.63	4.07 ± 1.91	2.75 ± 1.64	2.47 ± 1.45	4.93 ± 3.28	6.07 ± 4.86

There was no statistical significance between A and B, C and D, E and F, and G and H groups; *p < 0.05, vs A or B; #p < 0.05, vs C or D. Group A: patients with poor responses (age < 35 years' old); Group B: patients with poor responses treated with growth hormone (age < 35 years' old); Group C: patients with normal responses (age < 35 years); Group D: patients with normal responses treated with growth hormone (age < 35 years); Group E: patients with high responses (age < 35 years); Group F: patients with high responses treated with growth hormone (age < 35 years); Group G: infertile women of advanced age (age > 35 years); and Group H: infertile women of advanced age treated with growth hormone (age > 35 years); AMH — anti-Mullerian hormone, BMI — body mass index; IVF — in vitro fertilization

Table 2. The therapeutic outcome in the study population

Index	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
No. of collected oocytes	5.61 ± 4.56	7.72 ± 2.35	14.44 ± 3.28	15.87 ± 6.38	17.19 ± 8.62	13.13 ± 4.62	6.27 ± 5.22	8.80 ± 7.77
No. of MII oocytes	5.61 ± 4.56	7.56 ± 2.39	14.06 ± 3.29	14.87 ± 5.87	14.19 ± 7.38	12.23 ± 4.92	6.07 ± 5.01	8.60 ± 7.59
No. of fertilized oocytes	4.33 ± 3.37	7.28 ± 2.23*	12.13 ± 4.43	12.2 ± 6.42	13.44 ± 7.27	12.54 ± 5.18	5.67 ± 4.83	7.93 ± 7.08
No. of transferred embryos	1.39 ± 1.01	1.72 ± 0.65	1.88 ± 0.48	1.60 ± 0.80	1.50 ± 0.87	2.20 ± 0.40*	1.33 ± 1.14	1.73 ± 1.18
Chemical pregnancy, %	5 (42%)	11 (69%)	7 (46%)	11 (92%)	8 (67%)	12 (80%)	7 (33%)	7 (64%)
IVF rate	0.95 ± 0.09	0.82 ± 0.20*	0.84 ± 0.22	0.79 ± 0.31	0.84 ± 0.26	0.93 ± 0.12	0.93 ± 0.12	0.91 ± 0.15
Cleavage rate	0.94 ± 0.09	0.99 ± 0.03	0.98 ± 0.04	0.96 ± 0.05	0.97 ± 0.05	0.97 ± 0.06	0.97 ± 0.08	0.99 ± 0.04
High quality embryo rate	0.51 ± 0.30	0.35 ± 0.31	0.42 ± 0.28	0.45 ± 0.25	0.29 ± 0.26	0.58 ± 0.32*	0.30 ± 0.25	0.22 ± 0.22
Embryo implantation rate	0.44 ± 0.39	0.21 ± 0.25	0.30 ± 0.40	0.54 ± 0.32	0.38 ± 0.30	0.63 ± 0.41*	0.34 ± 0.64	0.29 ± 0.36
Pregnancy rate	0.63 ± 0.48	0.42 ± 0.49	0.40 ± 0.49	0.83 ± 0.37*	0.67 ± 0.47	0.80 ± 0.40	0.33 ± 0.47	0.47 ± 0.50

*p < 0.05 for group A vs B; C vs D; E vs F; G vs H; IVF — *in vitro* fertilization

old), GH significantly improved the number of fertilized oocytes. However, no significant difference was observed in normal ovarian reverse group, including the number of collected follicles, the number of MII oocytes, fertilization rate, cleavage rate, the number of transfer embryos, high-quality embryo rate and implantation rate. In high response population, high-quality embryo rates and embryo implantation rates were significantly increased. For patients over 35 years' old, the chemical pregnancy rate has been improved, but no significant difference was observed in all other data (Tab. 2).

Based on the randomized and double-blind principle, we collected the follicular fluid of the above eight groups. Western blot was utilized to measure the levels of IGF1 and IGFBP1 in the follicular fluid of the patients (Fig. 1). As only 70 patients agreed to perform this test, we obtained 70 sets of western blot results for 8 groups. Figure 1 listed all the Western blot bands from 70 patients. Table 3 summarized the quantitative results after gray-scale analysis. The results demonstrated that the IGF1 in follicular fluid of patients under 35 years' old showed an upward trend compared with groups of poor, normal and high ovarian reserves. However, there is no significant difference among poor, normal and high ovarian reserves groups. The level of IGF1 in patients' follicular fluid over 35 years' old was significantly decreased compared with that of patients under 35 years' old. There was no significant difference in IGFBP1 among each group. After GH induction, IGF1 in follicular fluid was significantly increased in patients over 35 years old, but there was no significant changed in other groups.

DISCUSSION

Growth hormone has been applied to improve clinical reproduction in ART for more than 30 years [13, 14]. However, the contradictory result from different researchers failed to confirm the benefits in terms of live birth rates with the use of adjuvant GH. The differences in clinical outcomes from GH application among different researchers may be related to individual differences in the complexity of GH-Insulin like Growth Factor (IGF)-1-gonadal axis. The fundamental theories of GH co-treatment remain to be further elucidated.

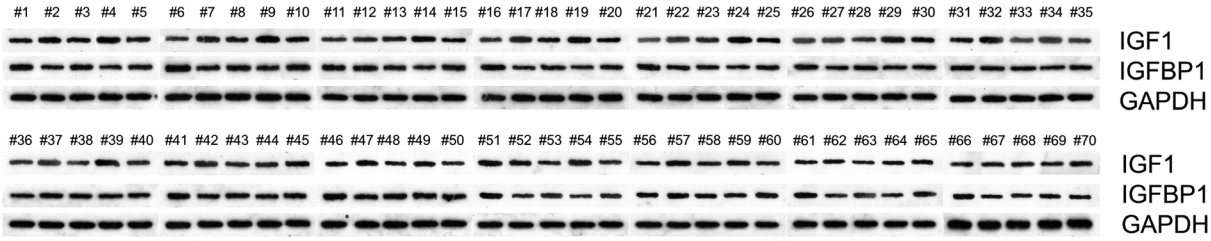

Figure 1. Protein level of IGF1 and IGFBP1 in follicular fluid; #2, #3, #7, #12, #17, #22, #32, #37, #42, #48, #52, #57, #58, #62, #67 were from group A; #8, #13, #18, #23, #27, #28, #33, #38, #43, #47, #53, #63, #68 were from group B; #69, #4, #5, #9, #14, #29, #35, #39, #40, #44, #49, #54, #59, #64 were from group C; #10, #15, #19, #20, #24, #34, #45, #50, #55, #60, #65, #70 were from group D; #31, #16, #21, #25, #30, #41, #56 were from group E; #51, #66 were from group F; #6, #36, #61 were from group G; #1, #11, #26, #46 were from group H

Table 3. Relative protein level of IGF1 and IGFBP1 in follicular fluid

Index	IGF1/GAPDH	IGFBP1/GAPDH
Group A	0.503 ± 0.062	0.611 ± 0.018
Group B	0.480 ± 0.067	0.674 ± 0.123
Group C	0.526 ± 0.022	0.682 ± 0.066
Group D	0.493 ± 0.054	0.710 ± 0.132
Group E	0.557 ± 0.091	0.570 ± 0.098
Group F	0.573 ± 0.114	0.544 ± 0.137
Group G	0.474 ± 0.073#	0.579 ± 0.087
Group H	0.578 ± 0.082*	0.544 ± 0.118

*p < 0.05 for Group H vs Group G, #p < 0.05 for Group E vs Group G

Our study focused on the levels of follicular fluid from different types of patients. We found that the level of IGF1 showed a decreasing trend from high-response patients to poor-response patients. A significant downregulation was observed in the older patients over 35 years' old in contrast with patients less than 35 years' old. After GH stimulation, different susceptibility was revealed in different types of patients. GH seems to have no upregulation effect in the level of IGF1 in follicular fluid from patients below age 35. However, a significant increase in IGF1 was found in patients over age 35. It indicated that there existed different functional mechanisms underlying younger and older patients in ART. Our study gave a reasonable explanation for previous contradictory findings and provided new clues for individual clinical treatment in ART.

The human genetic deficiency and animal model shed light on the roles in follicular development, ovarian response, and ovulation. Both GHRH or GH mutations in humans lead to puberty delayed and fertility declined. GHR knockout mice showed a decrease in the number of healthy and growing antral or pre-ovulatory follicles [10], which demonstrated that GH is necessary for optimal follicular maturation and survival. Previous studies have established that both in vivo and in vitro administration of GH could increase ovarian weight, follicular size, and promote human oocyte retrieval and fertilization rate [10]. Furthermore, GH was reported to improve the endometrial receptivity by increasing endometrial blood flow and cytokines release [15]. As a result, it is reasonable to conclude that GH could advance clinical reproduction. In this study, we found that co-treatment with GH in poor ovarian reserves could improve the chemical pregnancy rate, the number of fertilized oocytes and IVF rate. For older patient aged over 35, the increase of chemical pregnancy rate was confirmed, which is consistent with previous studies [12–14]. There are still some deviations in experimental results, which may be due to different treatment schemes. The oocyte quality decline with age could be resulted from the fact that functional mitochondria decrease led to impaired separation of chromosome. GH can also improve the mitochondria activity other than promoting proliferation and inhibiting apoptosis [16]. This could explain why GH promote chemical pregnancy rate in older patient.

Except for direct role in the oocyte, GH indirectly induces ovarian granulosa and thecal cells release IGF1, which raises the ovary sensitivity to gonadotropin [15]. IGF1 locally exerts the role in resuming meiosis of the oocytes via paracrine and autocrine modes, including DNA synthesis, steroidogenesis, aromatase activity, LH receptor synthesis, and inhibin secretion [17]. In synergy with FSH, IGF1 is considered to mediate growth-promoting actions of growth hormone. IGF1 is required for GH to stimulate oocyte maturation. In our study, the level of IGF1 in follicular fluid showed a decreasing trend from high response patients to poor response patients. Significant downregulation of IGF1 was found in older patients. It indicated that the baseline of IGF1 might be related to ovarian response and clinical outcome. GH seems to have the effects of selective upregulation of IGF1 in older patients rather than younger patients. This may result from the different responses from normally and highly reactive patients. The high background level of IGF1 may saturate the GH effect. However, IGF1 cannot completely mediate the GH function. There might be an unknown mechanism underlying the resistance in the effect of GH for PORs. IGF1 knockout

mice do not phenocopy the mutants with loss of GH and GHR. IGF1 cannot rescue the ovary deficiency caused by GHR mutants [18]. Loss of IGF1 in mice results in absence of antral follicles, infertility and fails to ovulate either spontaneously or under the influence of gonadotropins.

Different from GH, IGF1 plays a crucial role in the progression of the follicles from the non-gonadotropin sensitive to the gonadotropin sensitive stages [16]. It could be concluded that GH and IGF1 may synergistically function locally in follicular development. Our data show that the level of IGFBP1 in follicular fluid among each group did not change, suggesting that IGF1 is a local source rather than a circulation one. As IGF1 could bind with IGFBP1, lower IGF1 levels could increase the level of IGFBP1 in a certain time. In Table 3, we also found that IGF1 levels decreased, while IGFBP1 increased for group B compared with group A, and group D compared with group C. Follicular development is characterized by the proliferation and differentiation of the oocytes and granulosa cells. This process requires precise interaction between oocytes and granulosa cells. Through paracrine and autocrine ways, the balance of niche growth factors and cytokine steroids in follicles can be maintained. The good effect of granulosa cells on GH may help the older patients to achieve good clinical output in assisted reproduction.

Our study indicates a different mechanism underlying younger and older patients in ART and provides a new clue for individual clinical treatment in ART. However, as a limitation, our study did not identify the reason why GH did not regulate IGF1 in follicular fluid in POR patients but provided good clinical output. Further studies will be needed to investigate their inner associations and confirm the reasons and possible clinical outcomes. Due to space limitations and time constraints, this article did not include the plasma IGF1 experiments on this aspect. We will include the comparison between plasma IFG1 and IGF1 in the follicular fluid in the future research.

CONCLUSIONS

The study revealed that the chemical pregnancy rate was significantly increased in different degrees for groups with GH co-treatment compared to groups without GH co-treatment, suggesting that the application of GH may be beneficial to the pregnancy outcome in patients. The effects of GH in patients under 35 years of age might not be related to the expression of IGF1 and IGFBP1. GH application in patients older than 35 years might have a beneficial effect on pregnancy outcome via promoting the expression of IGF1. Our study indicates a different mechanism from GH application among younger and older patients in ART and provides a new clue for individual clinical treatment in ART.

Ethics approval and consent to participate

The study was approved by the Hunan Provincial Maternal and Children Health Hospital Ethics Committee. Written consents were obtained from the patients. The ethics approval number was provided (Identify Number: 2014005) by the ethics committee in 2014.

Conflict of interest

The authors declare that there is no conflict of interest.

Consent for publication

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Data availability statement

The authors confirm that all data underlying the findings are available. All relevant data are within the paper and its Supporting Information files.

Author Contributions

Luo Man performed the experiment and analyzed the data; Yuanyuan Chen, Nannan Li, Yan Luo and Xiangyang Pan performed the experiment; Shaoming Zhou guided the experiment, reviewed and edited the manuscript.

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REFERENCES

1. Fortin CS, Leader A, Mahutte N, et al. Gene expression analysis of follicular cells revealed inflammation as a potential IVF failure cause. J Assist Reprod Genet. 2019; 36(6): 1195–1210, doi: 10.1007/s10815-019-01447-4, indexed in Pubmed: 31001707.
2. García D, Brazal S, Rodríguez A, et al. Knowledge of age-related fertility decline in women: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2018; 230: 109–118, doi: 10.1016/j.ejogrb.2018.09.030, indexed in Pubmed: 30248536.
3. Nørgård BM, Larsen MD, Friedman S, et al. Decreased chance of a live born child in women with rheumatoid arthritis after assisted reproduction treatment: a nationwide cohort study. Ann Rheum Dis. 2019; 78(3): 328–334, doi: 10.1136/annrheumdis-2018-214619, indexed in Pubmed: 30636215.
4. Aguiar-Oliveira MH, Bartke A. Growth Hormone Deficiency: Health and Longevity. Endocr Rev. 2019; 40(2): 575–601, doi: 10.1210/er.2018-00216, indexed in Pubmed: 30576428.
5. Correa FA, Bianchi PHM, Franca MM, et al. Successful pregnancies after adequate hormonal replacement in patients with combined pituitary hormone deficiencies. J Endocr Soc. 2017; 1(10): 1322–1330, doi: 10.1210/js.2017-00005, indexed in Pubmed: 29264457.
6. Kucuk T, Kozinoglu H, Kaba A. Growth hormone co-treatment within a GnRH agonist long protocol in patients with poor ovarian response: a prospective, randomized, clinical trial. J Assist Reprod Genet. 2008; 25(4): 123–127, doi: 10.1007/s10815-008-9212-7, indexed in Pubmed: 18392675.
7. Norman RJ, Alvino H, Hull LM, et al. LIGHT investigators. Human growth hormone for poor responders: a randomized placebo-controlled trial provides no evidence for improved live birth rate. Reprod Biomed Online. 2019; 38(6): 908–915, doi: 10.1016/j.rbmo.2019.02.003, indexed in Pubmed: 30954433.

8. Dosouto C, Calaf J, Polo A, et al. Growth hormone and reproduction:
9. lessons learned from animal models and clinical trials. Front Endocrinol. 2019; 10, doi: 10.3389/fendo.2019.00404.
10. Carter-Su C, Schwartz J, Argetsinger LS. Growth hormone signaling pathways. Growth Horm IGF Res. 2016; 28: 11–15, doi: 10.1016/j.ghir.2015.09.002, indexed in Pubmed: 26421979.
11. List EO, Sackmann-Sala L, Berryman DE, et al. Endocrine parameters and phenotypes of the growth hormone receptor gene disrupted (GHR-/-) mouse. Endocr Rev. 2011; 32(3): 356–386, doi: 10.1210/er.2010-0009, indexed in Pubmed: 21123740.
12. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310(20): 2191–2194, doi: 10.1001/jama.2013.281053, indexed in Pubmed: 24141714.
13. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod. 2004; 19(3): 611–615, doi: 10.1093/humrep/deh127, indexed in Pubmed: 14998960.
14. Carter-Su C. Growth hormone signal transduction. Growth Hormone & IGF Research. 1998; 8(4): 311, doi: 10.1016/s1096-6374(98)80130-5.
15. Wondisford F. Growth Hormone. Essentials of Endocrinology and Metabolism. 2020: 229–240, doi: 10.1007/978-3-030-39572-8_26.
16. Cui N, Li AM, Luo ZY, et al. Effects of growth hormone on pregnancy rates of patients with thin endometrium. J Endocrinol Invest. 2019; 42(1): 27–35, doi: 10.1007/s40618-018-0877-1, indexed in Pubmed: 29671256.
17. Hrabia A, Sechman A, Gertler A, et al. Effect of growth hormone on steroid content, proliferation and apoptosis in the chicken ovary during sexual maturation. Cell Tissue Res. 2011; 345(1): 191–202, doi: 10.1007/s00441-011-1187-5, indexed in Pubmed: 21618141.
18. Hull KL, Harvey S. Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. Int J Endocrinol. 2014; 2014: 234014, doi: 10.1155/2014/234014, indexed in Pubmed: 25580121.
19. European Society for Paediatric Endocrinology (ESPE). 2016, doi: 10.1159/isbn.978-3-318-05958-8.

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Comparison of polymerase chain reaction method with culture method in antenatal *Group B Streptococcus* screening

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ABSTRACT

Objectives: The aim of this study was to investigate the prevalence of *Group B Streptococcus* (GBS) colonization in pregnancies between 35 and 37 weeks of gestation and to compare the effectiveness of polymerase chain reaction (PCR) method with gold standard technique of culture in antenatal GBS screening.

Material and methods: Vaginal and rectal swabs of a total of 106 pregnant women between 35th and 37th weeks of gestation, who were admitted to our clinic between January 2022 and August 2022, were evaluated using culture and PCR method. The prevalence of GBS was estimated. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the PCR method were analyzed.

Results: The prevalence of GBS was 10.4% and 21.69% using the culture and PCR method, respectively. Compared to the culture, the sensitivity, specificity, PPV, NPV and accuracy of PCR were found to be 100%, 87%, 47%, 100%, and 88%, respectively.

Conclusions: This study results suggest that the PCR method is a simple, effective and fast method with high sensitivity, specificity, PPV, and NPV in antenatal GBS screening.

Keywords: *Group B Streptococcus*; antenatal screening; culture; polymerase chain reaction

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INTRODUCTION

Streptococcus agalactiae, also known as *Group B Streptococcus* (GBS), is a facultative gram-positive bacterium. This microorganism naturally resides in the gastrointestinal and vaginal microbiome of some women. However, the potential pathogenic properties of GBS can lead to serious infections, especially during pregnancy and the neonatal period. This demonstrates that GBS is both a commensal and a pathogenic microorganism [1]. Maternal colonization is the primary risk factor for GBS infection in neonates and young infants and GBS infection in neonates and young infants is classified as early, late, and very lateonset. The early onset may be due to rupture of membranes, intraamniotic infection, or vaginal transmission in labor, occurring within 24 hours to 6 days postpartum and leading to generalized sepsis, pneumonia, meningitis, or pulmonary hypertension. Late onset, usually 7 to 89 days, presents as bacteremia, resulting in meningitis, pneumonia, septic arthritis and osteomyelitis. A very late onset is usually seen in infants older than 90 days [2].

In a meta-analysis including 37 countries in 2016, the prevalence of GBS was reported varied between 6.8 and 26.7% [3]. GBS screening for pregnant women is recommended between 36 0/7 and 37 6/7 weeks of gestation [4, 5] or 3 to 5 weeks before the expected delivery date [6]. Thanks to screening strategies, the incidence of infant GBS has decreased from 1.7 cases per 1000 live births to 0.5 cases per 1000 live births in (wihtin) the last 15 years [7].

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After 18 to 24 hours of incubation on blood agar at 37°C, a narrow zone of hemolysis forms large, oval-shaped, mucoid, gray-white colonies [8], which is evaluated after 24 hours of incubation and re-evaluated for another 24--hour incubation, if no growth occurs [9]. Rectovaginal swab specimens taken for screening in pregnant women should be cultivated in selective broth media, as they can be only directly identified with sheep blood agar, when there is extensive colonization [10]. The Centers for Disease Control and Prevention (CDC) recommends the use of Todd-Hewitt broth supplemented with either colistin, nalidixic acid, or gentamicin + nalidixic acid to suppress normal flora elements [11]. Techniques such as indirect immunofluorescence, reverse immunoelectrophoresis, staphylococcal coagglutination, and enzyme-linked immunosorbent assay (ELISA), most commonly latex agglutination test, are used to identify antigenic structures specific to GBS [12]. Molecular methods, such as real-time polymerase chain reaction (PCR) are used for rapid identification of GBSs and are commercially available [13]. Thus, genome sequencing, serotype, and antimicrobial resistance can be determined easily [14].

According to the ACOG and ASM guidelines, nucleic acid amplification testing (NAAT) can be also used as a rapid test for the detection of GBS with equivalent detection rates to culture-based screening [4, 5, 15]. For further detection, it is recommended that all vaginal and rectal swabs should be inoculated into selective enrichment broth medium and incubated for 18 to 24 hours at 35 to 37°C, 5% carbon dioxide (CO_2) conditions. Intrapartum NAAT without enrichment has a high false-negative rate ranging from 6.3 to 22%. Therefore, the use of intrapartum NAAT without enrichment is not recommended to rule out the need for prophylaxis. Vaginal and rectal specimens should be collected using a flocked swab and placed in a liquid-based transport medium such as Amies transport medium. Vaginal and rectal specimens should be transported to the testing laboratory within 24 hours [5].

In the literature, although it is recommended to perform screening with culture, it has certain drawbacks, such as yielding results within 24 to 48 hours and producing false-negative results in low colony counts [16]. In the present study, authors hypothesized that PCR, a rapid test, could be routinely used to screen for antenatal GBS. Therefore, aimed to investigate the prevalence of GBS between 35 and 37 weeks of gestation and to compare the effectiveness of culture and PCR method in antenatal GBS screening.

MATERIAL AND METHODS

Study design and study population

This single-center prospective study was conducted at the Department of Obstetrics and Gynecology of a tertiary care center between January 2022 and August 2022. A written informed consent was obtained from each participant. The study protocol was approved by the institutional Ethics Committee (No: 340 and Date: 26/05/2021). The study was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 106 antenatal pregnant women between the 35th and the 37th weeks of gestation were included in this study. The mean age of the patients was 29.14 ± 5.4 (range 17 to 40) years. The mean gestational age was 35.96 ± 0.62 (range 35.0 to 37.0) weeks. Among all women who applied to our clinic for a routine pregnancy control between January 2022 and August 2022, those with a pregnancy less than 35 weeks or older than 37 weeks, those who received antibiotherapy in the last month, had bleeding or refused to participate in the study were excluded. Data including demographic and clinical characteristics of the patients, gravidity, parity, number of abortions, previous cesarean-section (C/S) delivery and vaginal delivery, week of gestation, the presence of comorbidities such as gestational hypertension (GHT), gestational diabetes (GDM), and type 2 diabetes, smoking and alcohol use, education status and employment status were recorded. Demographic and clinical characteristics of the patients at the time of vaginal and rectal sampling are shown in Table 1.

Table 1. Demographic and clinical characteristics of patients at the time of vaginal and rectal sampling

Variable	
Age, year, median (IQR)	29 (8)
Body weight, kg median (IQR)	74 (17)
Height, cm median (IQR)	162 (9)
BMI, kg/m^2, median (IQR)	28.45 (5.06)
Gravidity, n median (IQR)	2 (2)
Parity, n, median (IQR)	1 (2)
Abortion, n median (IQR)	0 (1)
Previous C/S delivery, median (IQR)	0 (1)
Previous vaginal delivery, n, median (IQR)	0 (1)
Gestational age, week, median (IQR)	36.05 (1.10)
GHT, n [%]	3 (2.8)
GDM, n [%]	9 (8.4)
T2DM, n [%]	1 (0.94)
Smoking, n [%]	16 (15.09)
Alcohol use, n [%]	1 (0.94)
Education up to high school, n [%]	74 (69.8)
Undergraduate education, n [%]	32 (30.1)
Employment with income, n [%]	40 (37.7)

IQR— Interquartile Range; BMI— body mass index; C/S — cesarean delivery, GHT — gestational hypertension; GDM — gestational diabetes; T2DM — type 2 diabetes mellitus

Sample collection method

Vaginal and rectal swabs were collected without speculum. A single swab was used to obtain the specimen first from the lower vagina and then, from the rectum. The specimen first from the vagina (near the introitus) was collected by inserting the swab about 1.5 to 2 cm and then, from the rectum by inserting the same swab 1 cm through the anal sphincter. The vaginal and rectal specimens in a single medium (Stuart's transport medium) were transported to the testing laboratory immediately. The specimens were analyzed using both the culture and PCR method separately.

Culture method

All vaginal and rectal swabs were inoculated into the sheep blood agar (BD, Heidelberg, Germany) and incubated at 35 to 37°C in 5% CO_2 conditions.

The culture plates were examined at 24 and 48 hours. The Columbia agar has a high starch content and, thus, beta-hemolytic streptococci may show alpha rather than beta-hemolytic reactions or may exhibit week hemolytic reactions on media based on this formulation [9]. Therefore, the culture plates were assessed at 24 and 48 hours after incubation and large, gray, translucent colonies with or without narrow beta-hemolysis were examined. In suspected cases, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Biotyper®, USA) was used. After MALDI-TOF MS testing, colonies with GBS results were considered culture-positive.

BD™ MAX™ GBS PCR testing

After inoculation, the swabs were placed in selective LIM medium (Todd-Hewitt broth supplemented with 10 µg/mL of colistin and 15 µg/mL of nalidixic acid). The LIM medium (BD GmbH, Germany) was incubated at 37°C in 5% CO_2 conditions for minimum 18 hours.

Sample preparation

After incubation, the specimens in the LIM medium were vortexed. Using a pipette and a long pipette tip, 15 µL of the specimen was aspirated from the LIM medium and mixed with the sample preparation reagent included in the BD MAX GBS assay kit (BD GmbH, MD, Germany). A homogeneous mixture was obtained by pipetting several times. A GBS Master Mix, a GBS extraction reagent, and a BD MAX GBS unitized reagent strip included in the assay were used for each sample to be tested. The samples placed on racks in accordance with the manufacturer's recommendations were placed in the BD MAX device (BD GmbH, Germany). The results were recorded, and the device was operated.

Interpretation of results

Test results were automatically interpreted by the BD MAX System software as NEG (–), POS (+) or IND (indeterminate). Tests with positive results were interpreted as GBS DNA detected, negative results as GBS DNA not detected and IND results as PCR reaction, reagent failure or no sample process control amplification. Samples with IND results were re-run by applying the sample preparation procedure. Again, the examples that resulted in IND were indicated.

Statistical analysis

Statistical analysis was performed using the SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median and interquartile range (IQR) or number and frequency, where applicable. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the PCR method were analyzed.

RESULTS

The vaginal and rectal swabs were obtained from the patients and were analyzed using both the culture and PCR method, separately. The results are presented in Table 2. Accordingly, the prevalence of GBS was 21.69% (n = 23) using the PCR method.

Compared to the culture, the sensitivity, specificity, PPV, NPV and accuracy of the PCR are given in Table 3. Accordingly, these values were found to be 100%, 87%, 47%, 100% and 88%, respectively.

The records of the patients who underwent GBS screening were examined after delivery and those were called by phone to complete missing data and none of the infants were diagnosed with early-onset GBS.

Table 2. Comparison of culture and PCR results of vaginal and rectal samples

		Culture method		Total (n)
		Positive (n)	Negative (n)	
PCR method	Positive (n)	11	12	23
	Negative (n)	0	83	83
Total (n)		11	95	106

PCR — polymerase chain reaction

Table 3. Characteristics of PCR method compared to gold standard of culture method

Variable	%
Sensitivity	100%
Specificity	87%
PPV	47%
NPV	100%
Accuracy	88%

PCR — polymerase chain reaction; PPV — positive predictive value; NPV — predictive value

DISCUSSION

The colonization of GBS varies depending on race, geographical region, and sociocultural factors with a rate ranging from 4 to 40% in the literature [17]. The carriage rate has been reported as ranging from 0.4 to 32% in Türkiye [18, 19].

The gold-standard method for GBS screening is the culture method and many studies have compared the culture method with the PCR method. In a study, during the third trimester of pregnancy, the culture result was positive in 18.4% vaginal samples and positive in 18.1% rectal samples, while PCR yielded positive results in 22.6% vaginal samples and positive in 21.2% rectal samples [20]. The authors concluded that PCR was able to identify more colonized pregnant women than culture and was a fast and useful screening method with a shorter detection time.

In the light of these data, in this study, the authors compared the culture method with the PCR method, which can yield faster results and can also work with low colony counts. The prevalence of GBS was 10.4% and 21.69% using the culture and PCR method, respectively. The samples obtained were enriched with the LIM broth. Although this allowed to obtain results with higher sensitivity in both culture and PCR methods, time to enrichment limited the ability to obtain rapid results.

In a study including 204 pregnant women, the rate of GBS was found to be 26% with the PCR method and 22% with the culture method using vaginal and perianal swabs, and the sensitivity, specificity, PPV, and NPV were reported as 100%, 95.6%, 86.8% and 100%, respectively [21]. A high NPV, fast results, and high sensitivity are desirable characteristics of a screening test. In this study, the sensitivity of the PCR method was found to be 100%, specificity 87%, PPV 47%, NPV 100%, and accuracy 88%. Even if it is used with enrichment, its main advantages are that it yields results within 12 to 24 hours, compared to culture method and can detect smaller colonies. A PPV of 47% in this study can be attributed relatively low prevalence compared to previous studies.

In addition, although we consider the culture method as the gold standard, sensitivity may decrease due to reasons such as the need for living bacteria in the sample obtained and the overgrowth of microbiota-derived organisms that can inhibit the growth of GBS in the presence of a small number of living bacteria. The prevalence of GBS colonization may have been higher by the PCR than the culture method, as PCR can only detect bacterial genes and not viable bacterial colonies and, therefore, the culture method cannot detect them.

In the literature, there are several studies in which rectal and vaginal swabs are obtained separately or combined. In most studies comparing individual swabs, the sensitivity and specificity of both culture and PCR are reduced, particularly in vaginal specimens [16, 22]. According to these results, rectal sample culture seems to be more effective than vaginal culture [16]. In other words, there is a need for vaginal-rectal sampling to increase the chance of GBS isolation more effectively. In this study, vaginal and rectal sampling was performed.

The culture technique is a time-consuming method requiring at least 48 hours for the complete identification of GBS, whereas PCR is a sensitive and accelerated technique for detecting GBS with results available within 3 hours [16]. Compared to the culture method, PCR can be a fast and effective screening and diagnostic method with high sensitivity and NPV, and the ability to identify even low numbers of colonies, as it focuses on genetic material.

In Türkiye, there is no antenatal and/or intrapartum GBS screening guide for pregnant women issued by the Republic of Türkiye, Ministry of Health. By virtue of its focus on genetic material, PCR serves as a rapid and reliable screening and diagnostic tool characterized by high sensitivity and a strong negative predictive value (NPV). However, this test cannot determine antibiotic susceptibility. Within the population, the relatively high prevalence of penicillin intolerance, a mainstay of empiric therapy, may limit the test's applicability.

Two scenarios for employing the test can be considered:

1. Prenatal Screening: PCR may not be a cost-effective alternative for culture performance with positive results. This means that traditional culture tests might still be necessary for confirming positive results from PCR screening.
2. Intrapartum Testing: For patients who have not undergone prenatal screening, PCR can provide a rapid result. This can be particularly useful in emergency situations, where quick diagnostic results are needed. However, the relatively high cost of PCR limits the utilization of this screening test in our country.

Strengths and limitations

The design of this study was prospective, but the number of cases was small and GBS typing was not performed.

CONCLUSIONS

In conclusion, the prevalence of GBS detected by the culture and PCR methods in this study seems to be compatible with the national and global data. In Türkiye, there is no antenatal and/or intrapartum GBS screening guide for pregnant women issued by the Ministry of Health.

The PCR testing is a fast and effective method with high sensitivity, specificity, and NPV and can be used for GBS screening in pregnant women. However, enrichment with the LIM broth for PCR is a factor that limits obtaining rapid results. The authors believe that GBS screening in pregnant women would be faster and more effective with the use of molecular methods such as PCR, which can be studied directly from clinical samples with high sensitivity, specificity, and NPV, with faster results and lower cost, thanks to emerging technologies.

Article information and declarations

Data availability statement

The data and materials used in this study are available upon request. For access to the data or materials, please contact the corresponding author.

Ethics statement

The research protocol received approval from the Ethics Committee of Akdeniz University Faculty of Medicine, Türkiye, under the reference number 16.1.2019/38.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Doğukan Çopur, Özlem Koyuncu Özyurt, and Hülya Kandemir. The first draft of the manuscript was written by Hülya Kandemir, Doğukan Çopur, and Prof. Dr. Dilara Öğünç. Significant revisions were made by Prof. Dr. Dilara Öğünç and İnanc Mendilcioğlu. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

REFERENCES

1. Money D, Allen VM. No. 298-The Prevention of Early-Onset Neonatal Group B Streptococcal Disease. J Obstet Gynaecol Can. 2018; 40(8): e665–e674, doi: 10.1016/j.jogc.2018.05.032, indexed in Pubmed: 30103891.
2. Kimberlin DW, Barnett ED, Lynfield R, Sawyer MH. Red Book: 2021-2024 Report of the Committee on Infectious Diseases, 32nd Edition. American Academy of Pediatric 2021: 707–713.
3. Kwatra G, Cunnington MC, Merrall E, et al. Prevalence of maternal colonisation with group B streptococcus: a systematic review and meta-analysis. Lancet Infect Dis. 2016; 16(9): 1076–1084, doi: 10.1016/S1473-3099(16)30055-X, indexed in Pubmed: 27236858.
4. Prevention of Group B Streptococcal Early-Onset Disease in Newborns: ACOG Committee Opinion, Number 797. Obstet Gynecol. 2020; 135(2): e51–e72, doi: 10.1097/AOG.0000000000003668, indexed in Pubmed: 31977795.
5. Filkins L, Hauser JR, Robinson-Dunn B, et al. American Society for Microbiology Provides 2020 Guidelines for Detection and Identification of Group B . J Clin Microbiol. 2020; 59(1), doi: 10.1128/JCM.01230-20, indexed in Pubmed: 33115849.
6. Prevention of Early-onset Neonatal Group B Streptococcal Disease: Green-top Guideline No. 36. BJOG. 2017; 124(12): e280–e305, doi: 10.1111/1471-0528.14821, indexed in Pubmed: 28901693.
7. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant GBS Disease Investigator Group. Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017; 65(suppl_2): S160–S172, doi: 10.1093/cid/cix656, indexed in Pubmed: 29117326.
8. Gültepe B, Güdücüoğlu H, Özkaçmaz A, et al. Colonization of Group B Streptoccocal Bacteria In Pregnants and Their Antibiotic Susceptibility. Ortadoğu Tıp Dergisi. 2011; 3(2): 71.
9. Spellerberg B, Brandt C. Streptococcus. Manual ofClinical Microbiology. 2015: 383–402, doi: 10.1128/9781555817381.ch22.
10. Schuchat A. Group B streptococcus. The Lancet. 1999; 353(9146): 51–56, doi: 10.1016/s0140-6736(98)07128-1.
11. Prevention of Perinatal Group B Streptococcal Disease: A Public Health Perspective. PsycEXTRA Dataset. 1996, doi: 10.1037/e547302006-001.
12. Zumla A. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Lancet Infect Dis. 2010; 10(5): 303–304, doi: 10.1016/s1473-3099(10)70089-x.
13. Meehan M, Cafferkey M, Corcoran S, et al. Real-time polymerase chain reaction and culture in the diagnosis of invasive group B streptococcal disease in infants: a retrospective study. Eur J Clin Microbiol Infect Dis. 2015; 34(12): 2413–2420, doi: 10.1007/s10096-015-2496-5, indexed in Pubmed: 26433745.
14. Metcalf BJ, Chochua S, Gertz RE, et al. Active Bacterial Core surveillance team. Short-read whole genome sequencing for determination of antimicrobial resistance mechanisms and capsular serotypes of current invasive Streptococcus agalactiae recovered in the USA. Clin Microbiol Infect. 2017; 23(8): 574.e7–574.e14, doi: 10.1016/j.cmi.2017.02.021, indexed in Pubmed: 28257899.
15. Prevention of Group B Streptococcal Early-Onset Disease in Newborns: ACOG Committee Opinion, Number 797. Obstet Gynecol. 2020; 135(2): e51–e72, doi: 10.1097/AOG.0000000000003668, indexed in Pubmed: 31977795.
16. Bidgani S, Navidifar T, Najafian M, et al. Comparison of group B streptococci colonization in vaginal and rectal specimens by culture method and polymerase chain reaction technique. J Chin Med Assoc. 2016; 79(3): 141–145, doi: 10.1016/j.jcma.2015.06.021, indexed in Pubmed: 26803202.
17. Rick AM, Aguilar A, Cortes R, et al. Group B Streptococci Colonization in Pregnant Guatemalan Women: Prevalence, Risk Factors, and Vaginal Microbiome. Open Forum Infect Dis. 2017; 4(1): ofx020, doi: 10.1093/ofid/ofx020, indexed in Pubmed: 28480290.
18. Karaduman A, Doğruman AlF, Aksu G, et al. Gebelerde saptanan vajinal infeksiyon etkenlerinin dağılımı. İnfeksiyon Dergisi. 2006; 20: 171–175.
19. Kadanali A, Altoparlak U, Kadanali S. Maternal carriage and neonatal colonisation of group B streptococcus in eastern Turkey: prevalence, risk factors and antimicrobial resistance. Int J Clin Pract. 2005; 59(4): 437–440, doi: 10.1111/j.1368-5031.2005.00395.x, indexed in Pubmed: 15853861.
20. Gerolymatos G, Karlovasiti P, Sianou A, et al. Antenatal group B streptococcus detection in pregnant women: culture or PCR? J Infect Dev Ctries. 2018; 12(8): 631–635, doi: 10.3855/jidc.10367, indexed in Pubmed: 31958325.
21. Wollheim C, Sperhacke RD, Fontana SK, et al. Group B Streptococcus detection in pregnant women via culture and PCR methods. Rev Soc Bras Med Trop. 2017; 50(2): 179–183, doi: 10.1590/0037-8682-0454-2016, indexed in Pubmed: 28562753.
22. El Aila NA, Tency I, Claeys G, et al. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis. 2010; 10: 285, doi: 10.1186/1471-2334-10-285, indexed in Pubmed: 20920213.

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Prevalence of urinary incontinence and prolapse after hysterectomy for benign disease versus gynecologic malignancy

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ABSTRACT

Objectives: To estimate the prevalence of UI and POP after hysterectomy for benign disease and gynecologic malignancy.

This is a retrospective cohort chart review study.

Two major urban tertiary care centers between 2006–2010.

Women ≥ 18 years undergoing hysterectomy for benign or malignant indications.

Material and methods: Presence of UI and POP was based on patient report in clinic notes, ICD-9 UI and POP diagnosis codes, and CPT codes for treatment.

Prevalence of UI and POP after hysterectomy and time to development of UI and POP after hysterectomy.

Results: 1363 (55%) women underwent hysterectomy for benign disease while 1107 (45%) had a hysterectomy for malignancy. Postoperative prevalence of UI and POP in the benign versus the malignant group was 15.1% vs 11.1% ($p = 0.001$), and 12.1% vs 2.8%, ($p < 0.001$), respectively. The median time to development of UI in the subset of patients without preoperative UI was 3.5 years in the benign group vs 3 years in the malignant group ($p < 0.001$). The median time to development of POP in the subset of patients without preoperative POP was 5 years in the benign group and 3.5 years in the malignant group ($p < 0.001$). There was no significant difference in the risk of developing UI or POP between groups after adjusting for confounders or when accounting for pre-hysterectomy UI or POP.

Conclusions: When pre-hysterectomy UI or POP is taken into consideration, there is no difference in the prevalence of post-hysterectomy UI or POP.

Keywords: benign disease; gynecologic cancer; hysterectomy; pelvic organ prolapse; prevalence; urinary incontinence

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INTRODUCTION

Pelvic floor disorders (PFDs) are common conditions that negatively impact women's quality of life. Urinary incontinence (UI) and pelvic organ prolapse (POP) are two common PFDs, and their prevalence increases with age [1]. UI and POP symptoms after hysterectomy for benign indications, including POP correction, have been previously studied [2–7]. However, the prevalence of these conditions following hysterectomy for gynecologic cancer is still understudied. Most previous studies investigated the incidence of UI in cervical cancer patients undergoing radical hysterectomy (RH) [8–10]. Although these studies suggest UI symptoms may develop or worsen after RH, the majority have small sample sizes. The baseline prevalence of POP symptoms among women with gynecologic malignancy has been estimated to be 10.9%; however, the prevalence of POP after hysterectomy for malignant indications has not been reported [11].

A more recent systematic review estimated the prevalence of PFDs before and after treatment of various gynecologic malignancies including endometrial, ovarian, and cervical cancer [12]. However, this review did not distinguish between surgical and nonsurgical treatments or between hysterectomy and other surgical treatments for gynecologic

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cancer. It is unclear whether the prevalence of postsurgical PFDs differs between women who undergo hysterectomy for benign versus malignant indications.

Our primary objectives were to estimate the prevalence of UI and POP after hysterectomy for benign disease and gynecologic malignancy and to evaluate whether the prevalence of these conditions differed depending on the indication for hysterectomy. A secondary objective was to assess the time to development of these conditions after hysterectomy for those women who did not have POP or UI preoperatively.

MATERIAL AND METHODS

We conducted an IRB-approved retrospective cohort study of women who underwent hysterectomy for benign disease or gynecologic malignancy at two major tertiary care referral centers between 2006 and 2010 (Protocol #: 2017P001210). This period was chosen to allow for a relatively long follow up after hysterectomy to assess outcomes over time. We included women 18 years of age or older who underwent total or supracervical hysterectomy for benign disease including POP or gynecologic malignancy and who presented for at least one postoperative visit. Current procedural terminology (CPT) codes were used to identify women who had hysterectomies during the study period.

Patient data were abstracted from electronic medical record review. Demographic and clinical characteristics such as age, race, BMI, and relevant comorbidities were recorded from patient charts. Surgical characteristics including indication for hysterectomy, type of hysterectomy, concomitant surgeries, and perioperative data were gathered from surgical reports. For the malignant group, rates of neoadjuvant chemotherapy and adjuvant chemotherapy and radiation after hysterectomy were also noted.

The presence or absence of UI and POP and the subtype of UI (stress, urgency, or mixed), if present [13], were recorded at the baseline preoperative visit and again at the time of each postoperative visit. The presence of these conditions was based on patient report in clinic notes, ICD-9 UI and POP diagnosis codes, and CPT codes for treatment of these conditions. The primary outcome was the prevalence of UI after hysterectomy for benign disease versus hysterectomy for malignancy. The secondary outcome was the prevalence of POP after hysterectomy for benign disease versus hysterectomy for malignancy. Additional outcomes included the prevalence of UI subtypes after hysterectomy for benign disease versus hysterectomy for malignancy and time to development of UI and POP after hysterectomy for those who did not have UI or POP preoperatively.

Demographic data and clinical characteristics were summarized using descriptive statistics such as means, standard deviations, medians, and ranges for continuous variables, and frequencies and percentages for categorical variables. Comparisons between the prevalence of UI and POP after hysterectomy for benign disease versus hysterectomy for malignancy were assessed using Chi-square tests. Proportional hazards regression was used to analyze the development of UI and POP after surgery for the subset of patients without preoperative UI or POP adjusting for variables that were significantly different between groups. A p value < 0.05 was considered to indicate statistical significance.

The prevalence of UI 10 years after hysterectomy has been estimated to be 9% [14]. A preliminary review of outcomes following hysterectomy at our institution also suggested the prevalence of UI after hysterectomy was approximately 10–15%. Consequently, we estimated that a clinically significant difference in prevalence would be 5% or more in women who have hysterectomy for malignancy (15%) versus for benign (10%) conditions. Based on these estimates, approximately 725 women in each group were needed to detect a 5% difference with an alpha of 0.05 and beta of 0.80.

RESULTS

A total of 2,725 records of women who underwent hysterectomy between 2006 and 2010 were reviewed. Data were abstracted from 2,470 (90%) of these records, each of which contained at least one postoperative visit. Of these 2,470, 1363 (55%) underwent hysterectomy for benign disease while 1107 (45%) underwent hysterectomy for malignancy. Women in the malignant group were older (59.1 ± 12.1 vs 51.1 ± 11.2), obese (BMI 32.4 ± 9.8 vs 28.9 ± 7.7), and more likely to be Caucasian (77.1% vs 69.8%) compared with women in the benign group (Tab. 1, $p < 0.0001$ for all). A greater proportion of women in the malignant group had diabetes, heart disease, and obstructive sleep apnea while a greater proportion of those in the benign group had chronic constipation, anxiety, and depression (Tab. 1). Approximately 41% of patients had undergone previous pelvic surgery and there was no difference in previous POP or UI surgery between groups. In addition, there was no difference in tobacco or preoperative anticholinergic use between groups (Tab. 1).

The baseline prevalence of UI was 22.5% in women in the benign group versus 8.2% in women in the malignant group ($p < 0.001$). More women in both groups had stress urinary incontinence compared with urgency and mixed subtypes. Similarly, the baseline prevalence of urinary urgency, frequency, and nocturia were higher in the benign group compared with the malignant group. More women in the benign group had POP (Tab. 1).

Most women in the benign group had surgery for fibroids (33.3%) or POP (24.4%) and underwent either an open

Table 1. Baseline demographic data and clinical characteristics

Variable	Hysterectomy for benign disease n = 1363	Hysterectomy for malignancy n = 1107	p value
Age, years	51.1 ± 11.2	59.1 ± 12.1	< 0.0001
Race			
Asian	37 (2.7)	22 (2.0)	
Black/African American	107 (7.9)	39 (3.5)	
Hispanic	99 (7.3)	22 (2.0)	
White	951 (69.8)	853 (77.1)	< 0.0001
Other	5 (0.4)	1 (0.1)	
Unknown	164 (12.0)	170 (15.4)	
BMI, kg/m^2	28.9 ± 7.7	32.4 ± 9.8	< 0.0001
Parity	2.0 (0–12)	2.0 (0–13)	0.76
Comorbidities			
Neurologic disease	35 (2.6)	31 (2.8)	0.63
Diabetes	79 (5.8)	166 (15.0)	< 0.0001
Heart disease	112 (8.2)	123 (11.1)	0.03
COPD/asthma	147 (10.8)	102 (9.2)	0.2
Obstructive sleep apnea	21 (1.5)	35 (3.2)	0.007
Chronic constipation	58 (4.3)	30 (2.7)	0.04
Connective tissue disease	1 (0.1)	1 (0.1)	0.88
Anxiety/depression	287 (21.1)	167 (15.1)	0.0001
Tobacco use	139 (10.2)	85 (7.7)	0.08
Anticholinergic medication use	33 (2.4)	13 (1.2)	0.07
Urinary incontinence (UI)			
Stress UI	168 (12.3)	47 (4.2)	
Urgency UI	61 (4.5)	38 (3.4)	< 0.0001
Mixed UI	77 (5.6)	6 (0.5)	
Urinary urgency	206 (15.1)	29 (2.6)	< 0.0001
Urinary frequency	284 (20.8)	47 (4.2)	< 0.0001
Nocturia	160 (11.7)	13 (1.2)	< 0.0001
Previous pelvic surgery	559 (41.0)	456 (41.2)	0.66
Pelvic organ prolapse grade[a]			
Grade 0	2 (0.1)	0	
Grade 1	13 (1.0)	4 (0.4)	
Grade 2	113 (8.3)	12 (1.1)	< 0.0001
Grade 3	195 (14.3)	5 (0.5)	
Grade 4	12 (0.9)	0	

Data are n (%), mean ± SD, or median (interquartile range)
[a] Stage data not available; most prolapse during the study time period was quantified using Baden-Walker grade rather than POP-Q stage; additionally, quantification data not available for all patients with prolapse

abdominal (53.7%) or vaginal (21.6%) approach (Tab. 2). Most women (76.2%) in the malignant group had endometrial cancer. 28.1% in the malignant group underwent simple open abdominal hysterectomy while 26.9% underwent radical open hysterectomy and 41.7% underwent a laparoscopic or robotic approach. An apical suspension, either uterosacral/sacrospinous ligament or sacrocolpopexy, was performed in 12.9% in the benign group and in no patients in the malignant group ($p < 0.0001$, Tab. 2). A midurethral sling was placed in 12.2% in the benign group and in no patients in the malignant group ($p < 0.0001$).

More women in the benign group experienced intraoperative complications compared with the malignant group (10.9% vs 7.6%, $p = 0.006$, Tab. 2). There was no difference in blood transfusion rates or ureteral or bowel injuries between groups (Tab. 2). Although a greater proportion of women in the benign group experienced bladder injuries, the absolute number of women who suffered this complication was low (14, 1.1% vs 2, 0.2%, respectively, $p = 0.009$, Tab. 2). Most patients in the malignant group (58.4%) did not receive adjuvant treatment. Of those that did receive adjuvant treatment after surgery, 11.6% received chemotherapy, 17.1% radiation, and 13.0% both chemotherapy and radiation. The prevalence of UI after hysterectomy was 15.1% in the benign group and 11.1% in the malignant group, ($p = 0.001$, Tab. 3). Most patients with UI in both groups had urgency urinary incontinence. More women in the benign group experienced urinary urgency and frequency postoperatively and

Table 2. Intraoperative details

Variable	Hysterectomy for Benign disease n = 1363	Hysterectomy for malignancy n = 1107	p value
Indication for hysterectomy			
Abnormal uterine bleeding	161 (11.8)	–	
Fibroids	454 (33.3)	–	
Endometriosis	90 (6.6)	–	
Pelvic pain or dyspareunia	62 (4.5)	–	
Prolapse	333 (24.4)	–	
Cesarean hysterectomy	19 (1.4)	–	
Benign pelvic and/or adnexal mass	74 (5.4)	–	
Ovarian/primary peritoneal cancer	–	120 (10.8)	
Endometrial cancer	–	843 (76.2)	
Leiomyosarcoma	–	40 (3.6)	
Cervical cancer	–	99 (8.9)	
Vaginal cancer	–	5 (0.5)	
EIN	78 (5.7)	–	
Cervical dysplasia	27 (2.0)	–	
Other	65 (4.7)	–	
Hysterectomy route			
Simple open abdominal	732 (53.7)	311 (28.1)	
Radical open abdominal	15 (1.1)	298 (26.9)	
Vaginal	295 (21.6)	5 (0.5)	< 0.0001
Laparoscopic	241 (17.7)	334 (30.2)	
Robotic	27 (2.0)	128 (11.6)	
Laparoscopic assisted vaginal	53 (3.9)	31 (2.8)	
Supracervical hysterectomy	331 (24.3)	2 (0.2)	< 0.0001
McCall's culdoplasty	169 (12.4)	0	< 0.0001
Concomitant surgeries			
Unilateral or bilateral adnexectomy	701 (51.4)	998 (90.2)	< 0.0001
Lysis of adhesions	165 (12.1)	81 (7.3)	< 0.0001
Midurethral sling	166 (12.2)	0	< 0.0001
Cystocele repair	264 (19.4)	0	< 0.0001
Rectocele repair	275 (20.2)	0	< 0.0001
Uterosacral ligament suspension	98 (7.2)	0	< 0.0001
Sacrospinous ligament suspension	31 (2.3)	0	< 0.0001
Sacrocolpopexy	47 (3.4)	0	< 0.0001
Vaginal mesh	78 (5.7)	2 (0.2)	< 0.0001
Colpocleisis	2 (0.1)	0	0.2
Surgical staging	40 (2.9)	714 (64.5)	< 0.0001
EBL in mL	260.6 ± 367.3	223 ± 343.3	< 0.0001
Intraoperative complications			
EBL > 500 mL	118 (8.7)	72 (6.5)	0.046
Blood transfusion	20 (1.5)	10 (0.9)	0.2
Bladder injury	14 (1.1)	2 (0.2)	0.009
Ureteral injury	4 (0.3)	1 (0.1)	0.26
Bowel injury	2 (0.1)	3 (0.3)	0.49
Cardiopulmonary event	0	4 (0.4)	0.026

Data are n (%), mean ± SD; EBL — estimated blood loss; EIN — endometrial intraepithelial neoplasia

were prescribed anticholinergic medications after surgery compared with the malignant group although the proportion of women prescribed these medications was relatively low (4.9% vs 3.0%, respectively, $p < 0.0001$, Tab. 3).

The prevalence of POP after hysterectomy was 12.1% in the benign group and 2.8% in the malignant group ($p < 0.0001$, Tab. 3). The majority of those with POP had a cystocele. A small percentage in each group had vaginal vault prolapse (1.6% in the benign group vs 0.7% in the malignant group, $p = 0.04$, Tab. 3).

We performed two sub-analyses excluding those patients who had UI and POP at baseline prior to hysterectomy given these patients may be at higher risk for persistent or recurrent symptoms. In the first sub-analysis, 10.7% of patients who underwent hysterectomy for benign disease developed UI compared with 9.9% in the malignant group ($p = 0.514$, Tab. 4). There was a significant difference in the subtypes of postoperative UI between groups ($p = 0.008$, Tab. 4). In the second sub-analysis, 3.6% of patients who underwent hysterectomy for benign disease developed

Table 3. Postoperative urinary incontinence and pelvic organ prolapse data

Variable	Hysterectomy for benign disease n = 1363	Hysterectomy for malignancy n = 1107	p value
Urinary incontinence (UI) Stress UI Urgency UI Mixed UI	206 (15.1) 63 (4.6) 99 (7.3) 44 (3.2)	123 (11.1) 21 (1.9) 73 (6.6) 29 (2.6)	0.001
Urinary urgency	185 (13.6)	101 (9.1)	0.001
Urinary frequency	200 (14.7)	118 (10.7)	0.005
Nocturia	121 (8.9)	69 (6.2)	0.05
Anticholinergic medication use	67 (4.9)	33 (3.0)	< 0.0001
Pelvic organ prolapse	165 (12.1)	31 (2.8)	< 0.0001
Type of prolapse Cystocele Rectocele Vaginal vault prolapse	 132 (9.7) 65 (4.8) 22 (1.6)	 24 (2.2) 19 (1.7) 8 (0.7)	 < 0.0001 < 0.0001 0.04
Pelvic organ prolapse grade[a] Grade 1 Grade 2 Grade 3 Grade 4	 48 (29.1) 63 (38.2) 35 (21.2) 2 (1.2)	 9 (29) 13 (41.9) 7 (22.6) 0	 < 0.0001

Data are n (%). [a] Stage data not available; most prolapse during the study time period was quantified using Baden-Walker grade rather than POP-Q stage; additionally, quantification data not available for all patients with prolapse

Table 4. Prevalence of urinary incontinence and pelvic organ prolapse after hysterectomy excluding patients with preoperative urinary incontinence and prolapse

Variable	Hysterectomy for benign disease n = 1055	Hysterectomy for malignancy n = 1017	p value
Urinary incontinence (UI)	113 (10.7)	101 (9.9)	0.514
Stress UI Urgency UI Mixed UI	40 (3.79) 27 (2.56) 46 (4.36)	18 (1.77) 20 (1.97) 63 (6.19)	0.008
Variable	Hysterectomy for Benign Disease n = 1019	Hysterectomy for Malignancy n = 1085	p value
Pelvic organ prolapse	37 (3.63)	23 (2.12)	0.037

Data are n (%)

POP compared with 2.1% of patients in the malignant group (p = 0.037, Tab. 4).

The median study follow-up period was 87.0 months (IQR 0–156) for the benign group and 53.0 months (IQR 0–150) for the malignant group, p < 0.0001. The median time to development of UI in the subset of patients without preoperative UI was 42 months (IQR 12 – 78) in the benign group and 36 months (IQR 12–72) in the malignant group, p < 0.001. After adjusting for age, parity, BMI, constipation, diabetes, and midurethral sling placement, there was no significant difference in the risk of developing UI between groups (HR 1.13 [0.79–1.61], p = 0.499). We adjusted for these specific variables given that almost all of them were significantly different between groups and the fact that these variables were most likely to significantly affect the outcome of interest.

The median time to development of POP in the subset of patients without preoperative POP was 60 months (IQR 19.5–78.0) in the benign group and 42 months (IQR 11.5–87.0) in the malignant group, p < 0.001. There was no significant difference in the risk of developing POP between groups after adjusting for age, parity, BMI, and constipation (HR 1.70 [0.83–3.50], p = 0.15). We adjusted for these specific variables given that almost all of them were significantly different between groups and the fact that these variables were most likely to significantly affect the outcome of interest.

DISCUSSION

In our study, the prevalence of UI after hysterectomy for benign disease and malignancy was 15.1% and 11.1%, respectively. Similarly, the prevalence of POP was higher after hysterectomy for benign disease versus malignancy,12.1%

vs 2.8%, respectively. When we excluded patients with preoperative UI, it appeared that the prevalence of UI was not different between the two groups (10.7% in the benign vs 9.9% in the malignant group). Similarly, exclusion of patients with preoperative POP resulted in no difference in the prevalence of POP after hysterectomy between women in the benign and malignant group (3.6% vs 2.1%).

Strengths and limitations

The major strength of our study is the number of patients included (n = 2470) with a relatively balanced number of women in both the benign and malignant groups. Furthermore, we investigated the prevalence of UI and POP after hysterectomy for both benign and malignant indications, while most of the previous literature regarding prevalence estimates involves one group or the other. We also looked at the time to development of UI and POP after hysterectomy for the subset of patients without UI and POP preoperatively. Our study results add to the literature about PFDs in gynecologic oncology patients who undergo surgical treatment involving hysterectomy which is important as advancements in the diagnosis and treatment of oncology patients lead to higher survival rates. Moreover, data about the development of UI and POP after hysterectomy in oncology patients can inform preoperative counseling which may result in a better understanding of postoperative expectations and greater patient satisfaction after surgery.

This study did have some limitations including those inherent to studies with retrospective designs. Our study population was predominantly White, potentially limiting the generalizability of our findings. In addition, cases of UI and POP were based on medical record review and not using self-reported validated questionnaires. It is possible that patients who had symptoms of these conditions after surgery may not have reported them to their surgeons, or surgeons may not have documented patients' symptoms in either preoperative or postoperative notes. Oncologic patients may have been more focused on treatment of their primary disease and less likely to report symptoms of PFDs than patients who underwent hysterectomy for benign indications. It is possible that patients could have developed UI or POP postoperatively but did not follow up in our healthcare system after developing symptoms of these conditions.

Interpretation

Our prevalence estimates are lower than those reported previously [8–10, 12]. Two explanations may be considered regarding this finding. On one hand, our cohort is substantially larger than the cohorts included in other studies [8–10], but on the other hand, it is possible that we may have underestimated the prevalence of UI and POP. The presence or absence of these conditions in our study was based on documentation in medical notes and billing codes. It is worth noting that more recently, a large Swedish study reported the prevalence of de novo post-hysterectomy UI as 8.5%, which is similar to our findings [15]. Additionally, that study was conducted during an analogous time period (2006–2013) but included only those undergoing hysterectomy for benign indications. Interestingly, the authors of that study found de novo postoperative UI significantly reduces satisfaction 1 year after surgery. This finding should be considered when counseling patients planning hysterectomy for both benign and malignant indications.

A new study showed that among 169 women who underwent radical hysterectomy for cervical cancer, the prevalence of postoperative UI was as high as 39% [16]. In our study, most patients who underwent hysterectomy for malignancy had endometrial cancer and cervical cancer patients constituted only 9% (n = 99) of all patients in the malignant group. This may explain why our data differ from what was reported by Wang et al [1–6]. In another recent study querying women who underwent surgery for gynecologic cancer, 35% reported first-time postoperative UI [17]. That study was conducted during a similar period as ours (2008–2013 vs 2006–2010, respectively) and found that 57% had stress UI, 13% OAB and 31% mixed UI in the postoperative period. In contrast, we found 1.9% women had stress UI, 6.6% urgency UI and 2.6% mixed UI after hysterectomy for malignancy. However, our study had 10 times more participants in the malignant group compared to the study conducted by Nakayama et al [17].

Pelvic organ prolapse symptoms after total abdominal vs laparoscopic hysterectomy for endometrial cancer were recently studied by Higgs et al [18]. Patients in both groups experienced improvement in Pelvic Floor Distress Inventory (PFDI) scores at 6-months postoperatively, especially in the POP domain. This improvement in PFDI scores was sustained throughout the 4.5-year study period regardless of the mode of hysterectomy. In addition, pelvic floor symptoms did not differ between women who received or did not receive adjuvant therapy. However, only 54% of patients in this study completed PFDI questionnaires at 4.5 years postoperative. The prevalence of POP in the malignant group in our study was 2% after adjusting for confounders. It is worth noting that the median time to develop POP in our study was 42 months in women after gynecological cancer surgery.

CONCLUSIONS

When taking confounders and preoperative UI or POP into consideration, there is no difference in the prevalence of postoperative UI or POP. Future research should focus on prospectively evaluating UI and POP before and after hysterectomy in oncologic and general gynecologic populations

using both self-reported validated questionnaires and standardized objective outcome measures.

Article information and declarations

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

JMM was responsible for project development, data analysis, manuscript writing and editing. IG was responsible for project development, data collection, data analysis, and manuscript editing. MK was responsible for manuscript editing. AC was responsible for data analysis, manuscript editing. VAM was responsible for project development, data analysis, and manuscript editing.

Ethics statement

This study was approved by the Mass General Brigham Institutional Review Board (IRB) on 07/06/17 as an Exempt study (Protocol #: 2017P001210).

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REFERENCES

1. Wu JM, Vaughan CP, Goode PS, et al. Prevalence and trends of symptomatic pelvic floor disorders in U.S. women. Obstet Gynecol. 2014; 123(1): 141–148, doi: 10.1097/AOG.0000000000000057, indexed in Pubmed: 24463674.
2. Andersen LL, Møller LM, Gimbel H, et al. Danish Hysterectomy Trial Group. Lower urinary tract symptoms after subtotal versus total abdominal hysterectomy: exploratory analyses from a randomized clinical trial with a 14-year follow-up. Int Urogynecol J. 2015; 26(12): 1767–1772, doi: 10.1007/s00192-015-2778-6, indexed in Pubmed: 26215904.
3. Lakeman MME, van der Vaart CH, Roovers JP, et al. HysVA study group. Hysterectomy and lower urinary tract symptoms: a nonrandomized comparison of vaginal and abdominal hysterectomy. Gynecol Obstet Invest. 2010; 70(2): 100–106, doi: 10.1159/000297507, indexed in Pubmed: 20299800.
4. Farquhar CM, Sadler L, Stewart AW. A prospective study of outcomes five years after hysterectomy in premenopausal women. Aust N Z J Obstet Gynaecol. 2008; 48(5): 510–516, doi: 10.1111/j.1479-828X.2008.00893.x, indexed in Pubmed: 19032669.
5. Persson P, Brynhildsen J, Kjølhede P, et al. Hysterectomy Multicentre Study Group in South-East Sweden. Pelvic organ prolapse after subtotal and total hysterectomy: a long-term follow-up of an open randomised controlled multicentre study. BJOG. 2013; 120(12): 1556–1565, doi: 10.1111/1471-0528.12399, indexed in Pubmed: 24034602.
6. Altman D, Falconer C, Cnattingius S, et al. Pelvic organ prolapse surgery following hysterectomy on benign indications. Am J Obstet Gynecol. 2008; 198(5): 572.e1–572.e6, doi: 10.1016/j.ajog.2008.01.012, indexed in Pubmed: 18355787.
7. Gabriel I, Kalousdian A, Brito LG, et al. Pelvic organ prolapse after 3 modes of hysterectomy: long-term follow-up. Am J Obstet Gynecol. 2021; 224(5): 496.e1–496.e10, doi: 10.1016/j.ajog.2020.11.008, indexed in Pubmed: 33207236.
8. Selcuk S, Cam C, Asoglu MR, et al. Effect of simple and radical hysterectomy on quality of life - analysis of all aspects of pelvic floor dysfunction. Eur J Obstet Gynecol Reprod Biol. 2016; 198: 84–88, doi: 10.1016/j.ejogrb.2016.01.008, indexed in Pubmed: 26802255.
9. Cibula D, Velechovska P, Sláma J, et al. Late morbidity following nerve-sparing radical hysterectomy. Gynecol Oncol. 2010; 116(3): 506–511, doi: 10.1016/j.ygyno.2009.10.061, indexed in Pubmed: 19906412.
10. Laterza RM, Salvatore S, Ghezzi F, et al. Urinary and anal dysfunction after laparoscopic versus laparotomic radical hysterectomy. Eur J Obstet Gynecol Reprod Biol. 2015; 194: 11–16, doi: 10.1016/j.ejogrb.2015.08.005, indexed in Pubmed: 26313524.
11. Thomas SG, Sato HRN, Glantz JC, et al. Prevalence of symptomatic pelvic floor disorders among gynecologic oncology patients. Obstet Gynecol. 2013; 122(5): 976–980, doi: 10.1097/AOG.0b013e3182a7ef3c, indexed in Pubmed: 24104774.
12. Ramaseshan AS, Felton J, Roque D, et al. Pelvic floor disorders in women with gynecologic malignancies: a systematic review. Int Urogynecol J. 2018; 29(4): 459–476, doi: 10.1007/s00192-017-3467-4, indexed in Pubmed: 28929201.
13. Wojcik M, Jarzabek-Bielecka G, Merks P, et al. The role of visceral therapy, Kegel's muscle, core stability and diet in pelvic support disorders and urinary incontinence - including sexological aspects and the role of physiotherapy and osteopathy. Ginekol Pol. 2022; 93(12): 1018–1027, doi: 10.5603/GP.a2022.0136, indexed in Pubmed: 36602196.
14. Christiansen UJ, Hansen MJ, Lauszus FF. Hysterectomy is not associated with de-novo urinary incontinence: A ten-year cohort study. Eur J Obstet Gynecol Reprod Biol. 2017; 215: 175–179, doi: 10.1016/j.ejogrb.2017.06.022, indexed in Pubmed: 28633096.
15. Bohlin KS, Ankardal M, Lindkvist H, et al. Factors influencing the incidence and remission of urinary incontinence after hysterectomy. Am J Obstet Gynecol. 2017; 216(1): 53.e1–53.e9, doi: 10.1016/j.ajog.2016.08.034, indexed in Pubmed: 27593942.
16. Wang S, Wang R, Wen H, et al. Association of pelvic floor function with postoperative urinary incontinence in cervical cancer patients after the radical hysterectomy. Neurourol Urodyn. 2021; 40(1): 483–492, doi: 10.1002/nau.24587, indexed in Pubmed: 33305849.
17. Nakayama N, Tsuji T, Aoyama M, et al. Quality of life and the prevalence of urinary incontinence after surgical treatment for gynecologic cancer: a questionnaire survey. BMC Womens Health. 2020; 20(1): 148, doi: 10.1186/s12905-020-01012-7, indexed in Pubmed: 32680500.
18. Higgs P, Janda M, Asher R, et al. Pelvic floor functional outcomes after total abdominal vs total laparoscopic hysterectomy for endometrial cancer. Am J Obstet Gynecol. 2018; 218(4): 419.e1–419.e14, doi: 10.1016/j.ajog.2017.12.233, indexed in Pubmed: 29305254.

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Prenatal diagnosis of pure 1p36 terminal deletion by chromosome microarry analysis — clinical report of 3 new cases and review of the literature

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ABSTRACT

Objectives: Our objective was to present the experience on prenatal diagnosis of 1p36 terminal deletion, and further delineated the fetal presentation of the syndrome.

Material and methods: This was a retrospective analysis of three new prenatal cases with pure 1p36 terminal deletion detected by chromosome microarray analysis (CMA) at a single Chinese medical center. We also reviewed 11 published prenatal cases with similar deletion sizes. Clinical data of all cases including indications for invasive testing, sonographic findings, maternal factors, and pregnancy outcomes were reviewed and analyzed.

Results: Three new cases with pure 1p36 terminal deletion were prenatal diagnosed by CMA, the sizes of the deletion were 1.3 Mb, 5.0 Mb, and 4.9 Mb respectively. All cases were detected because of abnormal ultrasound findings, including central nervous system (CNS) abnormalities, congenital heart disease (CHD) and fetal growth restriction. Two pregnancies were terminated, and one was live-born but died three months after birth.

Conclusions: The 1p36 terminal deletion results in many clinical manifestations, but the specificity of clinical features are not high. Prenatal sonographic findings such as CNS, CHD may act as suggestive signs of 1p36 deletion or other microdeletion/duplication syndromes.

Keywords: prenatal diagnosis; 1p36 terminal deletion; chromosomal microarray analysis; prenatal ultrasound findings

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INTRODUCTION

Chromosome 1p36 deletion syndrome (OMIM 607872) is the most common subtelomeric terminal deletion syndrome, with an estimated incidence of 1/5000 births [1]. The previous study showed that the deletion size and breakpoints varies widely from 1.5 Mb– >10 Mb, about 40% of all breakpoints occur 3.0–5.0 Mb from the telomere, and 50% of individuals with 1p36 deletion syndrome have a terminal deletion [2]. The major clinical features of 1p36 deletion syndrome patients characterized by a variety of generalized hypotonia, seizure, prenatal or postnatal growth restriction, severe developmental delays, facial features and CHDs [3, 4] the patients may also show skeletal abnormalities, fetal akinesia, gastrointestinal malformations, cutis laxa, and biliary atresia [5–7]. Although 1p36 deletion syndrome was considered clinically recognizable, there was significant phenotypic variation among affected individuals, patients with this syndrome should undergo a multidisciplinary evaluation and receive comprehensive follow-up treatment.

The deletion sizes of about 40% 1p36 terminal deletion patients are 3.0–5.0 Mb, so traditional cytogenetic techniques including karyotype analysis and fluorescence in situ hybridization (FISH) are insufficient good diagnostic tools. In recent years, CMA is increasingly used in clinical genetics both in the postnatal and prenatal settings, several studies have focused specifically on the use of CMA in prenatal diagnosis of fetuses with abnormal ultrasound findings [8, 9], so more and more microdeletions and microduplications including 1p36 deletion were identified. However, only a few prenatal identified cases have been reported, prenatal diagnosis and genetic counseling are challenging. Sarah Guterman et al., reported ten new cases and reviewed

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22 prenatally diagnosed cases [10], Xun Zhang et al., also reported ten new prenatal cases of 1p36 deletion [11], but not all cases with pure 1p36 terminal deletion in previous studies. Therefore, more prenatal cases are necessary to delineate the fetal manifestations of the syndrome. In our present study, we present three new 1p36 terminal deletion cases in which prenatal ultrasonography demonstrated fetal manifestations, objective to present the prenatal features associated with the terminal chromosomal deletion. In addition, we also gathered 11 published prenatal cases of the deletion size less than 5 Mb and compared these new cases with the published data, further shed light on the genotypes and phenotypes.

MATERIAL AND METHODS

Case selection

This was a retrospective study, we reviewed three prenatal cases of 1p36 terminal deletion diagnosed at the First Affiliated Hospital of the Air Force Military Medical University, from January 2015 to August 2019. Only cases with pure 1p36 terminal deletion were included. We also reviewed 11 published prenatal cases of 1p36 terminal deletion with similar deletion sizes. Clinical data of all cases including maternal factors, sonographic findings, the indication for the invasive, duration of gestation at the time of diagnosis, and pregnancy outcomes were reviewed and analyzed.

Genomic DNA preparation

Amniotic fluid (20 mL) was sampled by amniocentesis after obtaining informed consent from the parents. Genomic DNA was extracted from amniotic fluid (10 mL) by a QIAamp DNA Blood Mini Kit (Qiagen, Venlo, The Netherlands). Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used for detecting the concentration and quality of genomic DNA.

CMA

Cytoscan 750k array (Thermo Fisher Scientifc, Santa Clara, CA, USA) with an average resolution of 100 kb for copy number variations (CNVs) was used according to the standard manufacturer's protocol. The public Databases such as OMIM (http://www.ncbi.nlm.nih.gov/omim), DECIPHER (http://decipher.sanger.ac.uk/), ISCA (https://www.iscaconsortium.org/), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) were used for the interpretation of the results and to analyze genotype-phenotype correlations.

RESULTS

In this study, only prenatal cases with a pure terminal deletion were enrolled. Three cases of 1p36 terminal deletion were detected because of abnormal ultrasound findings including CNS abnormalities and CHDs. G-band karyotyping of them showed normal. CMA demonstrated a deletion of 1.3 Mb between genomic positions 849,466 and 2,121,139 (hg19) at 1p36.33 including 32 OMIM genes in case 1. A deletion of 5.0 Mb and 4.9 Mb were detected in case 2 and case 3 respectively, and 56 OMIM genes were included in the region. The detailed descriptions of the new cases and published cases were summarized in Table 1. CMA results were showed in Figure 1.

We followed up all cases by telephone, cases 1 and 3 decided to terminate the pregnancy and case 2 remain selected to continue the pregnancy after detail genetic counseling. Case 2 was born by C/S at 38th week of gestation with an Apgar score of five at 1 min and five at 5 min, anoxia and respiratory weakness were observed, then the neonate was sent to the NICU for treatment. The case was observed to have hypertelorism, and deep-set ears, feeding difficulties, poor hearing, hypotonia, seizures and developmental delay. In addition, patent ductus arteriosus and patent foramen ovale were identified by postpartum echocardiography. Unfortunately, the patient would be more appropriate) died three months after birth.

DISCUSSION

Until now, few cases of prenatal diagnosis of pure 1p36 terminal deletion have been reported in the literature, especially those with a deletion less than 5 Mb, suggesting that some cases might have been underdiagnosed because of the low resolution karyotyping [12]. In recent years, with the wide application of genome-wide high-resolution CMA in prenatal diagnosis, more and more microdeletion or microduplication syndromes are detected [13, 14]. In the current study, we reported three new cases of prenatal diagnosis of pure 1p36 terminal deletion detected by CMA, and gathered 11 published prenatal cases of the deletion size within(less than) 5 Mb. We tried to identify the prenatal clinical manifestations and genotype-phenotype relationship of pure 1p36 terminal deletion.

It was noteworthy that CNS abnormalities are common ultrasonic manifestations of pure 1p36 terminal deletion fetuses. In our new cases and the published cases one or more CNS abnormalities including ventriculomegaly, agenesis of the corpus callosum and cerebellar hypoplasia were observed in six of the fourteen cases. Prenatal ultrasonography revealed CNS abnormalities for fetuses with the deletion include ventriculomegaly, hydrocephalus, interhemispheric cyst, abnormal sylvian fossa, cerebellar hypoplasia and agenesis of the corpus callosum. The most common CNS abnormality in the prenatal ultrasonic findings was ventriculomegaly, four fetuses were detected with isolated ventriculomegaly or combined with other abnormalities. These findings suggest that CMA should be offered to fetuses with ventriculomegaly, regardless of whether or

Table 1. Prenatal findings in fetuses with 1p36 terminal deletion in our study and literature

Case	Maternal age (years)	Gestation (w + d)	Ultrasound finding	G-band	CMA results	Size (Mb)	Inheritance	Pregnancy outcome
1	32	32 + 4	VSD	46, XX	arr[GRCh37] 1p36.33(849,466_2,121,139)x1	1.3	Unknown	TOP
2	33	23 + 1	ACC, polyhydramnios	46, XX	arr[GRCh37] 1p36.33p36.31(849,466_5,851,366)x1	5.0	De novo	Born, death
3	29	31 + 1	VMG, subependymal cyst, NVM	46, XY	arr[GRCh37] 1p36.33p36.31(849,466_5,708,006)x1	4.9	Unknown	TOP
Sarah Guterman et al. 1	21	13	Interhemispheric cyst, NT 4.8 mm, subcutaneous edema	46, XY	arr[GRCh37]1p36.33p36.32(759,762_2,879,963)x1	2.1	Unknown	TOP
Sarah Guterman et al. 4	36	13	Retrognathia	46, XX	arr[GRCh37] 1p36.33p36.2(779,727_4,859,438)x1	4	De novo	TOP
Sarah Guterman et al. 8	30	26	Bilateral VMG, abnormal sylvian fossa, cerebellar hypoplasia	46, XY	arr[GRCh37] 1p36.33(834101_2225782)x1	1.4	De novo	TOP
Xun Zhang et al. 3	45	11	NT: 1.4 mm	46, XY	arr[GRCh37]1p36.33p36.32(849,466_2,516,031)x1	1.7	De novo	TOP
Xun Zhang et al. 4	30	17	NT: 1.7 mm, cfDNA: high risk for 1p_	46, XX	arr[GRCh37]1p36.33p36.31(849,466_6,374,407)×1	5.5	De novo	TOP
Xun Zhang et al. 5	27	22	Ebstein anomaly	46, XX	arr[GRCh37]1p36.33p36.31(849,466_6,927,026)×1	6.1	De novo	TOP
Xun Zhang et al. 6	30	22	DORV, PVS, VSD	46, XX	arr[GRCh37]1p36.33p36.32(849,466_5,383,956)×1	4.5	De novo	TOP
Xun Zhang et al. 7	30	23	Ebstein anomaly,VSD	46, XY	arr[GRCh37]1p36.32p36.22(1,397,489_3,628,023)×1	2.3	De novo	TOP
Xun Zhang et al. 8	31	30	Bilateral VMG (13mm)	46, XY	arr[GRCh37]1p36.33p36.31(849,466_6,435,583)×1	5.6	De novo	Still born, death after report in reduced fetal movement
Masatake Toshimitsu et al.	28	32+5	Vascular ring, Ebstein's anomaly, VSD, and single umbilical artery, polyhydramnios, FGR,VMG,CPCs	NA	arr[GRCh37][hg]1p36.33p36.32(849466_3347420)x1	3	De novo	Died within 2 h after birth due to respiratory failure
Fang Fu et al.	NA	NA	VSD, DORV, PS	NA	arr[GRCh37][hg]1p36.33p36.32 (849,466_5,383,956)x1	4.5	De novo	NA

ACC — agenesis of the corpus callosum; AMA — advance maternal age; cfDNA — cell-free DNA; CPCs — choroid plexus cysts; DORV — double outlet right ventricle; FGR — fetal growth restriction; NVM — noncompaction of ventricular myocardium; NA — not available; NT — nuchal translucency; PS — pulmonary stenosis; PVS — pulmonary valve stenosis; TOP — termination of pregnancy; VSD — ventricular septal defect; VMG — ventriculomegaly

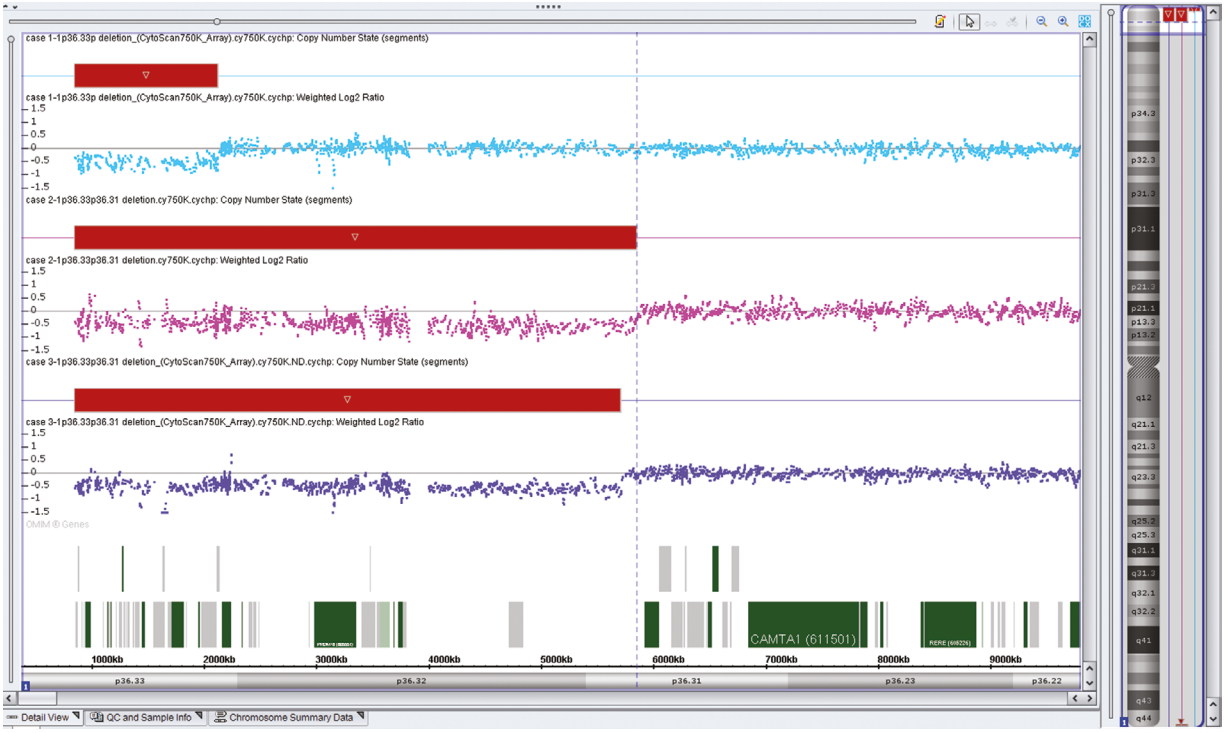

Figure 1. The CMA profile of the 1p36 microdeletions were represented by the bold red line. OMIM genes were listed at the bottom, dark green can be disease-causing. 39 OMIM genes were involved in the deletion region of case 1, 56 OMIM genes were involved in the deletion region of case 2 and 3

not other structural abnormalities are associated. We also identified a case with agenesis of the corpus callosum that was rare in the literature. Our findings support that a variety of brain abnormalities including ventriculomegaly, hydrocephalus, and agenesis of the corpus callosum associated with 1p36 deletion syndrome.

Congenital heart disease is also one of the most common clinical manifestations in prenatal echocardiography examination of fetuses with 1p36 deletion. Shino Shimada et al. [4], study showed that CHDs were observed in 69%, patent ductus arteriosus and ventricular septal defects were the most frequently observed patterns, but not all CHDs were prenatally identified. Seven cases were identified with various cardiac anomalies such as ventricular septal defect, noncompaction of ventricular myocardium, double outlet right ventricle, pulmonary valve stenosis in our new and the published cases. In case 3, noncompaction of ventricular myocardium was identified by prenatal echocardiography, this was consistent with the previous study. In addition, there were many reports that reveal the association between ebstein anomaly and 1p36 terminal deletion [15–17]. In the prenatal study, three fetuses with ebstein anomaly were detected, further provide the basis for 1p36 terminal deletion prenatal diagnosis. Therefore, the finding of cardiac abnormalities poses a high risk of 1p36 terminal deletion for the fetus. The ventricular septal defect was detected at 28th week of pregnancy in case 1, and CMA revealed a 1.3 Mb deletion in 1p36 terminal. To the best of our knowledge, this was the smallest deletion found before delivery, but 32 OMIM genes were included and many of them such as *GNB1, PRKCZ, GABRD* are well defined. Previously studies suggested that the gene *GNB1* implicated in neurological development [18]. The gene *PRKCZ* was thought to be necessary for regulating axonal differentiation and had been implicated in a variety of processes including cardiac muscle function [19, 20]. The gene *GABRD* encodes the subunit of the GABAA receptors, plays an important role in mammalian brain development, and haploinsufficiency may be responsible for neurologic features [21]. Previous studies showed that even a small deletion at the 1p36 terminal might lead to phenotypic alteration [22]. Considering the possible clinical manifestations such as developmental delay, mental retardation and CHDs, the couple decided to terminate the pregnancy after detailed genetic counseling. Characterization of the prenatal case with small deletion is helpful for narrowing critical intervals of 1p36 deletion syndrome.

The prenatal diagnosis of 1p36 terminal deletion is a challenge because of the variability clinical manifestations and depends largely on the gestational age when the diagnosis was made. Though CNS abnormalities and cardiac abnormalities could be the most common features in prenatal 1p36 terminal deletion cases, the prenatal ultrasound features of 1p36 terminal deletion remain non-specific. When cases with ultrasound findings such as congenital heart

defects, ventriculomegaly, agenesis of the corpus callosum, a clinical suspicion of 1p36 terminal deletion or other microdeletion/microduplication syndromes should be considered. In addition, isolated abnormalities such as ventriculomegaly may not attract the attention of pregnant women, so the best time for invasive prenatal diagnosis was missed. We suggest that cases with ultrasound findings such as CHDs, ventriculomegaly, agenesis of the corpus callosum should alert clinicians to the possibility of chromosome 1p36 deletion syndrome or other microdeletion/duplication syndromes.

In conclusion, CMA is an effective method for the precise diagnosis of 1p36 terminal deletion. We summarized the characteristics and prenatal clinical findings of fetuses with pure 1p36 terminal deletion, aim to emphasize the importance of CMA in detecting chromosomal abnormalities and provide a basis for prenatal diagnosis. Since 1p36 terminal deletion is associated with a poor prognosis in general and severe intellectual disability in particular, the prenatal diagnosis of this condition may help parents to decide whether to continue or to terminate the pregnancy.

Article information and declarations

Conflict of interest

All authors declare no conflict of interest.

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REFERENCES

1. Jordan VK, Zaveri HP, Scott DA. 1p36 deletion syndrome: an update. Appl Clin Genet. 2015; 8: 189–200, doi: 10.2147/TACG.S65698, indexed in Pubmed: 26345236.
2. Guterman S, Beneteau C, Redon S, et al. Prenatal findings in 1p36 deletion syndrome: New cases and a literature review. Prenat Diagn. 2019; 39(10): 871–882, doi: 10.1002/pd.5498, indexed in Pubmed: 31172545.
3. Greco M, Ferrara P, Farello G, et al. Electroclinical features of epilepsy associated with 1p36 deletion syndrome: A review. Epilepsy Res. 2018; 139: 92–101, doi: 10.1016/j.eplepsyres.2017.11.016, indexed in Pubmed: 29212048.
4. Shimada S, Shimojima K, Okamoto N, et al. Microarray analysis of 50 patients reveals the critical chromosomal regions responsible for 1p36 deletion syndrome-related complications. Brain Dev. 2015; 37(5): 515–526, doi: 10.1016/j.braindev.2014.08.002, indexed in Pubmed: 25172301.
5. Toshimitsu M, Nagaoka S, Kobori S, et al. Exome-first approach in fetal akinesia reveals chromosome 1p36 deletion syndrome. Case Rep Obstet Gynecol. 2019; 2019: 6753184, doi: 10.1155/2019/6753184, indexed in Pubmed: 31662930.
6. Zhang Z, Wang J, Li N, et al. Cutis laxa in a patient with 1p36 deletion syndrome. J Dermatol. 2018; 45(7): 871–873, doi: 10.1111/1346-8138.14311, indexed in Pubmed: 29611295.
7. Chawla V, Anagnost MR, Eldemerdash AE, et al. A novel case of biliary atresia in a premature neonate with 1p36 deletion syndrome. J Investig Med High Impact Case Rep. 2018; 6: 2324709618790613, doi: 10.1177/2324709618790613, indexed in Pubmed: 30057923.
8. Hu T, Zhang Z, Wang J, et al. Prenatal diagnosis of chromosomal aberrations by chromosomal microarray analysis in fetuses with ultrasound anomalies in the urinary system. Prenat Diagn. 2019; 39(12): 1096–1106, doi: 10.1002/pd.5550, indexed in Pubmed: 31461547.
9. Song T, Wan S, Li Yu, et al. Detection of copy number variants using chromosomal microarray analysis for the prenatal diagnosis of congenital heart defects with normal karyotype. J Clin Lab Anal. 2019; 33(1): e22630, doi: 10.1002/jcla.22630, indexed in Pubmed: 30047171.
10. Guterman S, Beneteau C, Redon S, et al. Prenatal findings in 1p36 deletion syndrome: New cases and a literature review. Prenat Diagn. 2019; 39(10): 871–882, doi: 10.1002/pd.5498, indexed in Pubmed: 31172545.
11. Zhang X, He P, Han J, et al. Prenatal detection of 1p36 deletion syndrome: ultrasound findings and microarray testing results. J Matern Fetal Neonatal Med. 2021; 34(13): 2180–2184, doi: 10.1080/14767058.2019.1660764, indexed in Pubmed: 31446820.
12. Campeau PM, Ah Mew N, Cartier L, et al. Prenatal diagnosis of monosomy 1p36: a focus on brain abnormalities and a review of the literature. Am J Med Genet A. 2008; 146A(23): 3062–3069, doi: 10.1002/ajmg.a.32563, indexed in Pubmed: 19006213.
13. Chang Q, Yang Y, Peng Y, et al. Prenatal detection of chromosomal abnormalities and copy number variants in fetuses with ventriculomegaly. Eur J Paediatr Neurol. 2020; 25: 106–112, doi: 10.1016/j.ejpn.2020.01.016, indexed in Pubmed: 32014392.
14. Fu F, Deng Q, Lei TY, et al. Clinical application of SNP array analysis in fetuses with ventricular septal defects and normal karyotypes. Arch Gynecol Obstet. 2017; 296(5): 929–940, doi: 10.1007/s00404-017-4518-2, indexed in Pubmed: 28905115.
15. Battaglia A, Hoyme HE, Dallapiccola B, et al. Further delineation of deletion 1p36 syndrome in 60 patients: a recognizable phenotype and common cause of developmental delay and mental retardation. Pediatrics. 2008; 121(2): 404–410, doi: 10.1542/peds.2007-0929, indexed in Pubmed: 18245432.
16. Digilio MC, Bernardini L, Lepri F, et al. Ebstein anomaly: Genetic heterogeneity and association with microdeletions 1p36 and 8p23.1. Am J Med Genet A. 2011; 155A(9): 2196–2202, doi: 10.1002/ajmg.a.34131, indexed in Pubmed: 21815254.
17. Hertz JM, Gregory-Evans CY, Moosajee M, et al. Translocation breakpoint maps 5 kb 3′ from TWIST in a patient affected with Saethre-Chotzen syndrome. Hum Mol Genet. 1997; 6(7): 1079–1086, doi: 10.1093/hmg/6.7.1079, indexed in Pubmed: 9215678.
18. Hemati P, Revah-Politi A, Bassan H, et al. C4RCD Research Group, DDD study. Refining the phenotype associated with GNB1 mutations: Clinical data on 18 newly identified patients and review of the literature. Am J Med Genet A. 2018; 176(11): 2259–2275, doi: 10.1002/ajmg.a.40472, indexed in Pubmed: 30194818.
19. Sentex E, Wang Xi, Liu X, et al. Expression of protein kinase C isoforms in cardiac hypertrophy and heart failure due to volume overload. Can J Physiol Pharmacol. 2006; 84(2): 227–238, doi: 10.1139/y05-120, indexed in Pubmed: 16900949.
20. Wu SC, Solaro RJ. Protein kinase C zeta. A novel regulator of both phosphorylation and de-phosphorylation of cardiac sarcomeric proteins. J Biol Chem. 2007; 282(42): 30691–30698, doi: 10.1074/jbc.M703670200, indexed in Pubmed: 17724026.
21. Gilsoul M, Grisar T, Delgado-Escueta AV, et al. Subtle brain developmental abnormalities in the pathogenesis of juvenile myoclonic epilepsy. Front Cell Neurosci. 2019; 13: 433, doi: 10.3389/fncel.2019.00433, indexed in Pubmed: 31611775.
22. Rosenfeld JA, Crolla JA, Tomkins S, et al. Refinement of causative genes in monosomy 1p36 through clinical and molecular cytogenetic characterization of small interstitial deletions. Am J Med Genet A. 2010; 152A(8): 1951–1959, doi: 10.1002/ajmg.a.33516, indexed in Pubmed: 20635359.

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Diagnostic potential of microRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia in the first trimester

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ABSTRACT

Objectives: MicroRNAs have been observed to play a major role in various physiological processes, for instance, programmed cell death, cell division, pregnancy development, and proliferation. With the help of profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems. The aim of the study was to evaluate the diagnostic potential of MicroRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia.

Material and methods: The study considered 53 patients who are in their first trimester of a singleton pregnancy. Participants have been divided into two study groups, one group with normal pregnancy and another group having the risk of developing preeclampsia or who developed hypertension or preeclampsia during follow-up constitute the study group. In order to collect data associated with circulating miRNAs in serum, blood samples have been collected from the participants of the study.

Results: Based on the univariate regression model, increased expression of Mi 517 and 526 and parity status (primapara/multipara) has been obtained. The multivariate logistic analysis shows that independent risk factors for hypertension or preeclampsia are the presence of an R527 and being a primipara.

Conclusions: The study's findings have revealed that R517s and R526s act as major indicative biomarkers in the first trimester for the detection of hypertension and preeclampsia. The circulating C19MC MicroRNA was examined as a potential early indicator of preeclampsia and hypertension in pregnant individuals.

Keywords: circulating microRNA; microRNAs; hypertension; biomarkers; pregnant women; pregnancy

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INTRODUCTION

Up to 10% of pregnancies can become complicated by hypertensive disorders of pregnancy, which include preexisting and gestational hypertension, eclampsia, and preeclampsia [1]. These conditions are a substantial source of perinatal and maternal mortality and morbidity. Preeclampsia/eclampsia and preeclampsia along with persistent hypertension are all considered hypertensive diseases of pregnancy (HDP). After 20 weeks of pregnancy, pre-eclampsia, manifests as hypertension and proteinuria. It appears in between 2 and 10% of pregnancies [2]. Pre-eclampsia is a serious pregnancy complication, and its clinical symptoms start to appear in the second trimester. Preeclampsia is described as the co-occurrence of hypertension 'proteinuria (> 0.3 g of protein in the 24-hour urine sample) and (blood pressure > 140/90 mmHg) in an initially normotensive woman after 20 weeks of pregnancy [3]. Pre-eclampsia is a significant factor in raising the risk of morbidity and mortality in fetuses or newborns and pregnant women due to the potential path leading to serious problems, such as proteinuria, convulsions (eclampsia), and hypertension. Preeclampsia may make future heart and blood vessel (cardiovascular) illnesses

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more likely. When a woman has experienced preeclampsia more than once, she is more vulnerable to developing cardiovascular problems in the future [4]. Pregnancy-related hypertension and preeclampsia carry concerns for lower placental blood flow. The fetus can receive less oxygen and fewer nutrients in case the placenta doesn't receive enough blood. Premature delivery, delayed growth (intrauterine growth restriction), and low birth weight can be consequential of the condition [5]. Before giving birth, the placenta in this syndrome separates from the uterus' inner wall. The risk of placental abruption is elevated by high blood pressure and preeclampsia. The life of both the mother and the unborn child may be in danger if there is a severe abruption that results in excessive bleeding. Slowed or reduced fetal growth may be a result of high blood pressure. The heart, brain, liver, eyes, kidneys, lungs, and other important organs might suffer damage from poorly controlled high blood pressure. It may even be fatal in extreme circumstances.

It is essential to identify a successful method to select out women who are likely to experience a severe case of preeclampsia for the prevention of this condition [3]. The prognostic indicators that are being considered include markers and biochemical aspects of maternal blood uterine activity, such as certain proteins, circulating microRNAs and circulating cell-free DNA, and ultrasonography [6]. The specific microRNAs from a group of non-coding RNAs that are relevant to this investigation are the main factors (ncRNAs). MicroRNAs (miRNAs) are single-stranded, 19–25 nucleotide-long RNA molecules. MicroRNAs are crucial in the post-transcriptional control of gene expression. In a process known as "silencing," microRNAs control the expression of more than 60% of all human genes.

MicroRNAs have been found to play a significant role in various physiological processes, such as programmed cell death, cell division, pregnancy development, and proliferation. It has been also found to play a role in a variety of pathological processes, including the development of myocardial infarctions, tumors, reactions to inflammation and infections, and the onset of chronic diseases [7]. In addition to that, for pre-eclampsia and other pregnancy-related disorders microRNAs have been identified as regulators of processes that occur throughout pregnancy. Pregnant women's serum microRNA profiles are different from those of non-pregnant women [8]. By contrasting the serum profile of microRNAs in non-pregnant and pregnant women, or between before and after labor, it is possible to distinguish microRNAs associated with pregnancy and the placenta [9]. Among pregnant women, the microRNA profile in the serum changes both qualitatively and quantitatively as the trophoblast develops and increases in volume and mass. According to recent findings, microRNAs may control 'cell migration in pre-eclampsia and apoptosis (programmed cell death)' [10]. Recent research on the pathogenesis of pregnancy complications linked to the presence of trophoblast (PIRCs, placental insufficiency-related complications) has revealed that microRNAs can be divided into four categories: placenta-associated, placenta-specific, circulating, uterine, and placenta-derived [11]. Through the profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems [12]. Thus, it is with the help of observing statistical differences in serum microRNAs expression levels among pregnant women with healthy pregnancies and women pregnant with pre-eclampsia, which enables selected, particular microRNAs to serve as safe clinical biomarkers of major complications at the time of the pregnancy. Studies have put forward the fact that up-regulation of miR-517-5p,miR-520h, and miR-518b are associated with the risk of later progression of preeclampsia [13]. It has also been identified in the screening of extracellular miR-517--5p during the first trimester a significant section of women develop subsequent preeclampsia. It has been mentioned in this context that the presence of elevated first-trimester plasma levels of miR-517-5p can be considered predictive of preeclampsia. In the study, it has been mentioned that miR-517-5p biomarker alone has been observed by the researchers as a predictive performance for preeclampsia [13]. The researchers also opined that C19MC microRNAs play a considerable role in the pathogenesis of complications associated with pregnancy. It has been reported in this context that in maternal circulation during the first trimester or early in the pregnancy C19MC microRNAs are dysregulated and it can play a role in stimulating preeclampsia and gestational hypertension [14].

Objectives

The objectives of the current study are to discuss the role of microRNAs and their biomarkers during pregnancy. It would also focus on identifying the role of microRNAs in pre-eclampsia development. The researcher would also evaluate the diagnostic potential of MicroRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia.

MATERIAL AND METHODS

Study design

Considering the need of the study, the researcher would be conducting a cohort study, wherein the researcher has considered two study groups, one group with normal pregnancy and another group having the risk of developing preeclampsia or who developed hypertension or preeclampsia during follow-up constitute the study group. This paper employs the STROBE guideline for reporting observational studies [15].

Participants

The study considered 53 patients who are in their first trimester, who were selected based on their availability and the stage of pregnancy of these patients. Herein, the study has considered only patients in their first trimester having a singleton pregnancy, which acted as an eligibility criterion for the selection of the participants. Among the chosen study group, 25 patients have been selected with normal pregnancies, who have been noted to not have risk factors associated with pre-eclampsia or who had not developed pre-eclampsia or hypertension at the time of follow-up. This section of the study group has been considered the control group for the study. Another 25 patients considered for the study comprised patients who had developed preeclampsia or hypertension at the time of follow-up or patients identified as having the risk factors of preeclampsia or hypertension during the follow-up. This section of the participants has been considered as the study group. The remaining three patients were excluded from the study analysis because of the absence of cDNA in the reverse transcription reaction, despite the various attempts. Four patients were further incorporated into the control group, which comprised a priori that they had no risk factors associated with developing preeclampsia or hypertension. Among these four patients, one patient had a stillbirth, and three patients developed gestational diabetes during pregnancy.

Sampling

For the purpose of determining the microRNA profile from participants of the study, sterile collection of 9.8 mL of peripheral venous blood (2 × 4.9 mL) has been performed into tubes with EDTA as an anticoagulant. Sampling has been performed at week 12 (first trimester).

Variables

Regarding the different variables associated with the study, the age of the patient has been identified as one of the variables. Herein, based on the age of the patients, the age group has been classified into two groups. One group comprised mothers below the age of 35 years and the other group comprised the advanced age of mothers who are of 35 years and above. Another variable is the risk of chromosomal aberrations, wherein, the study considered the risk of chromosomal aberrations: low risk > 1:1000, intermediate risk 1:300–1:1000; and high < 1:300. PAPP-A protein MoM is another variable considered for the study, for which, patients having PAPPA protein MoM less than 0.5–0.4 were identified to have elevated risk of developing preeclampsia.

Measurement

In order to collect data associated with circulating miRNAs in serum, blood samples have been collected from the participants of the study, which was the source of data for circulating miRNAs in serum. RNA isolation was the data source for collecting information associated with a fraction of short RNA fragments. RQ-PCR has been the data source for the miRNA expression profile, which provided information associated with 40 miRNAs that are associated with trophoblast. A group of miRNA molecules has been the data source for comparing statistically significant differences in expression levels between the control and study group.

Methodology for analyzing circulating miRNA's in serum

Blood samples have been collected from the participants under sterile conditions, followed by a 1-hour incubation period at room temperature. After collecting the blood samples, whole blood was centrifuged, for the purpose of separating separate serum from blood morphotic elements. Followed by collecting the serum which has been frozen at –80° C. RNA isolation has been conducted with the help of a ready-made RNA isolation kit that is specifically for the fraction of short RNA fragments. With the help of light absorption at 260 nm in a spectrophotometer the concentration and purity of RNA have been checked. In order to reduce the risk of DNA contamination, for a duration of 30 minutes incubation with DNase at 37° C has been performed. For determining the miRNA expression profile, the researcher has considered RQ-PCR based on Qiagen's dedicated kits. In this study, profiling of 40 miRNAs has been performed, the expression of which has been identified to be associated with the presence of trophoblastin in the pregnant population. After evaluating the results derived from the sample patients who developed preeclampsia and comparing the results of the patients having physiologically normal pregnancies, a group of miRNA molecules has been selected, for which, the study has demonstrated statistically significant differences in expression levels. Furthermore, with the help of qRT-PCR the expression level of the selected miRNA molecules in each group of women have been rechecked.

Calculations according to the formula R = 2-ΔΔCT

For the purpose of determining the relative expression levels of individual miRNAs at the mRNA level, the ΔΔCt comparative method has been implemented in this study, much like the assessment of ABI-1 gene expression. In this context, in order to implement the ΔΔCt comparative method, the endogenous control miR-423-5p has been implemented. In this alignment, it has been assumed that the value; of 0.8 < < R < 1.2 is indicative of a normal amplification range (N). For the value of R < 0.8 for the test group samples, it is indicative of a decrease in amplification (–). R > 1.2 for test group samples, it has been considered indicative of an increase in

amplification (+). For R = 0 for test group samples, it implies no amplification (0) for the expression level of individual miRNAs in the group of patients.

Statistical analysis

Quantitative data are presented as a median with an interquartile range, while qualitative ones are presented as a number of cases with a percentage value. To compare quantitative variables Mann Whitney U test was used due to the nonnormal distribution of the data assessed by Shapiro--Wilk test. Only for the age variable T-student test was used due to its normal distribution. To compare qualitative variables, the Chi-square test was used for data with more than two categories, while dichotomic variables were analyzed with the use of the Fisher test. To assess usefulness of investigated miRNA as predictive factors, a general linear model (GLM) and logistic regression were applied. The predictive ability of the obtained model was analyzed by the ROC curve with cut off values derived from Youden index. Analysis was performed using RStudio software (Integrated Development for R. RStudio, PBC, Boston, MA, USA).

RESULTS

The total number of participants who remained for the study is 48. These 48 participants have been equally grouped for the study into two groups, that is, the control group comprised of 24 patients, and the study group comprised of 24 patients. The mentioned number of participants for both groups continued till the end of the study. All the participants in both groups were subjected to follow-up and the sampling was performed at week 12, which is their first trimester of pregnancy.

Descriptive data

The study comprised women aged in two categories, one group was below the age of 35 years and another group of women was above the above of 35 years. The patients considered for the study were in the first trimester and had a singleton pregnancy. In regard to the clinical conditions of the control group, the sample has not developed hypertension or pre-eclampsia during follow-up, and they were free from any risk factors for pre-eclampsia. In the case of the study group, the participants had developed hypertension or preeclampsia during follow-up or were having risk of developing preeclampsia. In the case of three patients in the control group, they had developed gestational diabetes.

Outcome data

Based on the observed findings from the present study, it has been noted that among all the factors of PAPP-A protein, hypothyroidism, diabetes mellitus, and gravidity, it is only the factor of gravidity among the patients, which has been noted to have a statistically significant association with developed hypertension or preeclampsia among the patients during their first trimester. The other mentioned aspects associated with the patients have been noted to have no statistically significant association with developed hypertension or preeclampsia. The aspect of parity also demonstrated a statistically significant association with hypertension or preeclampsia among the patients, wherein univariate analysis showed a significantly decreased risk associated with a plurality. Univariate analysis showed a significantly increased risk of the development of hypertension or preeclampsia associated with the presence of R517s. The univariate analysis also demonstrated a significantly increased risk associated with the presence of R526s.

Main results

It has been noted that there exist significant differences in miRNA 517ddct, miRNA 526ddct, and gestational age of delivery between the control and study group. These variables have been noted to be higher in the study group (Tab. 1).

Based on the collected samples from the participants of the study, the association of PAPP-A protein, hypothyroidism, diabetes mellitus, and gravidity with the development of hypertension or preeclampsia during their first trimester was analyzed. With the help of the analysis, it has been noted that, for none of the factors, apart from gravidity and parity the association with the development of hypertension or is statistically significant. On conducting univariate analysis, in context to parity, it has been noted that parity has a statistically significant association with hypertension or preeclampsia during the first trimester among patients, with reduced risk of developing the conditions with pluriparity [odds ratio (OR) = 0.190; CI 0.04–0.81; p = 0.016]. This analysis demonstrated that gravidity significantly decreases the risk of developing hypertension or preeclampsia (OR = 0.050; CI 0.00–0.41; p = 0.001) (Tab. 2).

For R210s when the patients of both the control and the study group were compared it was noted that, for 14 patients in the control group the percentage derived is 58.33%, (–R210s), and for 7 patients it is 29.17% (+R210s), for the study group, among 8 patients it has been recorded to be 32.00% (–R210s), among 11 patients 44.00% (+R210s) based on which, it could be inferred that for R210s, no significant differences. For R517s as well, no significant differences were found between the control and the study group, wherein for 17 patients in the control group it has been recorded to be 70.83% (–R517s), for 1 patient 4.17% (+R517s) and when compared to study group it has been recorded to be 24.00% for 6 patients in the study group (–R517s), 40.00% for 10 patients in this group (+R517s), indicating no significant differences for R517s between the control and the study group, however, the percentage of patients having –R517s

Table 1. Characteristics of the study group

	Control group			Study group			p
	Median	q1	q3	Median	q1	q3	
miRNA 210ddct	0.79	0.577	1.32	1.19	0.66	2.23	0.13
miRNA 517ddct	0.158	0.128	0.284	2.14	0.603	3.36	**0.00**
miRNA 526ddct	0.178	0.093	0.42	1.3	0.465	3.94	**0.00**
bHCG_[MoM]	1.02	0.921	1.82	1.03	0.75	1.82	0.60
BMI	23.2	20.8	25.9	22.7	21	25.2	0.68
Gestational age of delivery [weeks]	38	38	39	39	38	40	**0.03**
Mean miRNA 210	33.2	32.8	34	32.6	32	33.7	0.19
Mean miRNA 517	34.7	33.7	35.9	35.3	20.3	36.1	0.73
Mean miRNA 526	35.1	33.8	36.4	35.4	33.7	36.5	0.91
Mean C425	27.6	26.9	28.5	27.4	26.7	29.3	0.98
PAPP-A_[MoM]	1.01	0.716	1.54	0.924	0.668	1.52	0.54
Age [years]	34.5	32.8	36.2	33	29	36	0.21

BMI — body mass index

Table 2. Univariate analysis of risk factors of hypertension or preeclampsia

	Control group		Study group		OR	95% CI		p
	n	%	n	%				
Hypothyroidism								
0	14	58.33	17	68.00				
1	10	41.67	8	32.00	0.66	0.17	2.46	0.561
Diabetes mellitus								
0	21	87.50	21	84.00				
1	3	12.50	4	16.00	1.33	0.20	10.18	0.100
Gravidity								
1	1	4.17	12	48.00				
> 1	23	95.83	13	52.00	0.05	0.00	0.41	**0.001**
Parity								
1	4	16.67	13	52.00				
> 1	20	83.33	12	48.00	0.19	0.04	0.81	**0.016**
Hypertension								
0	24	100.00	23	92.00				
1	0	0.00	2	8.00	–	–	–	0.490
Premature								
0	23	95.83	22	88.00				
1	1	4.17	3	12.00	3.067	0.23	171.51	0.609
Stillbirth								
0	23	95.83	25	100.00				
1	1	4.17	0	0.00	–	–	–	–
Age group								
0	12	50.00	15	60.00				
1	12	50.00	10	40.00	0.672	0.19	2.38	0.571

OR — odds ratio; CI — confidence interval

Table 3. Univariate analysis of risk factors of hypertension or preeclampsia

	Control group		Study group		OR	95% CI		p
	n	%	n	%				
R210s								
–	14	58.33	8	32.00	0.344	0.09	1.25	0.088
+	7	29.17	11	44.00	1.883	0.51	7.43	0.378
0	3	12.50	6	24.00				
R517s								
–	17	70.83	6	24.00	0.088	0.01	0.47	**0.001**
+	1	4.17	10	40.00	19.44	2.20	957.22	**0.001**
0	2	8.33	3	12.00				
R526s								
–	17	70.83	9	36.00	0.120	0.02	0.59	**0.004**
+	2	8.33	12	48.00	9.288	1.62	100.96	**0.004**
0	1	4.17	2	8.00				

OR — odds ratio; CI — confidence interval

Table 4. Multivariate model of risk factors of hypertension or preeclampsia

	estimate	aOR	95% CI		p
(Intercept)	0.6620	1.94	1.52	2.47	**0.001**
primapara	0.5340	1.71	2.21	1.32	**0.001**
R526s_+	0.4327	1.54	1.18	2.01	**0.003**
R517s_+	0.1487	1.16	0.86	1.57	0.339

aOR — adjusted Odds Ratio; CI — confidence interval

is significantly higher among the patients of the control group as compared to the study group, which is indicative of R517s have association with the development of hypertension or preeclampsia during the first trimester, which is statistically significant. The univariate analysis demonstrated a significantly increased risk of development of hypertension or preeclampsia associated with the presence of R517s (OR = 19.44; CI 2.20–957.22; p = 0.004). The univariate analysis showed also a significantly increased risk of developing hypertension or preeclampsia (OR = 9.28; CI 1.62–100.96; p = 0.004) associated with the presence of R526s (Tab. 3).

Based on the univariate logistic regression model, increased expression of Mi 517 and 526 and parity status (primapara/multipara) has been obtained. The analysis shows that independent risk factors for hypertension or preeclampsia are the presence of an R527 and being a primipara (Tab. 4). The model characterizes by 82.4% sensitivity and 94.4% specificity (Fig. 1).

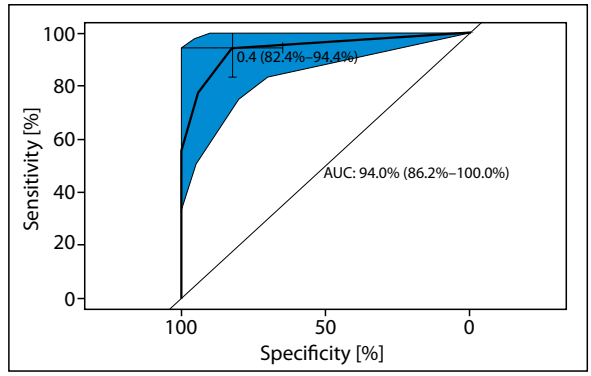

Figure 1. Receiver operating characteristic (ROC) curve for the multivariate model; AUC — area under the curve

DISCUSSION

Based on the results from the current study, it has been observed that R517s and R526s act as major indicative biomarkers in the first trimester for the detection of hypertension and preeclampsia. Herein based on the univariate analysis it has been observed that the presence of R517s and R526s significantly increases the risk of hypertension and preeclampsia among the patients.

Limitations

The limited number of patients considered for the study can be regarded as one of the limitations of this study.

Interpretation

Based on the findings from the current study it can be seen to be aligned with the study conducted by Hromadnikovaet al. [13], wherein the researchers have stated that through the profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems. The researchers have stated in this context that observing statistical differences in serum microRNAs expression levels among pregnant women with healthy pregnancies and women pregnant with pre-eclampsia, enables selected, particular microRNAs to serve as safe clinical biomarkers of major complications at the time of the pregnancy. The previous findings have provided the observations that up-regulation of miR-517-5p,miR-520h, and miR-518b is related to the risk of later progression of preeclampsia. It has also been identified in the screening of extracellular miR-517-5p during the first trimester a significant section of women develop subsequent preeclampsia [14]. It has been concluded in that study that the presence of elevated first-trimester plasma levels of miR-517-5p can be considered as predictive of preeclampsia [13]. The findings of the current study can be seen to be aligned with the mentioned findings wherein it has been noted that R517s act as a major indicative biomarker for the detection of hypertension and preeclampsia, wherein a similar finding has been reported that miR-517-5p biomarker alone has been observed by the researchers as a predictive performance for preeclampsia [13]. However, the finding of the present study on miRNA-210 can be seen to be contradicting with the study conducted by Jaszczuk et al. [16], wherein in the current study it has been noted that miRNA-210 is not statistically significant with the development of hypertension and preeclampsia. Currently, screening for preeclampsia is performed using biochemical tests, but the inclusion of new markers may lead to increased sensitivity and specificity of screening. The mRNA cannot be used to evaluate the entire population at present due to its expensive cost, but it may be applied to specific cases, such as high-risk. in addition, soon, we expect to reduce the cost of mRNA evaluation, after which it will be possible to use it in population screening. Therefore, research to reveal the mechanisms occurring is important to be able to use mRNA in further diagnostics.

CONCLUSIONS

In the conclusion, it can be stated that pregnant patients' use of biomarkers like C19MC MicroRNA would help define pregnancy monitoring and put therapies into place. The findings of the study revealed that independent risk factors for hypertension or preeclampsia are the presence of an R527s and being a primipara. In this work, the circulating C19MC MicroRNA was examined as a potential early indicator of preeclampsia and hypertension in pregnant individuals.

Article information and declarations

Ethical approval

The study was approved by the Bioethics Board of Medical University of Lublin (KE-0254/107/2019) and complied with the Declaration of Helsinki and good clinical practice guidelines. All participants provided written informed consent.

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Kondracka A, Jaszczuk I, Koczkodaj D, et al. Blood serum microRNA profiles of pregnant women as biomarkers of pre-eclampsia evaluation. J Pre-Clin Clin Res. 2020; 14(4): 174–177, doi: 10.26444/jpccr/131597.
2. Schiesser M, Holzgreve W, Lapaire O, et al. Sirenomelia, the mermaid syndrome — detection in the first trimester. Prenat Diagn. 2003; 23(6): 493–495, doi: 10.1002/pd.624, indexed in Pubmed: 12813764.
3. Park HJ, Shim SS, Cha DH. Combined Screening for Early Detection of Pre-Eclampsia. Int J Mol Sci. 2015; 16(8): 17952–17974, doi: 10.3390/ijms160817952, indexed in Pubmed: 26247944.
4. Leslie MS, Briggs LA. Preeclampsia and the Risk of Future Vascular Disease and Mortality: A Review. J Midwifery Womens Health. 2016; 61(3): 315–324, doi: 10.1111/jmwh.12469, indexed in Pubmed: 27155218.
5. Duley L. Pre-eclampsia and the hypertensive disorders of pregnancy. Br Med Bull. 2003; 67: 161–176, doi: 10.1093/bmb/ldg005, indexed in Pubmed: 14711762.
6. Tan MY, Syngelaki A, Poon LC, et al. Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol. 2018; 52(2): 186–195, doi: 10.1002/uog.19112.
7. Vickers NJ. Animal Communication: When I'm Calling You, Will You Answer Too? Curr Biol. 2017; 27(14): R713–R715, doi: 10.1016/j.cub.2017.05.064, indexed in Pubmed: 28743020.
8. Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. PLoS One. 2008; 3(9): e3148, doi: 10.1371/journal.pone.0003148, indexed in Pubmed: 18773077.
9. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol Hematol. 2011; 80(2): 193–208, doi: 10.1016/j.critrevonc.2010.11.004, indexed in Pubmed: 21145252.
10. Zhao G, Zhou X, Chen S, et al. Differential expression of microRNAs in decidua-derived mesenchymal stem cells from patients with pre-eclampsia. J Biomed Sci. 2014; 21(1): 81, doi: 10.1186/s12929-014-0081-3, indexed in Pubmed: 25135655.
11. Pantham P, Soncin F, Zhang-Rutledge K, et al. Innovations in Placental Pathology. Benirschke's Pathology of the Human Placenta. 2021: 837–867, doi: 10.1007/978-3-030-84725-8_31.
12. Martínez-Ibarra A, Martínez-Razo LD, Vázquez-Martínez ER, et al. Unhealthy Levels of Phthalates and Bisphenol A in Mexican Pregnant Women with Gestational Diabetes and Its Association to Altered Expression of miRNAs Involved with Metabolic Disease. Int J Mol Sci. 2019; 20(13), doi: 10.3390/ijms20133343, indexed in Pubmed: 31284700.
13. Hromadnikova I, Kotlabova K, Ivankova K, et al. First trimester screening of circulating C19MC microRNAs and the evaluation of their potential to predict the onset of preeclampsia and IUGR. PLoS One. 2017; 12(2): e0171756, doi: 10.1371/journal.pone.0171756, indexed in Pubmed: 28182660.
14. Bounds KR, Chiasson VL, Pan LuJ, et al. MicroRNAs: New Players in the Pathobiology of Preeclampsia. Front Cardiovasc Med. 2017; 4: 60, doi: 10.3389/fcvm.2017.00060, indexed in Pubmed: 28993808.
15. von Elm E, Altman DG, Egger M, et al. STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008; 61(4): 344–349, doi: 10.1016/j.jclinepi.2007.11.008, indexed in Pubmed: 18313558.
16. Jaszczuk I, Koczkodaj D, Kondracka A, et al. The role of miRNA-210 in pre-eclampsia development. Ann Med. 2022; 54(1): 1350–1356, doi: 10.1080/07853890.2022.2071459, indexed in Pubmed: 35543206.

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Effect of *Ureaplasma/Mycoplasma* genital tract infection on preterm labor

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ABSTRACT

Objectives: Genitourinary tract infections in pregnant women are one of the causes of abnormal pregnancy development including miscarriages, premature labor or premature rupture of membranes (PPROM). Atypical bacteria responsible for reproductive tract infections include *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*. Identification of pathogens and appropriately selected therapy can improve obstetric outcomes in patients with symptoms of threatened miscarriage or threatened preterm labor.

The purpose of our study is to analyze the impact of reproductive tract infections with *Ureaplasma* and *Mycoplasma* bacteria during pregnancy.

Material and methods: In the presented study, we retrospectively analyzed the cases of 201 pregnant patients hospitalized in the Obstetrics and Gynecology Department of Poznan Regional Hospital in 2019–2022, who had a swab taken from external os area of the cervix for atypical bacteria — *Ureaplasma* and *Mycoplasma*. Only patients with symptoms of threatened miscarriage or threatened preterm labor were included in the study group. Microbiological tests were performed in the hospital laboratory with the Mycoplasma IST 3 test from Biomerieux.

Results: We found a higher incidence of preterm labor in patients with symptoms of threatened preterm labor and a genital tract infection with *Ureaplasma/Mycoplasma* bacteria, compared to patients not infected with *Mycoplasma/Ureaplasma* — 31.1% vs 20% (p = 0.098). This observation in the case of *Ureaplasma/Mycoplasma* monoinfection group applied to 6 patients. This observation in the case of *Ureaplasma/Mycoplasma* monoinfection group applied to 6 patients — 75% of the group. Pregnant patients who had co-infection with other types of bacteria (48 patients in total) gave birth before 37 weeks of pregnancy in 27.1% of cases. We obtained a significant difference (p = 0.007) when comparing groups with positive and negative cultures for *Ureaplasma/Mycoplasma* by the presence of monoinfection/coinfection and the week of pregnancy in which delivery occurred. We also noted the effect of atypical bacterial infection for PPROM — this complication preceded preterm delivery in 40% of ureaplasma-positive patients, compared to 20% of PPROM without infection. We found a similar rate of preterm labor and pregnancy loss in *Ureaplasma/Mycoplasma*-positive patients who received antibiotic therapy (35.7%) compared to a group of pregnant women who did not receive treatment (31.6%).

Conclusions: Infection of the genital tract with atypical bacteria *Ureaplasma* and *Mycoplasma* has a negative impact on the course of pregnancy. Identification of the type of microorganisms in cervical canal secretions of pregnant patients with symptoms of threatened miscarriage or preterm labor seems crucial. The impact of antibiotic therapy though, requires further analysis.

Keywords: *Ureaplasma*; *Mycoplasma*; preterm labor; birth tract infection; premature preterm rupture of membranes (PPROM); miscarriage

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INTRODUCTION

One of the groups of pathogens responsible for genitourinary infections are atypical bacteria, such as *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*. They can be found both in the normal bacterial flora of the vagina in sexually active women and may be the causative agent of chorioamnionitis, inflammation of the appendages, bacterial vaginosis or endometritis in the puerperium [1–3]. They often cause infections with an asymptomatic course, making diagnosis and treatment difficult.

Ureaplasma bacteria are present in the vaginal secretions of about 50% of pregnant women, but only a fraction of them become infected by the ascending route, leading to intrauterine infection and preterm labor [4–6]. Each year, about 15 million babies worldwide are born prematurely, accounting for about 11% of all births. In Europe, the number of births before 37 weeks of pregnancy is gradually increasing [7]. Although *Ureaplasma* infection is an independent risk factor for adverse pregnancy outcome, the greatest risk is in patients with additional aggravating factors, such as bacterial vaginosis in pregnancy [8] or a previous history of preterm labor [9].

The exact mechanism of this phenomenon is not known. Abnormal vaginal bacterial flora can pave the way for ascending infection by *Ureaplasma* bacteria by weakening local immunity in the lower parts of the reproductive tract as well as affect the increase in the number of atypical bacteria [4]. At the same time, the urease produced by *Ureaplasma* breaks down urea to ammonia and *Mycoplasma* produces ammonia from arginine. These reactions lead to an increase in the pH of vaginal secretions and facilitate genital tract infections with other pathogens facilitating the development of, for example, bacterial vaginosis [10].

Ureaplasma are the most isolated pathogens in cervical secretions and of the amniotic cavity in patients who have had a preterm delivery or PPROM [11–13]. These pathogens found in upper respiratory tract secretions, blood serum or cerebrospinal fluid in premature babies, increase the risk of bronchopulmonary dysplasia, open ductus arteriosus, chronic lung disease, intraventricular brain hemorrhage leading to severe complications and increasing neonatal and child mortality [14, 15].

The gold standard in identification of *Ureaplasma* is the microbiological culture, which allows simultaneous determination of an antibiogram when the pathogen is found in the material under examination. A more modern and accurate method of pathogen identification uses polymerase chain reaction (PCR), which is particularly applicable for the determination of a specific bacterial species. The disadvantage of the method is the inability to perform an antibiogram. Treatment of asymptomatic vaginal infections in pregnancy remains a contentious issue. Given the prevalence of *Ureaplasma* infections in pregnant women, it seems that the intervention group should be more carefully selected [4]. At the same time, studies on the treatment of vaginal infections in pregnancy have shown that the inclusion of oral or vaginal antibiotic therapy prolongs the duration of pregnancy [16, 17]. The drugs of choice in pregnant women and children remain macrolides including erythromycin, azithromycin and clarithromycin [18–22].

Purpose

The purpose of our study is to analyze the impact of lower genital tract infections with *Ureaplasma* or *Mycoplasma* bacteria in pregnant patients with symptoms of threatened miscarriage or threatened preterm labor illustrated by pregnant women hospitalized at the Obstetrics and Gynaecology Department at Regional Hospital in Poznan in 2019–2022. We will retrospectively analyze the frequency of genital tract infections with atypical bacteria in the study group based on available medical records. We will check whether infection with *Ureaplasma/Mycoplasma* bacteria affects the subsequent course of pregnancy, including the incidence of preterm labor or premature preterm rupture of membranes (PPROM). We will compare the type of delivery, number of past pregnancies and type of pregnancy (single or multiple) in patients with positive and negative cultures for atypical bacteria. We will determine the prevalence of infections with aerobic, anaerobic bacteria or fungi in the lower genital tract in both groups of patients and their impact on the timing of delivery. We will also evaluate whether the lack of treatment for *Ureaplasma/Mycoplasma* in a swab taken from the external os area of the cervical canal significantly affects the subsequent course of pregnancy.

MATERIAL AND METHODS

In this study, we retrospectively analyzed the cases of 201 pregnant patients. hospitalized in the Obstetrics and Gynecology Department of Poznan Regional Hospital in 2019–2022, who were swabbed for atypical bacteria — *Ureaplasma* and *Mycoplasma* — from the external orifice of the cervical canal due to symptoms of threatened miscarriage or threatened preterm labor. These patients manifested the following clinical symptoms: lower abdominal pain, spotting or bleeding from the genital tract, uterine contraction activity, cervical shortening, dilation of the cervical canal or preterm premature rupture of the membranes.

Our department belongs to the second level of referral of perinatal care.

The analysis was based on medical records — results of bacteriological cultures and electronic records, including discharge cards available in the hospital integrated information system, as well as information obtained directly from

patients whose deliveries took place outside our center. The group of patients included both primiparous and multiparous women, in single and twin pregnancies.

Microbiological tests were performed in the hospital laboratory using the Mycoplasma IST 3 test from Biomerieux. The antibiogram included the reaction to:

- azithromycin, clarithromycin, erythromycin, ciprofloxacin, ofloxacin, doxycycline;
- tetracycline, iosamycin, and pristinamycin in tests performed in 2019 and 2020;
- erythromycin, levofloxacin, moxifloxacin, telithromycin, and tetracycline in tests performed in 2021 and 2022.

Microbiologists interpreted the antibiogram according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) bacteria v 9.0, 10.0, 11.0 and 12.0 depending on when the test was performed (2019–2022).

Positive culture results were considered those in which bacteria were present at the level of $\geq 10^4$/mL.

Statistical significance p was calculated using Pearson's Chi-square test or Fisher's exact test, depending on whether the relevant assumptions were met. A $p \leq 0.05$ value was considered as the cutoff point.

RESULTS

During the analyzed years (2019–2022), bacterial cultures were performed from the area of the external os of the cervical canal for *Ureaplasma* and *Mycoplasma* in 236 pregnant women. The inclusion criterion for the study was the known type and time of delivery. The studies of 61 positive and 140 negative (Fig. 1, 2) patients were used for further analysis (Fig. 1, 2). The remaining 35 patients were excluded from the study, due to incomplete data on the course of the pregnancy. In 4 patients at the time of writing the paper the pregnancy was still ongoing.

The gestational age of positive patients ranged from 13-36 completed weeks of pregnancy. Patients with threatened miscarriage accounted for 9.8% and the remaining 90.2% of cases included pregnant women with features of threatened preterm labor. The gestational age of the patients in whom we obtained a negative result for *Ureaplasma/Mycoplasma* was in the range of 12–36 weeks. In this group, signs of threatened miscarriage affected 11.4% and threatened preterm labor affected 88.6% of women (Tab. 1).

In the microbiological results obtained, *Ureaplasma* was responsible for the infection of the lower genital tract (96.7% of positive results), in two cases *Mycoplasma* was cultured (3.3% of positive results).

Among the analyzed group, preterm labor between 22 and 36 + 6 weeks of gestation occurred in 19 patients, representing 31.1% of pregnant women with positive smear results for atypical bacteria and known course of pregnancy. 3.3% of *Ureaplasma* positive patients had a miscarriage,

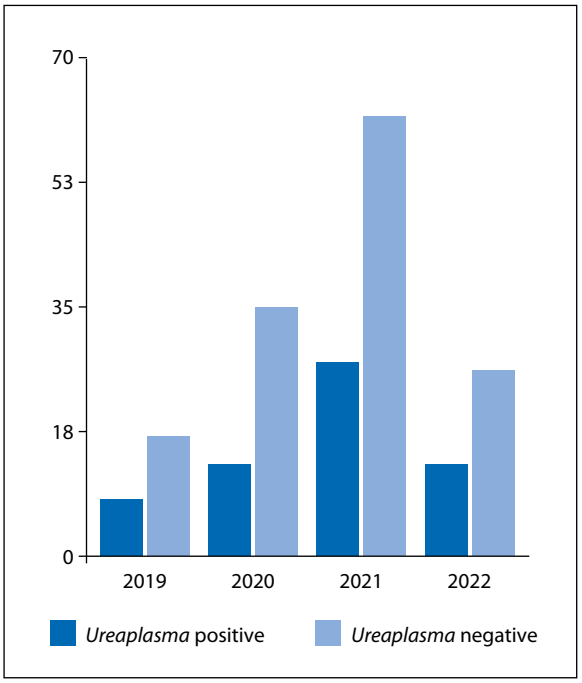

Figure 1. The number of patients with positive and negative culture results from the cervical canal for *Ureaplasma/Mycoplasma* bacteria, considering the calendar year in which the test was performed

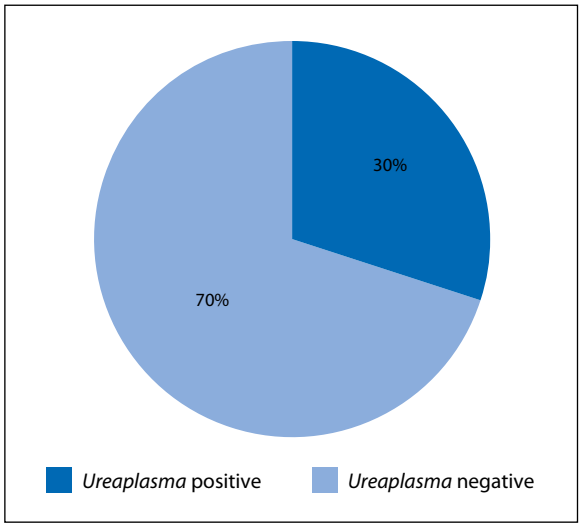

Figure 2. Total number [%] of positive and negative *Ureaplasma* patients in 2019–2022

in both cases at 17 weeks of pregnancy. Timely deliveries, above 36 + 6 weeks' gestation, occurred in 40 patients, accounting for 65.6% of cases (Tab. 1).

Patients were treated within the department or included in outpatient treatment. The most used drug was azithromycin — 36 cases, clarithromycin in two patients or clindamycin in two patients. In two cases, two drugs were used in therapy — azithromycin with clarithromycin and azithromycin

with clindamycin. In 19 patients, no information was found regarding the included treatment, which was mainly due to the lack of final culture results before discharge from the ward and failure to report for the test result within the indicated timeframe, transfer of the pregnant woman to a higher referral center, or the occurrence of miscarriage or preterm labor within a short period of time after the swab collection (Tab. 1).

We performed a similar analysis in patients with negative cultures from the area of the external os of the cervical canal for atypical bacteria. In the analyzed group, preterm labor occurred in 28 out of 140 patients with a known week and method of delivery, which is 20% of the group (Tab. 1).

Ureaplasma/Mycoplasma-positive patients. Our observations shows that regardless of the inclusion of treatment in patients with confirmed infection with atypical bacteria, the incidence of preterm labor or miscarriage in both groups is similar and it is, interestingly, 35.7% in the treated group and 31.6% in the group without antibiotic therapy (Tab. 1).

To broaden the diagnosis and try to determine the cause of symptoms of threatened miscarriage or preterm labor, we additionally performed cultures from the area of the external orifice of the cervical canal for aerobic bacteria, anaerobic bacteria and fungi. The positive results were obtained in 49 *Ureaplasma/Mycoplasma*-positive pregnant women, which accounted for 80.3%. results. In the group of *Ureaplasma/Mycoplasma*-negative patients, we found genital tract infection with other types of bacteria or fungi in 118 pregnant women which was 84.3%. In addition, we contrasted the above data with the weeks of gestation in which delivery occurred. In the group of *Ureaplasma/Mycoplasma*-positive patients, preterm labor occurred in 13 out of 48 cases of established infection with other types of bacteria and fungi (27.1%) and in 6 of 8 cases with monoinfection (75%). In the *Ureaplasma/Mycoplasma*-negative group, preterm labor occurred in 26 out of 117 patients with infection with aerobic bacteria, anaerobic bacteria or fungi (22.2%) and 2 out of 20 patients without genital tract infection (10%). When comparing these three characteristics, we obtained statistical significance at the level of $p = 0.007$. For the group with negative cultures for aerobic bacteria, anaerobic bacteria and fungi (*Ureaplasma/Mycoplasma* negative and positive, split by week of delivery), we achieved significance at the level $p = 0.002$ (Tab. 1).

On analyzing the medical records, it was found that *Ureaplasma/Mycoplasma*- positive patients had the complication of PPROM with subsequent delivery before 37 weeks of gestation, accounting for 47.4% of preterm deliveries in the group under study. In all of them, the delivery occurred between 33–36 weeks of gestation. In 3 cases, the situation involved twin pregnancies. Accordingly, in the *Ureaplasma/Mycoplasma*-negative group, PPROM occurred in 12 cases, of which in 11 patients' delivery occurred before 37 weeks of pregnancy, which accounts for 39.3% of preterm deliveries in this group. The situation in 3 cases involved twin pregnancies. In one of the pregnant women with PPROM found at 24 weeks of gestation, delivery took place on time (38 weeks of gestation).

When interpreting the results of the study, *Ureaplasma/Mycoplasma*-positive patients were also divided according to their obstetric history — 24 pregnant women were primiparous (39.3%), for the remaining 37 women it was a second or subsequent pregnancy (60.7%). The youngest of the analyzed patients was 16-years-old at the time of the study, while the oldest was 43. Among *Ureaplasma/Mycoplasma*-negative patients, the majority were also, accounting for 56.4% of patients in this group, 43.6% were primiparous. The age range in this group of patients was 22–46 years. No significant statistical difference was obtained in the compared groups ($p = 0.687$) (Tab. 1).

The group with positive cultures for atypical bacteria included both patients with single pregnancies — 90.2% and twin pregnancies — 9.8%. One of the multiple pregnancies was a monochorionic diamniotic twins, while the other 5 were dichorionic diamniotic twins. All multiple pregnancies ended prematurely (between 33–36 weeks).

In the group of *Ureaplasma*-negative patients, among the 7 dichorionic diamniotic twins (5% of the study group), 4 ended between 34–36 weeks of gestation, 3 patients gave birth after 36 + 6 weeks of gestation. Patients with singleton pregnancies made up 95% of the *Ureaplasma*-negative group (Tab. 1).

DISCUSSION

In the group of 201 of analyzed patients, which were hospitalized at the Obstetrics and Gynecology Department of Poznan Regional Hospital in 2019–2022, for threatened miscarriage or threatened preterm labor, there were 61 positive cultures from the area of the external outlet of the cervical canal for *Ureaplasma/Mycoplasma* and 140 negative results. Cases with a known course of pregnancy were included in the analysis. Patients with positive cultures accounted for about one-third of all pregnant women on the ward with a risk of preterm labor or pregnancy loss. In the literature, the percentage of at-risk of infection, pregnant women were as high as 57% [23]. A positive vaginal *Ureaplasma/Mycoplasma* culture is an independent predictive factor for preterm birth in patients with symptomatic threatened preterm labor and short cervix [23].

Ureaplasma accounted for 96.7% of positive culture results. The tests we use do not differentiate between *Ureaplasma* for *U. urealyticum* and *U. parvum*. Determination of

Table 1. Comparison of characteristics in groups of patients with positive and negative cultures for *Ureaplasma/Mycoplasma*. Groups were compared using Pearson's Chi-square test or Fisher's exact test (F), depending on whether assumptions were met

Attribute			Culture result for *Ureaplasma/Mycoplasma*		p value (statistical significance)
			Positive	Negative	
Number of cultures performed in 2019–2022			61 (100.0)	140 (100.0)	–
Gestational age at which culture was performed		< 22	6 (9.8)	16 (11.4)	0.931
		> 21 + 6	55 (90.2)	124 (88.6)	
Type of antibiotics used		Azithromycin	36 (59.0)	–	–
		Clarithromycin	2 (3.3)	–	
		Clindamycin	2 (3.3)	–	
		Azithromycin + clarithromycin	1 (1.6)	–	
		Azithromycin + clindamycin	1 (1.6)	–	
		No information available	19 (31.1)	–	
Time of pregnancy completion		< 22	2 (3.3)	2 (1.4)	0.098 (F)
		> 21 + 6 and < 37	19 (31.1)	28 (20.0)	
		> 36 + 6	40 (65.6)	110 (78.6)	
Time of pregnancy completion	Patients treated	< 37	15 (24.6)	–	–
		> 36 + 6	27 (44.3)	–	
	Patients who did not take treatment	< 37	6 (9.8)	–	
		> 36 + 6	13 (21.3)	–	
Type of delivery		Vaginal delivery	35 (59.3)	77 (55.8)	0.764
		Caesarean section	24 (40.7)	61 (44.2)	
Cervical culture result for aerobic bacteria, anaerobic bacteria and fungi		Positive	49 (80.3)	118 (84.3)	0.063 (F)
		Negative	8 (13.1)	21 (15.0)	
		Not performed	4 (6.6)	1 (0.7)	
The result of the cervical culture for aerobic bacteria, anaerobic bacteria and fungi, and the week of pregnancy in which the delivery occurred*	Positive	< 37	13 (23.2)	26 (19.0)	0.007
		> 36 + 6	35 (62.5)	91 (66.4)	
	Negative	< 37	6 (10.7)	2 (1.5)	
		> 36 + 6	2 (3.6)	18 (13.1)	
Obstetric history (number of current pregnancy)		I	24 (39.3)	61 (43.6)	0.687
		> I	37 (60.7)	79 (56.4)	
Type of current pregnancy		Singelton pregnancy	55 (90.2)	133 (95.0)	0220 (F)
		Multiple pregnancy	6 (9.8)	7 (5.0)	
Time of delivery		< 22	2 (3.3)	2 (1.4)	0.139
		> 21 + 6 i < 37	19 (31.1)	28 (20.0)	
		> 36 + 6	40 (65.6)	110 (78.6)	
Time of delivery		< 22 and > 21 + 6 and < 37	21 (34.4)	30 (21.4)	0.077
		> 36 + 6	40 (65.6)	110 (78.6)	
Cervical culture result for aerobic bacteria, anaerobic bacteria and fungi*		Positive	48 (85.7)	117 (85.4)	> 0.999
		Negative	8 (14.3)	20 (14.6)	
The week of pregnancy in which the delivery occurred*		< 37	19 (33.9)	28 (20.4)	0.072
		> 36 + 6	37 (66.1)	109 (79.6)	
Positive: Cervical culture results for aerobic bacteria, anaerobic bacteria and fungi, and the week of pregnancy in which the delivery occurred*		< 37	13 (27.1)	26 (22.2)	0.641
		> 36 + 6	35 (72.9)	91 (77.8)	
Negative: The result of the cervical culture for aerobic bacteria, anaerobic bacteria and fungi, and the week of pregnancy in which the delivery occurred*		< 37	6 (75.0)	2 (10.0)	0.002
		> 36 + 6	2 (25.0)	18 (90.0)	

*Excludes patients with miscarriage and patients without aerobic and anaerobic bacterial cultures

the specific bacterial genus could provide additional relevant information, especially considering reports of a higher risk of pregnancy complications with *U. parvum* infection [24].

We mostly used azithromycin to treat genital tract infections with atypical bacteria. The decision on treatment was primarily made based on the antibiogram (2019 and 2020 cultures) and in view of the relative safety of macrolides in pregnant women [20]. Interestingly, we noted no significant difference in the incidence of miscarriage or preterm labor in *Ureaplasma*-positive patients receiving antibiotic therapy compared to pregnant women who did not receive treatment. A team investigating the effects of treatment for *Ureaplasma/Mycoplasma* infection in patients with high-risk factors for preterm birth came to similar conclusions [25]. This observation warrants further analysis. Data obtained in other previously described studies indicate a positive correlation of antibiotic therapy on prolongation of pregnancy duration and successful neonatal outcomes [26].

Approximately 30% of patients with positive cultures for atypical bacteria had a preterm delivery, of which nearly half of the pregnancies were complicated by PPROM. The percentage of preterm deliveries in the group of *Ureaplasma/Mycoplasma*-negative patients was lower, accounting for 20%, of which 40% were associated with PPROM.

There was a higher percentage of pregnant women with symptoms of threatened miscarriage or preterm labor who additionally had a genital tract infection with aerobic and anaerobic bacteria or fungi. In *Ureaplasma/Mycoplasma*-positive patients, co-infection with other types of microorganisms occurred in 80.3% of the studied population and in *Ureaplasma/Mycoplsma*-negative patients in 84.3%. The presence of aerobic and anaerobic bacteria in the genital tract may influence the facilitation of *Ureaplasma* or *Mycoplasma* expansion and the incidence of pregnancy complications [4, 8, 9]. In the group of patients that we have studied, the risk of preterm delivery was paradoxically higher in patients with known monoinfection of the genital tract with *Ureaplasma* bacteria compared to patients with additional infection with other types of pathogens (75% vs 27%). The least frequent delivery before 37 weeks occurred in the case of negative results of both types of cultures — 10%. We obtained a statistically significant difference in the compared groups ($p = 0.007$). And for the group with negative cultures for aerobic bacteria, anaerobic bacteria and fungi the differences were also significant ($p = 0.002$).

There was no significant predominance of either type of delivery in patients with positive or negative cultures for *Ureaplasma/Mycoplasma*. In both groups of patients, differentiated based on the presence of atypical bacteria in the genital tract, the vaginal route of pregnancy completion prevailed. *Ureaplasma/Mycoplasma*-positive patients had a lower percentage of deliveries by cesarean section, 40.7%, than representatives of the other group, 44.2%.

Ureaplasma/Mycoplasma-positive patients were predominantly women with second or subsequent pregnancy (61%) and in 12% of cases these were multiple pregnancies. All patients in twin pregnancies with positive cultures gave birth prematurely.

CONCLUSIONS

Infection with *Mycoplasma/Ureaplasma* bacteria of the reproductive tract has a significant impact on the course of pregnancy. It increases the risk of pregnancy loss and preterm labor preceded by, among others, cervical shortening or PPROM. Identification of pathogens in cervical canal secretions is particularly important in patients with pregnancy risk symptoms. Although we were not able to obtain better obstetric outcomes in *Ureaplasma*-positive patients receiving treatment compared to pregnant women not receiving therapy, this observation requires further analysis on a larger number of cases.

Article information and declarations

Data availability statement

Available from the author.

Ethics statement

353/23.

Author contributions

Marcin Przybylski: concept, assumptions, study design, acquisition of data, analysis and interpretation of data, article draft, corresponding author. Ilona Wicher-Gozdur: concept, assumptions, study design, acquisition of data, analysis and interpretation of data, article draft. Joanna Kippen: concept, assumptions, study design, acquisition of data, analysis and interpretation of data, article draft. Sonja Millert-Kalinska: revised article critically. Agnieszka Zawiejska: revised article critically. Robert Jach: revised article critically. Dominik Pruski: concept, assumptions, study design, acquisition of data, analysis and interpretation of data, article draft.

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Conflict of interest

All authors declare no conflict of interest.

Supplementary material

None.

REFERENCES

1. Larsen B, Hwang J. Mycoplasma, Ureaplasma, and adverse pregnancy outcomes: a fresh look. Infect Dis Obstet Gynecol. 2010; 2010, doi: 10.1155/2010/521921, indexed in Pubmed: 20706675.
2. Chaim W, Horowitz S, David JB, et al. Ureaplasma urealyticum in the development of postpartum endometritis. Eur J Obstet Gynecol Reprod Biol. 2003; 109(2): 145–148, doi: 10.1016/s0301-2115(03)00007-1, indexed in Pubmed: 12860331.
3. Rumyantseva T, Khayrullina G, Guschin A, et al. Prevalence of Ureaplasma spp. and Mycoplasma hominis in healthy women and patients with flora alterations. Diagn Microbiol Infect Dis. 2019; 93(3): 227–231, doi: 10.1016/j.diagmicrobio.2018.10.001, indexed in Pubmed: 30344067.
4. Breugelmans M, Vancutsem E, Naessens A, et al. Association of abnormal vaginal flora and Ureaplasma species as risk factors for preterm birth: a cohort study. Acta Obstet Gynecol Scand. 2010; 89(2): 256–260, doi: 10.3109/00016340903418769, indexed in Pubmed: 19943819.
5. Who: Recommended Definitions, Terminology and Format for Statistical Tables Related to The Perinatal Period And Use of A New Certificate For Cause of Perinatal Deaths. Acta Obstet Gynecol Scand. 2011; 56(3): 247–253, doi: 10.3109/00016347709162009.
6. Bałajewicz-Nowak M, Kazimierz P, Małgorzata M. [Antioxidative system in pregnant women infected by Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum]. Ginekol Pol. 2011; 82(10): 732–737, indexed in Pubmed: 22379935.
7. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832): 2162–2172, doi: 10.1016/S0140-6736(12)60820-4, indexed in Pubmed: 22682464.
8. Jonduo ME, Vallely LM, Wand H, et al. Adverse pregnancy and birth outcomes associated with and : a systematic review and meta-analysis. BMJ Open. 2022; 12(8): e062990, doi: 10.1136/bmjopen-2022-062990, indexed in Pubmed: 36028274.
9. Vogel I, Thorsen P, Hogan VK, et al. The joint effect of vaginal Ureaplasma urealyticum and bacterial vaginosis on adverse pregnancy outcomes. Acta Obstet Gynecol Scand. 2006; 85(7): 778–785, doi: 10.1080/00016340500442423, indexed in Pubmed: 16817073.
10. Harada K, Tanaka H, Komori S, et al. Vaginal infection with Ureaplasma urealyticum accounts for preterm delivery via induction of inflammatory responses. Microbiol Immunol. 2008; 52(6): 297–304, doi: 10.1111/j.1348-0421.2008.00039.x, indexed in Pubmed: 18577163.
11. Sprong KE, Mabenge M, Wright CA, et al. species and preterm birth: current perspectives. Crit Rev Microbiol. 2020; 46(2): 169–181, doi: 10.1080/1040841X.2020.1736986, indexed in Pubmed: 32141797.
12. Yoon BoH, Romero R, Lim JH, et al. The clinical significance of detecting Ureaplasma urealyticum by the polymerase chain reaction in the amniotic fluid of patients with preterm labor. Am J Obstet Gynecol. 2003; 189(4): 919–924, doi: 10.1067/s0002-9378(03)00839-1, indexed in Pubmed: 14586326.
13. Skrzypczak J, Wirstlein PK, Wróbel M, et al. [Concentration of pro-inflammatory interleukins in cervical secretions of women with PROM and in the umbilical cord blood of their newborns]. Ginekol Pol. 2015; 86(6): 434–441, doi: 10.17772/gp/2400, indexed in Pubmed: 26255451.
14. Donders GGG, Ruban K, Bellen G, et al. Mycoplasma/Ureaplasma infection in pregnancy: to screen or not to screen. J Perinat Med. 2017; 45(5): 505–515, doi: 10.1515/jpm-2016-0111, indexed in Pubmed: 28099135.
15. Suzuki Yu, Horie K, Yada Y, et al. Vaginal Ureaplasma species increase chorioamnionitis in very preterm infants with preterm premature rupture of the membranes at < 28 weeks of gestation. Eur J Clin Microbiol Infect Dis. 2018; 37(12): 2371–2380, doi: 10.1007/s10096-018-3385-5, indexed in Pubmed: 30244363.
16. Larsson PG, Fåhraeus L, Carlsson B, et al. Premature study group of the Southeast Health Care Region of Sweden. Late miscarriage and preterm birth after treatment with clindamycin: a randomised consent design study according to Zelen. BJOG. 2006; 113(6): 629–637, doi: 10.1111/j.1471-0528.2006.00946.x, indexed in Pubmed: 16709205.
17. Lamont RF, Duncan SLB, Mandal D, et al. Intravaginal clindamycin to reduce preterm birth in women with abnormal genital tract flora. Obstet Gynecol. 2003; 101(3): 516–522, doi: 10.1016/s0029-7844(02)03054-5, indexed in Pubmed: 12636956.
18. De Francesco MA, Caracciolo S, Bonfanti C, et al. Incidence and antibiotic susceptibility of Mycoplasma hominis and Ureaplasma urealyticum isolated in Brescia, Italy, over 7 years. J Infect Chemother. 2013; 19(4): 621–627, doi: 10.1007/s10156-012-0527-z, indexed in Pubmed: 23192735.
19. Zhu C, Liu J, Ling Y, et al. Prevalence and antimicrobial susceptibility of Ureaplasma urealyticum and Mycoplasma hominis in Chinese women with genital infectious diseases. Indian J Dermatol Venereol Leprol. 2012; 78(3): 406–407, doi: 10.4103/0378-6323.95480, indexed in Pubmed: 22565456.
20. Redelinghuys MJ, Ehlers MM, Dreyer AW, et al. Antimicrobial susceptibility patterns of Ureaplasma species and Mycoplasma hominis in pregnant women. BMC Infect Dis. 2014; 14: 171, doi: 10.1186/1471-2334-14-171, indexed in Pubmed: 24679107.
21. Ikeda M, Oshima Y, Tsumura K, et al. Antibiotic administration reduced intra-amniotic inflammation 7 days after preterm premature rupture of the membranes with intra-amniotic infection. J Matern Fetal Neonatal Med. 2023; 36(2): 2286189, doi: 10.1080/14767058.2023.2286189, indexed in Pubmed: 38016702.
22. Zhang Su, Xu Ke, Liu SX, et al. Retrospective Analysis of Azithromycin-Resistant and Cervical Infection Among Pregnant Women. Infect Drug Resist. 2023; 16: 3541–3549, doi: 10.2147/IDR.S405286, indexed in Pubmed: 37305734.
23. Miyoshi Y, Suga S, Sugimi So, et al. Vaginal or and preterm delivery in women with threatened preterm labor. J Matern Fetal Neonatal Med. 2022; 35(5): 878–883, doi: 10.1080/14767058.2020.1733517, indexed in Pubmed: 32131651.
24. Kong F, Ma Z, James G, et al. Species identification and subtyping of Ureaplasma parvum and Ureaplasma urealyticum using PCR-based assays. J Clin Microbiol. 2000; 38(3): 1175–1179, doi: 10.1128/JCM.38.3.1175-1179.2000, indexed in Pubmed: 10699016.
25. Kawakita T, Waller J, DeYoung T, et al. Ureaplasma and Mycoplasma Screening for Pregnant Individuals Who Are at High Risk for Preterm Birth. Am J Perinatol. 2023 [Epub ahead of print], doi: 10.1055/s-0043-1771504, indexed in Pubmed: 37516118.
26. Antsaklis A, Daskalakis G, Michalas S, et al. Erythromycin treatment for subclinical Ureaplasma urealyticum infection in preterm labor. Fetal Diagn Ther. 1997; 12(2): 89–92, doi: 10.1159/000264438, indexed in Pubmed: 9218948.

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Expression of genes encoding galectin-1 and galectin-9 in placentas of pregnancies with preterm prelabor rupture of membranes

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ABSTRACT

Objectives: This study aims to elucidate the expression patterns of LGALS1 (galectin-1) and LGALS9 (galectin-9) genes in placental tissues of pregnancies affected by preterm prelabor rupture of membranes (PPROM). The overarching goal is to understand the potential roles of these galectins in the pathophysiology of PPROM, particularly in maternal-fetal immune tolerance and placental development.

Material and methods: Conducted as a prospective, single-center study at the Gynecology and Obstetrics Clinical Hospital in Poznan, Poland, from June 2021 to May 2023, the research involved 25 participants, including 12 with PPROM and 13 healthy controls. Placental tissues were obtained, and RNA extraction was performed. Galectin gene expression (LGALS1 and LGALS9) was analyzed using quantitative real-time PCR. Demographic and clinical data were collected, and statistical analyses were employed to assess correlations between galectin expression and clinical parameters.

Results: While significant differences were observed in gestational age at delivery and birth weight between the PPROM and control groups, the expression levels of LGALS1 and LGALS9 did not show statistically significant variations. Correlation analyses revealed no significant associations between galectin expression and various clinical parameters.

Conclusions: Contrary to the hypothesis, this study did not identify significant alterations in galectin-1 and galectin-9 expression in placentas affected by PPROM. Despite the limitations of a small sample size, these findings provide initial insights into the potential roles of galectins in PPROM. Further research on larger cohorts is warranted to comprehensively understand the implications of galectin involvement in the pathophysiology of PPROM.

Keywords: PPROM; galectins; placenta

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INTRODUCTION

Preterm prelabor rupture of membranes (PPROM) is a significant complication that profoundly impacts pregnancy. It is characterized by the untimely rupture of fetal membranes before the onset of labor and before reaching the 37th week of gestation [1, 2]. This clinical challenge contributes significantly to the prevalence of preterm births worldwide, posing substantial implications for neonatal morbidity and mortality [3, 4]. The etiology of PPROM is multifaceted, involving various risk factors such as infection, inflammation, mechanical stress, and hormonal imbalances. Among these, ascending bacterial infections, in particular, have been strongly associated with an increased risk of PPROM [5, 6]. Additionally, maternal and environmental factors, including smoking, maternal stress, and nutritional deficiencies, may also contribute to the occurrence of this condition [7]. Understanding these factors comprehensively is crucial to develop targeted interventions that can improve

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maternal and neonatal outcomes [5, 8]. Furthermore, the economic impact of PPROM-related preterm births, encompassing both immediate neonatal care and long-term medical support, calls for urgent attention from a public health perspective [8–12].

Galectins, a family of β-galactoside-binding lectins, play diverse roles in various physiological and pathological processes [13, 14]. During pregnancy, these multifunctional proteins are pivotal in regulating immune responses, cell adhesion, and tissue remodeling, significantly influencing key events such as implantation, placental development, and fetal growth [15, 16]. By interacting with glycoconjugates on the cell surface, galectins modulate various critical signaling pathways essential for successful pregnancies [17–19].

Among the galectins involved in pregnancy-related processes, galectin-1 and galectin-9 hold significant implications for pregnancy. Previous research has linked galectin-1 and galectin-9 to the establishment and maintenance of maternal-fetal immune tolerance, a fundamental component for successful pregnancies [20–23]. Additionally, both galectin-1 and galectin-9 have been implicated in promoting essential processes such as trophoblast invasion and angiogenesis in the placenta, thus exerting a notable influence on fetal growth and development [16, 24]. Investigating the expression and function of these galectins in the placentas of pregnancies affected by PPROM could provide novel perspectives on their potential contribution to the pathogenesis of this condition.

This study aims to elucidate the expression patterns of genes encodinggalectin-1 and galectin-9 in placental tissues of pregnancies affected by PPROM and explore their potential roles in the pathophysiology of this obstetric complication. Dół formularza

MATERIAL AND METHODS

This prospective, single-center study was conducted at the Gynecology and Obstetrics Clinical Hospital in Poznań, Poland, spanning from June 2021 to May 2023. A total of 25 women participated in the study, comprising 12 cases of preterm prelabor rupture of membranes occurring between 23 and 36 weeks of gestation, involving healthy women with a physiological course of pregnancy prior to PPROM. Additionally, 13 healthy mothers of full-term infants were included as the control group. Cases were recruited at the Delivery Ward following confirmation of PPROM and after a thorough assessment of the patients' general and obstetrical history. The control group consisted of pregnant women admitted to the Delivery Ward during the same period, who had no obstetric complications or other serious medical conditions.

Exclusion criteria were strictly defined and encompassed maternal age under 18 years, history of drug abuse or cigarette smoking, and serious maternal diseases, such as hypertension, preeclampsia, diabetes, cholestasis of pregnancy, and unstable thyroid disease. Other factors leading to exclusion included intrauterine infection, multiple pregnancies, detected fetal or placental abnormalities, fetal growth restriction, and cervical insufficiency. All participants were required to provide written, informed consent before their inclusion in the study. Medical records of the participants were obtained to gather more comprehensive information about the patients.

Gestational age was determined either based on the last menstrual period or through fetal crown-rump length measurement during the first trimester. The diagnosis of PPROM was established through speculum examination, wherein the visualization of amniotic fluid in the vagina served as an indicator. Alternatively, in cases requiring further confirmation, a placental insulin growth factor binding protein-1 (IGFBP-1) test (Amnioquick, Biosynex Swiss SA) was employed.

Patients diagnosed with PPROM were promptly hospitalized and received comprehensive management according to a specified protocol. For those with gestational age below 34 weeks, corticosteroids were administered to promote lung maturation. Additionally, prophylactic antibiotics were administered, and daily cardiotocographic monitoring and fetal assessments were conducted. Throughout the hospitalization, close monitoring for any signs of intrauterine infection was maintained, involving regular complete blood count (CBC) and C-reactive protein (CRP) assessments every second day, or more frequently if necessary.

For analysis, each participant provided a one-time 1 cm^3 sample of placenta, collected approximately 10 minutes after delivery. Two samples were acquired — one from the middle and one from the edge of the placenta, through its entire thickness. The samples were immediately stored in tubes containing RNAlater® (Sigma-Aldrich®, Merck Group, USA) and preserved at –20°C until analysis.

Total RNA was extracted from human placenta tissue samples using TRIzolTM Reagent (Thermo Fisher Scientific; Waltham; MA; USA) following the protocol. The quality and quantity of RNA samples were checked spectrophotometrically (DS-11; Wilmington, DE; USA), and each RNA sample was diluted to a final concentration of 100 ng/µl for cDNA synthesis using reverse PCR (High Capacity cDNA RT Kit; Thermo Fisher Scientific; Waltham; MA; USA). The expression of the *LGALS1* (galectin 1) and *LGALS9* (galectin 9) genes was evaluated by quantitative real-time PCR using the commercial TaqMan Gene Expression Assay (Hs00355202_m1 for *LGALS1* and Hs00371321_m1 for *LGALS9*; Thermo Fisher Scientific; Waltham; MA; USA) and LightCycler 480 (Roche; Basel; Switzerland). The *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *CYC1* (cytochrome c1) genes

Table 1. Demographic and clinical characteristics of the study population

	PPROM (n = 12)	Control (n = 13)	p value
Age [years]	30.17 ± 1.38	30.46 ± 1.34	0.88
Gravida	1 (1–3)	3 (1–2.25)	0.07
BMI at blood sampling [kg/m^2]	28.95 ± 1.51	29.59 ± 1.52	0.38
GA at delivery	34.5 ± 0.97	39.15 ± 0.39	0.00006
C-secection [%]	33.33%	23.07%	0.57
Female neonate [%]	16.67%	30.77%	0.41
Birth weight [g]	2428 ± 208	3579 ± 132	0.00004
pH of the neonate at birth	7.25 ± 0.02	7.24 ± 0.02	0.30
WBC count	11.87 ± 0.72	10.79 ± 0.55	0.12
CRP	4.4 ± 1.39	3.77 ± 1.11	0.39
LGALS1	0.01099 ± 0.00269	0.00913 ± 0.00111	0.52
LGALS9	0.00026 ± 0.00004	0.00033 ± 0.00007	0.40

BMI — body mass index; CRP — C-reactive protein; GA — gestational age; PPROM — preterm prelabor rupture of membranes; WBC — white blood cells

were selected as reference genes (Hs02758991_g1 for *GAPDH* and Hs00357717_m1 for *CYC1*; Thermo Fisher Scientific; Waltham; MA; USA). Relative quantification of the analyzed genes was performed based on the second derivative maximum method (Roche). All samples were analyzed in duplicate.

All analyses were conducted using PQStat Software 2022 (PQStat v.1.8.4, Poznan, Poland). To assess the distribution of all continuous variables, the Kolmogorov-Smirnov test was utilized. Variables with a normal distribution were estimated using means ± standard deviation (SD) and then compared using the independent samples Student's t-test. On the other hand, non-normally distributed variables were analyzed using the Mann-Whitney U-test, and the results were expressed as median and interquartile range. For categorical variables, frequency counts and percentages were used. The correlations between variables were evaluated through Spearman's correlation analysis. The one-way analysis of variance (ANOVA) was used to evaluate the differences between the groups. P value below 0.05 was considered statistically significant.

RESULTS

A prospective case-control study was undertaken, comprising 25 women, with 12 participants experiencing PPROM and 13 serving as controls. Age-matching was meticulously ensured between the cases and controls. Preterm prelabor rupture of membranes was specifically defined as the rupture of fetal membranes occurring between the 23rd and 36th week of gestation. The characteristics of the cases and controls are summarized in Table 1. Similar BMI at blood sampling, C-section rate, female neonate rate, and fetal outcomes at birth, as defined by the neonate's pH, were observed between the two groups. However, statistically significant differences were found in terms of gestational age at delivery and birth weight. No statistical significance was found regarding the expression of both LGALS1 and LGALS9 in both groups (0.01099 vs 0.00913; p = 0.52, shown in Figure 1 and 0.00026 vs 0.00033; p = 0.4 shown in Figure 2, respectively).

To further investigate the association between PPROM and LGALS1 and LGALS9 expression, we analyzed the correlations between galectins and other parameters (Tab. 2). However, no statistically significant correlations were found.

DISCUSSION

The aim of this study was to investigate the placental expression of LGALS1 and LGALS9 in pregnancies complicated with PPROM. Our hypothesis was that the placental expression of LGALS1 and LGALS9 would decrease in pregnancies with PPROM since they both act as anti-inflammatory molecules, contributing to the maintenance of pregnancy [25, 26]. However, we were unable to confirm our hypothesis.

Galectin-1's function has been extensively described in the literature due to its diverse impact on all stages of pregnancy. Abundant expression of LGALS-1 has been observed at the feto-maternal interface in humans, where it promotes maternal immune tolerance to the fetal semi-allograft. Galectin-1's tolerance-promoting mechanisms have been well-established for adaptive immune cells, such as T cells and dendritic cells [25]. This remarkable process begins early on, as murine, bovine, and human models have shown

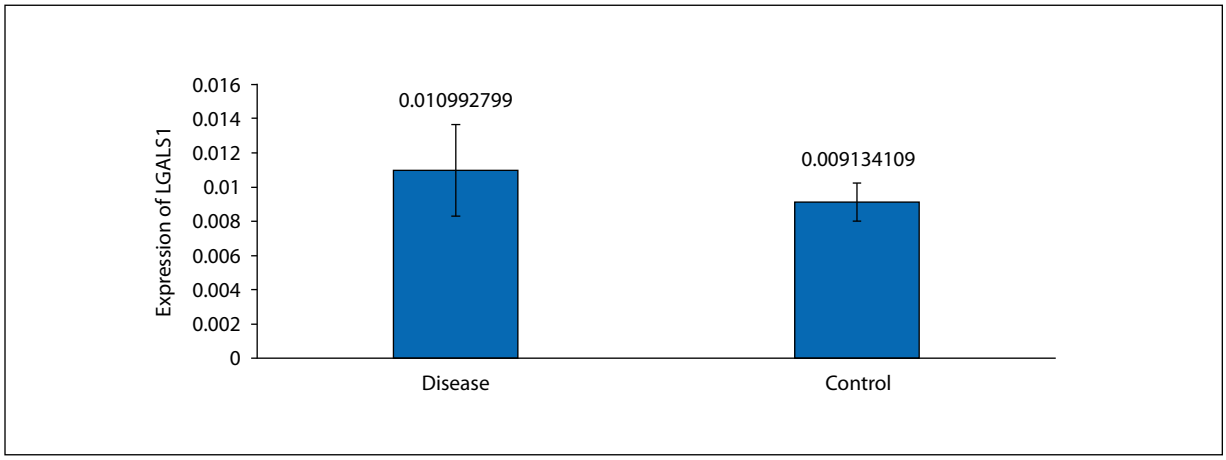

Figure 1. Distribution of LGALS1 expression study vs control group

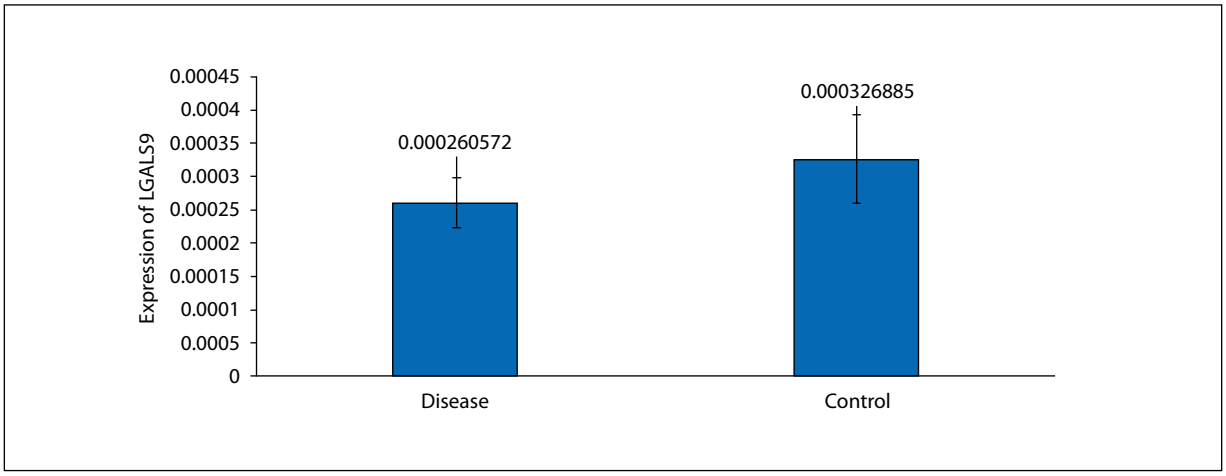

Figure 2. Distribution of LGALS9 expression in study vs control group

widespread expression of LGALS1 in the endometrium during the implantation window. Galectin-1 plays a crucial role in decidualization and creates an immune-privileged environment at the maternal-fetal interface, contributing to the low cytotoxic activity of decidual natural killer (dNK) cells [20, 27–30]. Some authors suggest that low LGALS1 expression and concentration might be associated with unexplained infertility [31]. Notably, LGALS-1 is believed to be expressed not only in the decidua and placenta but also in human embryos on the trophectoderm and inner cell mass. Interestingly, circulating galectin-1 levels were significantly decreased in women who later experienced a miscarriage [32]. Additionally, low LGALS1 decidual expression is thought to play a role in initiating parturition, both term and preterm [33]. On the contrary, an increased LGALS1 mRNA expression in chorioamniotic membranes in PPROM was associated with chorioamnionitis, suggesting that gal-1 may be involved in regulating inflammatory responses to chorioamniotic infection [21].

Various pregnancy pathologies have been investigated concerning LGALS1 expression in placenta and serum concentration. For instance, LGALS1 exhibits low expression in the serum and placenta of pregnant women with fetal growth restriction (FGR) and is hypothesized to be involved in its pathogenesis [34]. Moreover, placental LGALS1 expression is higher in severe preeclampsia (PE) than in normal pregnancy, regardless of the presence of small for gestational age (SGA) fetuses. However, it is not altered in SGA without PE, which might be due to the fetal response to an exaggerated systemic maternal inflammation [35]. Another serious pregnancy complication, HELLP syndrome, was also associated with increased circulating levels of gal-1 [36].

In cases of PPROM, galectin-1 women's serum concentration is believed to be decreased, similar to healthy individuals who deliver at term, thus facilitating the pro-inflammatory changes that lead to the onset of labor [33, 37]. However, the data are not consistent [38]. Considering the

Table 2. Correlations between LGALS1 and LGALS9 transcript levels and other parameters

	LGALS1	LGALS9
Age	R = –0.15	R = –0.25
	p = 0.24	p–0.12
BMI	R = 0.11	R = –0.16
	p = 0.31	p = 0.23
GA at blood sampling	R = 0.03	R = 0.17
	p = 0.45	p = 0.21
Birth weight	R = –0.07	R = 0.09
	p = 0.37	p = 0.33
pH of the neonate at birth	R = –0.15	R = –0.02
	p = 0.24	p = 0.45
WBC	R = –0.05	R = –0.30
	p = 0.40	p = 0.07
CRP	R = –0.16	R = 0.33
	p = 0.27	p = 0.10

GA — gestational age; BMI — body mass index; WBC — white blood cells; CRP — C-reactive protein

abovementioned reports and our own findings, in which we demonstrated that LGALS1 placental expression is not significantly different from healthy controls, we acknowledge that the studied group was relatively small to definitively confirm the hypothesis. Nonetheless, it does not discount the possibility, especially when considering all the previous data that supports it.

To date, galectin-9 has been less well-known, and the data regarding its exact function and characteristics are scarce. In addition to the endometrium, trophoblasts, and stromal cells of the decidua [29, 39], LGALS9 is also expressed by the placental endothelial cells and various types of immune cells [26, 40–42]. Several pregnancy pathologies have been linked to LGALS9 expression. Similar to galectin-1, galectin-9 concentrations were decreased in women with unexplained recurrent spontaneous abortion [26, 43, 44]. Moreover, the high expression of LGALS9 at uterodomes during the implantation window suggests that galectin9 can be considered as a marker of endometrial receptivity and may play an important role during the initial events of human embryo implantation [39]. It also immunomodulates the response in preeclampsia, with upregulation observed in decidual tissue and peripheral lymphocytes of preeclamptic pregnancies compared to normotensive pregnancies [45, 46]. During the course of pregnancy, the levels of galectin-9 in maternal blood rise in both concentration and expression, indicating its potential significance in maintaining the pregnancy [47]. Interestingly, some studies have indicated that galectin-9 serum levels are higher in women carrying a male fetus compared to a female fetus [48]. However, regarding PPROM, galectin-9 concentration did not show any significant differences in maternal serum [37]. In our study, we expected gal-9 to be downregulated similarly to gal-1, but we were unable to prove this.

This study is subject to several limitations, primarily due to the small number of participants and its single-center nature.

To conclude, there are no statistically significant differences in LGALS1 and LGALS9 expression in placentas with PPROM compared to healthy controls. However, further clinical studies on larger groups may be necessary to thoroughly investigate this topic.

Article information and declarations

Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Bioethics Committee of Poznan University of Medical Sciences, Poland, approval number 1158/19.

Author contributions

Conceptualization, Dorota Boron and Agnieszka Seremak-Mrozikiewicz; methodology, Dorota Boron; software, Joanna Mikolajczyk-Stecyna; validation, Joanna Mikolajczyk-Stecyna, Agata Chmurzynska and Grazyna Kurzawinska; formal analysis, Dorota Boron and Joanna Mikolajczyk-Stecyna; investigation, Dorota Boron; resources, Dorota Boron; data curation, Dorota Boron and Joanna Mikolajczyk-Stecyna; writing — original draft preparation, Dorota Boron; writing — review and editing, Agnieszka Seremak-Mrozikiewicz, Agata Chmurzynska; visualization, Dorota Boron; supervision, Agnieszka Seremak-Mrozikiewicz, Wieslaw Markwitz and Agata Chmurzynska; project administration, Dorota Boron; funding acquisition, Dorota Boron.

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Conflict of interest

The authors have no conflicts of interest to declare.

Supplementary material

None.

REFERENCES

1. Mercer BM. Preterm premature rupture of the membranes: current approaches to evaluation and management. Obstet Gynecol Clin North Am. 2005; 32(3): 411–428, doi: 10.1016/j.ogc.2005.03.003, indexed in Pubmed: 16125041.
2. Iams JD, Romero R, Culhane JF, et al. Epidemiology and causes of preterm birth. Lancet. 2008; 371(9606): 75–84, doi: 10.1016/S0140-6736(08)60074-4, indexed in Pubmed: 18177778.
3. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832): 2162–2172, doi: 10.1016/S0140-6736(12)60820-4, indexed in Pubmed: 22682464.
4. Walani SR. Global burden of preterm birth. Int J Gynaecol Obstet. 2020; 150(1): 31–33, doi: 10.1002/ijgo.13195, indexed in Pubmed: 32524596.
5. Romero R, Espinoza J, Kusanovic JP, et al. The preterm parturition syndrome. BJOG. 2006; 113(s3): 17–42, doi: 10.1111/j.1471-0528.2006.01120.x.
6. Richardson LS, Vargas G, Brown T, et al. Discovery and Characterization of Human Amniochorionic Membrane Microfractures. Am J Pathol. 2017; 187(12): 2821–2830, doi: 10.1016/j.ajpath.2017.08.019, indexed in Pubmed: 28939208.
7. Silverman RK, Wojtowycz M. Risk factors in premature rupture of membranes. Prim Care Update Ob Gyns. 1998; 5(4): 181, doi: 10.1016/s1068-607x(98)00092-4, indexed in Pubmed: 10838337.
8. Green NS, Damus K, Simpson JL, et al. March Of Dimes Scientific Advisory Committee On Prematurity. Research agenda for preterm birth: recommendations from the March of Dimes. Am J Obstet Gynecol. 2005; 193(3 Pt 1): 626–635, doi: 10.1016/j.ajog.2005.02.106, indexed in Pubmed: 16150253.
9. Costa ADe, Moller AB, Blencowe H, et al. Study protocol for WHO and UNICEF estimates of global, regional, and national preterm birth rates for 2010 to 2019. PLOS ONE. 2021; 16(10): e0258751, doi: 10.1371/journal.pone.0258751.
10. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008; 371(9608): 261–269, doi: 10.1016/S0140-6736(08)60136-1, indexed in Pubmed: 18207020.
11. Petrou S, Yiu HH, Kwon J. Economic consequences of preterm birth: a systematic review of the recent literature (2009-2017). Arch Dis Child. 2019; 104(5): 456–465, doi: 10.1136/archdischild-2018-315778, indexed in Pubmed: 30413489.
12. O'Connor AR, Wilson CM, Fielder AR. Ophthalmological problems associated with preterm birth. Eye (Lond). 2007; 21(10): 1254–1260, doi: 10.1038/sj.eye.6702838, indexed in Pubmed: 17914427.
13. Gabius HJ, André S, Kaltner H, et al. The sugar code: functional lectinomics. Biochim Biophys Acta. 2002; 1572(2-3): 165–177, doi: 10.1016/s0304-4165(02)00306-9, indexed in Pubmed: 12223267.
14. Blidner AG, Rabinovich GA. ‚Sweetening' pregnancy: galectins at the fetomaternal interface. Am J Reprod Immunol. 2013; 69(4): 369–382, doi: 10.1111/aji.12090, indexed in Pubmed: 23406009.
15. Than NG, Romero R, Balogh A, et al. Galectins: Double-edged Swords in the Cross-roads of Pregnancy Complications and Female Reproductive Tract Inflammation and Neoplasia. J Pathol Transl Med. 2015; 49(3): 181–208, doi: 10.4132/jptm.2015.02.25, indexed in Pubmed: 26018511.
16. Blois S, Dveksler G, Vasta G, et al. Pregnancy Galectinology: Insights Into a Complex Network of Glycan Binding Proteins. Frontiers in Immunology. 2019; 10, doi: 10.3389/fimmu.2019.01166.
17. Barondes SH, Cooper DN, Gitt MA, et al. Galectins. Structure and function of a large family of animal lectins. J Biol Chem. 1994; 269(33): 20807–20810, indexed in Pubmed: 8063692.
18. Rabinovich GA, Toscano MA. Turning ‚sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol. 2009; 9(5): 338–352, doi: 10.1038/nri2536, indexed in Pubmed: 19365409.
19. Jeschke U, Mayr D, Schiessl B, et al. Expression of Galectin-1, -3 (gal-1, gal-3) and the Thomsen–Friedenreich (TF) Antigen in Normal, IUGR, Preeclamptic and HELLP Placentas. Placenta. 2007; 28(11-12): 1165–1173, doi: 10.1016/j.placenta.2007.06.006.
20. Blois SM, Ilarregui JM, Tometten M, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007; 13(12): 1450–1457, doi: 10.1038/nm1680, indexed in Pubmed: 18026113.
21. Than NG, Kim SS, Abbas A, et al. Chorioamnionitis and increased galectin-1 expression in PPROM — an anti-inflammatory response in the fetal membranes? Am J Reprod Immunol. 2008; 60(4): 298–311, doi: 10.1111/j.1600-0897.2008.00624.x, indexed in Pubmed: 18691335.
22. Blois SM, Barrientos G. Galectin signature in normal pregnancy and preeclampsia. J Reprod Immunol. 2014; 101-102: 127–134, doi: 10.1016/j.jri.2013.05.005, indexed in Pubmed: 23953090.
23. Than NG, Romero R, Kim CJ, et al. Galectins: guardians of eutherian pregnancy at the maternal-fetal interface. Trends Endocrinol Metab. 2012; 23(1): 23–31, doi: 10.1016/j.tem.2011.09.003, indexed in Pubmed: 22036528.
24. Than NG, Romero R, Goodman M, et al. A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. Proc Natl Acad Sci U S A. 2009; 106(24): 9731–9736, doi: 10.1073/pnas.0903568106, indexed in Pubmed: 19497882.
25. Gómez-Chávez F, Castro-Leyva V, Espejel-Núñez A, et al. Galectin-1 reduced the effect of LPS on the IL-6 production in decidual cells by inhibiting LPS on the stimulation of IκBζ. J Reprod Immunol. 2015; 112: 46–52, doi: 10.1016/j.jri.2015.07.002, indexed in Pubmed: 26226212.
26. Heusschen R, Freitag N, Tirado-González I, et al. Profiling Lgals9 splice variant expression at the fetal-maternal interface: implications in normal and pathological human pregnancy. Biol Reprod. 2013; 88(1): 22, doi: 10.1095/biolreprod.112.105460, indexed in Pubmed: 23242525.
27. Mulac-Jeričević B, Šućurović S, Gulic T, et al. The involvement of the progesterone receptor in PIBF and Gal-1 expression in the mouse endometrium. Am J Reprod Immunol. 2019; 81(5), doi: 10.1111/aji.13104.
28. Kopcow HD, Rosetti F, Leung Y, et al. T cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1. Proc Natl Acad Sci U S A. 2008; 105(47): 18472–18477, doi: 10.1073/pnas.0809233105, indexed in Pubmed: 19011096.
29. von Wolff M, Wang X, Gabius HJ, et al. Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. Mol Hum Reprod. 2005; 11(3): 189–194, doi: 10.1093/molehr/gah144, indexed in Pubmed: 15681515.
30. Chaney HL, Grose LF, LaBarbara JM, et al. Galectin-1 induces gene and protein expression related to maternal-conceptus immune tolerance in bovine endometrium†. Biol Reprod. 2022; 106(3): 487–502, doi: 10.1093/biolre/ioab215, indexed in Pubmed: 34792096.
31. Dong L, Bai Q, Song W, et al. Abnormal expression of galectin-1, -3 leading to unexplained infertility by decreasing endometrial receptivity: a retrospective analysis. Am J Transl Res. 2023; 15(1): 493–501, indexed in Pubmed: 36777856.
32. Tirado-Gonzalez I, Freitag N, Barrientos G, et al. Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy. Mol Hum Reprod. 2013; 19(1): 43–53, doi: 10.1093/molehr/gas043, indexed in Pubmed: 23002109.
33. El-Azzamy H, Balogh A, Romero R, et al. Characteristic Changes in Decidual Gene Expression Signature in Spontaneous Term Parturition. J Pathol Transl Med. 2017; 51(3): 264–283, doi: 10.4132/jptm.2016.12.20, indexed in Pubmed: 28226203.
34. Jin XX, Ying X, Dong MY. Galectin-1 expression in the serum and placenta of pregnant women with fetal growth restriction and its significance. BMC Pregnancy Childbirth. 2021; 21(1): 14, doi: 10.1186/s12884-020-03477-8, indexed in Pubmed: 33407212.
35. Than NG, Erez O, Wildman DE, et al. Severe preeclampsia is characterized by increased placental expression of galectin-1. J Matern Fetal Neonatal Med. 2008; 21(7): 429–442, doi: 10.1080/14767050802041961, indexed in Pubmed: 18570123.
36. Schnabel A, Blois SM, Meint P, et al. Elevated systemic galectin-1 levels characterize HELLP syndrome. J Reprod Immunol. 2016; 114: 38–43, doi: 10.1016/j.jri.2016.02.002, indexed in Pubmed: 26956510.
37. Boroń DG, Świetlicki A, Potograbski M, et al. Galectin-1 and Galectin-9 Concentration in Maternal Serum: Implications in Pregnancies Complicated with Preterm Prelabor Rupture of Membranes. J Clin Med. 2022; 11(21), doi: 10.3390/jcm11216330, indexed in Pubmed: 36362558.
38. Kaya B, Turhan U, Sezer S, et al. Maternal serum galectin-1 and galectin-3 levels in pregnancies complicated with preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med. 2020; 33(5): 861–868, doi: 10.1080/14767058.2019.1637409, indexed in Pubmed: 31242786.
39. Shimizu Y, Kabir-Salmani M, Azadbakht M, et al. Expression and localization of galectin-9 in the human uterodome. Endocr J. 2008; 55(5): 879–887, doi: 10.1507/endocrj.k08e-111, indexed in Pubmed: 18506087.
40. Li Y, Feng J, Geng S, et al. The N- and C-terminal carbohydrate recognition domains of galectin-9 contribute differently to its multiple functions in innate immunity and adaptive immunity. Mol Immunol. 2011; 48(4): 670–677, doi: 10.1016/j.molimm.2010.11.011, indexed in Pubmed: 21146220.

41. Meggyes M, Miko E, Polgar B, et al. Peripheral Blood TIM-3 Positive NK and CD8+ T Cells throughout Pregnancy: TIM-3/Galectin-9 Interaction and Its Possible Role during Pregnancy. PLoS ONE. 2014; 9(3): e92371, doi: 10.1371/journal.pone.0092371.
42. Enninga EA, Harrington SM, Creedon DJ, et al. Immune checkpoint molecules soluble program death ligand 1 and galectin-9 are increased in pregnancy. Am J Reprod Immunol. 2018; 79(2), doi: 10.1111/aji.12795, indexed in Pubmed: 29205636.
43. Wyatt MA, Baumgarten SC, Weaver AL, et al. Evaluating Markers of Immune Tolerance and Angiogenesis in Maternal Blood for an Association with Risk of Pregnancy Loss. J Clin Med. 2021; 10(16), doi: 10.3390/jcm10163579, indexed in Pubmed: 34441875.
44. Wu, M.; Zhu, Y.; Zhao, J.; Ai, H.; Gong, Q.; Zhang, J.; Zhao, J.; Wang, Q.; La, X.; Ding, J. Soluble Costimulatory Molecule STim3 Regulates the Differentiation of Th1 and Th2 in Patients with Unexplained Recurrent Spontaneous Abortion. Int J Clin Exp Med. 2015; 8: 8812–8819.
45. Hao H, He M, Li J, et al. Upregulation of the Tim-3/Gal-9 pathway and correlation with the development of preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2015; 194: 85–91, doi: 10.1016/j.ejogrb.2015.08.022, indexed in Pubmed: 26342682.
46. Miko E, Meggyes M, Bogar B, et al. Involvement of Galectin-9/TIM-3 Pathway in the Systemic Inflammatory Response in Early-Onset Preeclampsia. PLoS ONE. 2013; 8(8): e71811, doi: 10.1371/journal.pone.0071811.
47. Meggyes M, Miko E, Polgar B, et al. Peripheral Blood TIM-3 Positive NK and CD8+ T Cells throughout Pregnancy: TIM-3/Galectin-9 Interaction and Its Possible Role during Pregnancy. PLoS ONE. 2014; 9(3): e92371, doi: 10.1371/journal.pone.0092371.
48. Enninga EA, Nevala WK, Creedon DJ, et al. Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period. Am J Reprod Immunol. 2015; 73(3): 251–262, doi: 10.1111/aji.12303, indexed in Pubmed: 25091957.

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Dietary patterns of Polish pregnant women in reference to prepregnancy BMI and gestational weight gain

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ABSTRACT

Objectives: During the life cycle of a woman, pregnancy is the period when she is most open to changing her behaviour and lifestyle for the benefit of the child's development. Lifestyle changes include also the diet correction. The objective of the study was to assess, through identified dietary patterns, the diets of women in the second and third trimester of pregnancy in relation to their nutritional status before and during pregnancy.

Material and methods: The study was conducted among pregnant women, participants of childbirth education classes at the Institute of Mother and Child, based on food frequency questionnaire. The study involved 392 women in the age 19–40 years (first single pregnancy without complications). Dietary patterns were identified using the k-means method, based on groups of products.

Results: Three dietary patters were identified in the study group of women: dietary pattern 1 — cereal-milk diet, dietary pattern 2 — vegetable-fruit diet and dietary pattern 3 — cottage cheese-vegetable diet. Dietary pattern 3 occurred in 43.9% of underweight women, in 45.5% of women with normal weight and in 43.1% of women with excess body weight. Dietary pattern 1 occurred in about one third of women and dietary pattern 2 in about 20% in each group. A greater diversity in the frequency of identified dietary patterns was observed in relation to weight gain during pregnancy. The identified dietary patterns differed significantly in terms of the profile of macronutrients, most minerals and vitamins — E, C and B group vitamins.

Conclusions: The identified dietary patterns and their energy and nutritional profile indicate the need for monitoring the diets and nutritional education of pregnant women.

Keywords: nutrition; pregnancy; dietary patterns

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INTRODUCTION

The issues related to the diets of pregnant women remain relevant and extremely important for the health status of future generations. The appropriate diet of a woman during pregnancy has an impact on her nutritional status, course of pregnancy and the health of the child — in utero, childhood, and in adulthood. According to the theory of metabolic programming, unfavourable environmental conditions, including nutritional conditions, related to the deficiency or excess of nutrients during the fetal life may cause changes in the structure, metabolism and functioning of the body, thus increasing the risk of developing diet-related diseases in adulthood [1–3]. The nutritional status of a pregnant woman is one of the main factors ensuring the maintenance of pregnancy, its appropriate course and minimizing the risk of complications in the fetus. Maternal undernutrition may contribute to the reduction of the weight of the placenta, thus causing its dysfunction, intrauterine growth restriction, low birth weight of infant and premature delivery, whereas overweight and obesity in the mother may be conducive to hypertension, gestational diabetes, urinary tract infections, fetal macrosomia and surgical delivery. Therefore, it is important to monitor the weight gain during pregnancy with reference to the body mass index before pregnancy [4–7].

During pregnancy, the need for energy, essential nutrients, vitamins and minerals changes. Therefore, the balance

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between increased nutritional needs and the nutritional value of the diet should be achieved by the appropriate selection of products, the introduction of fortified foods and/or appropriate dietary supplementation [8].

In recent years, in nutritional assessment, an increasing attention has been paid to dietary patterns of various population groups as a comprehensive method for determining the relationship between the diet and the nutritional status. The analysis of dietary patterns is considered a holistic, alternative and complementary approach to assessing the dependencies between the diet and the risk of chronic disease by evaluating the impact of the overall diet and its complexity. Dietary patterns are defined as a set of numerous specific, co-existing characteristics describing the human diet. These characteristics may define the type and amount of nutrients, foodstuffs or groups of products, the frequency of eating meals, food preferences or avoidance of certain foods [9, 10]. Optimal eating patterns, which represent the set of nutritional characteristics that are the most beneficial for health, are referred to as "dietary patterns" [11].

Objectives

The aim of the study was to assess, through identified dietary patterns, the diets of women in the second and third trimester of pregnancy in relation to their nutritional status before and during pregnancy.

MATERIAL AND METHODS

Study group

The study was conducted in the years 2012–2018 among participants of childbirth classes conducted at the Institute of Mother and Child in Warsaw. The courses took place four times a year during this period, in which 796 pregnant women attended the childbirth classes. All women were invited to participate in the study. Out of 796 pregnant women, 448 (56%) decided to participate in the study and completed the questionnaire. The main reason for not completing the questionnaire was the lack of interest to participating in the study and the failure to meet the criteria (44%) of inclusion and exclusion from the study group. The study involved women who agreed to complete an anonymous questionnaire. A total of 448 questionnaires were collected, of which 392 were included in the study, only those that were completed without missing data (Tab. 1). The inclusion criteria were: first and single pregnancy without complications, maternal age 19–40 years, and attending a childbirth education classes at the Institute of Mother and Child in Warsaw.

Table 1. The number of course participants and collected questionnaires

Year of the study	Number of courses*	Number of participants in 4 courses	Number of collected questionnaires
2012	4	112	76
2013	4	116	70
2014	4	112	60
2015	4	120	54
2016	4	112	51
2017	4	120	75
2018	4	104	62
7 years	28	796 (100%)	448 (56%)

*A maximum of 30 women could participate in one course due to practical classes and exercises

Analysis of the nutritional status

The nutritional status of pregnant women was examined based on body weight [kg], height [m] and body mass index BMI [kg/m^2] before and during pregnancy. Data were obtained from the questionnaire entries and current measurements. The recommendations of the American Institute of Medicine, which constitute the current medical standard, were used to assess the nutritional status of women before and during pregnancy [12].

Diet analysis

The diet was analysed using the data from the food frequency questionnaire (FFQ), which included the usual portion size, and the 24-hour dietary records (recall and record method) [13]. The diet record was used to verify the usual portion sizes of products using the product and food photography album [14]. The dietary patterns of pregnant women were identified using the k-means method based on product groups from the food frequency questionnaire. In accordance with adopted statistical procedure for identifying dietary patterns, an analysis was made of the grouping of products used in the diets of pregnant women at several levels of categorization in line with the grouping adopted in the Diet 6 dietary computer program. Then, the dimensions of the analysis were reduced using the factor analysis method, which allowed to reflect the acceptable level of overall variability. K-means clustering method was used to identify dietary patterns. Both the grouping parameters and the number of dietary patterns were analyzed in terms of quantitative parameters (group size) and qualitative parameters (interpretation possibilities). The standard is to identify 2–4 dietary patterns. The energy and nutritional value profile in the identified dietary patterns was also analysed using the Diet 6 nutritional computer programme [15]. The obtained results were compared with the current nutrition standards and medical standards [16–18].

Statistical analysis

The statistical analysis of the obtained results was performed using the IBM SPSS 21 statistical program. The median and quartile ranges were used to describe quantitative

variables due to the lack of conformity of the distributions of variables with the normal distribution. P < 0.05 was adopted as the level of statistical significance. To assess the relationship between the diet and the nutritional status of pregnant women, Student's t-tests and analysis of variance were used for quantitative variables fulfilling the conditions of normal distribution and homogeneity of variance. Non-parametric Mann-Whitney and Kruskal-Wallis tests were used for quantitative variables that did not meet the conditions of normal distribution and homogeneity of variance. The Chi-square test of independence was used to analyse the body mass index (BMI) and weight gain as categorical variables. The principal component analysis with Quatrimax rotation, including Bartlett sphericity tests, and k-means cluster analysis were used to identify the dietary patterns of the studied women. The energy value and the share of individual components in the average daily food ration of the studied women were expressed in the form of the mean and standard deviation as well as the median and quartiles 1 and 3. The percentage of women whose nutrient intake was below the average requirement of the group (EAR) and above the adequate intake (AI) was also calculated.

RESULTS

Characteristics of the study group

The study involved 392 women. Table 2 presents the characteristics of the studied group. The vast majority of the respondents had completed higher education and were residents of large cities. Most of the women were in the third trimester of pregnancy.

Assessment of the nutritional status of the studied women

Table 3 presents the nutritional status of the studied women, determined using prepregnancy BMI. The appropriate BMI values before pregnancy were recorded in 76.3% of the examined women. Based on the data on the current body weight and the body weight before pregnancy, the mean weight gain of the studied pregnant women was calculated with reference to the prepregnancy BMI (Tab. 4).

The mean weight gain during pregnancy was higher than recommended in all three subgroup. The highest mean gains were observed in the group of women with body weight deficiency before pregnancy (0.56 vs 0.51 kg/week), and the lowest in the group of women with excess body weight (0.40 vs 0.22–0.28 kg/week). In the group of women with appropriate body weight before pregnancy, the mean weight gain during pregnancy was 0.55 kg/week (norm 0.42 kg/week). There were statistically significant differences between the groups in the mean weight gain of pregnant women in relation to their prepregnancy BMI ($p < 0.05$).

Table 2. Characteristics of the studied group

Variables	Pregnant women (n = 392) n (%)
Women's age [years]	
• 19	1 (0.3)
• 20–30	205 (52.3)
• 31–40	186 (47.4)
Place of residence	
• city ≥ 100 tys.	366 (93.4)
• town < 100 tys.	18 (4.6)
• village	8 (2.0)
Education	
• lower than secondary	1 (0.3)
• secondary	46 (11.7)
• higher	345 (88.0)
Trimester of pregnancy	
• 2nd trimester	137 (35.0)
• 3rd trimester	255 (65.0)

Table 3. Prepregnancy BMI of the studied women

Variables	Studied group of women (n = 392) n (%)
Prepregnancy BMI [kg/m^2]	
• < 18.5	43 (10.9)
• 18.5–24.9	299 (76.3)
• 25.0–29.9	40 (10.2)
• ≥ 30.0	10 (2.6)

BMI — body max index

Assessment of the diets

As a result of the cluster analysis, three dietary patterns of the studied pregnant women were identified. Figure 1 shows the distribution of the mean values for each cluster (dietary pattern).

The identified patterns were defined as follows:

- **Dietary pattern 1** — with predominance of cereal products and butter, rennet cheeses and fermented milk drinks, defined as the cereal-milk diet.
- Dietary pattern 1 was characterised by high consumption of such products as cereals and butter, rennet cheeses and fermented milk drinks, as well as meat, poultry, cold cuts, and sugar. Dietary pattern 1 was characterized by low consumption of vegetables, including potatoes and fruit. Dietary pattern 1 was adhered to by 33.4% (n = 131) of the studied women.
- **Dietary pattern 2** — with a definite predominance of vegetables, fruit and potatoes, was defined as the vegetable-fruit diet. Dietary pattern 2 was characterized by high consumption of vegetables, including potatoes and fruit. The consumption of cereal products and butter, meat, poultry and cold cuts exceeded the average consumption for the whole group, but less than the products from the vegetables group, including potatoes and fruit. Milk consumption was also slightly above the average. This pattern also involved low consumption

Table 4. Average weight gain of the studied women depending on prepregnancy BMI

Prepregnancy BMI [kg/m^2]	Average weight gain in pregnancy [kg/week]					Recommended weight gain according to Institute of Medicine (2009)	p
	$\bar{x}$	SD	Me	1Q–3Q	Min–max		
Body weight deficiency: BMI ≤ 18.5	0.56	0.17	0.55	0.44–0.68	0.27–1.00	0.51	0.000
Normal body weight: BMI 18.5–24.9	0.55	0.20	0.53	0.43–0.65	–0.05–1.27	0.42	
Excess body weight: BMI ≥ 25.0	0.40	0.25	0.43	0.25–0.56	–0.21–1.08	0.22–0.28	

BMI — body max index; $\bar{x}$ — mean; SD — standard deviation; Me — median; 1Q–3Q — 1 quartile–3 quartile; min–max — range minimum–maximum; Kruskal-Wallis $p < 0.005$

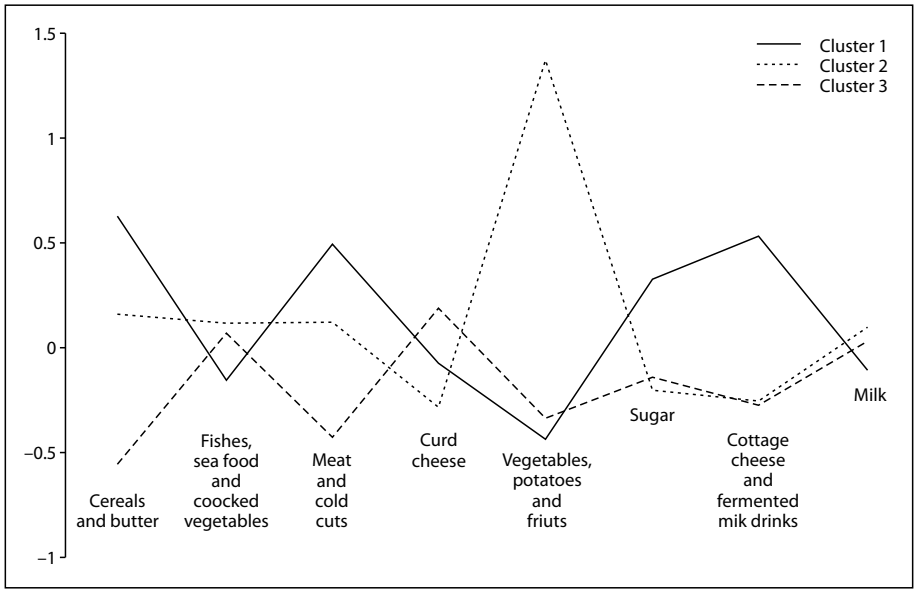

Figure 1. Distribution of mean values of the three identified clusters (dietary patterns) in the studied group of women

of cottage cheese and rennet cheeses, fermented milk drinks and sugar. Dietary pattern 2 was adhered to by 21.7% (n = 85) of the studied women.

- **Dietary pattern 3** — with predominance of cottage cheese, as well as a higher than average consumption of fish and cooked vegetables was defined as the cottage cheese-vegetable diet. Dietary pattern 3 was characterized by high consumption of cottage cheese. The majority of the analysed product groups did not differ significantly from the average for the entire group, but the consumption of fish and cooked vegetable dishes as well as the consumption of milk deserve attention. However, in the consumption structure of the identified patterns, the consumption of fish did not differ significantly, therefore dietary pattern 3 was named the cottage cheese-vegetable diet. This pattern also involved significantly lower consumption of cereal products and butter as well as meat, poultry and cold cuts. Dietary pattern 3 was adhered to by 44.9% (n = 176) of the studied women. Table 5 presents the characteristics of the identified dietary patterns based on the product consumption structure.

Figure 2 shows the frequency of the identified dietary patterns in the subgroups of pregnant women in relation to their nutritional status determined using the BMI before pregnancy. The most frequent dietary pattern in all groups of women was pattern 3, defined as the cottage cheese-vegetable diet. There was no statistically significant relationship between the dietary patterns and prepregnancy body mass index (BMI). The frequency of dietary patterns depending on weight gain during pregnancy and BMI before pregnancy was also analysed (Fig. 3).

Assessment of the frequency of dietary patterns depending on the weight gain in pregnancy and the BMI before pregnancy showed that dietary pattern 1 (cereal-milk diet) occurred most often in the group of women with excess body weight before pregnancy whose weight gain during pregnancy was above than recommended (75.0%). Dietary patterns 1 and 2 occurred most often in the group of women with underweight before pregnancy whose weight gain

Table 5. Characteristics of the identified dietary patterns based on the product consumption structure

Group of products [g]	Dietary pattern 1(A) cereal-milk diet	Dietary pattern 2(B) vegetable-fruit diet	Dietary pattern 3(C) cottage cheese-vegetable diet	p**
	$\bar{x} \pm SD$*** (Me, Q1–Q3)*			
Cereals (in terms of flour)	192.1 ± 64.1$^{B, C}$ (192.4, 146.2–223.9)	166.2 ± 70.7$^{A, C}$ (148.0, 119.0–201.7)	117.9 ± 52.2$^{A, B}$ (117.5, 79.7–148.0)	0.000
Milk	145.1 ± 114.5 (140.0, 70.0–250.0)	161.3 ± 136.5 (140.0, 70.0–250.0)	160.6 ± 145.4 (140.0, 70.0–250.0)	0.736
Fermented milk drinks	185.0 ± 144.0 (143.0, 85.0–250.0)	183.5 ± 147.2 (150.0, 85.0–280.0)	189.3 ± 143.9 (152.5, 85.0–280.0)	0.910
Cottage-cheese (in terms of milk)	352.5 ± 278.7^{B} (335.0, 201.0–402.0)	243.8 ± 183.9$^{A, C}$ (201.0, 113.9–402.0)	360.3 ± 327.2^{B} (288.1, 154.1–452.3)	0.011
Rennet cheese	45.6 ± 25.7$^{B, C}$ (40.0, 34.0–60.0)	21.5 ± 19.0^{A} (17.0, 6.0–35.0)	16.3 ± 11.3^{A} (11.0, 11.0–23.0)	0.000
Meat and poultry	219.6 ± 100.0^{C} (206.0, 158.0–273.0)	218.8 ± 91.6^{C} (218.0, 171.0–285.0)	156.6 ± 78.9$^{A, B}$ (158.0, 103.0–206.0)	0.000
Cold cuts	55.0 ± 32.1 48.6, (28.0–80.4)	38.2 ± 31.9 (39.1, 10.3–51.5)	24.9 ± 21.8 (20.7, 6.5–42.6)	0.000
Fish and seafood	47.9 ± 37.6 (60.0, 0.0–60.0)	46.9±36.0 (60.0, 0.0–60.0)	48.7 ± 37.2 (60.0, 0.0–60.0)	0.891
Butter and cream	20.8 ± 9.9$^{B, C}$ (20.0, 15.0–25.0)	16.3 ± 10.4$^{A, C}$ (15.0, 10.0–20.0)	10.4 ± 8.2$^{A, B}$ (10.0, 3.0–16.0)	0.000
Vegetables and fruits	659.2 ± 263.9$^{B, C}$ (599.8, 475.9–808.4)	1030.4 ± 420.8$^{A, C}$ (989.7, 671.3–1355.7)	795.2 ± 353.6$^{A, B}$ (775.3, 556.1–947.6)	0.000
Cooked vegetables	60.9 ± 69.9^{C} (43.0, 0.0–100.0)	90.4 ± 90.7 (80.0, 0.0–150.0)	83.2 ± 85.8^{A} (60.0, 10.0–128.0)	0.025
Cooked potatoes	92.4 ± 56.1$^{B, C}$ (85.0, 60.0–115.0)	198.4 ± 69.2$^{A, C}$ (170.0, 150.0–250.0)	64.6 ± 45.3$^{A, B}$ (60.0, 43.0–85.0)	0.000
Sugar	16.4 ± 13.2$^{B, C}$ (11.0, 6.0–23.0)	11.9 ± 10.9^{A} (11.0, 6.0–17.0)	12.3 ±10.3^{A} (11.0, 6.0–17.0)	0.006

* x ± SD (Me, Q1–Q3) — mean ± standard deviation (median; 1 quartile–3 quartile)
** statistically significant differences in the consumption of groups of products between the three clusters of the studied women (Kruskal-Wallis; $p < 0.005$)
*** statistically significant differences between clusters of studied women $^{A, B, C}$ calculated using a post hoc test (multiple two-sided comparisons)

during pregnancy was in the line with the recommendations and above (40.0% and 53.8% respectively). In the group of women with appropriate body weight before pregnancy, a more even distribution of the identified patterns was observed.

Assessment of the nutrient profile in the identified dietary patterns

Table 6 shows the energy value and nutrient profile of three identified dietary patterns in pregnant women.

The cereal-milk diet (dietary pattern 1) had the highest energy value (Me = 2487.3 kcal). The energy value of the vegetable-fruit diet (dietary pattern 2) was similar (Me = 2446.5 kcal). The cottage cheese-vegetable diet had the lowest energy value (Me = 2055.2 kcal). In the case of the first two dietary patterns, these values were close to nutritional norms, while in the third pattern they were significantly lower. The impact of pregnancy on energy expenditure varies by trimester of pregnancy and varies among women, e.g. depending on pre-pregnancy body weight and gestational weight gain. According to recommendations, gestational weight gain should be taken into account when determining energy needs for pregnant women. Energy requirement may be different in women whose gestational weight gain should be lower or higher than average values [16]. The protein and fat content was similar, being the highest in pattern 1 where it amounted to 128.6 g for proteins and 75.0 g for fat, and the lowest in pattern 3 — 101.8 g for proteins and 63.4 g for fat. The protein content significantly exceeded the recommended values in all dietary patterns. The highest content of carbohydrates was recorded in the vegetable-fruit diet (dietary pattern 2) (Me = 344.0 g), slightly lower in the cereal-milk diet (dietary pattern 1) (Me = 313.6 g) and the lowest in the cottage cheese-vegetable diet (dietary pattern 3) (Me = 265.8). The content of most minerals exceeded the recommended values in all dietary patterns, it was lower only in the case of iron and iodine. In the cottage cheese-vegetable diet

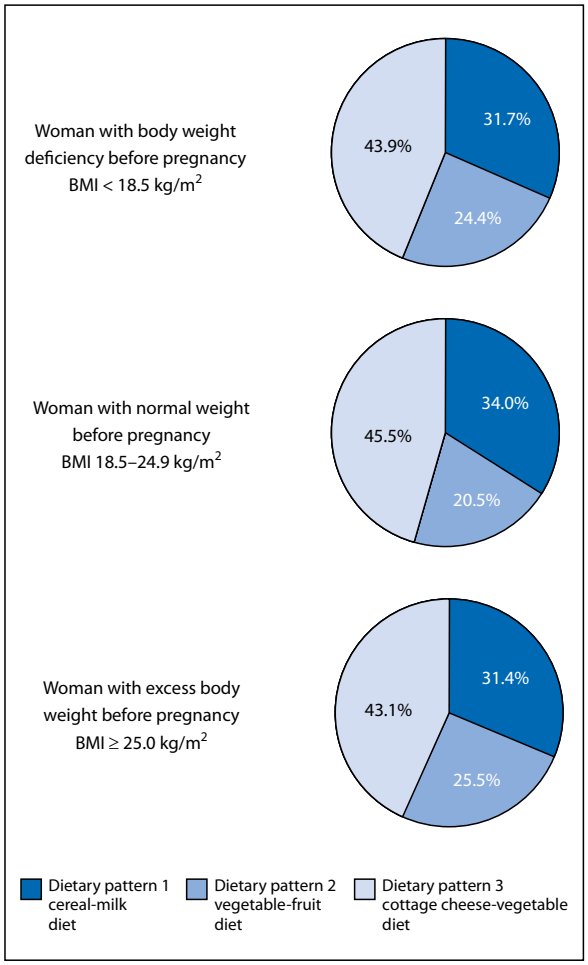

Figure 2. The frequency of the identified dietary patterns in the subgroups of pregnant women in relation to their nutritional status determined using the prepregnancy BMI

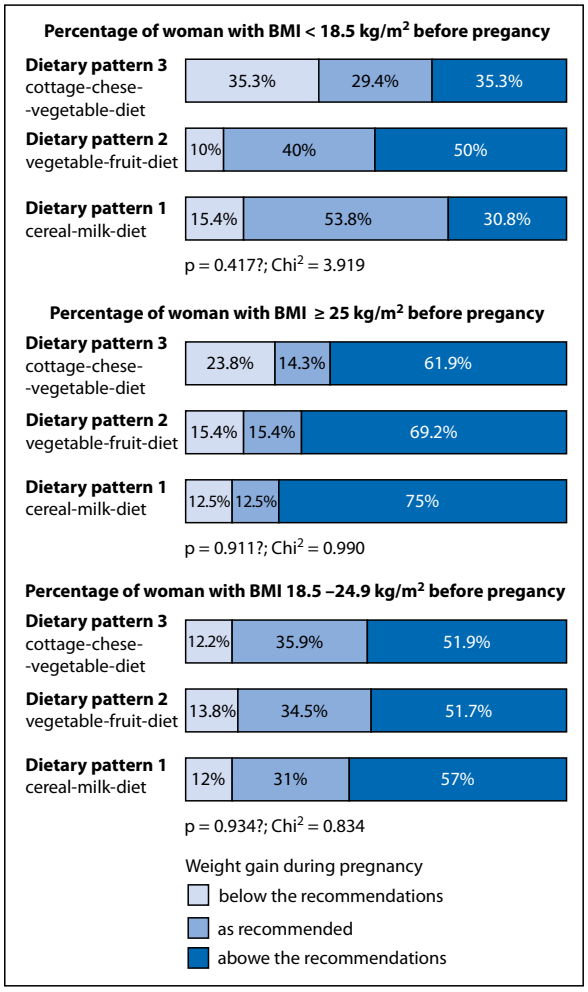

Figure 3. Frequency of dietary patterns depending on weight gain during pregnancy and prepregnancy BMI

(dietary pattern 3), the content of all analysed minerals was the lowest. The analysis of the consumption of fat-soluble vitamins revealed that the amounts of vitamins A and E were higher than recommended in all clusters. All dietary patterns were characterized by a lower than recommended content of vitamin D, which was approximately 3 µg. The content of water-soluble vitamins was higher than recommended in all dietary patterns. The content of vitamin C exceeded the nutritional standards four times. The percentage of energy from proteins slightly exceeded the recommended values, and the percentage of energy from fat and carbohydrates was within the norms.

DISCUSSION

During the life cycle of a woman, pregnancy is the period when she is most open to changing her behaviour and lifestyle for the benefit of the child's development. Lifestyle changes include, among others, also the diet correction. The nutritional factor is therefore one of the most important determinants of the appropriate development of pregnancy and the child. Nutritional needs should be analysed individually for each patient, taking into account her age, level of physical activity, lifestyle and, above all, nutritional status [2, 5]. A rational diet during pregnancy prevents complications and ensures appropriate development of the fetus. The implementation of nutritional recommendations in practice consists in adhering to a balanced and varied diet, thus reducing the incidence of both deficiency and excess of energy and nutrients [4–6]. The increased demand for energy (2nd and 3rd trimester) should be fulfilled by increasing the intake of mainly milk and dairy products, lean meat and its products, fish and additional portions of vegetables and fruit [16].

Numerous studies emphasize that the main complication caused by maternal underweight may be low birth weight of the child, which is often associated with an increased risk of cardiovascular diseases and type 2 diabetes [19–24], whereas maternal overweight and obesity may adversely affect the course of pregnancy and the child's development by increasing the risk of preeclampsia, preterm

Table 6. Comparison of energy value and nutrient profile of the three dietary patterns with the nutritional recommendations

Energy and nutrients	Dietary pattern 1 (A) cereal-milk diet			Dietary pattern 2 (B) vegetable-fruit diet			Dietary pattern 3 (C) cottage cheese-vegetable diet			p	NIZP-PZH (2020) [16], EFSA 2017 [17] EAR/AI
	$\bar{x} \pm SD$**	Me	1Q–3Q	$\bar{x} \pm SD$**	Me	1Q–3Q	$\bar{x} \pm SD$**	Me	1Q–3Q		
Basic nutrients											
Energy [kJ]	10645.7 ± 1982.4^{C}	10423.5	9437.8–11560.9	10716.8 ± 2074.3^{C}	10271.0	9240.0–12138.4	8581.8 ± 1810.4$^{A, B}$	8637.9	7426.5–9971.0	0.000	–
Energy [kcal]	2531.8 ± 470.9^{C}	2487.3	2243.1–2743.9	2547.3 ± 493.1^{C}	2446.5	2197.5–2882.9	2040.3 ± 430.6$^{A, B}$	2055.2	1766.8–2371.2	0.000	2200+85 (I trymestr) 2200+285 (II trymestr) 2200+475 (III trymestr)
Total protein [g]	135.2 ± 29.6^{C}	130.9	115.5–151.4	128.1 ± 28.9^{C}	128.6	111.5-144.9	104.4 ± 26.7$^{A, B}$	101.8	83.8–126.6	0.000	44–78
Animal protein [g]	102.5 ± 27.1^{C}	97.8	85.1–114.6	91.,4 ± 25.9^{C}	90.5	78.1–106.7	76.8 ± 25.2$^{A, B}$	74.7	58.4–94.1	0.000	–
Plant-based protein [g]	32.3 ± 8.1^{B,C}	31.0	25.8–37.0	36.1 ± 10.3$^{A, C}$	34.6	30.0–40.8	26.9 ±7.3$^{A, B}$	26.8	21.9–31.5	0.000	–
Fat [g]	84.2 ± 18.0^{B,C}	81.9	71.0–96.8	75.8 ± 18.5$^{A, C}$	75.0	62.5–88.6	64.0 ± 17.7$^{A, B}$	63.4	51.9–76.2	0.000	+3 (I trimester) +10–11 (II trimester)* +16–19 (III trimester)
Cholesterol [mg]	380.0 ± 103.1^{C}	371.2	304.9–449.8	349.5 ± 117.0^{C}	348.7	258.6–400.1	287.7 ± 97.2$^{A, B}$	266.8	221.0–348.8	0.000	–
Saturated faty acids [g]	33.3±7.5	32.4	28.4–37.8	26.9 ± 8.1^{C}	27.3	22.1–32.0	22.2 ± 6.8 B	22.0	16.9–27.2	0.000	As low as achievable in a diet that provides adequate nutritional value
Monounsaturated fatty acids [g]	31.8 ± 8.2^{C}	30.6	25.8–37.2	29.3 ± 8.9^{C}	28.7	23.7–34.1	24.8 ± 8.1$^{A, B}$	24.3	19.2–29.1	0.000	–
Polyunsaturated fatty acids [g]	13.1 ± 4.5	12.4	10.0–15.5	13.8 ± 4.6	13.8	10.8–16.8	12.4 ± 5.4	11.3	8.7–15.8	0.110	Linoleic acid 4% en. α-linolenic acid 0,5%
Linoleic acid [g]	9.6 ± 3.3^{C}	8.9	7.2–11.1	10.0 ± 3.3^{C}	9.6	7.8–12.0	8.7 ± 4.1$^{A, B}$	7.8	5.8–11.1	0.001	4% of energy
α-linolenic acid [g]	2.3 ± 0.9	2.1	1.7–2.7	2.4 ± 0.9	2.4	1.7–3.0	2.3 ± 1.4	2.0	1.4–2.8	0.101	α–linolenic acid 0,5%
Long chain polyunsaturated fatty acids [g]	1.0 ± 1.1	0.3	0.1–2.0	1.1 ± 1.2	0.3	0.1–2.1	1.2 ± 1.3	0.3	0.1–2.0	0.908	DHA+EPA 250+100–200 mg DHA
Omega 3 fatty acids [g]	3.4 ± 1.7	3.0	2.1–4.4	3.6 ± 1.8	3.4	2.1–4.7	3.5 ± 2.1	3.4	1.8–4.5	0.708	–
Omega 6 fatty acids [g]	9.8 ± 3.4^{C}	9.1	7.3–11.4	10.2 ± 3.3^{C}	9.8	8.0–12.3	8.9 ± 4.1$^{A, B}$	7.9	6.0–11.4	0.001	Linoleic acid 4% of energy
Docosaheksaenoic acid (DHA) [g]	0.7 ± 0.7	0.2	0.1–1.3	0.7 ± 0.8	0.2	0.1–1.3	0.8 ± 0.8	0.2	0.1–1.3	0.913	100–200 mg
Eicosapentaenoic acid (EPA) [g]	0.2 ± 0.2	0.1	0.0–0.4	0.3 ± 0.3	0.1	0.0–0.4	0.3 ± 0.3	0.1	0.0–0.4	0.801	250 mg
Carbohydrates [g]	323.3 ± 75.5$^{B, C}$	313.6	273.5–360.5	355.5 ± 82.7$^{A, C}$	344.0	302.9–401.0	275.5 ± 68.9$^{A, B}$	265.8	228.7–330.1	0.000	–
Saccharose [g]	57.6 ± 26.2	52.6	40.7–71.2	63.1 ± 28.3^{C}	57.6	43.9–76.2	53.9 ± 25.2 B	50.9	34.7–67.3	0.030	–

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Table 6. (cont.). Comparison of energy value and nutrient profile of the three dietary patterns with the nutritional recommendations

Energy and nutrients	Dietary pattern 1 (A) cereal-milk diet			Dietary pattern 2 (B) vegetable-fruit diet			Dietary pattern 3 (C) cottage cheese-vegetable diet			p	NIZP-PZH (2020) [16], EFSA 2017 [17] EAR/AI
	$\bar{x} \pm SD$**	Me	1Q–3Q	$\bar{x} \pm SD$**	Me	1Q–3Q	$\bar{x} \pm SD$**	Me	1Q–3Q		
Glucose [g]	16.9 ± 7.7 B	15.7	11.7–20.2	24.8 ± 11.2$^{A, C}$	23.0	17.0–33.3	18.2 ± 7.6 B	17.0	13.0–22.8	0.000	–
Fructose [g]	22.8 ± 10.12 B	20.7	15.7–27.4	31.4 ±13.0$^{A, C}$	31.2	21.6–40.6	23.8 ± 10.2 B	21.7	17.3–28.1	0.000	–
Starch [g]	155.9 ± 45.7^{C}	148.7	124.0–181.5	156.5 ± 53.5^{C}	156.5	123.5–179.1	111.0 ± 37.3$^{A, B}$	110.6	82.8–137.6	0.000	–
Fibre [g]	29.8 ± 8.9 B	28.7	22.6–36.5	34.2 ± 10.9$^{A, C}$	33.0	25.5–42.2	27.1 ± 8.6 B	26.3	20.8–32.3	0,000	– Level to be agreed with doctor or dietitian
Minerals											
Sodium [mg]	3383.1 ± 719.0^{C}	3244.5	2876.3–3899.7	3231.0 ± 846.3^{C}	3158.8	2647.1–3787.0	2358.6 ± 615.3$^{A, B}$	2332.4	1950.7–2643.2	0.000	1500 (AI)
Potassium [mg]	4743.9 ± 1058.2^{B,C}	4492.2	4023.5–5424.8	5799.2 ± 1307.6$^{A, C}$	5608.7	4901.9–6579.0	4400.4 ± 1068.5$^{A, B}$	4310.6	3673.8–5120.8	0.000	3500 (AI)
Calcium [mg]	1290.5 ± 424.5$^{B, C}$	1278.2	999.2–1515.5	1156.7 ± 387.1^{A}	1174.8	923.0–1337.9	1091.2 ± 336.7^{A}	1098.3	836.5–1288.1	0.000	800
Phosphorus [mg]	2115.2 ± 465.7^{C}	2035.5	1822.2–2405.1	2042.1 ± 437.0^{C}	2090.7	1780.7–2239.4	1723.9 ± 446.5$^{A, B}$	1654.5	1408.7–2036.0	0.000	580
Magnesium [mg]	462.0 ± 102.4$^{B, C}$	454.7	388.5–521.0	510.3 ± 108.5$^{A, C}$	500.7	430.6–554.9	410.2 ± 95.0$^{A, B}$	406.1	341.1–479.3	0.000	300
Iron [mg]	14.8 ± 3.5^{C}	14.7	12.2–16.8	16.1 ± 4.1^{C}	15.8	13.5–18.4	12.7±3.2$^{A, B}$	12.6	10.3–14.4	0.000	23
Zinc [mg]	16.0 ± 3.5^{C}	15.8	13.4–18.5	14.9 ± 3.3^{C}	14.8	12.4–16.8	12.7 ± 3.1$^{A, B}$	12.0	9.9–14.2	0.000	9.5
Copper [mg]	1.5 ± 0.4^{B,C}	1.5	1.3–1.8	1.8 ± 0.5$^{A, C}$	1.8	1.5–2.2	1.4 ± 0.4$^{A, B}$	1.4	1.1–1.6	0.000	0.8
Manganese [mg]	7.4 ± 2.6^{C}	7.4	5.4–8.9	7.1 ± 2.4^{C}	7.1	5.3–8.4	6.2 ± 2.4$^{A, B}$	5.9	4.5–7.7	0.000	2.0 (AI)
Iodine [µg]	127.6 ± 42.9$^{B, C}$	122.8	99.9–152.9	147.3 ± 43.5$^{A, C}$	140.3	119.7–170.0	113.7±39.1$^{A, B}$	111.1	82.2–140.0	0.000	160
Fat soluble vitamins											
Vitamin A (retinol equvalent) [µg]	1344.9 ± 729.8	1191.0	819.5–1660.1	1424,4 ± 831,1	1207,1	834.3–1758.3	1206.6 ± 665.2	1085.1	707.0–1574.5	0.88	530
Retinol [µg]	493.0 ± 219.0$^{B, C}$	454.0	374.0–547.5	395.6 ± 207.3$^{A, C}$	373.9	283.4–459.8	336.9 ± 151.1$^{A, B}$	313.1	235.7–398.0	0.000	–
B–carotene [µg]	5112.5 ± 4303.2	4143.7	2010.8–7018.9	6157.9 ± 4693.7	4840.7	2651.9–7965.8	5216.8 ± 3819.1	4298.3	2334.1–7207.1	0.213	–
Vitaminum E [mg]	13.0 ± 4.2 B	12.5	10.0–15.1	**15.? ±** 5.4$^{A, C}$	14.5	11.6–18.1	12.6 ± 4.4 B	12.2	9.5–14.9	0.000	10
Vitaminum D [µg]	5.7 ± 4.6	3.1	1.8–9.5	5.8 ± 4.8	3.0	1.7–9.2	5.9 ± 5.2	3.5	1.5–9.0	0.377	15
Water soluble vitamins											
Vitaminum B_1 [mg]	2.0 ± 0.4^{C}	1.9	1.7–2.2	2.1 ± 0.6^{C}	2.0	1.8–2.4	1.6 ± 0.4$^{A, B}$	1.6	1.3–1.8	0.000	1.2
Vitaminum B_2 [mg]	2.5 ± 0.6^{C}	2.4	2.1–2.9	2.5 ± 0.6^{C}	2.5	2.1–2.8	2.2 ± 0.6$^{A, B}$	2.1	1.7–2.7	0.000	1.2
Vitaminum PP [mg]	31.1 ± 9.8^{C}	29.5	24.7–35.2	33.1 ± 9.7^{C}	32.8	26.9–38.9	24.1 ± 7.3$^{A, B}$	23.8	19.6–28.3	0.000	14
Vitaminum B_6 [mg]	3.2±0.8^{B,C}	3.1	2.7–3.7	3.8 ± 1.0$^{A, C}$	3.8	3.2–4.4	2.9 ± 0.8$^{A, B}$	2.8	2.3–3.3	0.000	1.6

→

Table 6 (cont.). Comparison of energy value and nutrient profile of the three dietary patterns with the nutritional recommendations

Energy and nutrients	Dietary pattern 1 (A) cereal-milk diet			Dietary pattern 2 (B) vegetable-fruit diet			Dietary pattern 3 (C) cottage cheese-vegetable diet			p	NIZP-PZH (2020) [16], EFSA 2017 [17]
	$\bar{x}$ ± SD**	Me	1Q–3Q	$\bar{x}$ ± SD**	Me	1Q–3Q	$\bar{x}$ ± SD**	Me	1Q–3Q		EAR/AI
Folates [µg]	398.1 ± 103.5 B	398.4	321.8–458.4	477.7 ± 149.3[A, C]	452.3	351.2–575.6	387.4 ± 109.9 B	376.9	314.2–457.7	0.000	520
Vitaminum B_{12} [µg]	6.7 ± 2.5[C]	6.4	4.8–8.1	6.4 ± 2.8	5.8	4.5–7.7	6.0 ± 2.6[A]	5.7	4.0–7.6	0.028	2.2
Percentage of energy from basic nutrients											
Energy from protein [%]	21.7 ± 3.3 B	21.3	19.5–23.6	20.4 ± 3.4[A]	21.3	19.5–23.6	20.8 ± 3.8	20.7	18.4–23.1	0.036	10–20
Energy from fat [%]	29.7 ± 4.2[B, C]	29.3	27.1–31.5	26.5 ± 4.5[A]	29.3	27.1–31.5	27.9 ± 5.1[A]	27.9	24.4–31.3	0.000	20–35
Energy from carbohydrates [%]	46.4 ± 5.1[B, C]	46.5	43.2–49.8	50.5 ± 5.8[A]	46.5	43.2–49.8	48.8 ± 6.2[A]	48.6	44.8–53.0	0.000	45–65
Other											
Caffeine [mg]	133.9 ± 82.4	110.0	91.5–170.0	123.1 ± 79.8	110.0	91.5–170.0	121.8 ± 81.0	110.0	60.0–170.0	0.418	< 300

$\bar{x}$ ± SD — mean ± standard deviation; Me — median; 1Q–3Q – 1 quartile – 3 quartile
p — statistically significant differences in energy and nutrients intakes between dietary patterns (Kruskal–Wallis $p < 0.05$)
EAR — Estimated Average Requirement; AI — Adequate Intake
*for women weighing 65 kg, ≥ 30 years fat requirement is assumed 49–86 g, at the level of physical activity PAL = 1.6 [nutritional standards for Poland] [16]
** statistically significant differences between clusters of studied women [A, B, C] calculated using a post hoc test (multiple two-sided comparisons)

labour and gestational diabetes. In the case of excess body weight in a pregnant woman, it is more often necessary to perform a caesarean section. Some research reports suggest that maternal overweight or obesity during pregnancy may increase the risk of obesity in a child in later life [25–32]. The nutritional status determined using prepregnancy BMI of the majority of women (76.3%) in our study was appropriate. To assess the nutritional status of the studied women during pregnancy, their weight gain in kg per week was calculated. In all subgroups, the weight gain was above the values recommended by the Institute of Medicine [12]. The highest average weight gain was recorded in the group of women who were underweight before pregnancy (0.56 vs 0.51 kg/week). Weight gain in women with an appropriate body mass index before pregnancy was on average 0.55 kg/week, which was 0.13 kg/week higher than recommended. The lowest average gains of 0.40 kg/week were observed in women with excess body weight before pregnancy, but they still exceeded the recommendations (0.22–0.28 kg/week). The literature describes extensively the relationship between abnormal weight gain in pregnancy and the risk of health complications, both in the mother and in the child [19–21, 23, 33, 34]. In the studied group of women who were underweight before pregnancy, body weight gains were higher than recommended and the highest in the entire group of women. This suggests that during pregnancy they changed their diet, ate larger amounts of food to provide all the necessary nutrients to the developing child. In the studied group of women with overweight and obesity, weight gain during pregnancy was the lowest, but still higher than recommended. This may mean that these women have a more conscious attitude towards the nutritional factor and pay more attention to the selection of products in their diet. Studies by Oken et al. suggest that weight gain in obese women which is lower than recommended has a positive effect on pregnancy outcomes, *i.e.* it reduces the risk of macrosomia in a child, the risk of premature birth, and even obesity in their offspring at the age of 3 years. [27]. At the same time, weight gain lower than the American guidelines should not be routinely recommended for obese pregnant women. These recommendations should be individualized, taking into account risk factors [35].

The nutritional status is related to the diet. The diets of different population groups can be assessed using various methods, one of them being the identification of dietary patterns, which has attracted an increasing attention in recent years. As a comprehensive method, it is used to determine the relationship between the diet and the nutritional status, which gives a more complete picture of the impact of consumption of various dietary components on health status indicators. In the Polish literature, dietary patterns of women during pregnancy have not been widely described,

in contrast to foreign literature, where this topic is more popular. Dietary patterns of pregnant women are most often described in the context of the course of pregnancy, the risk of complications, including gestational diabetes, pregnancy-induced hypertension, birth weight of the newborn, and also in the long-term perspective, *i.e.* nutritional programming [36–40]. Researchers identifying dietary patterns try to indicate the pattern which is most similar to the nutritional recommendations for pregnant women and compare it with other patterns that significantly deviate from the recommendations. The dietary pattern compliant with the recommendations is most often described as healthy or prudent [41–44], but also health conscious [45]. Thus, defined dietary patterns are characterized by a high intake of vegetables, fruit, oils, whole grains and fish. The patterns which deviate from the recommendations are referred to as traditional or Western and are characterized by a high intake of red meat and its products, potatoes, sugar and sweets, cereal products, fat, except for olive oil, salty snacks, eggs, sauces and sweet drinks. For example, in a Canadian study of 1,545 pregnant women, four dietary patterns were identified. The first one, *i.e.* healthy pattern, was characterized by high intake of vegetables, including green vegetables, fruit, orange vegetables, oils, white and brown pasta, brown rice, fish and tomatoes. In the second one, named the meat and refined carbohydrates pattern, the more frequent intake of red meat, processed meat, fries, roast and boiled potatoes, and white bread was recorded. The third pattern was characterized by a high intake of beans and pulses, cheese, and vegetable salads and was named the beans, cheese and salad pattern. Women adhering to the fourth pattern named tea and coffee pattern more frequently drank coffee and tea, including with added reduced-fat milk, cream and sugar. The results showed that women adhering to healthy dietary patterns before pregnancy had a lower risk of developing complications such as hypertension [44]. In the Greek study, two dietary patterns were identified in pregnant women and named health conscious and Western. The Western pattern was characterised by a high intake of meat and meat products, potatoes, sugar and sweets, cereals, fats except olive oil, salty snacks, eggs, sauces and sweet beverages [45]. For the sake of comparison, in the Japanese study, the Western dietary pattern was described as a pattern with low intake of non-alcoholic beverages and sweets [46]. This shows that different dietary patterns may have the same names in different studies and vice versa. Some patterns, which were characterised by similar products in different studies, may be named differently. For example, there are many names for the pattern called the Mediterranean diet, such as the Mediterranean-type diet, the Mediterranean diet index, and the alternative Mediterranean diet, but the main components of this diet are the same [47–51]. A study conducted in 10 Mediterranean countries proved that adherence to the Mediterranean pattern of eating during pregnancy results in a better glucose tolerance and a lower incidence of gestational diabetes [51]. The authors of a Dutch prospective study proved that adherence to a traditional dietary pattern during pregnancy, in comparison with a Mediterranean pattern, has a negative impact on blood pressure parameters causing their increase [52]. In Denmark, the adherence to a Mediterranean dietary pattern by pregnant women was associated with a reduced risk of preterm labour [47]. A prospective study involving 13.110 American women showed that the Western dietary pattern was positively correlated with the risk of gestational diabetes, and the prudent dietary pattern, characterised by a high intake of fruit, green leafy vegetables, poultry and fish, showed a negative correlation [41]. Another study found that a prudent dietary pattern was associated with a lower risk of gestational diabetes, especially among women with excess body weight [43]. He et al. also proved that the dietary pattern defined as vegetable pattern was correlated with a lower risk, while the sweets and seafood pattern with a significantly higher risk of gestational diabetes mellitus [53]. It has also been shown that consumption of the DASH diet (high in fruit, vegetables, whole grain cereals, low-fat dairy products, and lower in saturated fat, cholesterol, and sodium 2400 mg/day) for 4 weeks by pregnant women had a beneficial effect on glucose tolerance and lipid profile compared to the control diet [54]. A Norwegian study has shown that pregnant women eating a diet with a high intake of vegetables and plant-based products, including oils, had a lower risk of pre-eclampsia, while a diet with a high intake of meat, sweet drinks and snacks increased this risk [55].

In our study, it can be concluded that none of the identified dietary patterns was fully compliant with the health-promoting pattern described in the current literature. Dietary pattern 1 defined as the cereal-milk diet seems to be the closest to the patterns described in the literature as traditional or Western, due to a high intake of cereals and butter, hard cheese, meat, poultry, cold cuts, and sugar and a low intake of vegetables and fruit. Dietary pattern 2 defined as the vegetable-fruit diet stood out due to the intake of this group of products, but at the same time it was characterized by a higher intake of potatoes, meat and poultry, and a lower intake of cottage cheese, rennet cheese and fermented milk drinks. Dietary pattern 3, defined as the cottage cheese-vegetable diet, was characterized by a higher intake of cottage cheese, as well as milk, cooked vegetables and fish, but also a lower intake of cereal products and butter, as well as meat, poultry and cold cuts.

The frequency of the identified dietary patterns in the three subgroups of the studied women, depending on their pre-pregnancy body mass index, was similar. The differences

were slight and statistically insignificant. It should be noted, however, that the most common pattern was dietary pattern 3 defined as the cottage cheese-vegetable diet with the lowest energy value. The analysis of the frequency of individual patterns in the studied women depending on the weight gain during pregnancy revealed a greater differentiation. Dietary pattern 1, *i.e.* the cereal-milk diet with higher energy value occurred most often in the group of women with excess bodyweight before pregnancy whose weight gain during pregnancy was above the recommendations (75.0%). It means that they should pay more attention to their diet during pregnancy due to the higher risk of complications related to excess body weight in pregnancy. Dietary patterns 1, *i.e.* the cereals-milk diet and dietary pattern 2, *i.e.* vegetable-fruit diet occurred most often in the group of women with underweight before pregnancy whose weight gain during pregnancy was in the line with the recommendations and above (40.0% and 53.8% respectively). It is possible that women in this group, who were slim before pregnancy, paid more attention to their diet during pregnancy.

The study has several strengths. All data regarding the diets of studied pregnant women were collected by one qualified person, and verified with the product and food photography album [14], which reduces the risk of incorrect estimation of the size of the consumed portions of food. The study focused on the identification of dietary patterns as a comprehensive method used to determine the relationship between the diet and nutritional status. Diet pattern analysis is considered as a holistic, alternative and complementary approach to assessing the relationship between diet and chronic disease risk by assessing the impact of the overall diet and its complexity. Therefore, the analysis of dietary patterns can be helpful in evaluating dietary recommendations and in explaining the relationship between the consumption of individual dietary components and health when these relations may depend on the dietary pattern. Moreover, the identification of dietary patterns of pregnant women can be the basis for shaping educational programs in childbirth classes. In the literature, there are only few studies describing the dietary patterns of Polish women during pregnancy [56, 57]. Only our study includes the nutrient profile of identified feeding patterns of pregnant women.

However, this study also has limitations. The group of studied pregnant women is not representative, but it is a large sample (n = 392). The studied women are participants of childbirth classes, mostly with higher education. Despite this, the results of the conducted research suggest the need for educational activities in the field of healthy eating patterns during pregnancy.

CONCLUSIONS

Although pregnancy should not differentiate the dietary procedures, including the selection of products, different dietary patterns were identified. In each group of women, regardless of their nutritional status before pregnancy as determined by BMI, the percentage of women adhering to the identified patterns was similar. In the studied women, weight gain during pregnancy was higher than in recommendations/guidelines, being the highest in women who were slim before pregnancy, and lower in women with excess body weight, which was related to their dietary patterns. The method of analysing the nutritional management through identified dietary patterns of pregnant women, correlated with their weight gain, can be a fast and effective way to convince this group of women to adjust their diets for the benefit of the health of the mother and the child, as well as for long-term prediction of the risk of diet-related disease in adulthood of the child. The analysis of the dietary patterns of pregnant women who are participants of childbirth education classes may be helpful in education and modification of the dietary recommendations for this population group, also with regard to supplementation.

Article information and declarations

Data availability statement

All data generated or analyzed during this study are included in this article. The datasets are not publicly available but are available from the corresponding author on reasonable request.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Mother and Child (Opinion No 10/2019).

Author contributions

Malgorzata Wiech: corresponding author, concept, methodology, acquisition of data, analysis and interpretation of data, visualization, manuscript writing, editing and approval the final manuscript. Ewa Kawaik-Jawor: data curation, formal analysis methodology, visualization, review, editing and approval the final manuscript. Marta Baranska: writing original draft, review and approval the final manuscript. Julia Zareba-Szczudlik: revised article critically. Halina Weker: concept, methodology, writing original draft, review and approval the final manuscript.

Consent for publication

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Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

1. Schwarzenberg SJ, Georgieff MK. Committee on Nutrition. Advocacy for Improving Nutrition in the First 1000 Days to Support Childhood Development and Adult Health. Pediatrics. 2018; 141(2), doi: 10.1542/peds.2017-3716, indexed in Pubmed: 29358479.
2. Moreno-Fernandez J, Ochoa JJ, Lopez-Frias M, et al. Impact of Early Nutrition, Physical Activity and Sleep on the Fetal Programming of Disease in the Pregnancy: A Narrative Review. Nutrients. 2020; 12(12), doi: 10.3390/nu12123900, indexed in Pubmed: 33419354.
3. Del Castillo-Matamoros SE, Poveda NE. Importance of nutrition in pregnant women. Rev Colomb Obstet Ginecol. 2021; 72(4): 339–345, doi: 10.18597/rcog.3825, indexed in Pubmed: 35134281.
4. Scherer-Adami F, Dutra-Rosolen M, Schedler F, et al. Nutritional status and dietary intake of pregnant women. Rev Salud Publica (Bogota). 2020; 22(1): 27–33, doi: 10.15446/rsap.V22n1.72795, indexed in Pubmed: 36753136.
5. Grenier LN, Atkinson SA, Mottola MF, et al. Be Healthy in Pregnancy: Exploring factors that impact pregnant women's nutrition and exercise behaviours. Matern Child Nutr. 2021; 17(1): e13068, doi: 10.1111/mcn.13068, indexed in Pubmed: 32705811.
6. Marshall NE, Abrams B, Barbour LA, et al. The importance of nutrition in pregnancy and lactation: lifelong consequences. Am J Obstet Gynecol. 2022; 226(5): 607–632, doi: 10.1016/j.ajog.2021.12.035, indexed in Pubmed: 34968458.
7. Harrison CL, Teede H, Khan N, et al. Weight management across preconception, pregnancy, and postpartum: A systematic review and quality appraisal of international clinical practice guidelines. Obes Rev. 2021; 22(10): e13310, doi: 10.1111/obr.13310, indexed in Pubmed: 34312965.
8. Most J, Dervis S, Haman F, et al. Energy Intake Requirements in Pregnancy. Nutrients. 2019; 11(8), doi: 10.3390/nu11081812, indexed in Pubmed: 31390778.
9. Wirfält E, Drake I, Wallström P. What do review papers conclude about food and dietary patterns? Food Nutr Res. 2013; 57, doi: 10.3402/fnr.v57i0.20523, indexed in Pubmed: 23467387.
10. Wądołowska L. Zasady obliczania i interpretacji wyników w: Gronowska-Senger A. [red.] Przewodnik metodyczny badań sposobu żywienia. Komitet Nauki o Żywieniu Człowieka Polskiej Akademii Nauk, Warszawa; 2013; 57-65. https://www.researchgate.net/publication/271645174_Zasady_obliczania_i_interpretacji_wynikow.
11. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol. 2002; 13(1): 3–9, doi: 10.1097/00041433-200202000-00002, indexed in Pubmed: 11790957.
12. Weight Gain During Pregnancy. 2009, doi: 10.17226/12584.
13. Jagtap S. Codesign in Low Resource Settings. Design and Engineering for Low Resource Settings. 2024: 31–45, doi: 10.1007/978-3-031-66156-3_3.
14. Szponar L, Wolnicka K, Rychlik E. Album fotografii produktów i potraw. Instytut Żywności i Żywienia, Warszawa; 2000.
15. Wajszczyk B, Chwojnowska Z, Nasiadko D, Rybaczuk M. Program Dieta 6 do planowania i bieżącej oceny żywienia indywidualnego. Instytut Żywności i Żywienia, Warszawa; 2021. https://www.pzh.gov.pl/wp-content/uploads/2022/02/Instrukcja-korzystania-z-Diety-6.0-2021.pdf (publication in polish).
16. Jarosz M, Rychlik E, Stoś K, Charzewska J. [ed.] Normy żywienia dla populacji Polski i ich zastosowanie. Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny, Warszawa 2020. https://www.pzh.gov.pl/wp-content/uploads/2020/12/Normy_zywienia_2020web-1.pdf.
17. Dietary Reference Values for nutrients Summary report. EFSA Supporting Publications. 2017; 14(12), doi: 10.2903/sp.efsa.2017.e15121.
18. Zimmer M., Sieroszewski P., Oszukowski P., Huras H., Fuchs T., Pawłosek A. Rekomendacje Polskiego Towarzystwa Ginekologów i Położników dotyczące suplementacji u kobiet ciężarnych. Ginekologia i Perinatologia Praktyczna 2020;5:170-181. https://www.ptgin.pl/sites/scm/files/2022-01/07.2020 - Rekomendacje Polskiego Towarzystwa Ginekologów i Położników dotyczące suplementacji u kobiet ciężarnych_0.pdf.
19. Davis RR, Hofferth SL, Shenassa ED. Gestational weight gain and risk of infant death in the United States. Am J Public Health. 2014; 104 Suppl 1(Suppl 1): S90–S95, doi: 10.2105/AJPH.2013.301425, indexed in Pubmed: 24354832.
20. Galjaard S, Pexsters A, Devlieger R, et al. The influence of weight gain patterns in pregnancy on fetal growth using cluster analysis in an obese and nonobese population. Obesity (Silver Spring). 2013; 21(7): 1416–1422, doi: 10.1002/oby.20348, indexed in Pubmed: 23408453.
21. Hunt KJ, Alanis MC, Johnson ER, et al. Maternal pre-pregnancy weight and gestational weight gain and their association with birthweight with a focus on racial differences. Matern Child Health J. 2013; 17(1): 85–94, doi: 10.1007/s10995-012-0950-x, indexed in Pubmed: 22322428.
22. Goldstein RF, Abell SK, Ranasinha S, et al. Association of Gestational Weight Gain With Maternal and Infant Outcomes: A Systematic Review and Meta-analysis. JAMA. 2017; 317(21): 2207–2225, doi: 10.1001/jama.2017.3635, indexed in Pubmed: 28586887.
23. Zhao R, Xu L, Wu ML, et al. Maternal pre-pregnancy body mass index, gestational weight gain influence birth weight. Women Birth. 2018; 31(1): e20–e25, doi: 10.1016/j.wombi.2017.06.003, indexed in Pubmed: 28716548.
24. Goldstein RF, Abell SK, Ranasinha S, et al. Gestational weight gain across continents and ethnicity: systematic review and meta-analysis of maternal and infant outcomes in more than one million women. BMC Med. 2018; 16(1): 153, doi: 10.1186/s12916-018-1128-1, indexed in Pubmed: 30165842.
25. Hedderson MM, Weiss NS, Sacks DA, et al. Pregnancy weight gain and risk of neonatal complications: macrosomia, hypoglycemia, and hyperbilirubinemia. Obstet Gynecol. 2006; 108(5): 1153–1161, doi: 10.1097/01.AOG.0000242568.75785.68, indexed in Pubmed: 17077237.
26. Stotland NE, Cheng YW, Hopkins LM, et al. Gestational weight gain and adverse neonatal outcome among term infants. Obstet Gynecol. 2006; 108(3 Pt 1): 635–643, doi: 10.1097/01.AOG.0000228960.16678.bd, indexed in Pubmed: 16946225.
27. Oken E, Taveras EM, Kleinman KP, et al. Gestational weight gain and child adiposity at age 3 years. Am J Obstet Gynecol. 2007; 196(4): 322.e1–322.e8, doi: 10.1016/j.ajog.2006.11.027, indexed in Pubmed: 17403405.
28. Poston L. Gestational weight gain: influences on the long-term health of the child. Curr Opin Clin Nutr Metab Care. 2012; 15(3): 252–257, doi: 10.1097/MCO.0b013e3283527cf2, indexed in Pubmed: 22406744.
29. Catalano P, deMouzon SH. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. Int J Obes (Lond). 2015; 39(4): 642–649, doi: 10.1038/ijo.2015.15, indexed in Pubmed: 25777180.
30. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. J Hum Nutr Diet. 2015; 28 Suppl 1: 1–14, doi: 10.1111/jhn.12212, indexed in Pubmed: 24479490.
31. Tebbani F, Oulamara H, Agli A. Effects of gestational weight gain on pregnancy complications. Nutrition Clinique et Métabolisme. 2018; 32(1): 27–32, doi: 10.1016/j.nupar.2017.09.011.
32. Szamotulska K. [red.] Przeprowadzenie kompleksowych badań epidemiologicznych dotyczących sposobu żywienia i stanu odżywienia kobiet ciężarnych wraz z identyfikacją czynników ryzyka zaburzeń odżywiania, oceną poziomu aktywności fizycznej, poziomu wiedzy żywieniowej oraz występowania nierówności w zdrowiu. Raport końcowy z badania 2017-2020. Instytut Matki i Dziecka. Warszawa; 2020. https://archiwum.mz.gov.pl/zdrowie-i-profilaktyka/narodowy-program-zdrowia/poprawa-sposobu-zywienia-stanu-odzywienia-oraz-aktywnosci-fizycznej-spoleczenstwa/3-1-przeprowadzenie-kompleksowych-badan-epidemiologicznych-dotyczacych-sposobu-zywienia-i-stanu-odzywienia-kobiet-ciezarnych-wraz-z-identyfikacja-czynnikow-ryzyka-zaburzen-odzywiania-ocena-poziomu/.
33. Krukowski RA, Bursac Z, McGehee MA, et al. Exploring potential health disparities in excessive gestational weight gain. J Womens Health (Larchmt). 2013; 22(6): 494–500, doi: 10.1089/jwh.2012.3998, indexed in Pubmed: 23751164.
34. Faucher MA, Barger MK. Gestational weight gain in obese women by class of obesity and select maternal/newborn outcomes: A systematic review. Women Birth. 2015; 28(3): e70–e79, doi: 10.1016/j.wombi.2015.03.006, indexed in Pubmed: 25866207.
35. Kapadia MZ, Park CK, Beyene J, et al. Can we safely recommend gestational weight gain below the 2009 guidelines in obese women? A systematic review and meta-analysis. Obes Rev. 2015; 16(3): 189–206, doi: 10.1111/obr.12238, indexed in Pubmed: 25598037.

36. Chen X, Zhao D, Mao X, et al. Maternal Dietary Patterns and Pregnancy Outcome. Nutrients. 2016; 8(6), doi: 10.3390/nu8060351, indexed in Pubmed: 27338455.
37. Raghavan R, Dreibelbis C, Kingshipp BL, et al. Dietary patterns before and during pregnancy and birth outcomes: a systematic review. Am J Clin Nutr. 2019; 109(Suppl_7): 729S–756S, doi: 10.1093/ajcn/nqy353, indexed in Pubmed: 30982873.
38. da Mota Santana J, de Oliveira Queiroz VA, Pereira M, et al. Associations between Maternal Dietary Patterns and Infant Birth Weight in the NISAMI Cohort: A Structural Equation Modeling Analysis. Nutrients. 2021; 13(11), doi: 10.3390/nu13114054, indexed in Pubmed: 34836305.
39. Kebbe M, Flanagan EW, Sparks JR, et al. Eating Behaviors and Dietary Patterns of Women during Pregnancy: Optimizing the Universal‚Teachable Moment'. Nutrients. 2021; 13(9), doi: 10.3390/nu13093298, indexed in Pubmed: 34579175.
40. Schwedhelm C, Lipsky LM, Temmen CD, et al. Eating Patterns during Pregnancy and Postpartum and Their Association with Diet Quality and Energy Intake. Nutrients. 2022; 14(6), doi: 10.3390/nu14061167, indexed in Pubmed: 35334823.
41. Zhang C, Schulze MB, Solomon CG, et al. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia. 2006; 49(11): 2604–2613, doi: 10.1007/s00125-006-0422-1, indexed in Pubmed: 16957814.
42. Englund-Ögge L, Brantsæter AL, Sengpiel V, et al. Maternal dietary patterns and preterm delivery: results from large prospective cohort study. BMJ. 2014; 348: g1446, doi: 10.1136/bmj.g1446, indexed in Pubmed: 24609054.
43. Tryggvadottir EA, Medek H, Birgisdottir BE, et al. Association between healthy maternal dietary pattern and risk for gestational diabetes mellitus. Eur J Clin Nutr. 2016; 70(2): 237–242, doi: 10.1038/ejcn.2015.145, indexed in Pubmed: 26350393.
44. Jarman M, Mathe N, Ramazani F, et al. APrON and ENRICH study teams. Dietary Patterns Prior to Pregnancy and Associations with Pregnancy Complications. Nutrients. 2018; 10(7), doi: 10.3390/nu10070914, indexed in Pubmed: 30018227.
45. Chatzi L, Melaki V, Sarri K, et al. Dietary patterns during pregnancy and the risk of postpartum depression: the mother-child ‚Rhea' cohort in Crete, Greece. Public Health Nutr. 2011; 14(9): 1663–1670, doi: 10.1017/S1368980010003629, indexed in Pubmed: 21477412.
46. Miyake Y, Okubo H, Sasaki S, et al. Maternal dietary patterns during pregnancy and risk of wheeze and eczema in Japanese infants aged 16-24 months: the Osaka Maternal and Child Health Study. Pediatr Allergy Immunol. 2011; 22(7): 734–741, doi: 10.1111/j.1399-3038.2011.01176.x, indexed in Pubmed: 21539616.
47. Mikkelsen TB, Osterdal ML, Knudsen VK, et al. Association between a Mediterranean-type diet and risk of preterm birth among Danish women: a prospective cohort study. Acta Obstet Gynecol Scand. 2008; 87(3): 325–330, doi: 10.1080/00016340801899347, indexed in Pubmed: 18307073.
48. Haugen M, Meltzer HM, Brantsaeter AL, et al. Mediterranean-type diet and risk of preterm birth among women in the Norwegian Mother and Child Cohort Study (MoBa): a prospective cohort study. Acta Obstet Gynecol Scand. 2008; 87(3): 319–324, doi: 10.1080/00016340801899123, indexed in Pubmed: 18307072.
49. Tobias DK, Hu FB, Chavarro J, et al. Healthful dietary patterns and type 2 diabetes mellitus risk among women with a history of gestational diabetes mellitus. Arch Intern Med. 2012; 172(20): 1566–1572, doi: 10.1001/archinternmed.2012.3747, indexed in Pubmed: 22987062.
50. Tobias DK, Zhang C, Chavarro J, et al. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. Am J Clin Nutr. 2012; 96(2): 289–295, doi: 10.3945/ajcn.111.028266, indexed in Pubmed: 22760563.
51. Timmermans S, Steegers-Theunissen RPM, Vujkovic M, et al. Major dietary patterns and blood pressure patterns during pregnancy: the Generation R Study. Am J Obstet Gynecol. 2011; 205(4): 337.e1–337.12, doi: 10.1016/j.ajog.2011.05.013, indexed in Pubmed: 21855845.
52. He JR, Yuan MY, Chen NN, et al. Maternal dietary patterns and gestational diabetes mellitus: a large prospective cohort study in China. Br J Nutr. 2015; 113(8): 1292–1300, doi: 10.1017/S0007114515000707, indexed in Pubmed: 25821944.
53. Asemi Z, Tabassi Z, Samimi M, et al. Favourable effects of the Dietary Approaches to Stop Hypertension diet on glucose tolerance and lipid profiles in gestational diabetes: a randomised clinical trial. Br J Nutr. 2013; 109(11): 2024–2030, doi: 10.1017/S0007114512004242, indexed in Pubmed: 23148885.
54. Brantsaeter AL, Haugen M, Samuelsen SO, et al. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with reduced risk of preeclampsia in nulliparous pregnant Norwegian women. J Nutr. 2009; 139(6): 1162–1168, doi: 10.3945/jn.109.104968, indexed in Pubmed: 19369368.
55. Wesołowska E, Jankowska A, Trafalska E, et al. Sociodemographic, Lifestyle, Environmental and Pregnancy-Related Determinants of Dietary Patterns during Pregnancy. Int J Environ Res Public Health. 2019; 16(5), doi: 10.3390/ijerph16050754, indexed in Pubmed: 30832307.
56. Association between Bone Mineral Density and Dietary Patterns in Elderly Women in Guangxi: A Cross-Sectional Study in China. International Journal of Frontiers in Medicine. 2024; 6(5), doi: 10.25236/ijfm.2024.060511.

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Assessment of emotions in pregnancy: introduction of the Pregnancy Anxiety and Stress Rating Scale (PASRS) and its application in the context of hospitalization

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ABSTRACT

Objectives: Pregnancy is a special time that brings both joy and challenges. Among these challenges, anxiety and stress are emotions that can affect the mental wellbeing of the pregnant woman as well as the developing baby.

Material and methods: In response to these challenges, we present the Pregnancy Anxiety and Stress Rating Scale (PASRS), an innovative tool that aims to identify and assess anxiety and stress levels among pregnant women.

Results: The PASRS contains 15 questions that are more comprehensive and cover various aspects of pregnancy, including the health of the baby, the health of the mother, body changes, finances and social support.

Conclusions: In an era where mental health is just as important as physical health, SOLiSC is a step forward in recognising and addressing the unique mental challenges that pregnant women may face.

Keywords: emotions; pregnancy; anxiety; stress

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INTRODUCTION

Pregnancy is a unique period in every woman's life, characterised by intense physiological, hormonal and emotional changes. During this time, a woman not only experiences the joy associated with the impending birth of her child, but also faces challenges and pressures that can affect her wellbeing and mental health.

The hormonal changes that occur in a woman's body during pregnancy naturally have a direct impact on her mood and emotions. Fluctuations in oestrogen and progesterone levels can lead to mood swings, the appearance of anxiety and sometimes concerns about motherhood and the health of the baby [1]. A changing body, associated with weight gain, skin stretching and other physical changes, also affects a woman's mood, self-esteem and self-image [2].

Additionally, pregnancy brings with it an awareness of increased responsibility not only for oneself, but also for the life and health of the developing baby. This increased responsibility can also be a source of stress and anxiety, especially for mothers-to-be who are experiencing motherhood for the first time [3].

Modern society, dominated by social media and body worship, also plays a role in shaping the experience of pregnancy. Societal pressures, related to expectations about appearance, behaviour and the role of the mother, can increase the psychological burden on a pregnant woman [4].

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Statistically, lack of acceptance of one's own appearance during pregnancy is reported by one in three women [5]. The ease with which people judge each other, especially in terms of appearance and lifestyle, can lead to an increase in negative feelings such as insecurity, anxiety and decreased self-esteem.

Social media has many contradictions within it, although it can be both a source of support and information, it can also contribute to the build-up of pressure, expectations and is a place for easy judgement and hectoring. Comparisons with others, idealised images of motherhood, curved photos and pregnancy presented online can be a source of stress and anxiety [6].

With the increasing number of people affected by anxiety disorders and depression worldwide, the attention of researchers is increasingly turning to specific risk groups. Pregnant women are one of these groups where both anxiety and depression can have far-reaching consequences for maternal and child health [7, 8]. It is estimated that by 2030, one in four people worldwide will suffer from some type of mental disorder [9].

According to WHO data, depression is the leading cause of disability and anxiety disorders are the most diagnosed mental disorders [10]. In the context of pregnancy, these disorders may be exacerbated by hormonal changes, stress and other psychosocial factors [11]. It follows that the prevalence of suicidal thoughts during pregnancy ranges from 2.73% to 39% in various studies [12], and approximately 20% of postnatal deaths are attributed to suicide [13].

One study has shown that women who have experienced preterm birth are at increased risk of psychiatric hospitalisation in the short and long term [14]. Furthermore, there is a strong association between a woman's mental health status during hospitalisation for childbirth and the risk of re-hospitalisation within 42 days postpartum [15]. Several studies have focused on this association and found that mental health conditions including depression, anxiety, bipolar affective disorder and conditions associated with chronic stress or trauma increase the risk of hospital readmission after childbirth [15, 16].

Anxiety disorders include a variety of disorders, including generalised anxiety disorder, specific phobias, social anxiety disorder and post-traumatic stress disorder. Prevalence rates of these disorders vary by region, but overall, they are very common. In the United States, for example, the National Comorbidity Survey Replication (NCS-R) estimated the 12-month prevalence rate of anxiety disorders to be approximately 18.1% [7].

It is worth realising that depression is a very common mental disorder. The WHO estimates that depression affects more than 264 million people worldwide [8]. The concept of depression as an illness must therefore not only apply to mental health services, but also to primary care physicians and other specialties, including obstetrics and gynaecology. It is important to remember that depression is an illness that leads to significant suffering and impaired functioning and increases the risk of suicide.

The increase in the number of people affected by anxiety disorders and depression can be attributed to several factors, including increased awareness and recognition of these disorders, lifestyle changes, stress and social pressure, as well as biological, epigenetic and genetic factors.

Objectives

Sudden and unexpected hospital admissions during pregnancy can significantly increase anxiety levels, which in turn can affect maternal and child health. Extensive research in this area is needed to understand these relationships and develop effective support strategies [17].

MATERIAL AND METHODS

In developing the Anxiety Rating Scale, we considered various aspects of pregnancy-related anxiety. The questions were carefully selected to reflect these aspects, using the latest reports from the fields of psychiatry and obstetrics and gynaecology [18].

The Pregnancy Anxiety and Stress Scale was developed to comprehensively assess anxiety and stress experienced by pregnant women. The selection of questions in the scale was based on a thorough analysis of the scientific literature and consultation with experts in psychiatry, psychology and gynaecology [18, 19]. There are no ethics concerns. The study due to its observational nature did not require Ethics Committee approval. There was no financial support for the study.

Concerns about the child's health

Questions about concerns about the health of the baby were included because research shows that these are among the most common concerns among pregnant women [20]. It is therefore crucial to understand and quantify these concerns to develop effective psychological support interventions.

Social support

Social support is an important component of coping with anxiety and stress in pregnancy. Research has shown that social support can have a significant impact on reducing anxiety in pregnancy [21]. The questions in this category aim to determine the level of social support and its impact on perceived anxiety.

Concerns about childbirth

Anxiety related to childbirth is a common phenomenon and understanding it can contribute to developing strategies

Table 1. Pregnancy Anxiety and Stress Scale (PASRS)

No.	Question	0 — Not at all	1 — Rarely	2 — Sometimes	3 — Often	4 — Always
1	Worried about your child's health?	0	1	2	3	4
2	Do you feel anxious about your own health during pregnancy?	0	1	2	3	4
3	Do you have concerns about childbirth?	0	1	2	3	4
4	Do you happen to feel anxious before medical appointments or examinations?	0	1	2	3	4
5	Are you worried about your future role as a mother?	0	1	2	3	4
6	Do you feel stressed about possible changes to your body?	0	1	2	3	4
7	Do you have concerns about your child's finances or support?	0	1	2	3	4
8	Do you happen to feel overwhelmed by the amount of information about pregnancy and motherhood?	0	1	2	3	4
9	Do you have concerns about your partner's/partner's reaction and support?	0	1	2	3	4
10	Are you experiencing work-life balance anxiety?	0	1	2	3	4
11	Do you feel lonely or isolated?	0	1	2	3	4
12	Do you find it difficult to relax and unwind?	0	1	2	3	4
13	Do you sometimes feel anxious about being judged by others as a mother?	0	1	2	3	4
14	Do you have concerns about the impact of stress on your child's health?	0	1	2	3	4
15	Do you happen to think about the risks involved in childbirth, for you or your baby?	0	1	2	3	4

to reduce the anxiety and stress associated with childbirth [22]. Tokophobia, or fear of pregnancy and childbirth, affects approximately 10% of women and can be a serious condition that very often leads to the decision to have a caesarean section. Symptoms of tocophobia can range from sleep disturbances to panic attacks [23]. Questions in this category focus on fears of pain, unpredictability and possible complications.

Fear of complications

Pregnant women are often concerned about possible complications that may affect their mental health [24]. The questions in this category aim to understand and assess these concerns.

Concerns about one's own health

The mother's health status has a direct impact on her experience of anxiety during pregnancy [25]. Questions about the mother's reported health concerns are designed to assess how the mother's current health status affects her anxiety levels.

Fear of death

Although rarely talked about out loud, death anxiety can occur and has an impact on a woman's psychological wellbeing [26]. Understanding these fears is important for a comprehensive assessment of anxiety in pregnancy.

Additional questions

Additional questions were included in the scale to explore other aspects of anxiety and stress that may be relevant in the context of pregnancy. These questions include work-life balance, perceived anxiety about being judged by others as a mother or concerns about the impact of stress on the health of the baby [27].

Scale validation

The PASRS scale will undergo a validation process that will include factor analysis, assessment of reliability and validity, and correlational studies with other scales measuring anxiety and stress in pregnancy [28]. The validation process aims to ensure that the scale is a reliable and valid tool for assessing anxiety and stress in pregnancy.

Importance of research

The development of the PASRS scale is crucial for understanding and supporting pregnant women who experience anxiety and stress. This will enable the development of effective interventions and support strategies that are tailored to the individual needs of pregnant

women, helping to improve their mental health and overall wellbeing [29].

Interpretation of results

0–15: Low levels of anxiety and stress. Further monitoring and support as needed is recommended.
16–30: Medium level of anxiety and stress. Consulting a specialist and seeking support is recommended.
31–45: High levels of anxiety and stress. Immediate consultation with a specialist and intensive support is recommended.

RESULTS

The development of accurate tools to assess anxiety in pregnancy, considering the specificity of emergency and unexpected hospital admissions, is crucial to understanding and supporting pregnant women. A new scale, based on a sound scientific and clinical foundation, can make a significant contribution to progress in this area.

In Poland, despite growing awareness of the importance of mental health among pregnant women, there is still no widely used screening tool for anxiety disorders in this group of patients.

This phenomenon is challenging, especially in the context of statistical data. According to studies published in international scientific journals, between 15% and 25% of women experience significant levels of anxiety during pregnancy [30]. Other studies indicate that up to 10% of pregnant women may experience symptoms related to depressive disorders [31].

Anxiety disorders and stress, especially in pregnancies at risk and complicated by maternal illness or fetal complications, are an issue that cannot be ignored.

In response to this gap, we propose the introduction of PASRS, a tool to quickly and effectively identify women who may need specialist support. The importance of this tool is even greater as there is currently no standard method of assessing and monitoring the mental health of pregnant women in Poland, which is a barrier to accessing appropriate care and support.

The development and implementation of the scale has the potential to revolutionise the approach to mental health care for pregnant women in Poland, enabling early detection and therefore intervention for anxiety and stress disorders, which in turn can improve health outcomes for both mothers and their babies.

Other scales for assessing anxiety and stress in pregnancy

There are a number of scales worldwide that assess anxiety and stress in pregnancy. One of these is the Pregnancy Anxiety Scale (PAS) [32], which focuses on assessing anxiety related to the birth process itself. Another popular scale, the Pregnancy Stress Scale (PSS) [33], focuses on stress related to various aspects of pregnancy, including body changes, baby's health and relationship changes.

Comparison of other anxiety scales with the Pregnancy Anxiety and Stress Scale (PASRS)

Scope of questions

The PASRS contains 15 questions that are more comprehensive and cover various aspects of pregnancy, including baby health, maternal health, body changes, finances and social support. Other scales, such as the PAS and PSS, are more limited in scope and focus on specific areas of anxiety and stress.

Emotional and social assessment

The PASRS also considers emotional and social aspects such as loneliness, social evaluation and work-life balance. This holistic approach is rarely found in other scales.

Customisation

Thanks to its versatility, PASRS can be tailored to each pregnant woman's individual needs and concerns, enabling more personalised assessment and support.

DISCUSSION

Pregnancy is a special time that brings both joy and challenges. Among these challenges, anxiety and stress are emotions that can affect the mental wellbeing of the pregnant woman as well as the developing baby. In response to these challenges, we present the Pregnancy Anxiety and Stress Assessment Scale, an innovative tool that aims to identify early women at risk of developing depression during the perinatal period.

In the context of the development and implementation of the PASRS scale, it becomes crucial to understand how pregnant women perceive their experiences and needs, which differs significantly from postpartum, postpartum women's perceptions of the same aspects. As noted by Podolska and co-authors [34], even despite depressive symptoms, postpartum women experience a positive change in the assessment of the real self-image, which may be related to adaptation to the maternal role or a reduction in anxiety related to fear for the life of the child. This observation highlights the complexity of adaptation mechanisms in the psychological context and points to the need to take these variables into account when constructing diagnostic tools such as the PASRS, which are specifically dedicated to pregnant women. Appropriate adaptation of the scale can contribute to a better understanding and support of pregnant women,

capturing the full range of their unique emotional and psychological experiences.

In the context of developing the scale we presented, it was important to highlight the importance of using appropriate diagnostic tools in identifying women at increased risk of postpartum depression. As noted by Kossakowska-Petrycka and co-authors [35], high-risk pregnancy and the accompanying stress can significantly influence the occurrence of postpartum depression. The authors highlight that women with high-risk pregnancies experience significantly higher levels of stress and negative emotions compared to women with normal pregnancies.

Such observations emphasise the need for special attention in monitoring the mental state of pregnant women, especially in at-risk groups. The proposal of the PASRS scale, focused on the assessment of anxiety and stress in pregnancy, aims to identify early symptoms of depression. This enables the implementation of appropriate interventions that can contribute to better management and prevention of major depressive conditions after childbirth. The integration of this scale with existing scientific knowledge is crucial for the effective support of pregnant women.

Attention should also be paid to the emotional response aspect of coping with stress, in which individuals focus on their feelings and negative emotions. This approach differs from active problem-solving strategies or seeking social support. It appears that women who focus on their emotions in response to stress may experience a higher risk of postpartum depression. Focusing on negative feelings may exacerbate psychological discomfort and increase the risk of depression [36]. Such an observation only confirms that the emotional response to stress is a specific way of responding that involves focusing on the negative feelings caused by the stressor, rather than trying to resolve or minimise its impact.

In the context of these observations, Libera and co-authors [37] also highlight the emotional aspects of coping with stress, defining it as a strategy in which reactions such as expressing anxiety, worry, or sadness predominate. Such emotional focus in response to stress, especially in life situations such as premature birth, delivery of a child with a birth defect, *etc.*, can significantly affect mental health.

The PASRS scale is distinguished by its holistic approach, taking into account a broad spectrum of questions related to the pregnancy itself and the emotions surrounding it. It has been designed to provide a concrete, evidence-based assessment method that can be used by both medical professionals and pregnant women themselves. A number of questions have been chosen to ensure that answering them is not tiring and at the same time that they provide as much information as possible. This tool can serve as a link for open and honest communication between patient and doctor, enabling a personalised approach and tailored support strategies.

In a digital age where information is available at our fingertips, PASRS can also serve as a self-help resource for pregnant women who are looking for ways to understand and manage their emotions. This can be particularly valuable in the context of the social pressures and expectations surrounding pregnancy and motherhood that are often portrayed on social media.

The scale is not only a diagnostic tool, but also an educational platform. It can help pregnant women understand that their fears and anxieties are part of a shared experience, and that support is available. It can also inspire the medical and research community to further explore the impact of anxiety and stress on maternal and child health, leading to the development of more targeted and timely interventions or therapies.

In the context of global mental health, PASRS can be adapted and applied across cultures and societies, helping to identify and support women who experience anxiety and stress in pregnancy around the world. This can lead to a global shift in attitudes towards the mental health of pregnant women, with an emphasis on empathy, understanding and support.

CONCLUSIONS

In an era where mental health is as important as physical health, PASRS is a step forward in recognizing and managing the unique mental challenges that pregnant women may face. The tool not only diagnoses, but also educates, supports and connects, opening the door to more integrated mental health care for pregnant women.

Article information and declarations

Data availability statement

The data is available after reasonable request to the first author.

Ethics statement

There are no ethics concerns. The study due to observational nature did not require Ethics Committee approval.

Author contributions

KZK — concept, assumptions, methods, protocol, analysis, data interpretation, final acceptance; OS — analysis, litarature search, visualization; MK — analysis, assumption; PO — analysis; KW — methods, analysis; PG — methods, analysis; MG — conducting, literature search.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

No supplementary material.

REFERENCES

1. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. Child Dev. 2010; 81(1): 131–148, doi: 10.1111/j.1467--8624.2009.01385.x, indexed in Pubmed: 20331658.
2. Silveira M, Ertel K, Dole N, et al. The role of body image in prenatal and postpartum depression: a critical review of the literature. Archives of Women's Mental Health. 2015; 18(3): 409–421, doi: 10.1007/s00737-015-0525-0.
3. Glover V. Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. Best Pract Res Clin Obstet Gynaecol. 2014; 28(1): 25–35, doi: 10.1016/j.bpobgyn.2013.08.017, indexed in Pubmed: 24090740.
4. Fredrickson B, Roberts TA. Objectification Theory: Toward Understanding Women's Lived Experiences and Mental Health Risks.Psychol Women Quart. 1997; 21(2): 173–206, doi: 10.1111/j.1471-6402.1997.tb00108.x.
5. Przybyła-Basista H, Kwiecińska E, Ilska M. Body Acceptance by Pregnant Women and Their Attitudes toward Pregnancy and Maternity as Predictors of Prenatal Depression. Int J Environ Res Public Health. 2020; 17(24), doi: 10.3390/ijerph17249436, indexed in Pubmed: 33339240.
6. Bartholomew MK, Schoppe-Sullivan SJ, Glassman M, et al. New Parents' Facebook Use at the Transition to Parenthood. Fam Relat. 2012; 61(3): 455–469, doi: 10.1111/j.1741-3729.2012.00708.x, indexed in Pubmed: 23671354.
7. Kessler RC, Chiu WT, Demler O, et al. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005; 62(6): 617–627, doi: 10.1001/archpsyc.62.6.617, indexed in Pubmed: 15939839.
8. WHO fact sheet on infertility. Global Reproductive Health. 2021; 6(1): e52–e52, doi: 10.1097/grh.0000000000000052.
9. Hock RS, Or F, Kolappa K, et al. A new resolution for global mental health. Lancet. 2012; 379(9824): 1367–1368, doi: 10.1016/S0140-6736(12)60243-8, indexed in Pubmed: 22500865.
10. Smith MV, et al. Anxiety in pregnancy and postpartum: course, predictors and co-occurrence with postpartum depression. Acta Obstet Gynecol Scand. 2021; 100(5): 852–859.
11. Gavin NI, Gaynes BN, Lohr KN, et al. Perinatal depression: a systematic review of prevalence and incidence. Obstet Gynecol. 2005; 106(5 Pt 1): 1071–1083, doi: 10.1097/01.AOG.0000183597.31630.db, indexed in Pubmed: 16260528.
12. Legazpi PC, Rodríguez-Muñoz MF, Olivares-Crespo ME, et al. Review of suicidal ideation during pregnancy: risk factors, prevalence, assessment instruments and consequences. Psicol Reflex Crit. 2022; 35(1): 13, doi: 10.1186/s41155-022-00220-4, indexed in Pubmed: 35606474.
13. Chin K, Wendt A, Bennett IM, et al. Suicide and Maternal Mortality. Curr Psychiatry Rep. 2022; 24(4): 239–275, doi: 10.1007/s11920-022-01334-3, indexed in Pubmed: 35366195.
14. Côté-Corriveau G, Paradis G, Luu TM, et al. Longitudinal risk of maternal hospitalization for mental illness following preterm birth. BMC Med. 2022; 20(1): 447, doi: 10.1186/s12916-022-02659-9, indexed in Pubmed: 36397055.
15. Brown CC, Kuhn S, Stringfellow K, et al. Association Between Mental Health Conditions at the Hospitalization for Birth and Postpartum Hospital Readmission. J Womens Health (Larchmt). 2023; 32(9): 982–991, doi: 10.1089/jwh.2022.0481, indexed in Pubmed: 37327368.
16. Kumar A, Rao A, O'Rourke K, et al. Relationship Between Depression and/or Anxiety and Hospital Readmission Among Women After Childbirth. J Obstet Gynecol Neonatal Nurs. 2019; 48(5): 552–562, doi: 10.1016/j.jogn.2019.07.001, indexed in Pubmed: 31356766.
17. Wenzel A, Haugen E, Jackson L, et al. Anxiety symptoms and disorders at eight weeks postpartum. J Anx Disord. 2005; 19(3): 295–311, doi: 10.1016/j.janxdis.2004.04.001.
18. Biaggi A, Conroy S, Pawlby S, et al. Identifying the women at risk of antenatal anxiety and depression: A systematic review. J Affect Disord. 2016; 191: 62–77, doi: 10.1016/j.jad.2015.11.014, indexed in Pubmed: 26650969.
19. Thompson, R. . The interplay between maternal and infant health: Implications for anxiety and stress. J Prenatal Psychol. 2021; 35(3): 210–220.
20. Fawcett EJ, Fairbrother N, Cox ML, et al. The Prevalence of Anxiety Disorders During Pregnancy and the Postpartum Period: A Multivariate Bayesian Meta-Analysis. J Clin Psychiatry. 2019; 80(4), doi: 10.4088/JCP.18r12527, indexed in Pubmed: 31347796.
21. Leach L, Poyser C, Fairweather-Schmidt K. Maternal perinatal anxiety: A review of prevalence and correlates. Clinical Psychologist. 2020; 21(1): 4–19, doi: 10.1111/cp.12058.
22. Bayrampour H, Heaman M, Duncan KA, et al. Advanced maternal age and risk perception: a qualitative study. BMC Pregnancy Childbirth. 2012; 12: 100, doi: 10.1186/1471-2393-12-100, indexed in Pubmed: 22988825.
23. Ramalingappa P, Gowda R, Srinivasamurthy S. A study of factors associated with tokophobia and its effect on pregnancy. Internat J Reproduct, Contracept, Obstet Gynecol. 2022; 11(3): 798, doi: 10.18203/2320-1770.ijrcog20220559.
24. Rubertsson C, Hellström J, Cross M, et al. Anxiety in early pregnancy: prevalence and contributing factors. Arch Womens Ment Health. 2014; 17(3): 221–228, doi: 10.1007/s00737-013-0409-0, indexed in Pubmed: 24442712.
25. Dunkel Schetter C, Tanner L. Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. Curr Opin Psychiatry. 2012; 25(2): 141–148, doi: 10.1097/YCO.0b013e3283503680, indexed in Pubmed: 22262028.
26. Radoš SN. Anxiety During Pregnancy and Postpartum: Course, Predictors and Comorbidity with Postpartum Depression. Acta Clinica Croatica. 2018; 57(1): 39–51, doi: 10.20471/acc.2018.57.01.05.
27. Thompson, R. The interplay between maternal and infant health: Implications for anxiety and stress. J Prenatal Psychol. 2021; 35(3): 210–220.
28. Kang J. A Study on Fear and Anxiety of Pregnant Women.J Nurs Academ Soc. 1980; 10(2): 1, doi: 10.4040/jnas.1980.10.2.1.
29. Comprehensive Review of Management of Hypertension in Pregnancy. Obstetrics & Gynecology: Open Access. 2022; 6(1), doi: 10.29011/2577-2236.100148.
30. Fawcett EJ, Fairbrother N, Cox ML, et al. The Prevalence of Anxiety Disorders During Pregnancy and the Postpartum Period: A Multivariate Bayesian Meta-Analysis. J Clin Psychiatry. 2019; 80(4), doi: 10.4088/JCP.18r12527, indexed in Pubmed: 31347796.
31. Bennett HA, Einarson A, Taddio A, et al. Prevalence of depression during pregnancy: systematic review. Obstet Gynecol. 2004; 103(4): 698–709, doi: 10.1097/01.AOG.0000116689.75396.5f, indexed in Pubmed: 15051562.
32. Dave D, Yang M. Maternal and Fetal Health Effects of Working during Pregnancy. 2019, doi: 10.3386/w26343.
33. Rini CK, Dunkel-Schetter C, Wadhwa PD, et al. Psychological adaptation and birth outcomes: the role of personal resources, stress, and sociocultural context in pregnancy. Health Psychol. 1999; 18(4): 333–345, doi: 10.1037//0278-6133.18.4.333, indexed in Pubmed: 10431934.
34. Podolska MZ, Majkowicz M, Sipak-Szmigiel O, et al. Personality profiles in pregnant and postpartum women with symptoms of perinatal depression: differences in self-image in the area of psychological needs. Ginekol Pol. 2009; 80(5): 343–34, indexed in Pubmed: 19548453.
35. Kossakowska-Petrycka K, Walecka-Matyja K. [Psychological causative factors in postpartum depression amongst women with normal and high-risk pregnancies]. Ginekol Pol. 2007; 78(7): 544–548, indexed in Pubmed: 17915411.
36. Podolska MZ, Majkowicz M, Sipak-Szmigiel O, et al. [Ways of coping in stressful situations and anxiety-state or anxiety-trait among women with symptoms of perinatal depression]. Ginekol Pol. 2009; 80(3): 201–206, indexed in Pubmed: 19382612.
37. Libera A, Leszczyńska-Gorzelak B, Oleszczuk J. [Applicability of the Polish equivalent of Neonatal Unit Parental Stress Scale [Skala Stresu Rodziców: OItn; SSR: OITN] in clinical diagnosis of women after preterm delivery]. Ginekol Pol. 2013; 84(4): 281–285, indexed in Pubmed: 23700861.

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Severe novel coronavirus infection in late pregnancy: a case report

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ABSTRACT

This study reported the diagnosis, treatment and perinatal outcome of a novel coronavirus infection patient at 29^{+6} weeks pregnancy. The patient case came to the hospital with persistent fever and cough for 6 days. Patient's chest CT diagnosis showed double pneumonia, and viral infection was considered. Blood gas analysis revealed type I respiratory failure, and a throat swab nucleic acid test confirmed the novel coronavirus infection (critical type). After 13 days of isolation and supportive treatment, the patient recovered and was discharged from hospital after two consecutive negative nucleic acid tests. After discharge, the patient delivered a baby girl successfully by cesarean section on March 16, 2023. The newborn weighing 2050 g, with an Apgar score of 9–10 points/1–5 minutes. The newborn was transferred to the neonatology department for hospitalization and discharged 10 days later. The patient and her baby were followed up for nearly 1 year. Both mother and daughter were in good health.

Keywords: severe type of novel coronavirus infection; pregnancy; virus; pneumonia; infant; follow-up visit

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CASE DATA

Basic data

Patient case: female, 32 years old, company staff. The patient presented to the hospital on January 22, 2023 with persistent fever and cough.

Six days before admission, the patient had persistent fever without obvious cause, and the highest temperature of the patient reached 39°C. The patient's obvious symptoms are cough, sputum, dry cough, chest tightness, choking, obvious after activity, cannot sleep at night, with fatigue and muscle pain. The patient had no abdominal pain, diarrhea, chills, chest pain, hemoptysis, palpitation, or edema of both lower limbs. When patients take "acetaminophen tablets" treatment, body temperature can be reduced to normal, but normal body temperature can only be maintained for 4 hours, and then the fever may return. The patient was admitted to Weifang Maternal and Child Health Hospital on January 22, 2023. Chest CT plain scan in hospital showed double pneumonia, self-tested positive for novel coronavirus antigen, and then took "Nirmatrelvir (150 mg, 2 tablets)/ /Ritonavir (100 mg, 1 tablets)" for treatment. The patient underwent further treatment in the emergency department of our hospital and the blood oxygen saturation was measured at 89%, and the blood gas analysis results show that PH 7.49 PCO_2 28 mm Hg, PO_2 58 mm Hg% FiO_2 21%. Emergency Department diagnosed "severe pneumonia and type I respiratory failure" and admitted the patient to the Respiratory and Critical Care Medicine Department.

Previous history: previous good health

Basic inspection: The test results show that body temperature 36.5°C, pulse 116 times/min, respiration 16 times/min, blood pressure 118/59 mm Hg. The patient had clear consciousness, breathless appearance, normal thorax, quiet sound on percussion, low respiratory sound on auscultation, audible moist rales on both lungs. Further examination showed that the heart boundary of the patient was not large, the heart rate was 116 beats/min, regular heart rhythm, the sound of the heart beat was low and dull, and no abnormal murmurs were heard in each valve area, and there was no edema in both lower limbs, and bilateral pathological signs were negative.

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Medical pathological examination

January 22, 2023: the novel coronavirus nucleic acid was positive.

January 30, 2023 and February 1, 2023: the nucleic acid of the novel coronavirus was negative.

January 22, 2023: D dimer, 1.77 μg/mL.

January 22, 2023: Blood routine, blood images, and indicators of fast C-reactive protein. The blood routine examination results are shown in Table 1; C-reactive protein results showed 91.7 mg/L, exceeding the normal index; the results of blood imaging showed that the leukocytosis under the microscope was mainly classified as neutral granulation.

January 22, 2023 to January 28, 2023: Interleukin-6 tests were performed daily and the results were shown in Table 2.

January 23, 2023: Erythrocyte sedimentation rate index: 65 mm/h.

January 23, 2023: Electrocardiogram showed sinus tachycardia.

January 31, 2023: Chest orthograph bedside photography, imaging results combined with the doctor's diagnosis to consider double pneumonia (Fig. 1).

Admission diagnosis

Combined with the results of various auxiliary examinations, the patient was diagnosed as: Severe pneumonia; Novel coronavirus infection (critical type); Viral pneumonia; Bacterial pneumonia; Type I respiratory failure. In addition, the patient was 29^{+6} weeks pregnant.

Hospitalization

Routine treatment: The patient was admitted to Respiratory intensive care unit (RICU) in our hospital on January 22, 2023, and given patients continuous Electrocardiograph (ECG) oximetry monitoring and high flow humidifying oxygen therapy.

Drug treatment: The specific drug treatment methods (drug dosage and duration) are shown in Table 3.

Treatment outcome

The patient's clinical symptoms gradually improved one week after admission, and the oxygen concentration and oxygen flow rate of the patient's high-flow oxygen intake decreased from 60% oxygen concentration and 40 L/min at admission to 37% oxygen concentration and 30 L/min. At the same time, the oxygen and index of the patient gradually increased after hospitalization (Fig. 2).

Imaging examination showed that the patient's bedside chest radiograph and chest CT gradually improved absorption of double pneumonia (Fig. 3).

Follow-up visit

The patient was discharged from the hospital on February 4, 2023, with no obvious chest tightness, breath-holding, cough or sputum, and two consecutive nucleic acid tests were negative. Then a patient case delivered a baby girl by cesarean section on March 16, 2023. The baby girl weighed 2050 g with an Apgar score of 9–10 points /1–5 minutes. After delivery, the baby girl was transferred to the neonatal department for observation and treatment for 10 days before discharge. At present, the telephone follow-up for nearly one year, the prognosis of both the baby girl and her mother are well.

DISCUSSION

Susceptibility of pregnant women to novel coronavirus

Pregnant women are more susceptible to various viruses infection due to weakened immune function, changes in physiological adaptability and increased physical burden [1–3] . For example, in pregnant women, the diaphragm rises, the

Table 1. Blood routine examination

No.	Project Name	Result	Units
1	Leukocyte Count	13.02	10^9/L
2	Lymphocyte ratio	3.7	%
3	Monocyte ratio	1.3	%
4	Neutrophil ratio	95	%
5	Eosinophilic cell ratio	0.0	%
6	Neutrophil ratio	12.37	10^9/L
7	Lymphocyte absolute value	0.48	10^9/L
8	Eosinophils absolute value	0.0	10^9/L
9	Erythrocyte count	3.53	10^{12}/L
10	Hemoglobin	113	g/L
11	Hematokrit	33.7	%

Table 2. Interleukin-6 test results

No	Date	Result	Units
1	January 22, 2023	203.4	pg/mL
2	January 23, 2023	66.46	pg/mL
3	January 24, 2023	28.69	pg/mL
4	January 25, 2023	17.05	pg/mL
5	January 27, 2023	16.49	pg/mL
6	January 28, 2023	54.85	pg/mL
7	January 29, 2023	39.42	pg/mL
8	January 31, 2023	24.63	pg/mL

Table 3. Drug therapy

Date (2023)	Medicine	Medicine classification	Medicine dosage	Continuous days
January 22	Cefotaxime	Anti-infection	3.0 g, twice/day	13
January 22	Nirmatrelvir/Ritonavir	Antiviral	150 mg, 2 tablets/100 mg, 2 tablets, twice/day	5
January 22, January 26–February 4	Methylprednisolone sodium succinate	Hormone	40 mg, once/day	10
January 23–January 24	Dexamethasone	Hormone	5 mg, once/12 h	2
January 22	Budesonide	Nebulization	2 mg, once/12 h	13
January 22	Low molecular heparin	Anti-freezing	4250 IU, once/day	13
January 23	Tocilizumab		400 mg, once/day	1
January 24	Human serum albumin		20 g, once/day	10
January 24	Gamma globulin		5 g, once/day	10

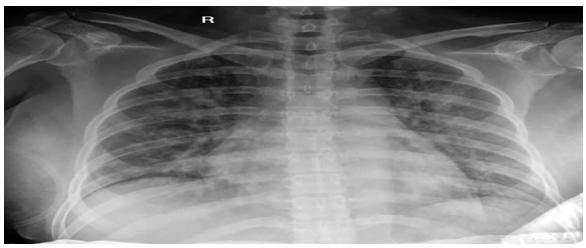

Figure 1. Bedside photography of patient's chest in front position

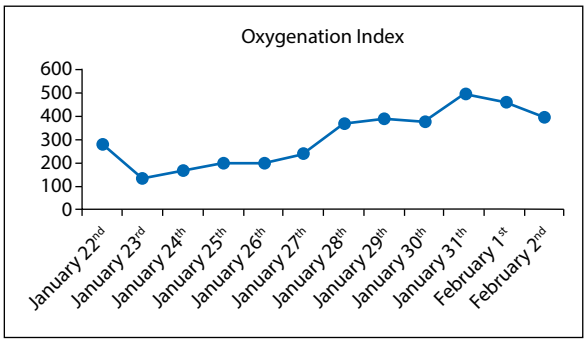

Figure 2. Blood oxygen indexes after hospitalization

respiratory mucosa becomes congested and edema, and the oxygen consumption increased, which not only affects the respiratory movement of the pregnant women's lungs, but also makes them more tolerant to hypoxia, making them more susceptible to respiratory pathogens and developing into severe pneumonia [4–7]. Therefore, pregnant women may have an increased chance of developing severe infections compared to non-pregnant women of the same age. In addition, due to psychological factors of pregnant women [8], such as concerns about mother-to-child transmission, limited drug treatment during pregnancy or the effects of drugs on the fetus [9], pregnant women often do not receive timely treatment after illness. At present, it is generally believed that pregnancy may aggravate the clinical course of novel coronavirus infection and may also lead to aggravation of the disease [10–12]. Also, a study suggests that pregnant women with COVID-19 are at higher risk of complications during pregnancy and their newborns are more likely to be sent to the neonatal intensive care unit (NICU) and born prematurely [13].

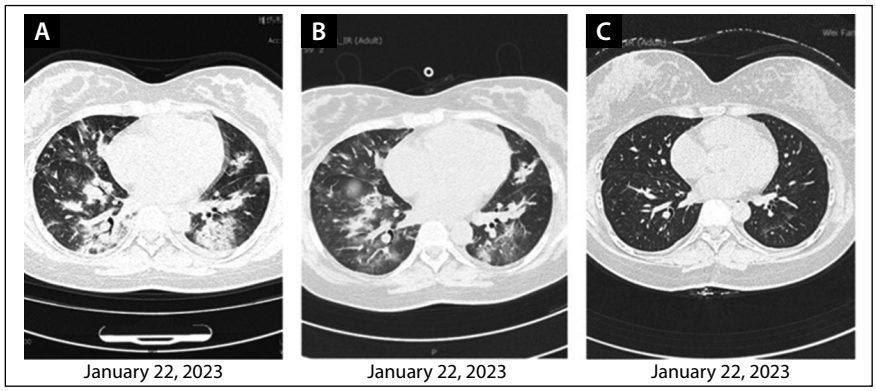

Figure 3. Chest CT of patient on different dates

Identification of severe disease in pregnancy with novel coronavirus infection

Because of women during pregnancy are generally younger, and most of them do not have underlying diseases, and their conditions are relatively stable, there are fewer severe patients at this stage, and critical cases are rare. Therefore, when we receive pregnant patients, we must accurately identify and actively deal with the disease of patients. Pregnant patients in the third trimester may have the following symptoms, examples include hypoxemia, progressive exacerbation of respiratory distress, deterioration of tissue oxygenation indicators (oxygen saturation, oxygenation index), progressive increase of lactic acid, progressive decrease of peripheral blood lymphocyte count, and progressive increase of inflammatory factors such as interleukin-6, C-reactive protein, and ferritin [14–16]. If a patient develops the above symptoms, medical workers should be alert to the possibility of deterioration and progression to severe disease [17].

Chest low-dose X-ray and CT examination are important bases for evaluating lung injury in patients infected with COVID-19 [18, 19]. Under perfect radiological protection conditions, low dose X-ray or CT examination of the chest can be performed in the second and third trimester of pregnancy. A study has shown that in the third trimester of pregnancy complicated with COVID-19, timely termination is recommended for patients with term or rapid progression of lung lesions, and termination has a good outcome for the mother and child [20, 21].

Pregnancy with novel coronavirus infection medication precautions

However, the safety and effectiveness of some drugs for mother and child has not been proven, thus the risk should be fully informed when using to ensure that it will not affect the health of the mother and the baby. At present, numerous studies and clinical data show that antiviral drugs can effectively reduce the risk of infected patients with mild to moderate COVID-19 evolving into severely infected patients [22, 23]. The National Health Commission of China recommended oral antiviral drugs including Nirmatrelvir /Ritonavir combination package, Azvudine and Molnupiravir in the Diagnosis and Treatment Protocol for Novel Coronavirus Infection [24]. However, the main cause of death in patients with severe COVID-19 pneumonia is multiple complications caused by lung damage [25, 26]. In addition, interleukin-6 receptor antagonist monoclonal therapy with tocilizumab (TCZ) has shown potential therapeutic efficacy in patients with COVID-19 [27]. However, currently available data on TCZ in pregnant women are limited and insufficient to determine whether there is a drug-related risk of major birth defects and miscarriage. Therefore, for pregnant women with novel coronavirus infection, the use of TCZ should be carefully considered, and the situation of the pregnant women and the fetus should be closely monitored.

Attention to the mental health of women during pregnancy

During the treatment of this patient, we found that pregnant women infected with the novel coronavirus may have psychological problems, such as insomnia, anxiety (including panic attacks), depression, hypochondria, compulsion, *etc*. [28, 29]. Thus, screening and intervention of maternal psychological symptoms should be standardized. For high-risk pregnant women with a history of depression or anxiety and a family history of mental illness, obstetricians can cooperate with psychologists to provide online or offline pregnancy education to pregnant women, introducing pregnancy-related knowledge, examination contents at different gestational weeks, and precautions during childbirth. According to the specific conditions of the pregnant women, timely multi-disciplinary hierarchical diagnosis and treatment should be carried out in psychological specialist clinics and obstetric outpatient clinics. Pregnant women with mild psychological disorders, non-drug intervention should be given in time in combination with gynecological and psychological specialists. Pregnant women with moderate to severe psychological disorders can be hospitalized in specialized wards and specialized hospitals, and the psychological diagnosis and treatment mode of multidisciplinary teams can be initiated, and non-drug intervention and/or drug intervention comprehensive treatment can be given [30, 31]. Drug intervention should refer to the classification of psychiatric drugs in pregnancy and follow the principle of individuation [32].

In summary, the clinical characteristics and prognosis of pregnant women with novel coronavirus infection may not be worse than that of the general population. And in most cases, maternal and fetal and neonatal outcomes observed in the third trimester appear to be favorable. However, the pathogenesis of novel coronavirus infection and its long-term effects on mother and their infants still need further study. It is hoped that the diagnosis and treatment experience of this case can provide reference for the subsequent diagnosis and treatment of related cases.

Article information and declarations

Author contributions

JL carried out the research design and conception; ZY analyzed and interpreted the data regarding; BW performed the examination of sample; NY and BW contributed essential reagents or tools; JZ and ZY authors wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics statement

The experimental procedures were all in accordance with the guideline of the Ethics Committee of Weifang People's Hospital and has approved by the Ethics Committee of Weifang People's Hospital. This study complies with the Declaration of Helsinki.

A signed written informed consent was obtained from each patient.

Availability of data and materials

The data used and analyzed can be obtained from the corresponding author under a reasonable request.

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Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

1. Röbl-Mathieu M, Kunstein A, Liese J, et al. Vaccination in Pregnancy. Dtsch Arztebl Int. 2021; 118(15): 262–268, doi: 10.3238/arztebl.m2021.0020, indexed in Pubmed: 34114547.
2. Albrecht M, Arck PC. Vertically Transferred Immunity in Neonates: Mothers, Mechanisms and Mediators. Front Immunol. 2020; 11: 555, doi: 10.3389/fimmu.2020.00555, indexed in Pubmed: 32296443.
3. Khan L. Immunization Considerations in Pregnancy. Pediatr Ann. 2019; 48(7): e251–e254, doi: 10.3928/19382359-20190611-01, indexed in Pubmed: 31305940.
4. Lim WS, Macfarlane JT, Colthorpe CL, et al. Pneumonia and pregnancy. Thorax. 2001; 56(5): 398–405, doi: 10.1136/thorax.56.5.398, indexed in Pubmed: 11312410.
5. Goodnight WH, Soper DE. Pneumonia in pregnancy. Crit Care Med. 2005; 33(10 Suppl): S390–S397, doi: 10.1097/01.ccm.0000182483.24836.66, indexed in Pubmed: 16215363.
6. Goodrum LA. Pneumonia in pregnancy. Semin Perinatol. 1997; 21(4): 276–283, doi: 10.1016/s0146-0005(97)80070-5, indexed in Pubmed: 9298716.
7. Laibl VR, Sheffield JS. Influenza and pneumonia in pregnancy. Clin Perinatol. 2005; 32(3): 727–738, doi: 10.1016/j.clp.2005.04.009, indexed in Pubmed: 16085029.
8. Pearlstein T. Depression during Pregnancy. Best Pract Res Clin Obstet Gynaecol. 2015; 29(5): 754–764, doi: 10.1016/j.bpobgyn.2015.04.004, indexed in Pubmed: 25976080.
9. Van den Bergh BRH, van den Heuvel MI, Lahti M, et al. Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. Neurosci Biobehav Rev. 2020; 117: 26–64, doi: 10.1016/j.neubiorev.2017.07.003, indexed in Pubmed: 28757456.
10. Collier ARY, McMahan K, Yu J, et al. Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women. JAMA. 2021; 325(23): 2370–2380, doi: 10.1001/jama.2021.7563, indexed in Pubmed: 33983379.
11. Nunes MC, Madhi SA. COVID-19 vaccines in pregnancy. Trends Mol Med. 2022; 28(8): 662–680, doi: 10.1016/j.molmed.2022.04.012, indexed in Pubmed: 35610123.
12. Wainstock T, Yoles I, Sergienko R, et al. Prenatal maternal COVID-19 vaccination and pregnancy outcomes. Vaccine. 2021; 39(41): 6037–6040, doi: 10.1016/j.vaccine.2021.09.012, indexed in Pubmed: 34531079.
13. Moro F, Buonsenso D, Moruzzi MC, et al. How to perform lung ultrasound in pregnant women with suspected COVID-19. Ultrasound Obstet Gynecol. 2020; 55(5): 593–598, doi: 10.1002/uog.22028, indexed in Pubmed: 32207208.
14. Safiabadi Tali SH, LeBlanc JJ, Sadiq Z, et al. Tools and Techniques for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)/COVID-19 Detection. Clin Microbiol Rev. 2021; 34(3), doi: 10.1128/CMR.00228-20, indexed in Pubmed: 33980687.
15. Habas K, Nganwuchu C, Shahzad F, et al. Resolution of coronavirus disease 2019 (COVID-19). Expert Rev Anti Infect Ther. 2020; 18(12): 1201–1211, doi: 10.1080/14787210.2020.1797487, indexed in Pubmed: 32749914.
16. Majumder J, Minko T. Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19. AAPS J. 2021; 23(1): 14, doi: 10.1208/s12248-020-00532-2, indexed in Pubmed: 33400058.
17. Zambrano LD, Ellington S, Strid P, et al. CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020; 69(44): 1641–1647, doi: 10.15585/mmwr.mm6944e3, indexed in Pubmed: 33151921.
18. Filchakova O, Dossym D, Ilyas A, et al. Review of COVID-19 testing and diagnostic methods. Talanta. 2022; 244: 123409, doi: 10.1016/j.talanta.2022.123409, indexed in Pubmed: 35390680.
19. Erenler AK, Baydin A. Challenges in COVID-19 diagnosis. Bratisl Lek Listy. 2020; 121(12): 864, doi: 10.4149/BLL_2020_142, indexed in Pubmed: 33300355.
20. Juan J, Gil MM, Rong Z, et al. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. Ultrasound Obstet Gynecol. 2020; 56(1): 15–27, doi: 10.1002/uog.22088, indexed in Pubmed: 32430957.
21. Vidal MJ, Martínez-Solanas È, Mendoza S, et al. Impact of SARS-CoV-2 infection in pregnant women and their babies: clinical and epidemiological features. Gac Sanit. 2023; 37: 102332, doi: 10.1016/j.gaceta.2023.102332, indexed in Pubmed: 38007961.
22. von Delft A, Hall MD, Kwong AD, et al. Accelerating antiviral drug discovery: lessons from COVID-19. Nat Rev Drug Discov. 2023; 22(7): 585–603, doi: 10.1038/s41573-023-00692-8, indexed in Pubmed: 37173515.
23. Yuan Y, Jiao B, Qu L, et al. The development of COVID-19 treatment. Front Immunol. 2023; 14: 1125246, doi: 10.3389/fimmu.2023.1125246, indexed in Pubmed: 36776881.
24. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). Chin Med J (Engl). 2020; 133(9): 1087–1095, doi: 10.1097/CM9.0000000000000819, indexed in Pubmed: 32358325.
25. Jum'ah H, Kundrapu S, Jabri A, et al. Cardiac macrophage density in Covid-19 infection: relationship to myocyte necrosis and acute lung injury. Cardiovasc Pathol. 2022; 60: 107447, doi: 10.1016/j.carpath.2022.107447, indexed in Pubmed: 35718082.
26. Swenson KE, Swenson ER, Swenson KE, et al. The Pathophysiology and Dangers of Silent Hypoxemia in COVID-19 Lung Injury. Ann Am Thorac Soc. 2021; 18(7): 1098–1105, doi: 10.1513/AnnalsATS.202011-1376CME, indexed in Pubmed: 33621159.
27. Aabakke AJM, Petersen TG, Wøjdemann K, et al. Risk factors for and pregnancy outcomes after SARS-CoV-2 in pregnancy according to disease severity: A nationwide cohort study with validation of the SARS-CoV-2 diagnosis. Acta Obstet Gynecol Scand. 2023; 102(3): 282–293, doi: 10.1111/aogs.14512, indexed in Pubmed: 36695168.
28. Verma K, Prasad DN, Kumar B, et al. Brain and COVID-19 Crosstalk: Pathophysiological and Psychological Manifestations. ACS Chem Neurosci. 2020; 11(20): 3194–3203, doi: 10.1021/acschemneuro.0c00446, indexed in Pubmed: 33006881.
29. Masood S. What We Do Not Know about COVID-19. Prepr. 2020, doi: 10.20944/preprints202005.0045.v1.
30. Kołomańska D, Zarawski M, Mazur-Bialy A. Physical Activity and Depressive Disorders in Pregnant Women-A Systematic Review. Medicina (Kaunas). 2019; 55(5), doi: 10.3390/medicina55050212, indexed in Pubmed: 31130705.
31. Keskin DD, Keskin S, Bostan S. Mental disorders among pregnant women during the COVID-19 pandemic. A cross-sectional study. Sao Paulo Med J. 2022; 140(1): 87–93, doi: 10.1590/1516-3180.2021.0356.27052021, indexed in Pubmed: 34468634.
32. Gimbel LA, Allshouse AA, Neff D, et al. Mental health symptom changes in pregnant individuals across the COVID-19 pandemic: a prospective longitudinal study. BMC Pregnancy Childbirth. 2022; 22(1): 897, doi: 10.1186/s12884-022-05144-6, indexed in Pubmed: 36463120.

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Uterine artery embolization for arteriovenous malformation of the cervix

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INTRODUCTION

In women of reproductive age, vaginal bleeding is common but may indicate serious underlying pathologies, such as cervical arteriovenous malformation (AVM). AVM, defined by abnormal connections between arterial and venous systems in the cervix, can lead to severe hemorrhage, anemia, hemodynamic instability, and even life-threatening outcomes. According to the International Society for the Study of Vascular Diseases (ISSVA) 2018 classification, vascular malformations are categorized into simple and mixed types, including capillary, venous, lymphatic, and arteriovenous subtypes [1]. While these abnormalities can occur in various anatomical sites, pelvic AVMs are rare, with fewer than 150 reported uterine cases from 1926 to 2023 [2]. Uterine AVMs are either congenital or acquired, and the latter are often associated with gynecological interventions like delivery, cesarean section, gestational trophoblastic disease, diagnostic curettage, placement of intrauterine devices, or specific infections [2, 3].

This article presents a case of a 27-year-old woman with recurrent, heavy vaginal bleeding due to AVM, located in the cervix without prior risk factors. Considering her medical history, symptoms, and imaging findings, a congenital uterine AVM was likely. We detail her diagnosis and successful treatment with uterine artery embolization (UAE), which preserved her fertility, and discuss the causes, symptoms, diagnosis, and treatment options for AVM, as well as the advantages and disadvantages of UAE.

CASE PRESENTATION

Basic information

A 27-year-old woman with no history of abortion, delivery, or pelvic surgery was admitted repeatedly for severe vaginal bleeding episodes, often with shock symptoms (as depicted in Table 1). Initial ultrasounds showed no abnormal blood flow. After a fourth bleeding episode, a congenital vascular malformation was suspected. During the preparation for oocyte cryopreservation, an arteriogram revealed tortuous uterine arteries, and immediate bilateral UAE was performed.

Treatment

Under local anesthesia and sedation, a catheter was inserted through the femoral artery to the uterine arteries, where metal coils were placed to block blood flow to the AVM. The procedure was successful, with no complications.

Prognosis and follow-up

Nine days post-procedure, the patient was discharged without signs of abnormal vaginal bleeding. Her oocytes were cryopreserved to protect fertility. During follow-up, no abnormal bleeding occurred, and she successfully conceived.

DISCUSSION AND CONCLUSION

The patient was admitted after her third episode of vaginal bleeding. Urgent pelvic Doppler ultrasound showed no abnormal blood flow. Suspecting abnormal uterine bleeding due to anovulation or endometrial irregularity,

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we performed a curettage, which temporarily stopped the bleeding. However, another episode followed, highlighting the severity of her condition. Despite normal coagulation tests and pathology results, GnRH-a treatment was ineffective. Consequently, we investigated further and ultimately suspected cervical vascular malformations.

Vascular malformations, known for their progressive enlargement and potential for life-threatening hemorrhage, are complex to diagnose and treat. Under the PALM-COEIN classification, uterine AVMs are categorized as AUB-N [4], often presenting with sudden heavy bleeding. Doppler ultrasound is the preferred diagnostic tool, while MRI and CT serve as adjuncts for comprehensive assessment [5]. Treatment options include medications, UAE, and hysterectomy. UAE, a minimally invasive approach, effectively controls bleeding while preserving uterine function, offering reduced risks and faster recovery than hysterectomy. Studies show that UAE does not impact ovarian reserves and allows for future conception [6].

This case underscores the importance of considering pelvic vascular malformations in cases of recurrent, sudden vaginal bleeding, particularly when aiming to preserve reproductive function. Cervical artery malformations are seldom discussed in the literature, highlighting the necessity of clinical experience for accurate diagnosis. Furthermore, this case reinforces the value of prompt intervention in safeguarding the reproductive potential of affected women.

Article information and declarations

Ethics statement

The studies involving human participants were reviewed and approved by Affiliated Hospital of Guangdong Medical University, Zhanjiang, China. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

Supervision: Ying Zhang; writing — original draft: Shangao Huang; writing — review & editing: Yueling Wu.

Conflict of interest

The authors declare that they have no competing interests.

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Supplementary material

Table S1, Figures S1–S3.

REFERENCES

1. Dimassi K, Ben Amor A, Halouani A, et al. Successful management of acquired uterine arteriovenous malformation. Tunis Med. 2018; 96(7): 445–447, indexed in Pubmed: 30430490.
2. Hoang VT, Van HA, Trinh CT, et al. Uterine arteriovenous malformation: a pictorial review of diagnosis and management. J Endovasc Ther. 2021; 28(5): 659–675, doi: 10.1177/15266028211025022, indexed in Pubmed: 34142901.
3. Rosen A, Chan WV, Matelski J, et al. Medical treatment of uterine arteriovenous malformation: a systematic review and meta-analysis. Fertil Steril. 2021; 116(4): 1107–1116, doi: 10.1016/j.fertnstert.2021.05.095, indexed in Pubmed: 34130801.
4. Hill S, Shetty MK. Abnormal uterine bleeding in reproductive age women: Role of imaging in the diagnosis and management. Semin Ultrasound CT MR. 2023; 44(6): 511–518, doi: 10.1053/j.sult.2023.10.002, indexed in Pubmed: 37832698.
5. Samadi K, Salazar GM. Role of imaging in the diagnosis of vascular malformations vascular malformations. Cardiovasc Diagn Ther. 2019; 9(Suppl 1): S143–S151, doi: 10.21037/cdt.2018.08.06, indexed in Pubmed: 31559160.
6. El Shamy T, Amer SAK, Mohamed AA, et al. The impact of uterine artery embolization on ovarian reserve: A systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2020; 99(1): 16–23, doi: 10.1111/aogs.13698, indexed in Pubmed: 31370100.

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