POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

Edited since 1922

no **12**/vol **91**/**2020**

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

IF: **0.941,** MNiSW: **40**

GINEKOLOGIA POLSKA 2020, VOL 91, NO 12, PAGES 719–780

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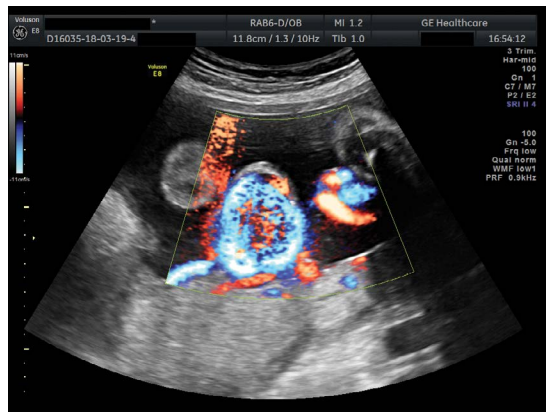

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THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS ISSN 0017–0011

Ginekologia Polska is published monthly, twelve volumes a year, by VM Media sp. z o.o. VM Group sp.k., 73 Świętokrzyska St, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94, fax: (+48 58) 320 94 60, e-mail: redakcja@viamedica.pl, marketing@viamedica.pl, http://www.viamedica.pl

Editorial office address: Woman's Health Institute, School of Health Sciences, Medical University of Silesia in Katowice, 12 Medyków St, 40–752 Katowice, e-mail: ginpol@viamedica.pl

Indexed in: CrossRef, DOAJ, Index Copernicus, Ministry of Science and Higher Education (40), POL-Index, Polish Medical Bibliography, PubMed, Science Citation Index Expanded (0.941), Scimago Journal Rank, Scopus, Ulrich's Periodicals Directory

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19-0202.012.001

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2020, vol. 91, no. 12, 719–725

ISSN 0017–0011

DOI 10.5603/GP.a2020.0135

FokI vitamin D receptor polymorphism as a protective factor in intrahepatic cholestasis of pregnancy

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ABSTRACT

Objectives: Intrahepatic cholestasis in pregnancy (ICP) is a pregnancy-specific liver disorder. Its etiology is not fully understood. Increasing evidence indicates the important role of vitamin D and the vitamin D receptor (VDR) in this disorder. The presence of polymorphic variants in the VDR gene could influence its activity and susceptibility to ICP development. The goal of the study was to investigate the role of four genetic polymorphisms of the VDR gene — *Fok* (rs731236), *Bsm* (rs1544410), *Apa* (rs7975232), and *Taq* (rs731236) — in the etiology of ICP in Polish women.

Material and methods: Ninety-eight women with confirmed ICP and 215 healthy pregnant women as a control group were recruited to the study. We examined four SNPs of the *VDR* gene: *BsmI* (rs7975232), *TaqI* (rs1544410), *ApaI* (rs228570), *FokI* (rs731236). Genotyping was performed using the PCR/RFLP method.

Results: We observed higher frequency (borderline significant) of the Ff-ff genotypes containing at least one mutated allele of the *VDR FokI* polymorphism in the control group compared to the ICP group (p = 0.045, OR = 1.71, 95% CI 1.01–2.88). The frequency of the mutated f allele was slightly higher in controls (49.1%) than in the ICP group (43.4%) (OR = 1.26, 95% CI 0.90–1.77), but the difference was not statistically significant (p = 0.196).

Conclusions: Our results showed that the maternal *VDR FokI* polymorphism could play a protective role in ICP development and probably modulate the risk of ICP occurrence in pregnant women in the Polish population. In the future, to confirm these observations, research in larger, ethnically stratified and clinically analyzed groups is necessary.

Key words: intrahepatic cholestasis in pregnancy; vitamin D receptor; genetic polymorphism

Ginekologia Polska 2020; 91, 12: 719–725

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP), affecting 0.2–2% of pregnancies, is a pregnancy-specific liver disorder that typically presents in the third trimester. It is characterized by pruritus associated with elevated serum bile acids and/or aminotransferase levels. The etiology of ICP is complex and still not fully understood. Evidence suggests that it is caused by a combination of hormonal changes, genetic variations, environmental factors and nutritional deficiencies [1]. Also seasonal variation in the frequency of ICP has been observed with a higher prevalence noted in winter months in Scandinavia, Chile and Portugal [2, 3].

Vitamin D (VD) has many important biologic functions, including mineral balance and skeletal maintenance, control of cell proliferation, regulation of differentiation, inhibition of tumor growth and induction of apoptosis [4, 5]. The expression of more than 2000 genes (3% of the human genome) is regulated by the vitamin D signaling pathway [6, 7].

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Maternal vitamin D deficiency (VDD) during pregnancy is a global concern that may have important implications for offspring metabolic health. There is increasing evidence that vitamin D plays a role in hepatobiliary homeostasis and in various liver diseases, including ICP [8, 9].

The role of vitamin D in fetal development has been demonstrated, especially in early pregnancy, in skeletal development, and the maturity of the immune system [10, 11]. Vitamin D deficiency has been related to a higher incidence of preeclampsia, fetal hypotrophy, gestational diabetes, preterm labor and bacterial vaginosis in pregnant women [12–16]. Some reports indicate a possible relationship between vitamin D and intrahepatic cholestasis in pregnant women [8, 10]. In addition, it has been proven that VDR is expressed in different parts of the uteroplacental unit and performs different functions in physiological pregnancy. It regulates implantation, affects hormone secretion, and modulates the immunological functions of the placenta, especially in early pregnancy [17, 18].

The vitamin D receptor (VDR), which maps on chromosome 12q12-q14, a member of the nuclear hormone receptor superfamily of ligand-inducible transcription factors, is one of the candidate genes of vitamin D deficiency. Polymorphism of the VDR receptor gene may reduce the response to vitamin D and significantly change the expression of the genes regulated by this vitamin [19]. Currently, more than 200 genes modulated by VDR receptors are known, which indicates the pleiotropic effect of vitamin D. The VDR receptor, activated by 1.25-(OH)D, together with the retinoic acid receptor (RXR) forms a heterodimer that acts as a transcription factor. The VDR-RXR heterodimer binds to the promoter of the selected genes, which enables the initiation/inhibition of the transcription process [20]. Increasing evidence shows the role of vitamin D and the VDR receptor in intrahepatic hemostasis, by inhibiting expression of the cholesterol 7alpha-hydroxylase gene (CYP7A1) and therefore modulating the synthesis of bile acids in hepatocytes, thereby protecting liver cells in humans. One of the possible pathways affecting the modulation of VDR activity and in the same way susceptibility to disease occurrence, is the presence of genetic variants in its gene [21, 22].

Therefore, the purpose of this study was to investigate the effect of four genetic polymorphisms of the *VDR* gene — *Fok* (rs731236), *Bsm* (rs1544410), *Apa* (rs7975232), and *Taq* (rs731236) — on the etiology of ICP in Polish women.

MATERIALS AND METHODS

Subjects

A total of 313 women were recruited to the study: 98 women with confirmed ICP and 215 randomly selected healthy pregnant women who comprised the control group. The research was performed in the years 2013–2017, in two medical centers: the Division of Perinatology and Women's Diseases of Poznan University of Medical Sciences in Poznan and the Department of Gynecology and Obstetrics with Gynecological Oncology Subdivision of Regional Hospital in Zielona Góra. The study was approved by the Poznan University of Medical Sciences Bioethics Committee, Poland, and informed consent was obtained from all the participants. All women included in our study were of Polish ancestry.

In the course of the study the detailed demographic profiles and clinical characteristics were collected from all patients. ICP was diagnosed based on clinical and laboratory criteria: characteristic pruritus without rashes and/or increase in serum bile acids (TBA) ≥ 10 µmol/L in fasting state, increase level of alanine aminotransferase (ALT) ≥ 33 IU/L and aspartate aminotransferase (AST) ≥ 32 IU/L. The women with ICP were observed within 2–3 weeks after delivery when the symptoms had resolved. We excluded women with any causes of hepatic impairment, such as infection with hepatitis viruses (HAV, HBV and HCV), autoimmune diseases, excessive alcohol consumption, HIV infection, biliary obstruction, and other liver and dermatological diseases that cause skin itching. We also excluded those with multiple pregnancies, chromosomal abnormalities, fetal anomalies, maternal infections, pregnancies complicated by thyroid disease, hypertension, and diabetes mellitus, from the study groups. Blood for laboratory tests was secured in all women with ICP before initiating treatment.

Genotyping

We examined four SNPs (rs7975232, rs1544410, rs228570, rs731236) in the *VDR* gene, and all of the SNPs had minor allele frequencies (MAF) greater than 5%. Traditionally these allelic variants have been designated by the upper and lower case of the starting initial of the named loci, *e.g. BsmI* (b and B), *TaqI* (t and T), *ApaI* (a and A) and *FokI* (f and F). Genomic DNA was isolated from whole blood collected in K3-EDTA tubes using the Qiagen DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions and was stored at minus 80°C. A NanoDrop 2000 spectrophotometer (Wilmington, DE, USA) was used to evaluate the quality and quantity of DNA. Genotyping was performed in the Molecular Biology Laboratory of Poznan Medical Science University using the PCR/RFLP method as described previously [23–25]. Products were analyzed by electrophoresis on 2.5% agarose gel with Midori Green Advanced DNA Stain (Nippon Genetics, Europe GmbH) (Tab. 1).

Statistical analysis

Statistical analyses were conducted using R version 3.6.0 [26]. For each SNP, the Hardy-Weinberg equilibrium (HWE) was assessed using Pearson's goodness-of-fit χ^2 statis-

Table 1. SNPs genotyped in *VDR* gene

Reference SNP ID	rs2228570	rs1544410	rs7975232	rs731236
Traditional Name	FokI	BsmI	ApaI	TaqI
Localization	Exon 2	Intron 8	Intron 8	Exon 9
Allele (Traditional Name Variant)	C/T (F/f)	G/A (b/B)	A/C (A/a)	T/C (T/t)
Amino Acid	Met-Thr	Non-coding	Non-coding	Ile-Ile

Table 2. Clinical, biochemical, obstetrical and perinatal characteristics of the women with ICP and healthy controls

Parameters at mothers	ICP n = 98	Controls n = 215	p
Maternal age [years]	30.40 ± 4.38	30.66 ± 4.66	0.65
Gestational age at delivery [weeks]	36.87 ± 2.73	39.03 ± 1.23	< 0.0001
Systolic blood pressure [mmHg]	110.13 ± 10.71	108.65 ± 10.23	0.26
Diastolic blood pressure [mmHg]	67.57 ± 8.89	66.42 ± 7.83	0.27
Before pregnancy BMI [kg/m^2]	22.93 ± 4.53	21.89 ± 3.59	0.04
Total bile acids [µmol/L]	20.94 ± 27.05	2.80 ± 2.10	< 0.0001
Aspartate aminotransferase [IU/L] AST	255.51 ± 230.50	N.A.	–
Alanine amino transferase [IU/L] ALT	155.33 ± 142.58	N.A.	–
Placenta weight [g]	585.90 ± 154.55	621.18 ± 112.04	0.04
Caesarean section, n (%)	44 (45.83%)	71 (33.02%)	0.03
Primipara, n (%)	43 (44.79%)	83 (38.60%)	0.31
Parameters at neonates			
Neonate birth weight [g]	3094.09 ± 631.30	3418.37 ± 434.21	< 0.0001
Apgar score at 5 min	9.74 ± 0.74	9.97 ± 0.22	< 0.0001
Baby sex (son), n (%)	49 (51.04%)	125 (58.14%)	0.24

N.A. — not accomplished

tic. Continuous variables were expressed as mean ± standard deviation whereas categorical variables were expressed as numbers or percentages. Significant differences between two groups were analyzed by Student's *t*-test. Differences in allele and genotype frequencies between the case and control subjects, odds ratios (ORs) and associated 95% confidence intervals (95% CIs) were evaluated using the SNPassoc package for R [27]. The data were analyzed using codominant, recessive and dominant inheritance models. Haplotype analysis was performed using the Haplo.stats R package [28]. Two-tailed p values less than 0.05 were accepted to be statistically significant.

RESULTS

Demographic and clinical characteristics of subjects

In our study the mean age of cases and controls was 30.40 ± 4.38 and 30.66 ± 4.66 years, respectively (non-significant). ICP women delivered at 36.87 ± 2.73 weeks, whereas controls delivered at term (39.3 ± 1.23 weeks) ($p < 0.0001$). Significant differences in total bile acids (20.94 ± 27.05 vs 2.80 ± 2.10 µmol/L, $p < 0.0001$), neonate birth weight (3094.09 ± 631.30 g vs 3418.37 ± 434.21 g, $p < 0.0001$), and neonate Apgar score (9.74 ± 0.74 vs 9.97 ± 0.22, $p < 0.0001$) were observed. The demographic and clinical characteristics of ICP patients and controls are shown in Table 2.

Association of *VDR* gene polymorphisms with risk of ICP

We explored the *VDR* gene polymorphisms with risk of ICP under codominant, dominant and recessive model, and there were no significant differences in the codominant model (rs2228570, rs1544410, rs797523 and rs731236, all results show $p > 0.05$).

The most interesting observation was the higher frequency of the Ff-ff genotypes containing at least one mutated allele of the VDR FokI polymorphism in the control group compared to the ICP group with the difference of borderline statistical significance ($p = 0.045$, OR = 1.71, 95% CI 1.01–2.88). The frequency of the mutated f allele was slightly higher in controls (49.1%) than in the ICP group (43.4%) (OR = 1.26, 95% CI 0.90–1.77), but the difference was not statistically significant ($p = 0.196$).

For both *VDR BsmI* and *VDR TaqI* polymorphisms a slightly higher frequency of genotypes containing at least one mutated allele in the ICP group was found, but without statistical significance. There was also no contribution of the *VDR ApaI* polymorphism to ICP etiology. Genotype analysis of the *VDR* polymorphisms did not show any significant deviation from Hardy-Weinberg equilibrium in ICP and control groups. The allele and genotype frequencies in ICP women and healthy controls are presented in Table 3.

Haplotype analyses

For four-locus haplotypes six main variants (fbaT, FBAt, fBAt, fbAT, FbaT and FbAT) were constructed for rs2228570,

Table 3. Allele and genotype frequencies of VDR SNPs in ICP women and controls

		ICP n = 98 (%)	Control n = 215 (%)	OR	95%CI	p
rs2228570 (FokI)	CC (FF)	34 (34.7)	51 (23.7)	1.00		
	CT (Ff)	43 (43.9)	117 (54.4)	1.81	1.04–3.17	0.112
	TT (ff)	21 (21.4)	47 (21.9)	1.49	0.76–2.92	
	CC vs CT–TT	64 (65.3)	164 (76.3)	1.71	1.01–2.88	0.045
	CC–TC vs TT	77 (78.6)	168 (78.1)	1.03	0.57–1.83	0.931
Allele	C (F)	111 (56.6)	219 (50.9)	1.00		0.196
	T (f)	85 (43.4)	211 (49.1)	1.26	0.90–1.77	
rs1544410 (BsmI)	GG (bb)	31 (31.6)	91 (42.3)	1.00		
	GA (bB)	48 (49.0)	90 (41.9)	0.64	0.37–1.09	0.191
	AA (BB)	19 (19.4)	34 (15.8)	0.61	0.30–1.22	
	GG vs GA–AA	67 (68.4)	124 (57.7)	0.63	0.38–1.04	0.070
	GG–GA vs AA	79 (80.6)	181 (84.2)	0.78	0.42–1.45	0.439
Allele	G (b)	110 (56.1)	272(63.3)	1.00		0.094
	A (B)	86 (43.9)	158(36.7)	0.74	0.53–1.05	
rs797523 (ApaI)	AA (AA)	26 (26.5)	50 (23.3)	1.00		
	AC (Aa)	51 (52.0)	115 (53.5)	1.17	0.66–2.09	0.811
	CC (aa)	21 (21.4)	50 (23.3)	1.24	0.62–2.48	
	AA vs AC–CC	72 (73.5)	165 (76.7)	1.19	0.69–2.06	0.533
	AA–AC vs CC	77 (78.6)	165 (76.7)	1.11	0.62–1.98	0.719
Allele	A (A)	103 (52.6)	215 (50.0)	1.00		0.605
	C (a)	93 (47.4)	215 (50.0)	1.11	0.79–1.55	
rs731236 (TaqI)	TT (TT)	32 (32.7)	93 (43.3)	1.00		
	TC (Tt)	54 (55.1)	94 (43.7)	0.60	0.36–1.01	0.150
	CC (tt)	12 (12.2)	28 (13.0)	0.80	0.37–1.76	
	TT vs TC–CC	66 (67.3)	122 (56.7)	0.64	0.39–1.05	0.074
	TT–TC vs CC	86 (87.8)	187 (87.0)	1.07	0.52–2.21	0.848
Allele	T (T)	118 (60.2)	280 (65.1)	1.00		0.245
	C (t)	78 (39.8)	150 (34.9)	0.81	0.57–1.15	

rs1544410, rs797523 and rs731236 polymorphisms of the VDR gene. The most frequent variant in ICP patients was FBAt haplotype (28.5%), whereas the estimated prevalence of this haplotype in controls was only 23.9%. When looking at the three-locus haplotypes (*BsmI*, *ApaI*, and *TaqI*), there were also no apparent associations with ICP. Our results indicate that the most common haplotype for the *VDR* gene is baT (47.4% in ICP women and 49.3% in controls). There were no significant differences in the frequency of investigated haplotype variants between ICP women and healthy controls (Tab. 4).

DISCUSSION

Numerous studies have analyzed the influence of vitamin D disturbed metabolism on pregnancy outcome. Several studies analyzed vitamin D levels in groups of women with ICP.

In the Swedish population, reduced VD levels were observed in women with ICP, regardless of the level of bile acids (22 ICP women, 11 healthy pregnant women). In this study statistically significantly lower VD in serum from the ICP group (76.4 ± 23.1 vs 112.0 ± 40 ng/L in controls, p = 0.0041) was observed [8].

Also in the study performed by Gençosmanoğlu Türkmen et al. [9] (40 pregnant women with ICP and 40 healthy pregnant women) vitamin D levels were significantly lower in women with ICP compared to the controls (8.6 ± 4.9 vs 11.3 ± 6.1 ng/mL in controls, p = 0.033). In addition, the authors observed lower VD levels in serum from patients with the severe form of ICP (6.9 ± 2.1 vs 10.3 ± 6.2 ng/mL in severe ICP, p = 0.029) [9].

Although it is known that etiology of ICP is multifactorial, including the genetic basis of the disease, there is not much

Table 4. Haplotype analysis results among SNPs in VDR locus

Haplotype	ICP (freq)	Control (freq)	OR (95% CI)	p value
FBAt	56 (0.285)	103 (0.239)	1.269 (0.867–1.858)	0.218
FbaT	48 (0.244)	93 (0.216)	1.175 (0.789–1.75)	0.426
fbaT	45 (0.229)	119 (0.276)	0.778 (0.525–1.155)	0.213
fBAt	22 (0.112)	43 (0.100)	1.137 (0.66–1.96)	0.641
fbAT	12 (0.061)	42 (0.097)	0.602 (0.309–1.171)	0.131
FbAT	5 (0.025)	14 (0.032)	0.777 (0.276–2.19)	0.633
baT	93 (0.474)	212 (0.493)	0.928 (0.662–1.302)	0.667
BAt	78 (0.397)	146 (0.339)	1.285 (0.907–1.822)	0.157
bAT	17 (0.086)	56 (0.130)	0.634 (0.358–1.122)	0.115
BAT	8 (0.040)	12 (0.027)	1.482 (0.596–3.686)	0.394

haplotypes with frequency < 0.03 are ignored

research focused on this problem. However, there is research demonstrating the importance of *VDR* genetic variants in etiology of primary biliary cirrhosis (PBC) in different populations. The results of these investigations are inconclusive but suggest the possibility of the involvement of genetic variants in the ICP pathomechanism and their influence on liver dysfunction [29-33]. One of them is a meta-analysis of six studies (672 cases and 1148 total controls) showing that the *VDR ApaI* polymorphism is associated with the risk of PBC especially in Asians, while the *VDR TaqI* polymorphism may affect the risk of PBC in Caucasians. However, no significant association was observed between *VDR BsmI* polymorphism and PBC risk [30].

Another meta-analysis of Li et al. [31] (6 studies, 1322 subjects with PBC and 2264 controls) demonstrated that VDR TaqI (rs731236) polymorphism significantly correlated with the risk of PBC (for allele T vs allele t OR = 0.75, p = 0.001; TT + Tt vs tt OR = 0.62, p = 0.005; OR = 0.74, p = 0.016 for recessive model), while for *VDR ApaI* (rs7975232) and *VDR BsmI* (rs1544410) polymorphisms such correlation was not confirmed [31].

The study of 334 PBC patients (195 Japanese and 139 Italians), and 334 healthy sex- and age-matched subjects (179 Japanese and 156 Italians) showed that the BB genotype of *VDR BsmI* polymorphism was significantly associated with PBC risk (OR = 1.80, p = 0.005), both in the Japanese (OR = 13.77, p = 0.001) and Italian (OR = 1.83, p = 0.019) population, but not significantly in the Italian group after Bonferroni correction. The frequency of the *VDR BsmI* polymorphism B allele also was significantly higher in the PBC group (OR = 1.27, p = 0.040), indicating the importance of both BB genotype and the B allele in PBC development [32].

In contrast, a meta-analysis performed by Mo et al. suggests that the *VDR ApaI*, *BsmI*, and *TaqI* polymorphisms do not correlate with increased risk of PBC (*ApaI*: allele A vs allele a OR = 1.132, p = 0.355; BsmI: allele B vs allele b OR = 1.148, p = 0.589; TaqI: allele t vs allele T OR = 1.1432, p = 0.584). Moreover, also in the subgroups separated by ethnicity no relationship was found between the *VDR ApaI*, *BsmI*, and *TaqI* polymorphisms and the occurrence of PBC for the Caucasian or Asian race [33].

As mentioned above, the involvement of *VDR* polymorphisms in ICP etiology is not fully understood, and to the best of our knowledge, this study is the first analysis of this type performed in the population of Polish women. In our study the most interesting result was the higher frequency of genotypes containing at least one mutated allele in the ICP group compared to controls. The frequency of both Ff and ff genotypes was higher in the control group of healthy women with borderline statistical significance (p = 0.045, with OR = 1.71). This observation indicated the protective role of both Ff and ff genotypes of VDR FokI polymorphism in ICP development. This stimulating observation indicated the direction for future research on the mechanism of ICP to prevent the clinical signs of ICP and its serious consequences for the fetus. Considering the haplotypes analysis we have not observed the significance of VDR gene haplotype settings in the etiology of ICP in Polish women. Interestingly, in our population of Polish women the most common haplotype for the *VDR* gene was baT, followed by BAt and bAT, as has been previously described for Caucasians [34].

A limitation of our study is the relatively small number of patients enrolled in the ICP group. On the other hand, these patients were recruited fulfilling the precise criteria for assignment to the study group including the laboratory and clinical signs of ICP. This number of subjects is not sufficient to draw definitive conclusions indicating a direct relationship between VDR polymorphic variants and ICP occurrence but shows the way for further investigations in ICP etiology. Secondly, VDR polymorphisms are unlikely to be the only variants affecting susceptibility to disease and ICP development. No less important is to identify other genetic markers in order to determine the risk groups of

patients predisposed to the development of ICP, as well as to determine the severity of the disease and the possibility of its progression [35–38]. Finally, it is important to note that the exact pathomechanism of the impact of the presence of individual VDR polymorphic variants on ICP etiology remains unexplained. It is known that ICP etiology is multifactorial, with hormonal, genetic and environmental components. Several studies point to the possible role of vitamin D in regulating steroid metabolism, as well as the importance of VDR polymorphisms for maintaining intrahepatic hemostasis. It seems crucial to identify the full ICP pathomechanism that links the presence of VDR genetic variants and serum vitamin D levels to steroid metabolism, bile acid levels, and ICP prevalence. All these findings together could have important clinical implications for our patients and could improve current knowledge about the genetic determinants of ICP.

CONCLUSIONS

To our knowledge this is the first study that suggests an association between maternal VDR FokI variant (rs2228570) and increased risk for ICP in Polish women. This interesting observation noted in relatively small number of patients merit future research to indicate whether this relationship could modulate the ICP development.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 726–732

ISSN 0017–0011

DOI 10.5603/GP.2020.0119

Comparison of Misoprostol versus Dinoprostone for delivery induction among pregnant women without concomitant disease

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ABSTRACT

Objectives: Induction of labour is a part of an active prenatal care nowadays and the ideal method of that procedure still remains to be identified. The purpose of this study was to evaluate effectiveness of misoprostol vaginal insert as compared to dinoprostone gel for delivery induction in pregnant women without any comorbidities.

Material and methods: It was a retrospective cohort study of 240 pregnant women. The primary study outcome was successful delivery. Other analysed parameters included time to delivery of a baby, time to the beginning of the first stage of labour, time to vaginal delivery, and duration of all delivery stages. We compared both methods regarding maternal complications during and after delivery. We also reviewed neonatal outcomes such as birth weight, birth length and 1-minute Apgar scores.

Results: The patients' basic characteristics were similar regarding their age, gravidity, parity, height, weight and Bishop score. Time to any delivery and to the onset of a labour in the misoprostol group versus in the dinoprostone group was 14.5 vs 35.6 h ($p < 0.001$) and 9.9 h vs 25.3 h ($p < 0.001$) respectively. The chance of the beginning of labour and the baby's delivery over time has been observed to be approximately two times higher for misoprostol as compared to dinoprostone.

Conclusions: Our study showed that using misoprostol vaginal insert in comparison to dinoprostone seems to shorten the time to beginning of the first stage of labour as well as the time to the delivery itself. Some lower Apgar scores observed in the misoprostol group requires further investigation.

Key words: misoprostol; dinoprostone; induction of labor

Ginekologia Polska 2020; 91, 12: 726–732

INTRODUCTION

Finding a safe, quick and effective method of labour induction is a crucial part of a growing need for active prenatal care. Decision on the right moment to complete a pregnancy that would be the best for both the baby and the mother has been always a debatable issue. Modern medicine has developed only a few tools to manage an unfavourable cervix which may be shortly divided into mechanical and pharmacological methods of preinduction. In the final step, induction of delivery involves oxytocin administration which strengthens the uterine contractions leading to childbirth. In current practice, no cervical ripening method appears superior to the others considering their overall effectiveness and safety outcomes [1], which indicates that the ideal method of labour preinduction remains to be identified. Prostaglandins are commonly used in obstetrics nowadays. Dinoprostone is a natural E2 prostaglandin produced by decidua and amnion, it causes relaxation of cervical collagen and develops uterine fibres contractions [2]. Misoprostol is a synthetic analogue of E1 prostaglandin and, although it was originally registered as a drug in the prevention and treatment of stomach ulcer disease [3], nowadays it is widely used in obstetrics also in preinduction of labour [4].

Objectives

The purpose of this study was to evaluate effectiveness and safety of misoprostol vaginal insert at a dose of 0.2 mg (Misodel, Ferring Pharmaceuticals Poland sp. z o.o) as compared to dinoprostone gel at a dose of 0.5 mg (Prepidil, Pfizer Polska Sp. z o.o.), administered in daily clinical practice for delivery induction in pregnant women without any comor-

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bidities. Additionally, we evaluated whether mothers' age affected clinical outcomes.

MATERIAL AND METHODS

It was a retrospective cohort study that included pregnant women requiring labour induction for either medical or obstetric indications, hospitalized at the Obstetric and Perinatology Department at the University Hospital in Cracow between January 2015 and April 2019.

Inclusion criteria were (1) singleton gestation, (2) cephalic foetus presentation, (3) full-term pregnancy, (4) Bishop's score ≤ 4, (5) reactive foetal heart rate (FHR) pattern, and (6) lack of spontaneous uterine contractions before administration of the drug.

Patients were excluded for the following reasons: (1) malpresentation, (3) estimated foetal weight > 4500 g (4) placenta previa or unexplained vaginal bleeding, (5) vasa previa, (6) other known contraindications to vaginal delivery, (7) any contraindication to receiving prostaglandins, (8) previous caesarean delivery or uterine surgery, (9) preterm delivery. Other exclusion criteria included maternal comorbidities such as: hypertension, diabetes mellitus, intrahepatic cholestasis of pregnancy, asthma, and thrombocytopenia.

The primary study outcome was successful vaginal delivery. Other analysed parameters included time from drug administration to vaginal delivery, to any (vaginal or by Caesarean section) delivery, and time to the onset of labour defined as regular uterine contradictions at least every ten minutes with evidence of change in cervical dilatation or cervical effacement, and duration of all delivery stages. We also reviewed neonatal outcomes such as birth weight, birth length and 1-minute Apgar score. Finally, we analysed potential delivery complications like a necessity of emergency Caesarean section, placenta abruption, placenta arrest and a necessity of uterine curettage after vaginal birth, as well as episiotomy and anaemia requiring blood transfusion.

Statistical analysis

The study group consisted of women who received misoprostol, dinoprostone, and both (after dinoprostone was ineffective the misoprostol was used in some women). Thus, there were three groups of mothers. The first part of the analysis, which is intended to compare successful labour inductions after the implementation of one drug only, presents differences between 'misoprostol only' and 'dinoproston only' groups. In order to compare basic characteristics of the study groups for interval scale variables [age, time, weight, body mass index (BMI)], first normal distribution in groups was assessed by Shapiro-Wilk test, and then for normally distributed variables the t-test for equal or unequal variances, and for skewed variables the U-Mann-Whitney test were used to assess significance. Chi-squared Person's test was used for nominal or ordinal scale variables provided that the expected value of at least five was observed in each cell, otherwise the exact Fisher's test was performed. As a next step, to reveal whether there is a difference in the effectiveness between implemented drugs an intention to treat (ITT) approach was implemented — meaning mothers started with misoprostol were considered as the first group, and mothers started with dinoproston as the second group irrespective of whether the mother was given later the other drug for induction purposes or not. The proportional Cox regression model was performed to assess the strength of difference between misoprostol and dinoprostone in the effectiveness of delivery upon time. The calculated hazard ratio shows how many times the delivery is more or less likely in a specified amount of time. Models were created as both unadjusted and adjusted for the clinically important covariates. In addition, it was tested a possible impact of woman's age on the efficacy of the treatment. For that purpose, firstly, a linear regression of the mother's age on the time between drug implementation and the beginning of labour or the delivery of a baby, and additionally the second stage of labour, with the treatment type covariate were performed. Secondly, to check whether the difference between misoprostol and dinoprostone depended on mother's age (the test for a modification effect of the mother's age < 35, and 35+) the interaction terms between categorised mother's age and a group type variable in the ANOVA models were used. All the analyses were done using the IBM SPSS Statistics version 25. Pairwise procedure was used for missing data. Results (differences) were considered statistically significant if the p-value was less than 0.05.

RESULTS

There were 560 pregnant women identified in medical records as admitted to the Obstetric and Perinatology Department at the University Hospital in Cracow, Poland for induction of delivery in the period from January 2015 till April 2019. Out of 560, 320 were excluded due to the presence of any of the co-morbidity mentioned under the exclusion criteria. In the remaining 240 women there were 93 women who received misoprostol and 147 who were given dinoprostone. In the last-mentioned group, however, there were 39 (26.5%) which were observed as not-reacting to the drug, and they received after some time misoprostol additionally. Thus "one drug successful labour induction" groups included 201 pregnant women, out of whom 93 (46.3%) were treated with misoprostol and 108 (53.7%) were given dinoprostone.

The basic characteristics of mothers were similar across study groups regarding their age, gravidity, parity, weight, and BMI at admission. There were no statistically significant differences in pre-ripening cervical characteristics either, or the initial Bishop score in all patients was ≤ 4 (Tab. 1). Consid-

Table 1. Clinical characteristics of the study groups (across "after one drug successful delivery" groups)

	Misoprostol [n = 93]	Dinoprostone [n = 108]	Significance
Maternal age [years] Mean, (SD) Median (Q1–Q3)	 31.1 (4.4)* 31.0 (28.5–34.0)	 30.6 (4.9) 30.0 (28.0–34.0)	$p^{MW} = 0.293$
Weight at admission [kg] Mean, (SD) Median (Q1–Q3)	[n = 47]* 76.8 (10.9) 75.0 (69.0–83.0)	[n = 63] 78.8 (13.1) 77.0 (70.0–86.0)	$p^{MW} = 0.385$
Height [cm] Mean, (SD) Median (Q1–Q3)	[n = 84] 165.7 (5.7) 166.5 (162.0–170.0)	[n = 97] 167.3 (5.9) 168.0 (164.0–171.0)	$p^{t-e} = 0.054$
Body mass index at admission [kg/m^2] Mean, (SD) Median (Q1–Q3)	[n = 46] 28.5 (3.7)* 27.4 (25.9–30.4)	[n = 63] 27.9 (4.3) 27.1 (24.8–30.1)	$p^{MW} = 0.406$
Number of pregnancies [n, (%)]			df = 2 $p^{chi2} = 0.713$
1	52 (55.9%)	66 (61.1%)	
2	20 (21.5%)	22 (20.4%)	
≥ 3	21 (22.6%)	20 (18.5%)	
Parity history (current delivery included) [n (%)]			df = 2 $p^{chi2} = 0.854$
1	64 (68.8%)	77 (71.3%)	
2	18 (19.4%)	21 (19.4%)	
3	11 (11.8%)	10 (9.3%)	
Nulliparous [n, (%)]	62 (66.7%)	76 (70.4%)	df = 1 $p^{chi2} = 0.648$
Miscarriage history [n, (%)]			df = 1 $p^{chi2} = 0.305$
No	70 (75.3%)	88 (81.5%)	
Yes	23 (24.7%)	20 (18.5%)	
Pre–ripening cervical characteristics [n (%)]			
Dilatation ≤ 1 cm	86 (92.5%)	99 (91.7%)	df = 1 $p^{chi2} = 0.999$
Effacement ≤ 50%	85 (91.4%)	101 (93.5%)	df = 1 $p^{chi2} = 0.600$
Gestational age [weeks]# Mean, (SD) Median (Q1–Q3)	 39.7 (1.8)* 40.6 (38.9–40.9)	 40.6 (0.7)* 40.9 (40.5–41.0)	$p^{MW} < 0.001$
Estimated birth weight [g] Mean, (SD) Median (Q1–Q3)	[n = 54] 3325 (552)* 3450 (3000-3748)	[n = 37] 3588 (413) 3682 (3233-3883)	$p^{MW} = 0.027$

*p < 0.05 by the Shapiro-Wilk test for normal distribution; #at a time of administration of the first dose of the drug; MW — the U-Mann-Whitney test; t-e — the Student's t-test for equal variances; chi2 — the chi-squared test, df — degrees of freedom; F — the exact Fisher's test

ering gestational age, it was slightly lower in the misoprostol group as compared to dinoprostone group (medians: 40.6 vs 40.9 weeks respectively, p < 0.001), additionally, estimated birth weight was also lower in the misoprostol group (medians: 3450 vs 3682 g, respectively, p = 0.027). The indications for labour induction did not differ significantly between the two groups, although Rh incompatibility was observed slightly more frequently in the dinoprostone group (4.1% vs 1.1%) and foetal indications (including foetal growth restriction) were noticed more frequently in the misoprostol group (16.1% vs 8.2%). Most inductions were initiated due to prolonged pregnancy exceeding the term date. There was no operative vaginal delivery.

The proportion of mothers who underwent vaginal delivery was comparable between the groups, as it was 68.8% in the misoprostol and 76.9% in the dinoprostone group (p = 0.207). There were also no differences between the rate of Caesarean section or indications for such delivery between the two groups. The most often causes of Caesarean section were foetal distress seen in cardiotocography

Table 2. Mode of delivery and indications for caesarean section across "after one drug successful delivery" groups

	Misoprostol [n = 93]	Dinoprostone [n = 108]	Significance
Cesarean section	29 (31.2%)	25 (23.1%)	df = 1 $P^{chi2} = 0.207$
Emergency Caesarean delivery out of total deliveries	19 (20.4%)	10 (9.3%)	df = 1 $P^{chi2} = 0.028$
Emergency Caesarean delivery out of total Caesarean sections	[n = 29] 19 [65.5%]	[n = 25] 10 [40.0%]	df = 1 $P^{chi2} = 0.100$
Vaginal delivery	64 (68.8%)	83 (76.9%)	df = 1 $P^{chi2} = 0.207$
Indications for Caesarean section	[n = 29]	[n = 25]	$p^F = 0.207$
Foetal distress	18 (62.1%)	10 (40.0%)	
Labor arrest during first stage (First-stage Caesarean)	6 (20.7%)	10 (40.0%)	
Labor arrest during second stage (Second-stage Caesarean)	4 (13.8%)	5 (20.0%)	
Fetal hand prolapse	1 (3.4%)	0 (0.0%)	

chi^2 — the chi-squared test; df — degrees of freedom; F — the exact Fisher's test

Table 3. Postpartum complications among study participants

	Misoprostol [n = 93]	Dinoprostone [n = 108]	Dinoprostone + misoprostol [n = 39]	Significance
Any complication	14 (15.1%)	12 (11.1%)	9 (23.1%)	df = 2 $P^{chi2} = 0.196$
Blood transfusion	2 (2.2%)	2 (1.9%)	2 (5.1%)	$P^F = 0.470$
Uterine hyper-stimulation	2 (2.2%)	0	2 (5.1%)	$P^F = 0.059$
Curettage after delivery	8 (8.6%)	9 (8.3%)	3 (7.7%)	$P^F = 0.999$
Episiotomy	26 (28.0%)	42 (38.9%)	17 (43.6%)	df = 1 $P^{chi2} = 0.137$
Rupture of perineum (any type)	15 (16.1%)	24 (22.2%)	4 (10.3%)	df = 1 $P^{chi2} = 0.224$
Rupture of perineum No rupture I-stage II-stage III-stage	 78 (83.9%) 14 (15.1%) 1 (1.1%) 0	 84 (77.8%) 20 (18.5%) 2 (1.9%) 2 (1.9%)	 35 (89.7%) 3 (7.7%) 0 1 (2.6%)	$P^F = 0.427$

chi^2 — the chi-squared test; df — degrees of freedom; F — the exact Fisher's test

tracing or lack of the labour progress, although there was significantly more often emergency Caesarean delivery out of all deliveries (p = 0.028) in the misoprostol group (Tab. 2). For the analysis of the safety, the three groups, *i.e.*, misoprostol, dinoprostone and dinoprostone followed by misoprostol were considered. Delivery complications were categorized into the following: anaemia with blood transfusion need, uterine hyperstimulation, uterus curettage after delivery, shoulder dystocia or the perineum rupture needing surgical suturing. There were no significant differences in postpartum complications between groups (Tab. 3).

The oxytocin usage to accelerate the contraction activity of the uterine muscle was necessary in eight patients (8.6%) from the misoprostol group, and in 55 patients (37.4%) from dinoprostone group, which reached a statistically significant difference ($p < 0.001$).

The misoprostol use appeared to significantly shorten the time to any delivery compared to dinoprostone gel (medians: 14.5 vs 35.6 h, $p < 0.001$), it also shortened the time to beginning of delivery and vaginal delivery. There were no significant differences found in duration of any stage of labour between the two groups (Tab. 4).

There were statistically significant differences in Apgar scores of the baby (Tab. 5). The birth weight and length were statistically different, which may reflect the previously noted difference in gestational age at the delivery (Tab. 1 and 5).

As a next step, we checked whether woman's age had been associated with the analyzed time periods. After the

Table 4. Time intervals to delivery intention to treat groups

	Misoprostol first [n = 93]	Dinoprostone first [n = 147]	Significance
Time admission to delivery (vaginal or Caesarean section) [h] Mean, (SD) Median (Q1–Q3)	 47.0 (69.3)* 25.8 (14.0–44.0)	 67.3 (73.0)* 51.2 (31.3–81.3)	$P^{MW} < 0.001$
Time drug application to delivery (vaginal or Caesarean section) [h] Mean, (SD) Median (Q1–Q3)	 14.5 (13.8)* 11.0 (8.0–17.4)	 35.6 (25.0)* 28.8 (13.5–51.9)	$P^{MW} < 0.001$
Time drug application to vaginal delivery (Caesarean sections excluded) [h] Mean, (SD) Median (Q1–Q3)	[n = 64] 15.1 (15.6)* 11.0 (8.7–17.8)	[n = 107] 31.3 (24.4)* 26.0 (12.0–47.2)	$P^{MW} < 0.001$
Time drug application to the beginning of a labor [h] Mean, (SD) Median (Q1–Q3)	[n = 68] 9.9 (15.0)* 6.2 (3.9–11.5)	[n = 115] 25.3 (23.0)* 20.9 (5.8–40.8)	$P^{MW} < 0.001$
I stage of labor duration [h] Mean, (SD) Median (Q1–Q3)	[n = 68] 4.8 (2.2)* 4.8 (3.0–6.0)	[n = 115] 5.4 (2.7)* 5.0 (3.0–7.0)	$P^{MW} = 0.181$
II stage of labor duration [min] Mean, (SD) Median (Q1–Q3)	[n = 68] 30.0 (28.6)* 20.0 (10.0–40.0)	[n = 111] 37.7 (33.4)* 30.0 (15.0–60.0)	$P^{MW} = 0.058$
III stage of labor duration [min] Mean, (SD) Median (Q1–Q3)	[n = 63] 8.8 (5.6)* 10.0 (5.0–10.0)	[n = 106] 8.6 (4.2)* 10.0 (5.0–10.0)	$P^{MW} = 0.771$

*p < 0.05 by the Shapiro-Wilk test for normal distribution; MW — the U-Mann-Whitney test

Table 5. Neonatal outcomes across intention to treat misoprostol and dinoprostone groups

	Misoprostol first [n = 93]	Dinoprostone first [n = 147]	Significance
Apgar score [points] Mean, (SD) Median (Q1–Q3)	 9.4 (1.6)* 10.0 (10.0–10.0)	 9.8 (0.6)* 10.0 (10.0–10.0)	$P^{MW} = 0.041$
Apgar score ≤ 6 points at the 1st min (n, %) Apgar score 7–8 points at the 1st min (n, %) Apgar score 9–10 points at the 1st min (n, %)	8 (8.6%) 4 (4.3%) 81 (87.1%)	2 (1.4%) 4 (2.7%) 141 (95.9%)	$p^{F} = 0.018$
Birth weight [g] Mean, (SD) Median (Q1–Q3)	[n = 93] 3385 (530) 3420 (3045–3750)	[n = 146] 3623 (417) 3625 (3320–3955)	$p^{t-ue} < 0.001$
Birth length [cm] Mean, (SD) Median (Q1–Q3)	[n = 93] 54.9 (3.4) 55.0 (53.0–57.0)	[n = 146] 56.0 (3.0)* 56.0 (54.0–58.0)	$p^{MW} = 0.008$
Female [n, %]	44 (47.3%)	68 (46.3%)	df = 1 $P^{chi2} = 0.895$

*p < 0.05 by the Shapiro-Wilk test for normal distribution; MW — the U-Mann-Whitney test; t-ue — the Student's t-test for unequal variances

woman's age was regressed on the time from the drug implementation to the beginning of a labour (to the first stage of labour), to the delivery of a baby (to the end of second stage of labour) and additionally on the duration of the second stage of a labour only, no one result was statistically significant (p values: 0.114; 0.308; 0.131, respectively). Additionally, when the differences in the considered time periods between drug types were analysed with interaction terms between woman's age categorical variable no result had been significant either (p values: 0.970; 0.757; 0.800).

Finally, Cox regression models were performed to assess whether there were differences between misoprostol and dinoprostone upon time in the labour induction. The results showed that misoprostol increased more than twice the chance of beginning the labour or delivery of a baby in comparison to dinoprostone. Even after adjustment for the covariates which might influence the process, like mother's age, number of pregnancies, cervical state, and oxytocin use, the probability still was significantly higher (Tab. 6 and 7).

Table 6. The relative probability (assessed by the hazard ratio) for induction of a labor for misoprostol compared to dinoprostone

	Hazard ratio	95% CI	p value
Beginning of labor	2.57	1.85–3.57	p < 0.001
Beginning of labor[1]	2.44	1.75–3.40	p < 0.001
Beginning of labor[2]	2.42	1.73–3.39	p < 0.001
Beginning of labor[3]	2.10	1.49–2.97	p < 0.001

The proportional Cox regression model; [1]Adjusted for number of pregnancies, mother's age; [2]Adjusted for the covariates as in (1) and additionally for cervical effacement; [3]Adjusted for the covariates as in (2) and additionally for oxytocin use; CI — confidence interval

Table 7. The relative probability (assessed by the hazard ratio) for baby's delivery for misoprostol as compared to dinoprostone

	Hazard ratio	95% CI	p value
Baby's delivery	3.21	2.40–4.29	p < 0.001
Baby's delivery[1]	3.07	2.28–4.13	p < 0.001
Baby's delivery[2]	3.09	2.29–4.16	p < 0.001
Baby's delivery[3]	2.59	1.90–3.53	p < 0.001

The proportional Cox regression model; [1]Adjusted for number of pregnancies, mother's age; [2]Adjusted for the covariates as in (1) and additionally for cervical effacement; [3]Adjusted for the covariates as in (2) and additionally for oxytocin use; CI — confidence interval

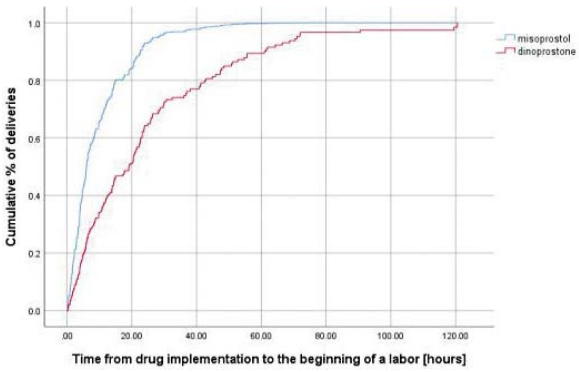

Figure 1. Cox regression model — from drug implementation to the beginning of labor

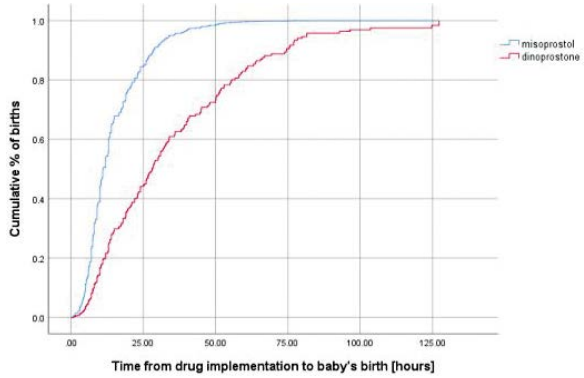

Figure 2. Cox regression model — from drug implementation to baby's delivery

DISCUSSION

The use of pharmaceutical induction of labour increased in many European countries during the last decades, for example in Norway the rate increased from 12.5% in 2003 to 20.3% in 2013. The rate of caesarean section in the induced patients' group did not change, and it remained stable at 17.1 and 17.4%, respectively [5]. It might be connected with introducing into the contemporary obstetrics the use of prostaglandins, which facilitate cervical ripening and accelerate uterine contractions like in the natural course of delivery.

The purpose of this study was a comparison of two prostaglandins used in everyday clinical practice, which are, among others, recommended by the Polish Society of Gynaecologists and Obstetricians Guidelines for labour induction [6]. We verified that vaginally implemented misoprostol insert was more effective than the dinoprostone gel in induction of labour in pregnant women without any comorbidities at term. Our findings are in line with the results of Sharp et al. [7] who demonstrated a statistically shorter time to the delivery when using misoprostol vaginal insert rather than dinoprostone vaginal gel, namely means 18.2 h (11.6–27.6) vs 21.8 h (19.0–23.9), respectively. In our study, the mean interval between the implementation of misoprostol to the time of delivery was even shorter (14.5 h). We have shown that misoprostol, in comparison to dinoprostone, resulted in twice higher chance of successful beginning of delivery regardless the number of previous pregnancies, gestational age or cervical state. Even if additionally, oxytocin use or mother's age were considered, the chance of labour initiation across time was still 1.9 times higher in this group (Tab. 6, Fig. 1). We observed similar effects on the time to baby's delivery (Tab. 7, Fig. 2). Those results indicated that misoprostol was more effective than dinoprostone in the induction of labour, which also further supported the findings of the other studies [8–9,12]. It is worth mentioning that most of available studies are based on different routes of administration of misoprostol (oral tablets, tablets implemented into the posterior vaginal fornix, vaginal insert) or dinoproston (vaginal insert, vaginal gel), which may interrupt the direct comparison between result and conclusions of those studies. One study which compared misoprostol vaginal insert (MVI) and misoprostol vaginal tablets (MVT) for induction of labour in term pregnancies [10] showed that MVI achieved a more vaginal delivery rate within 24-hours and shorter time from induction to vaginal delivery than MVT, with no influence on caesarean section rate, postpartum haemorrhage, Apgar score below 7. Remarkably similar conclusions were made after comparing of the misoprostol vaginal insert with oral misoprostol tablets in favour of vaginal rout [11].

The results of our study suggested that use of misoprostol vaginal insert shorten the time intervals from medicament implementation to active labour and to delivery itself when compared with preinduction with dinoprostone.

Incidence of vaginal deliveries after induction of labour vary widely in the literature; it was 92.5% [7], 88% [12], 73.3% [8] in the misoprostol group, compared with dinoprostone 89,1%, 74%, 71,6% respectively. In our study, the misoprostol intervention ended in the vaginal delivery in 68.8% cases in comparison to 76.9% in the dinoprostone group.

We observed lower mean birth weight of the neonates in the misoprostol group, which we assumed that was mostly connected with the lower gestational age in this group (40.6 vs 40.9 $p < 0.001$). However, we believe that it had no influence on the effectiveness of misoprostol, since there was another study where the misoprostol group had higher mean birth weight and gestational age compared to the dinoprostone group and the time interval to delivery was still shorter [12] or in another one in which the time from drug application to onset of labour was also significantly reduced [13].

As the prostaglandins' administration may result in many adverse outcomes, in both the mother and the infant, we analysed the results thoroughly. In both groups we have noticed disturbances in the foetal heart rate pattern described as foetal distress needing emergency caesarean delivery, with no statistical difference. In the end of our observation, we have shown that the caesarean delivery rates and its indications, as well as the maternal complications were not significantly different between studied groups.

In our study the analysis of the factors that potentially might have influenced the effect of each intervention showed no differences between the groups regarding maternal age, parity, prior vaginal delivery, miscarriage history, patients' body mass index, Bishop's scale score before the drug administration or estimated foetal weight.

There were a few limitations of this work that should be acknowledged. First, the study was retrospective and the enrolment of subjects for this study was not randomised. Additionally, we reviewed medical records as the source of information, and there were several missing data which were not possible to retrieve. Moreover, our sample size was limited; however, it was big enough to reach statistical significance for some of our results. It was not observed in our study any significant difference in the complication rate between different drugs, but, in our opinion, it should be confirmed on large sample size study. Thus, further studies on bigger groups are still needed.

CONCLUSIONS

Our study showed that using the misoprostol vaginal insert in comparison to dinoprostone gel seems to increase the chance of delivery and to shorten the time to beginning of the first stage of labour as well as the time to the delivery itself regardless the way, vaginal birth or caesarean section with no influence on maternal complications. Some lower Apgar scores observed in the misoprostol group requires further investigation.

Acknowledgments

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Financial disclosure

The authors receive no financial support for the study conduct.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 733–739

ISSN 0017–0011

DOI 10.5603/GP.a2020.0124

Are serum Netrin-4 levels predictive of preeclampsia?

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ABSTRACT

Objective: To investigate the levels of anti-angiogenic factors, namely sFlt-1 and Netrin-4, in patients with preeclampsia (PE).

Material and methods: Cord-blood (UC) sFlt-1 and Netrin-4 concentrations were measured in 30 patients with severe PE, 30 patients with PE and 30 control infants and their mothers (MS).

Results: Maternal sFlt-1 levels were significantly higher in the severe PE and PE groups than in the control group. There were no statistical differences among the three groups in maternal and fetal Netrin-4 levels. But Netrin-4 levels were found to be the lowest in the control group and higher in the PE and severe PE groups. The correlation analysis revealed a positive correlation between maternal sFlt-1 levels and maternal Netrin-4 levels ($p = 0.012$, and $r = 0.263$), maternal sFlt-1 levels and fetal sFlt-1 levels ($p = 0.012$, and $r = 0.263$).

Conclusions: There was a positive correlation found between maternal sFlt-1 levels and maternal Netrin-4 levels. We are of the opinion that elevation in levels of Netrin-4 might be secondary to placental hypoxia occurring in PE. The present study led to the consideration of anti-angiogenic biomarkers (sFlt-1 and Netrin-4) on automated platforms for clinical use as an aid in establishing the diagnosis and prognosis of PE.

Key words: sFlt-1; Netrin-4; preeclampsia; anti-angiogenic effects

Ginekologia Polska 2020; 91, 12: 733–739

INTRODUCTION

The exact mechanism of preeclampsia (PE) has yet to be fully unraveled; however, healthcare professionals consider PE to be a condition arising from placental insufficiency caused by insufficient remodeling of the maternal vascular bed. Angiogenetic imbalance assumes an important role in the pathophysiology of PE [1]. When angiogenic factors' functions are hindered by anti-angiogenic factors in patients with PE, hypertension, endothelial dysfunction, and proteinuria occur [2]. Despite it being a prominent feature, the presence of proteinuria is no longer considered to be the ultimate diagnostic criterion for PE. PE is defined by hypertension accompanied by one or more of the following conditions: thrombocytopenia (a platelet count lower than 100,000/microliter), impaired liver function, new-onset renal failure, pulmonary edema, and new-onset cerebral and visual disturbances [1]. It has serious potential to aggravate maternal mortality and morbidity rates in the short-, medium-, and long-term. In addition, it might bring about serious consequences for the fetus, including prenatal mortality, intrauterine growth restriction, and premature delivery [3]. The reported frequency of PE varies from 3–10% [4, 5].

Vascular endothelial growth factor (VEGF) is an endothelial-specific mitogen that plays a key role in promoting angiogenesis [6]. The VEGF family has five members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF). VEGF-A binds with high affinity to fms-like tyrosine kinase-1 (Flt-1), also known as VEGFR-1. Soluble fms-like tyrosine kinase 1 (sFlt-1), a soluble form of VEGF receptor Flt-1, is anti-angiogenic [3]. sFlt-1 is significantly involved in the etiology of PE. One study reported that the effects of VEGF on arterioles were inhibited by sFlt-1 elevation [2]. Another study reported that excess amounts of sFlt-1 released by trophoblasts contributed significantly to the development of PE [7]. One group of researchers reported that concentrations of anti-angiogenic factors, including serum-soluble fms-like tyrosine kinase 1 (sFlt-1), were elevated in patients with PE compared to healthy control subjects. In contrast, concentrations of significant angiogenic factors, including serum VEGF, were diminished

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in patients with PE compared to healthy control subjects [8]. Maternal sFlt-1 concentration is known to be elevated in patients with PE. The concentration of sFlt-1 is increased in PE in direct correlation with the severity of the condition [2].

Netrins are bifunctional and assume either pro-angiogenic or anti-angiogenic roles depending on the receptor and its expression [9]. Netrins have been shown to play a key role in angiogenic processes. They are located in the placenta and are important for both placental development and placental vascular development [10]. Koch et al. [11] were the first to define Netrin-4, a member of evolutionarily conserved extracellular protein family netrins. It is also referred to as β-netrin because it is more closely related to the laminin chains than the other netrins that are known to exhibit homology to the short arms of laminin chains. It is also defined as basement membrane component found in the basement membranes of the vasculature, kidneys, and ovaries. It does not play an active role in normal vascular development; however, its expression may have a major effect on pathological blood vessel formation and increased hypoxia. Netrin-4 also plays a key role in angiogenesis and is anti-angiogenic [12]. Finally, several studies have reported that it significantly inhibits VEGF-induced angiogenesis [13, 14].

The purpose of the present study was to investigate the levels of anti-angiogenic factors-namely, sFlt-1 and Netrin-4 in patients with PE, a condition that lacks a thorough etiological and pathogenetic explanation. The results of the study might improve the prediction and treatment of PE.

MATERIAL AND METHODS

Patients and study groups

This prospective clinical study was conducted between March 2015 and February 2017 at a tertiary centre. A total of 90 patients were included in this study; 30 were severe PE, 30 were PE and 30 were a control group. Before the study, approval was obtained from the local ethics committee (approval no: 2015/178).

Inclusion criteria were patients ages 16 to 44 years with PE diagnosis. Diagnosis of severe PE and PE was established using the ACOG criteria [1]. Exclusion criteria included the following: additional systemic diseases, twin pregnancy, fetal abnormalities, HELLP syndrome and eclampsia. The control group consisted of the patients who delivered in our clinic without any additional disease.

Serum measurements of blood samples and anti-angiogenic biomarkers

Maternal peripheral venous blood samples were taken in the first stage of the delivery in patients undergoing normal vaginal delivery, right before the administration of anesthesia in patients undergoing C-section under general anesthesia, and right before the administration of spinal anesthesia in patients undergoing C-section under spinal anesthesia. Fetal cord blood samples were taken from the umbilical artery after the umbilical cord was clamped and cut. Sera were isolated by centrifugation and kept at –80°C until further analysis. Hematological parameters (Hemoglobin, hematocrit and platelet) were measured by means of an automated hematology analyzer (Abbott Cell-Dyn Sapphire; Abbott Laboratory, Abbott Park, IL, USA). Levels of urea, creatinine, aspartate transaminase (AST) and alanine aminotransferase (ALT) were determined by means of an automated chemistry analyzer by the Abbott Architect C 16000 autoanalyzer (Architect C16000 auto-analyzer; Abbott Laboratory, Abbott Park, IL, USA) using commercial kits (Abbott) in the peripheral venous blood samples. Proteinuria was assessed using an automated urinalysis system by IRIS IQ 200 ELITE (Iris Diagnostics Chatsworth, CA, USA). Serum sFlt-1 and Netrin-4 levels were determined by means of an enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Sunred Biological Technology Co., Shanghai, China) according to the manufacturer's instructions. The serial numbers were 201-12-0228 and 201-12-1298, respectively. All the measurements were performed by the same investigator in a blinded manner.

Statistical analysis

All statistical analyses were performed by SPSS-21. Data were expressed as mean ± sd. According to our study results for sFlt-1 and Netrin-4 levels, sample size of the study population was calculated to be 30 patients per group ($\alpha = 0.05$ and the study power = 80%). According to distribution assumptions for the continuous variables, the categorical data are given using numbers and percentages. The distribution of the data was evaluated using the Kolmogorov-Smirnov test. Group comparisons were performed using the Mann-Whitney U test. Correlations were tested using the Spearman correlation analysis. A p value smaller than 0.05 was considered statistically significant.

RESULTS

No significant differences were observed among the three groups in terms of age or parity. Gestational age, fetal weight, and one- and five-minute Apgar scores were significantly lower in the severe PE group compared to the PE and control groups. There were significant differences among the three groups in terms of systolic and diastolic blood pressure (Tab. 1). Hb and Htc levels were significantly higher in the severe PE group than they were in the control group. Urea and creatinine levels were significantly higher in the severe PE and PE groups than they were in the control group. ALT and AST levels were significantly higher in the severe PE group than in the PE and control groups. Mater-

Table 1. Comparison of the demographic characteristics of the groups

	Control (n = 30)	Preeclampsia (n = 30)	Severe preeclampsia (n = 30)	p
Maternal age (years)	30.10 ± 5.30	30.33 ± 7.11	30.53 ± 30.77	α: 0.807 β: 0.756 €: 0.941
Parity	2.23 ± 1.75	2.23 ± 2.70	2.13 ± 3.07	α: 0.464 β: 0.248 €: 0.539
Gestational age (weeks)	36.37 ± 2.52	36.37 ± 2.54	34.43 ± 3.09	α: 0.009 β: 0.010 €: 0.970
Systolic blood pressure [mm Hg]	117.33 ± 11.72	144.67 ± 5.07	172.33 ± 18.51	α: 0.000 β: 0.000 €: 0.000
Diastolic blood pressure [mm Hg]	74.33 ± 7.73	91.00 ± 7.58	172.33 ± 18.51	α: 0.000 β: 0.000 €: 0.000
Birth weight [g]	2827.17 ± 620.75	2777.30 ± 637.82	2293.17 ± 813.89	α: 0.008 β: 0.004 €: 0.923
1-min Apgar score	6.80 ± 1.49	6.27 ± 1.14	5.47 ± 1.33	α: 0.027 β: 0.001 €: 0.075
5-min Apgar score	8.47 ± 1.04	8.23 ± 0.97	7.57 ± 1.13	α: 0.021 β: 0.003 €: 0.360

α — comparison of severe and preeclampsia; β — comparison of severe preeclampsia and the control group; € — comparison of preeclampsia and control group; values shown are means ± SEM

nal sFlt-1 levels were significantly higher in the severe PE and PE groups than they were in the control group. However, no significant difference was found between maternal sFlt-1 values in the severe PE versus PE groups. In contrast, fetal sFlt-1 levels were significantly higher in the severe PE group than they were in the control group. There were no statistically significant differences among the three groups in terms of maternal and fetal Netrin-4 levels. However, maternal and fetal Netrin-4 levels were lowest in the control group and higher in the PE and severe PE groups (Tab. 2). In all three groups, fetal Netrin-4 levels were higher than maternal Netrin-4 levels. The correlation analysis revealed a positive correlation between maternal sFlt-1 levels and maternal urea levels ($p = 0.010$, $r = 0.269$*) (Fig. 1A) as well as a negative correlation between maternal sFlt-1 levels and maternal platelet counts ($p = 0.040$, $r = –0.217$*) (Fig. 1B). The correlation analysis revealed a positive correlation between maternal sFlt-1 levels and maternal Netrin-4 levels ($p = 0.012$, $r = 0.263$*) (Fig. 1C). Lastly, there was a positive correlation between maternal sFlt-1 levels and fetal sFlt-1 levels ($p = 0.012$, $r = 0.263$*) (Fig. 1D).

DISCUSSION

This study investigated levels of anti-angiogenic biomarkers (sFlt-1 and Netrin-4) in the diagnosis of PE. Maternal sFlt-1 and Netrin-4 levels were higher in the severe PE and PE groups than they were in the control group. Further, there was a positive correlation between maternal sFlt-1 levels and maternal Netrin-4 levels.

For patients with PE, the earlier the gestational week, the higher the chances of maternal and prenatal mortality and morbidity. There is a known association between abnormal placentation and PE, which is thought to arise from insufficient trophoblast invasion in the maternal spiral arteries in the early stages of gestation [15]. It is for this reason that generally, gestation is at an earlier stage and fetal weight is lower when patients develop severe PE. In the present study, fetal weight was lower in the severe PE group than it was in the PE and control groups. Gestation was also at an earlier stage in the severe PE group than it was in the PE and control groups. However, there was no correlation between fetal and maternal sFlt-1 levels and gestational week.

Catherine et al. investigated pregnancies complicated by PE and reported higher sFlt-1 levels in both maternal and fetal sera compared to control sera. Elevated levels in the fetal sera were limited compared to levels in the maternal sera, but they were still considered to be high. In this respect, there was a positive correlation between maternal and fetal sFlt-1 levels in patients with PE. The researchers found that maternal sFlt-1 levels were 29 times higher than fetal

Table 2. Comparison of the biochemical parameters of the groups

	Control (n = 30)	Preeclampsia (n = 30)	Severe preeclampsia (n = 30)	p
Haemoglobin [g/dL]	11.85 ± 1.91	11.85 ± 1.48	12.84 ± 1.81	α: 0.011 β: 0.018 €: 0.807
Hematocrit (%)	36.84 ± 4.99	37.89 ± 3.91	39.49 ± 5.47	α: 0.183 β: 0.061 €: 0.473
Platelet count [mm^3]	259.47 ± 72.45	273.40 ± 59.87	219.27 ± 59.92	α: 0.001 β: 0.059 €: 0.290
Urea [mg/dL]	17.77±5.15	22.33±6.61	23.63±7.92	α: 0.615 β:0.000 €: 0.000
Creatinine [mg/dL]	0.54 ± 0.06	2.73 ± 11.57	0.62 ± 0.07	α: 0.695 β: 0.000 €: 0.000
ALT [U/L]	12.20 ± 3.50	12.77 ± 5.74	25.23 ± 27.36	α: 0.028 β: 0.026 €: 0.841
AST [U/L]	19.13 ± 5.51	21.60 ± 7.64	33.90 ± 32.89	α: 0.020 β: 0.001 €: 0.147
Maternal Netrin- 4 [pg/mL]	177.00 ± 71.76	192.67 ± 68.09	224.47 ± 182.50	α: 0.836 β: 0.442 €: 0.487
Fetal Netrin-4 [pg/mL]	264.93 ± 120.86	295.40 ± 240.31	359.37 ± 282.47	α: 0.089 β: 0.730 €: 0.110
Maternal sFlt-1 [pg/mL]	4220.97 ± 1804.13	7184.47 ± 6934.28	8363.40 ± 5303.40	α: 0.061 β: 0.000 €: 0.010
Fetal sFlt-1 [pg/mL]	423.37 ± 172.75	509.60 ± 136.97	570.63 ± 106.13	α: 0.243 β: 0.001 €: 0.061

α — comparison of severe and preeclampsia; β — comparison of severe preeclampsia and the control group; € — comparison of preeclampsia and control group; values shown are means ± SEM

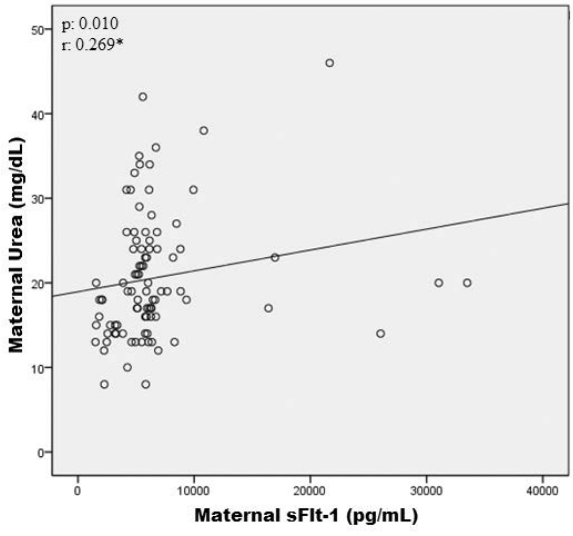

Figure 1A. Correlation analysis between maternal sFlt-1 levels and maternal urea levels

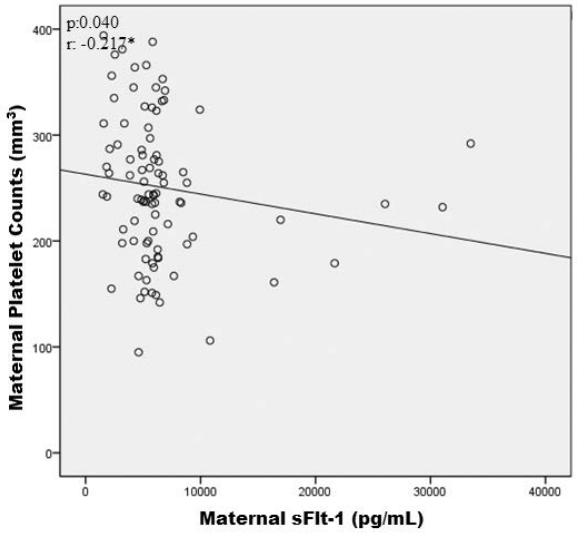

Figure 1B. Correlation analysis between maternal sFlt-1 levels and maternal platelet counts

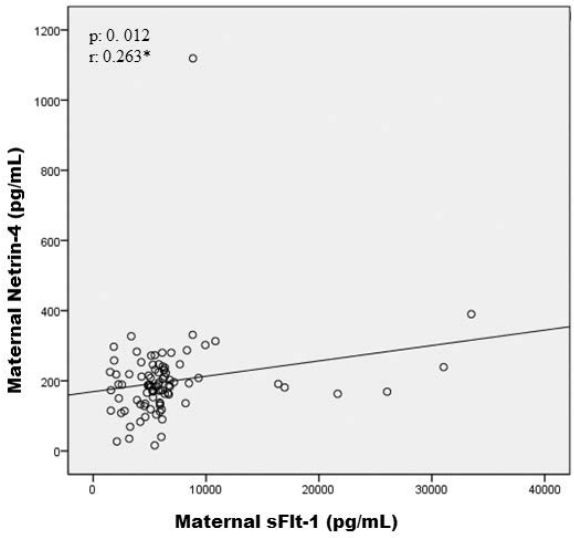

Figure 1C. Correlation analysis between maternal sFlt-1 levels and maternal Netrin-4 levels

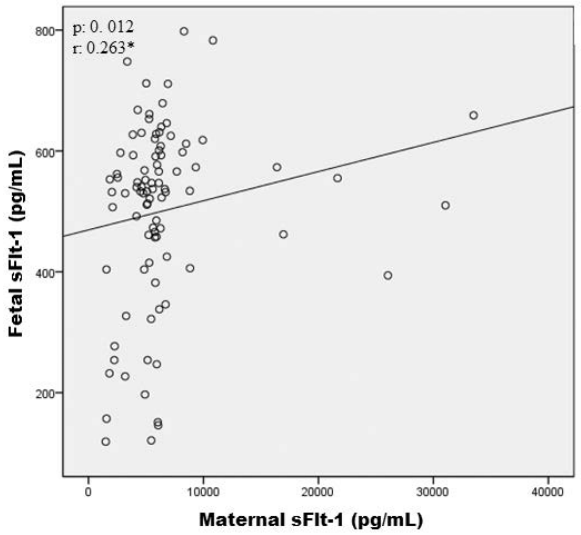

Figure 1D. Correlation analysis between maternal sFlt-1 levels and fetal sFlt-1 levels

sFlt-1 levels. This specific study observed that sFlt-1 elevation in PE was not sourced from fetal sFlt-1 levels; however, sFlt-1 elevation was higher in severe PE patients compared to mild PE patients, and maternal sFlt-1 elevation correlated with the severity of the condition [16]. Similarly, a study evaluating late-onset PE reported that the level of maternal sFlt-1 in the PE group was significantly higher than it was in the control group, but unlike this, height was not proportional to the severity of the condition [17]. In the present study, maternal sFlt-1 levels were two times higher, whereas fetal sFlt-1 levels were 1.5 times higher, than the respective levels in the control group. In addition, there was a positive correlation between maternal and fetal sFlt-1 levels. In all three groups, maternal sFlt-1 levels were higher than fetal sFlt-1 levels (10 times higher in the control group, 14 times higher in the PE group, and 16 times higher in the severe PE group), indicating the absence of a significant association between elevation in maternal sFlt-1 levels and fetal sFlt-1 levels. In the present study, both maternal sFlt-1 and fetal sFlt-1 levels were significantly higher in the severe PE and PE groups than they were in the control group. In addition, sFlt-1 levels were higher in the severe PE group than they were in the PE group. However, the difference between the groups was not statistically significant, and no significant difference was found between the severity of the disease and the level of sFlt-1. This could be attributed to the small sample size of the study.

Several studies have reported the involvement of placental insufficiency in the etiopathogenesis of PE. The clinical manifestation is marked by placental hypoxia; hypoperfusion, hypoxia, and ischemia are critical components of the pathogenesis of PE. Hypoperfusion appears to be both a cause and a consequence of abnormal placental development. In response to placental hypoxia, placenta produces pathogenic factors to be released into the maternal circulation. These factors disrupt maternal endothelial cell functions and cause signs and symptoms of PE. Previous studies reported that anti-angiogenic factors were increased in PE. One of these factors, sFlt-1, was involved in the pathogenesis of PE [2, 16, 18]. One study reported increased Netrin-4 release by Müllerian cells in hypoxic patients. Netrin-4 stimulates angiogenesis through the deleted in colorectal cancer (DCC) receptor; it was found to be highly anti-angiogenic [19]. It was further reported to exhibit a dose-dependent inhibitory effect on cellular proliferation and assume anti-angiogenic roles in human placenta [20]. In agreement with the literature, in the present study, sFlt-1 was high in the PE group and even higher in the severe PE group. Further, there was a positive correlation between maternal sFlt-1 levels and maternal Netrin-4 levels. The present authors opine that elevation in Netrin-4 levels might be secondary to placental hypoxia occurring in PE. Moreover, that elevation Netrin-4 levels is correlated with elevation in sFlt-1 levels, an anti-angiogenic agent, indicates that Netrin-4 might assume an anti-angiogenic role in conditions such as PE. This finding supports previous findings in the literature.

When sFlt-1 levels are elevated in the maternal circulation, free VEGF levels ensuring the continuity of angiogenetic and endothelial functions drop accordingly. Similarly, increased Netrin-4 levels decrease VEGF levels [21]. In the present study, maternal Netrin-4 levels were positively correlated with maternal sFlt-1 levels. This suggests that these two anti-angiogenic factors concurrently increase due to the

decreasing pro-angiogenic factors in PE; further, it suggests that Netrin-4 is an anti-angiogenic factor in PE.

VEGF itself and VEGFR play roles not only in physiological but also in pathologic angiogenesis [7]. The overexpression of Netrin-4 has been reported to affect VEGF/basic fibroblast growth factor-induced angiogenesis [13]. In a previous study, Netrin-4 was shown to have anti-angiogenic features in high levels and be present in vasculature, kidneys, and ovaries [11]. It was further reported to stimulate endothelial cell activities related to angiogenesis. In addition, it acted as an anti-angiogenic agent when present in high levels; these levels tended to increase secondary to hypoxia [22]. In a study by Boutsikou et al. that investigated intrauterine growth restriction (IUGR), which is characterized by abnormal feto-placental angiogenesis, maternal Netrin-4 levels were lower than fetal Netrin-4 levels in all of the study groups. The authors suggested that elevation in these levels was an indicator of hypoxia. Also, as the severity of IUGR increases, the level of maternal Netrin-4 is found to be high [9]. Hypoxia has been found to increase Netrin-4 mRNA expression [19]. In the present study, maternal Netrin-4 levels were lower than fetal Netrin-4 levels; this is in accordance with the literature. Although no statistically significant difference was detected, maternal and fetal Netrin-4 levels were lowest in the control group and higher in the PE and severe PE groups — hypoxia is known to play a role in this pathogenesis. Also, as the severity of the disease increased, Netrin-4 was also present in higher levels. Based on this study's observation that elevation in Netrin-4 levels was secondary to hypoxia, and that such elevation inhibited the expression of VEGF, which is involved in angiogenesis, the authors propose that Netrin-4 might also be involved in the etiology of PE. The positive correlation between sFlt-1 and Netrin-4 also supports this hypothesis.

The small sample size was a limitation of the present study. However, its prospective design and correlation analysis make the study strong.

CONCLUSIONS

In the last decade, the molecular mechanisms of PE have been explained. Anti-angiogenic factors, such as sFlt-1, are known to play an important role in the pathogenesis of PE and be significantly associated with negative outcomes associated with PE. This study found a positive correlation between maternal sFlt-1 and Netrin-4 levels. Elevation in levels of Netrin-4 might be secondary to placental hypoxia within PE. The present study contributes to practice by urging healthcare practitioners to consider the possibility of using anti-angiogenic biomarkers (sFlt-1 and Netrin-4) on automated platforms for clinical use to aid in establishing the diagnosis and prognosis of PE. Further studies are needed to evaluate the effect of vaginal delivery or cesarean delivery on Netrin-4 levels.

Conflict of interest

We declare that we have no conflict of interest.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 740–747

ISSN 0017–0011

DOI 10.5603/GP.a2020.0125

Outcome dependent growth curves for singleton pregnancies based on birth weight of babies for Polish population

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ABSTRACT

Objectives: To create outcome dependent fetal growth curves and birth weight standards that can be analyzed for use in clinic specifically for Western European populations.

Material and methods: We conducted a retrospective study on fetal growth and birth weight trends from live birth singleton pregnancies between 2005 and 2018 at one of the largest tertiary gynecologic-obstetric hospitals in Poland. The inclusion criteria were at least 22 weeks of gestation at birth regardless of delivery mode (vaginal or C-section), no congenital anomalies diagnosed before and after delivery and an Apgar score of at least 7 in the first minute. The final sample had a total of 39,413 cases (18,562 girls and 20,851 boys). We presented 7 (for all fetuses in the 5th, 10th, 25th, 50th, 75th, 90th and 95th percentiles) and 6 (for boys and girls each at 10th, 50th and 90th percentiles) fetal growth curves between 25 and 40 weeks of gestation. Birth weight trends were obtained and analyzed from all babies in the 5th, 10th, 25th, 50th, 75th and 95th percentiles born between 22 to 42 weeks of gestation with also separate trends for boys and girls.

Results: The largest differences in fetal growth curves were observed in the 10th and 50th percentiles between 22 and 34 weeks of gestation. A decreasing fetal weight gain pattern was observed between 27 and 30 weeks and after 38 weeks of gestation, the decrease was more drastic in female. A significant increase from 2009 to 2017 was observed in the weight of 50th percentile babies born at or after 35 weeks. We found significant discrepancies between our results and the most used European fetal growth curves particularly in the 10th and 90th percentile weights at 30 weeks.

Conclusions: Separate scales for boys and girls were implied and given the overall difference form commonly used references. We believe there is significant value in using these unique patterns found in fetal growth curves and birth weights of ethnically homogenous population (such as Poland) at everyday clinical practice for more opportunities of safe obstetric care and higher chances of delivering a healthy child.

Key words: outcome dependent growth curves, singleton pregnancy

Ginekologia Polska 2020; 91, 12: 740–747

INTRODUCTION

In everyday clinical practice, we make extremely important decisions regarding the continuation of pregnancy or delivery. In our decisions, most often we rely on the gestational age and biophysical assessment of the fetus. Sometimes they result from the analysis of biochemical or hematological results concerning both the mother and the fetus. However, the estimated weight of the fetus and its implied maturity play a key role. If we are dealing with fetal growth disorders, the reference of the week of pregnancy, confirmed in the first trimester of pregnancy, to that determined based on ultrasound imaging, is one of the most important factors in our analysis. In pregnancy after the 34th week, decisions are much easier, but when faced with prematurity, with extremely low fetal weight, making such a decision is one of the most difficult challenges in obstetrics. We have various fetal growth curves included in the programs that support our ultrasound devices, but none of these scales is based on the Polish population. Each of them, based on the same fetal measurements, shows incoherent weeks of pregnancy as well as difference in estimated fetal weight [1–3]. Not without significance are differences in the

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body proportions between individual races and different ways of creating growth curves.

There is no national birth registry in Poland, hence the creation of a full database is impossible. The last data showing the birth weight of the fetuses come from about 30 years ago, since then the living conditions have changed dramatically. All foreign, available databases contain an image of a very mixed racial population. In view of the very uniform nationality structure of our country, it seems necessary to create our own database.

The aim of this study was to create an outcome-dependent growth curves and birth weight standards for Polish population, basing on database of biggest tertiary care hospital in Poland.

MATERIAL AND METHODS

Data for the study were retrieved from the database of a tertiary care woman hospital in western Poland, for all patients that delivered between 01.01.2005 and 31.03.2018. Both the patients that electively chose our center as a first line care as well as the patients transferred from the other hospitals, including primary and secondary care, were included in the study.

The database included: date of birth, gestational age (completed weeks of pregnancy), sex, birth weight (rounded to 10 g), mother's parity and age, mode of delivery and Apgar scores at 1, 3, 5 and 10 minutes. The database did not have information about pre and postnatal care, maternal health condition and whether the pregnancy resulted from assisted reproduction techniques.

The gestational age was based on LMP and was confirmed on ultrasound examination at the first trimester of pregnancy. The weight of each child was acquired by qualified personnel on electronic scale after calibration.

Inclusion criteria for the study were: (i) Babies born from singleton pregnancies at least in 22nd gestational week without regard to the way of delivery (natural vs. C-section); (ii) No congenital anomalies.

Because this study was intended to be outcome-dependent and the database did not yield any information about postnatal course, all children that might have had high risk of unfavorable outcome had to be identified. Thus, following exclusion criteria was used: an Apgar scored in the first minute less than 7 or deteriorating in consecutive measurements [4, 5].

Due to the statistical nature of this study we identified and excluded outliers from the sample. Because the fetal mass within each gestational age did not have the normal distribution, we chose to identify the outliers without any method referring to a standard deviation. Since all the data were acquired by trained personnel, we decided to remove only extreme outliers within each gestational week, after applying all fore mentioned criteria. All fetuses within gestational week that had a mass below the 3rd or above the 97th percentile was identified as extreme outliers, which were 0.5% of the cases.

After applying all criteria, 2769 out of 42,182 records were excluded. The final sample had 39,413 cases (18,562 girls and 20,851 boys).

Statistical analysis

The Shaphiro-Wilk test revealed a non-normal distribution of weight and sex regarding the week of gestation. To alleviate this, we used the Generalized Additive Model for Location Scale and Shape (GAMLSS) which has been applied for data that has lost normality, for example when the distributions are skewed or kurtotic. This non-linear model was used to create growth curves by the World Health Organization [6, 7].

Prior to using GAMLSS, the distribution and smoothing method for tested groups were applied by fitting all relevant distributions and choosing the one fitting the best. Correctness was checked by visual inspection of theoretical and calculated percentiles and worm plots regarding gestational age.

All the calculations were performed in Microsoft Windows, with GAMLSS package ver. 5.0-6 for R ver. 3.4.3 in RStudio ver. 1.1.419 framework. Methods are described in our manuscript "Growth curves for twins for polish population" — in press).

RESULTS

According to the statistical nature of the study all results are presented on histograms and tables. On the five histograms we have shown distribution of the groups according to the week of delivery (Fig. 1), sex of the baby in consecutive weeks (Fig. 2), fetal mass according to week of delivery (Figs. 3 and 4) and finally centile curves using Box-Cox Power Exponential for the entire study group. Table 1 presents percentiles of the fetal birth weight for the entire study group. Distribution of the centiles separately for girls and boys are presented on Figure 5 and in Tables 2 and 3. Table 4 was created to show annual changes.

DISCUSSION

Seventy percent of infants born below the 10th percentile are not at risk for adverse outcomes [8]. Therefore, we search for this 30% of babies with growth restriction and severe risk of adverse outcome. The Society of Obstetricians and Gynecologists of Canada (SGOC) and the Royal College of Obstetricians and Gynecologists (RCOG) define

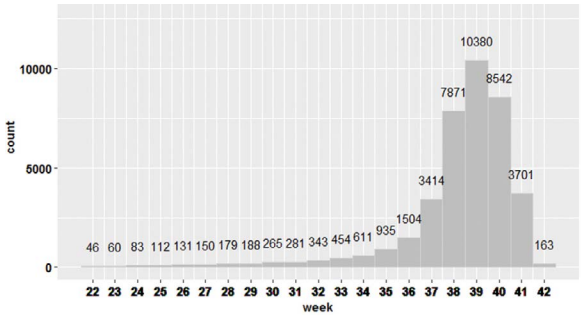

Figure 1. Histogram of the final group according to the week of delivery

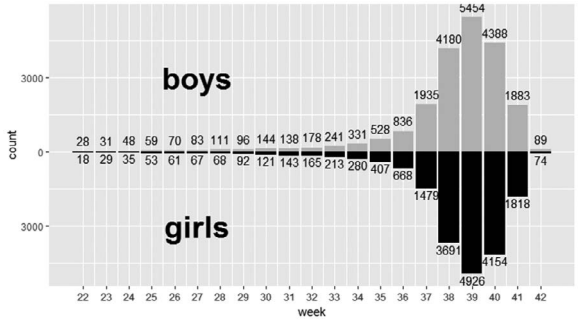

Figure 2. Histogram of the final group according to the sex of the babies and completed weeks at the delivery

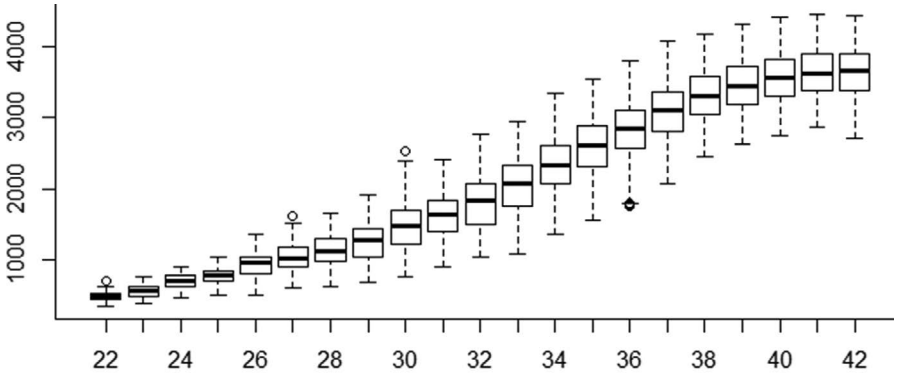

Figure 3. Distribution of the fetal mass after delivery when estimated rough to completed weeks

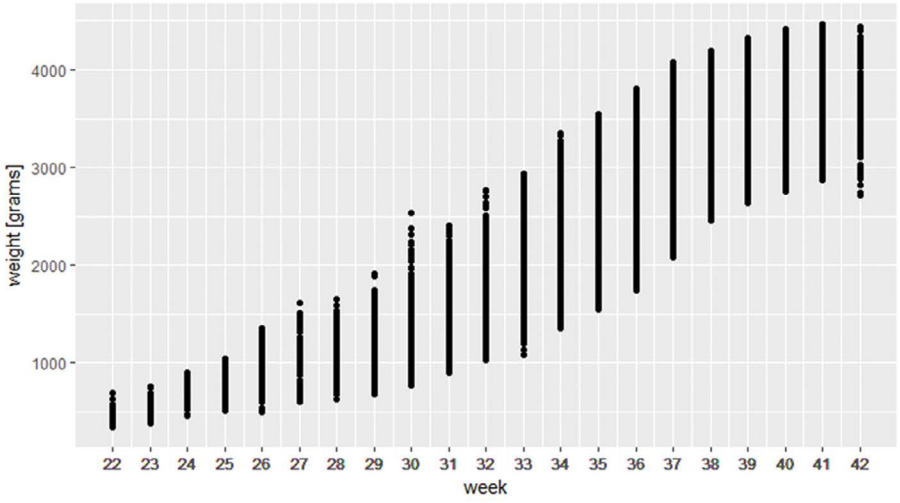

Figure 4. Distribution of the fetal mass after delivery when estimated rough to completed weeks

fetal growth restriction (FGR) as an estimated fetal weight of < 10th percentile on ultrasound in a fetus that, because of a pathologic process, has not attained its biologically determined growth potential [9]. American College of Obstetricians and Gynecologists (ACOG) recommends "If the ultrasonographically estimated fetal weight is below the 10th percentile for gestational age, further evaluation should be considered, such as amniotic fluid assessment

Table 1. Percentiles of fetal birth weight with regard to the week of gestation for the entire study group

Gestational age [week]	Percentiles						
	C5	C10	C25	C50	C75	C90	C95
22	368	395	440	485	532	578	607
23	436	464	515	577	639	690	718
24	508	542	604	682	758	817	849
25	572	619	700	796	890	966	1008
26	623	687	794	915	1035	1136	1194
27	674	746	873	1026	1177	1300	1368
28	726	803	946	1130	1310	1445	1515
29	800	886	1047	1255	1463	1622	1707
30	923	1018	1194	1416	1649	1847	1961
31	1062	1164	1355	1599	1855	2070	2193
32	1207	1319	1530	1807	2086	2304	2420
33	1392	1521	1757	2053	2342	2562	2679
34	1692	1779	2037	2335	2622	2854	2983
35	1867	2032	2305	2607	2894	3129	3262
36	2107	2282	2560	2850	3126	3364	3502
37	2412	2560	2811	3098	3379	3615	3750
38	2714	2824	3033	3308	3590	3813	3933
39	2868	2971	3173	3448	3732	3953	4071
40	2984	3086	3286	3559	3841	4057	4172
41	3082	3181	3375	3642	3917	4130	4242
42	3090	3203	3408	3666	3930	4147	4270

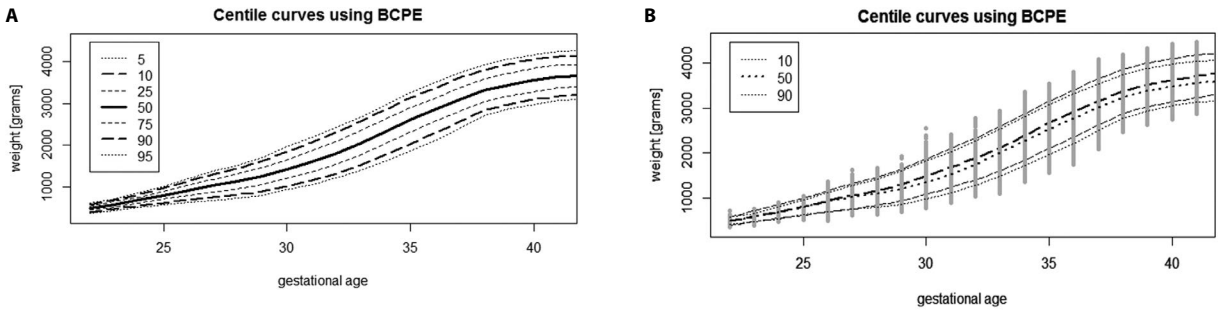

Figure 5. A. Centile curves for the entire study group; **B.** Centile curves separately for the boys and girls); BCPE — Box-Cox Power Exponential

and Doppler blood flow studies of the umbilical artery." [10]. In all guidelines, detection of the fetuses with estimated fetal weight below 10th percentile is the first step. Further, searching for Doppler abnormalities should be performed in the carefully selected group of fetuses. It is worth mentioning a publication by Gordijn et al. [11] which presented a more precise expert consensus definition of fetal growth restriction. The experts agreed upon between early and late FGR (demarcation at 32 weeks of gestation), exclusion of congenital abnormalities, lower cut-offs size measurement of < 3rd centile and functional parameters (solitary- absent end-diastolic flow in the UA, contributory UA-PI or Ut A-PI > 95th centile or CPR < 5th centile). However, even for the group of true experts guided by Delphi procedure it is difficult to choose uniform and simple definition of fetal growth restriction. On the other

Table 2. Percentiles of fetal birth weight with regard to the week of gestation for girls

Gestational age [week]	Percentiles						
	C5	C10	C25	C50	C75	C90	C95
22	372	398	440	484	526	565	588
23	428	457	506	559	612	659	686
24	499	534	593	660	727	786	821
25	575	619	695	781	868	945	992
26	641	697	793	905	1019	1121	1181
27	681	747	866	1007	1152	1277	1349
28	709	785	922	1095	1270	1416	1499
29	778	855	1003	1200	1412	1591	1694
30	900	976	1128	1345	1594	1817	1950
31	1027	1112	1282	1524	1797	2033	2169
32	1170	1271	1470	1743	2033	2265	2391
33	1345	1468	1700	1999	2298	2528	2651
34	1574	1713	1963	2270	2575	2819	2953
35	1804	1958	2220	2522	2821	3075	3221
36	2055	2215	2480	2771	3057	3310	3459
37	2368	2504	2741	3019	3300	3544	3687
38	2671	2773	2968	3229	3509	3744	3878
39	2838	2932	3118	3376	3654	3883	4010
40	2951	3046	3231	3486	3759	3981	4103
41	3040	3133	3313	3560	3826	4047	4171
42	3047	3151	3345	3592	3850	4066	4189

hand, clinically relevant is the determination of children over 90 centiles in terms of perinatal complications.

We are aware of all the inaccuracies that ultrasound carries — overestimation in large fetuses and underestimation of small fetuses, differences between races, and most often — human error. However, we believe that the determination of the fetal weight gain for individual weeks of pregnancy should be constructed based on the population in which we operate and, on the births, already performed. How can we know the actual weight of the fetus by ultrasound if errors cannot be eliminated? In the opposite, Lackman et al. [12], have advocated that "...intrauterine growth curves derived from ultrasonographically estimated fetal weight of continuing pregnancies are more appropriate than neonatal growth curves to discriminate fetuses and neonates at higher risk for adverse outcome."

In our opinion, the weight of newborns is the only objective assessment method. However, in order to create growth curves reflecting the undisturbed development of children with different growth curves, we rejected extreme outliers and children with: an Apgar scored in the first minute less than 7 or deteriorating in consecutive measurements. There is also a risk of incorrect selection of fetuses born in extremely low weeks. How often does a child born in 22 weeks have Apgar 7 or more?

Another necessary element is separate analysis of fetuses of different sexes. The Polish population is very homogeneous, hence there is no need for separate analysis of individual race. This need is indicated, for example, by the data provided by the National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies which demonstrates the differences in fetal growth between different races and ethnicities [13]. These differences concern both 10^{th} and 90^{th} percentile. For example, according to Hadlock et al. [1] the 10^{th} percentile for 30^{th} week is 1294 g, according to Duryea et al. [14] — 1068 g. We estimated this to be 1018 g. Assessing gender separately, the value for girls is 976, and for boys 1018. In the higher weeks of pregnancy these proportions are reversed. For week 40 they are 3004 g, 3005 g and 3086 g, respectively. For the girls we achieved in our chart 3046 g and for the boys — 3138 g. Comparing 90^{th} centile for 40^{th} week the differences seem high. Thus,

Table 3. Percentiles of fetal birth weight with regard to the week of gestation for boys

Gestational age [week]	Percentiles						
	C5	C10	C25	C50	C75	C90	C95
22	368	390	430	480	532	576	602
23	441	471	524	590	656	710	740
24	504	543	613	697	778	843	878
25	560	611	701	806	907	985	1027
26	620	686	800	933	1059	1156	1208
27	678	756	892	1051	1201	1317	1378
28	738	825	977	1155	1325	1455	1526
29	831	928	1096	1294	1485	1634	1715
30	964	1071	1257	1478	1694	1865	1959
31	1111	1229	1433	1674	1909	2099	2204
32	1266	1396	1618	1877	2128	2331	2444
33	1449	1595	1838	2112	2374	2588	2707
34	1672	1835	2099	2382	2649	2871	2997
35	1920	2097	2374	2661	2929	3154	3283
36	2183	2357	2631	2917	3183	3407	3535
37	2482	2631	2881	3164	3434	3654	3777
38	2751	2875	3097	3373	3644	3855	3969
39	2912	3025	3239	3517	3792	4000	4109
40	3029	3138	3349	3629	3906	4111	4216
41	3134	3239	3444	3719	3990	4187	4287
42	3213	3316	3514	3781	4042	4231	4326

Hadlock et al. [1] estimated this for 4234 g, Duryea et al. [14] — 4057 g. We have obtained the same value as Duyrea et al. [14] — 4057 g. However, comparing the girls and boys we have 3981g and 4111 g, respectively.

It is not difficult to notice differences reaching almost 250 g depending on the evaluated curves, as well as comparing them shows quite different fetal growth curves. Therefore, is it possible to make key decisions for obstetricians in children with Small for Gestational Age (SGA) or with macrosomia, without knowing what the correct fetal mass is for a given population? It was stressed already in data from the Global Survey which showed that birthweight at 40 completed weeks of gestation varied between 2790 g in India and 3511 g in Algeria, which is well below the mean birthweight for the women from the European Continental Ancestry Group in the original study sample used to develop the ultrasound reference by Hadlock et al. [15]. An interesting study was presented by Nicolaides et al. [16], where reference population for birth weight charts were derived from all babies — born and those still *in utero*. They assumed that median for birth weight is the same as estimated fetal weight done shortly before birth. They believe strongly that the sonographic estimation is fully accurate. Is it true? This is what we are afraid of. As for us, the underlined strength of the study seems its weakness. The other study — INTERGROWTH 21st project [17] encompasses population from 8 countries from 5 different continents. The authors applied many exclusion criteria: maternal age younger than 18 or over 35, maternal height shorter than 153, body mass index ≥ 30 kg/m^2 or ≤ 18.5 kg/m^2, current smoker, medical history, birth of any previous baby weighing less than 2.5 kg or more than 4.5 kg, past 2 pregnancies ending in miscarriage, any previous stillbirth or neonatal death, or congenital malformation. However, if the week of pregnancy was allowed to be based on biparietal diameter done before 24 weeks and most of the living condition are completely different between each other in aspects of geography, ethnicity and most of all life standard, we have some doubts concerning usefulness of such birth charts.

Table 4. Fetal birth weight (50th percentile) with regard to the week of gestation for the consecutive years

Year	Gestational age [week]																			
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
2009	358	460	525	650	630	864	704	890	1038	1040	1137	1403	1850	1746	1926	2365	2710	2902	2886	3143	3182
2010	390	484	470	624	624	660	736	715	1087	950	1110	1294	1720	1694	2052	2100	2560	2800	2886	2988	3080
2011	422	474	471	550	697	896	830	870	835	1120	1255	1420	1552	2130	1993	2379	2702	2630	2928	2870	2903
2012	476	456	559	588	693	723	888	823	1233	1258	1235	1383	1696	1894	2220	2320	2701	2846	2930	3010	2804
2013	476	510	659	540	545	680	742	855	1135	1209	1230	1418	1726	2000	2252	2460	2790	2910	3060	3172	3126
2014	448	410	650	715	594	668	893	815	1078	1280	1373	1420	1810	1970	2250	2575	2792	2940	3030	3130	3321
2015	455	548	540	593	515	640	770	1075	878	1060	1138	1396	1516	2010	2323	2508	2799	2920	3063	3140	3349
2016	412	518	656	705	681	627	734	703	965	1160	1247	1560	1898	1900	2310	2506	2780	2920	3070	3140	2980
2017	358	364	622	702	696	745	889	744	1062	1233	1566	1514	1612	2045	2218	2490	2780	2920	3040	3101	3232

Analyzing the birth curves, we obtained, it seems extremely important to analyze them weekly. Comparison of the 10th percentile and 50 percentiles for both boys and girls shows some analogies. The largest differences in growth curves (centiles 10th vs. 50th) were obtained between weeks 22 and 34. After this time, the fetuses from the 10th percentile compared to the fetuses from the 50th percentile show a larger mass gain. It can be assumed that if there was an earlier delivery due to Intrauterine Growth Restriction (IUGR) or other underlying pathologies, then hypotrophic fetuses tend to make up for the mass required.

It can therefore be assumed that pregnancy is relatively safe during this period. Our previous feelings seemed different. The above-described group of hypotrophic fetuses with accelerated growth after 35 weeks may be just a group of healthy fetuses with constitutionally lower weight.

It is worth emphasizing the slowing down of weight gain for both sexes and for both percentiles analyzed above between 27 and 30 weeks and after 38 weeks of pregnancy. If the correct weight gain for the 10th percentile at week 33 is about 200 g for girls and boys, between 34 and 37 is for both sexes and centiles between 240 and 289 g, then for the following weeks it is about 150 g at week 39, in 40 — around 115, and in the following weeks it decreases drastically, especially for female fetuses. The above data indicate the need to create customized fetal growth charts with separate scales for boys and girls.

The improvement of living conditions, or maybe differently — the improvement of society's health, and at the same time a change in eating habits can have a significant impact on accelerating the secular trend in the birth weight of newborns. An analysis of our material, where we compared 50 percentiles over the 9 years studied, indicates a slight increase in newborns mass, especially those born after 35 weeks of pregnancy (Table 4).

The overriding goal of our research is to determine the birth curve of fetal masses that can be used in everyday clinical work. By rejecting extreme outliers and children born in moderate or poor condition, we excluded a significant proportion of obstetric pathologies that could have a significant effect on fetal weight. Despite the methodological conditions and imperfections in the construction of each of the scales, we have proposed one that we believe will allow us to safely conduct obstetric supervision, giving the best chance of giving birth to a healthy child.

When assessing the weight of the fetus, and at the same time maintaining common sense, we should have in mind the ACOG hint on the assessment of fetal weight in macrosomia. "An accurate diagnosis of macrosomia can be made only by weighing the newborn after delivery." [18].

CONCLUSIONS

Week-to-week weight gain equal to or higher than 200 g at week 33 and minimum 240 g between 34 and 37 weeks seems to be good predictor of favorable outcome in absence of the other pathologies for both sexes.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 748–754

ISSN 0017–0011

DOI 10.5603/GP.a2020.0126

The significance of maternal blood pregnancy-associated plasma protein A (PAPP-A) and free beta-subunit of human chorionic gonadotropin (β-hCG) levels for the risk assessment of fetal trisomy 18 during the first prenatal testing between 11 and 13^{+6} weeks of pregnancy

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ABSTRACT

Objective: The aim of the study was to evaluate the significance of the maternal blood level of pregnancy-associated plasma protein A (PAPP-A) and free beta-subunit of human chorionic gonadotropin (β-hCG), to estimate the risk of fetal trisomy 18 and their correlation with the assessment of nuchal translucency (NT) during the first prenatal testing.

Material and methods: Examinations of 93 pregnant women between 11 and 13^{+6} weeks of pregnancy were conducted, which included determination of β-hCG and PAPP-A concentrations in the maternal serum and ultrasound assessment of fetal nuchal translucency. Concentrations of biochemical parameters were expressed as multiples of median (MoM) for the appropriate gestational age. The risk assessment of trisomy 18 was analyzed using Astraia software. Pregnant women with a high (≥ 1:300) risk of trisomy 18 were offered a genetic amniocentesis with an examination of fetal karyotype. Twenty cases were healthy and 23 with trisomy 18.

Results: PAPP-A and β-hCG MoM values < 0.3 were found in 61% cases of fetal trisomy 18. In 26% of cases, PAPP-A and β-hCG MoM values < 0.2 were NT-independent risk factors for trisomy 18. There were no significant differences between groups with normal fetal karyotype (40%) and trisomy 18 (35%) in PAPP-A and β-hCG MoM 0.2–0.5 range.

Conclusions: Maternal free β-hCG MoM was found to change parallelly to fetal NT widening in case of trisomy 18 diagnosis. Maternal β-hCG and PAPP-A MoM results presented less then 0.2 might be used independently of NT widening in fetus for trisomy 18 risk evaluation. Above 0.2 for PAPP-A and β-hCG MoMs, fetal NT measurement was an requirment.

Key words: trisomy 18, pregnancy-associated plasma protein A (PAPP-A), free beta-subunit of human chorionic gonadotropin (β-hCG), nuchal translucency

Ginekologia Polska 2020; 91, 12: 748–754

INTRODUCTION

Prenatal screening based on non-invasive diagnostics allows to determine the risk of chromosome aberrations in the fetus and limit the use of invasive procedures [1–5]. Since the 1990s, the diagnostic scheme for pregnant women between 11 and 13^{+6} weeks of pregnancy has been in force, consisting of the evaluation of free beta-subunit of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum, as well as an ultrasonographic evaluation of fetal anatomy with measurement of nuchal translucency (NT), which may contribute to the detection of 94% cases of trisomy 18

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[5, 6–12]. Results of PAPP-A and β-hCG concentrations are converted into multiples of the median (MoM) by dividing the concentration of the examined parameter by the median for a given week of pregnancy. In a normal pregnancy, the concentration of PAPP-A increases exponentially, while the concentration of β-hCG, after the initial increase, decreases between 10 and 14 weeks of pregnancy [5]. A PAPP-A MoM decrease below 0.500 on average, along with β-hCG MoM decrease below 0.300 in maternal serum is considered to characterize trisomy 18 [7–20].

The discussion on the advisability of concurrent ultrasound and biochemical tests and their role in the diagnosis of chromosome aberrations has been an ongoing topic for over 20 years [5]. In this study, we analyzed the values of MoM for PAPP-A and β-hCG to be essential for calculating the risk of Edwards' syndrome (trisomy 18) occurrence and how fetal neck translucency ultrasound assessment influences the effectiveness of the syndrome diagnosis in the first trimester of pregnancy.

The aim of the study was to evaluate the significance of the maternal blood level of biochemical parameters: PAPP-A and free β-hCG, to estimate the risk of fetal trisomy 18 and their correlation with the assessment of NT during the first prenatal testing.

MATERIAL AND METHODS

The study included 93 pregnant women from the Prenatal Diagnostic Center of Gynecology and Obstetrics Hospital of Poznan University of Medical Sciences, who were assessed for the risk of chromosome aberrations occurrence between 11 and 13^{+6} weeks of pregnancy. The study included ultrasonographic evaluation of fetal anatomy, including NT and fetal heart function, as well as the measurement of PAPP-A and β-hCG concentrations in maternal serum.

Then in 43 pregnant women with increased risk of trisomy 18 (≥ 1:300) occurrence, genetic amniocentesis was performed, in accordance with the recommendations of the Ultrasonography Division of the Polish Society of Gynecologists and Obstetricians. The control group consisted of 50 pregnant women with a low (< 1:1000) risk of trisomy 18 occurrence. In the group with increased risk of trisomy 18 occurrence, the mean age was 34.17 years (from 19 to 43) and body mass index (BMI) — 23.79 (SD ± 3.35), while in the control group — 33 years (from 17 to 41) and BMI — 23.86 (SD ± 4.5) respectively, and did not differ statistically.

The ultrasound examination was performed using the Voluson E8. GE instrument (USA) and included evaluation of fetal anatomy and heart function, along with measurement of fetal NT as a marker of risk of chromosome aberrations (trisomy 21, 18, 13) — according to Fetal Medicine Foundation standards and based on recommendations of the Polish Society of Gynecologists and Obstetricians, Ultrasonography Division [1, 2, 5, 6, 21].

The concentrations of PAPP-A and β-hCG in maternal serum were evaluated using the immunofluorescence (time-delayed fluorescence) method, by means of the Delfia Xpress device (PerkinElmer Life and Analytical Sciences, Waltham, USA) [5, 12].

The calculation of the risk of chromosome aberrations, including biochemical and ultrasound markers, was performed using the ©2000–2016 Astraia software (Astraia Software Gmbh, Munich, Germany) [1, 5, 6, 21].

Amniocentesis was performed after taking about 20 mL of amniotic fluid. Amniocytes were cultured *in vitro* after obtaining cytogenetic slides, following by karyotype analysis utilizing the metaphase G-banding method.

The analysis of PAPP-A and β-hCG MoMs was carried out in both studied groups by dividing the concentration of the examined parameter by the median for a given week of pregnancy, with following ranges determined for them: 0.001–0.200; 0.201–0.300; 0.301–0.500; > 0.501.

The NT values of the fetuses in the group with trisomy 18, that were classified into the predefined ranges of: 1.0–2.0 mm, 2.1–3.0 mm, 3.1–5.0 mm and > 5.1 mm.

The statistical analysis was performed using the PQStat software. The variable distribution normality was analyzed by Shapiro-Wilk tests. Groups were compared with Mann-Whitney's U test, and analyzed with Pearson correlation coefficient and receiver operating characteristic (ROC) curves.

RESULTS

In 93 cases examined, an increased (≥ 1:300) risk of trisomy 18 occurrence was observed in 43 cases with genetic amniocentesis performed. Edwards syndrome was reported in 23 cases, while in 20 pregnant women the fetal karyotype was normal. The number of pregnant women over 35 years old was evaluated in the study groups: 60.87% of subjects with trisomy 18 diagnosed in newborns (n = 23), 75% of subjects at increased risk but normal fetal karyotype in newborns (n = 20) and 58% of controls (n = 50). In the analyzed groups, the pregnant woman's age had no significant impact on the results of the tests, and this parameter was not determined as an evaluation criterion.

In the group of patients with diagnosed fetal trisomy 18 (n = 23), lower values of β-hCG MoM and PAPPA-A MoM were observed, in comparison to patients with healthy fetuses (n = 70) (Fig. 1, 2).

Analysis of ROC curves for the assessed classifiers: PAPP-A MoM and β-hCG MoM allowed to distinguish pregnant women with fetal trisomy 18 in the examined material (Fig. 3, 4). PAPP-A MoM of 0.154 was indicated as a cut-off value with 98.6% sensitivity and 43.5% specificity estab-

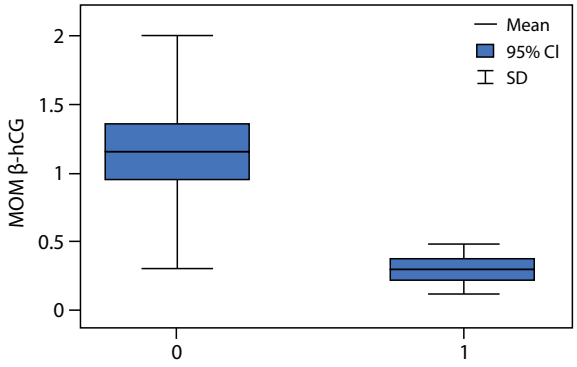

Figure 1. Comparison of free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) in the patient group with fetal trisomy 18 (1) and in the group with with a normal karyotype (0)

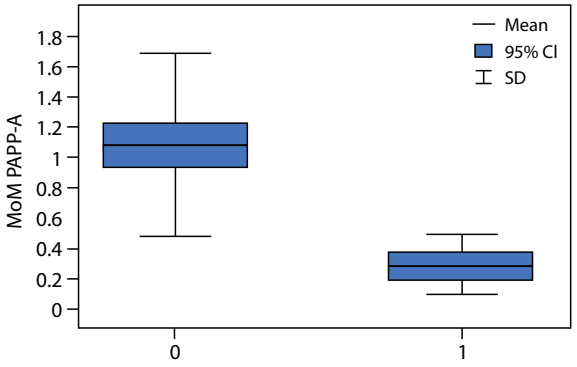

Figure 2. Comparison of associated plasma protein A (PAPP-A) multiples of median (MoM) in the patient group with fetal trisomy 18 (1) and in the group with a normal karyotype (0)

Table 1. Analysis of receiver operating characteristic curve parameters for pregnancy-associated plasma protein A (PAPP-A) multiples of median (MoM) and free beta-subunit of human chorionic gonadotropin (β-hCG) MoM — comparison of pregnant women with fetal trisomy 18 (n = 23) and pregnant women with a normal fetal karyotype (n = 70)

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	p
PAPP-A MoM	0.154	98.6	43.5	0.849	< 0.001
β-hCG MoM	0.369	95.7	69.6	0.892	< 0.001

AUC — area under the curve; p — statistical significance

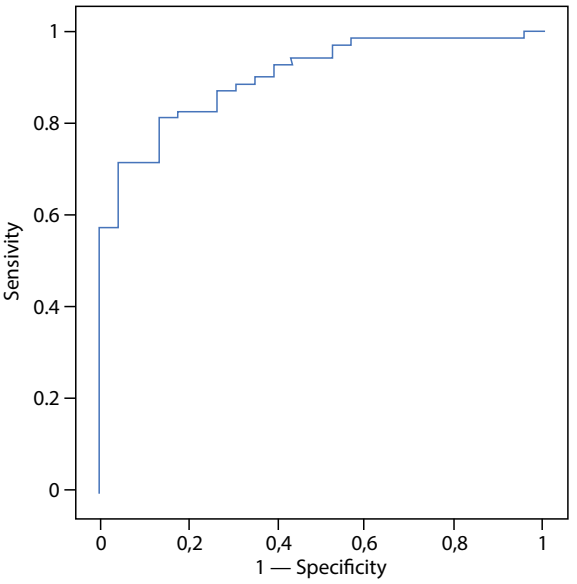

Figure 3. Receiver operating characteristic curve — sensitivity and specificity for associated plasma protein A (PAPP-A) multiples of median

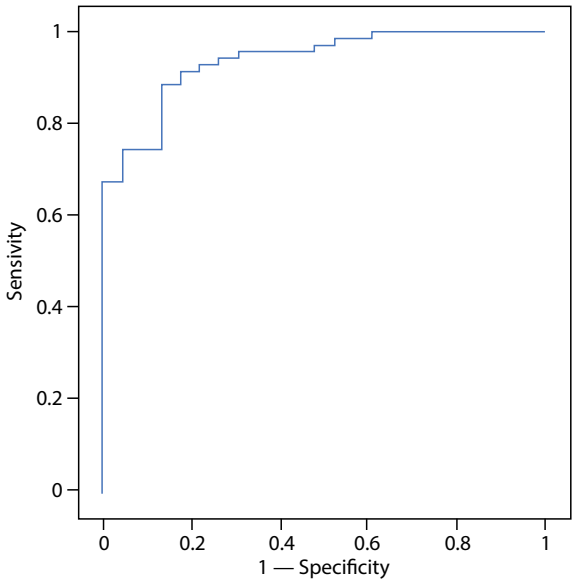

Figure 4. Receiver operating characteristic curve — sensitivity and specificity for free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median

lished. As the PAPP-A MoM decreases from the cut-off point, the sensitivity increases and the probability of diagnosing trisomy 18 increases. β-hCG MoM of 0.369 was assumed as a cut-off point with 95.7% sensitivity and 69.6% specificity established. A decrease of β-hCG MoM from the cut-off point increases the probability of diagnosing trisomy 18 (Tab. 1, Fig. 3, 4).

In the group of pregnant women with fetal trisomy 18, PAPP-A MoM concentration values were 0.277 ± 0.215 and were lower than the group with normal fetal karyotype — 0.720 ± 0.677 ($p < 0.05$).

The PAPP-A MoM analysis showed that the highest percentage of patients with low (< 1:1000) trisomy 18 risk was in the > 0.501 MoM range and was 96%. The high-

est percentage of patients with fetal trisomy 18 (52%) was in the < 0.2 PAPP-A MoM range, and the lowest (13%) in > 0.501 MoM, however, 35% of patients with fetal trisomy 18 and 40% of pregnant women with normal fetal karyotype were in the 0.2–0.5 PAPP-A MoM range, which indicate light differences between these groups (Fig. 5).

The mean value of the β-hCG MoM in the group of pregnant women with fetal trisomy 18 (0.298 ± 0.180) was lower than in the group with normal fetal karyotype (1.04 ± 1.232) ($p < 0.05$). Detailed analysis of β-hCG MoM showed that — similarly to PAPP-A MoM values — 13% of pregnant women with fetal trisomy 18 were presented with > 0.500 β-hCG MoM and 39.13% of them were presented with < 0.200 MoM of β-hCG. In the control group (described by < 1:1000 risk of fetal trisomy 18) 94% of pregnant women were found > 0.500 MoM of β-hCG and 6% of them were found within 0.301–0.500 MoM of β-hCG. However, 47.8% of pregnant women with fetal trisomy 18 and 35% of pregnant women with normal fetal karyotype were presented with 0.200–0.500 MoM range of β-hCG, adequately to similar results obtained for PAPP-A MoMs (Fig. 6).

The mean value of NT in the group of pregnant women with fetal trisomy 18 was 4.66 ± 2.514 mm (median 4.4) and was higher compared to the group with normal fetal karyotype, 1.836 ± 0.308 mm ($p < 0.05$) (median 1.8).

The correlation between NT and biochemical parameters PAPP-A MoM and β-hCG MoM was analyzed in the study groups.

The positive relationship between NT and β-hCG MoM ($R = 0.40$, $p = 0.012$) was observed only in pregnant women with fetal trisomy 18 (Fig. 7).

Detailed analysis of NT values within selected PAPP-A MoM ranges showed that in pregnant women with fetal trisomy 18 presented with < 0.200 PAPP-A MoM 26% of subjects were found NT > 3.1 mm and 26% of them were found NT < 3.1 mm, which may suggest higher diagnostic

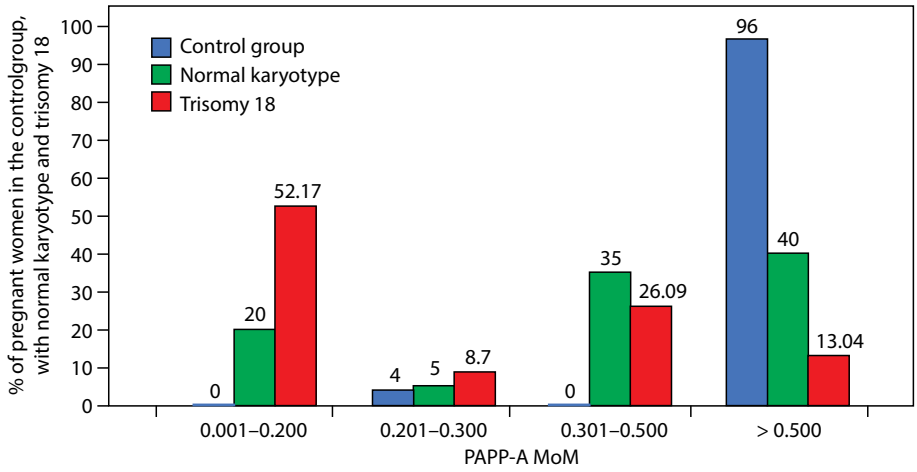

Figure 5. Percentage of pregnant women in the control group, a group with increased risk of trisomy18 but normal fetal karyotype and confirmed trisomy 18 within the analyzed associated plasma protein A (PAPP-A) multiples of median (MoM) ranges

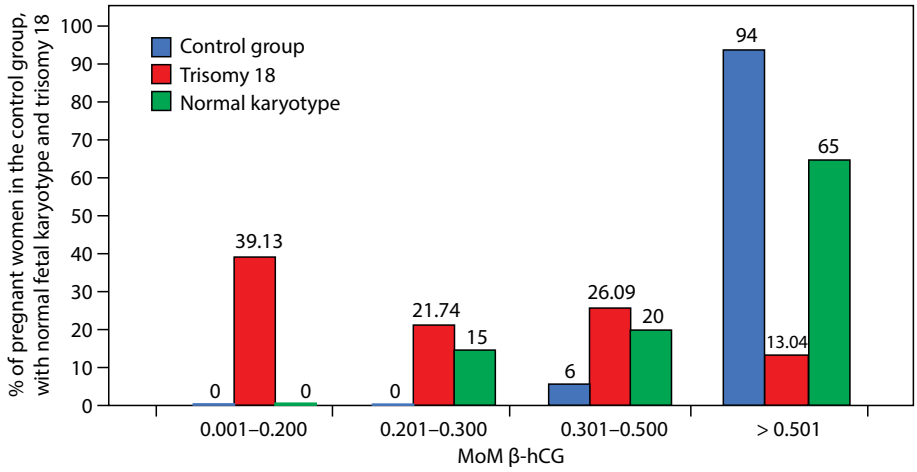

Figure 6. Percentage of pregnant women in the control group, group with increased risk of trisomy 18 but normal fetal karyotype and confirmed trisomy 18 in the analyzed free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) ranges

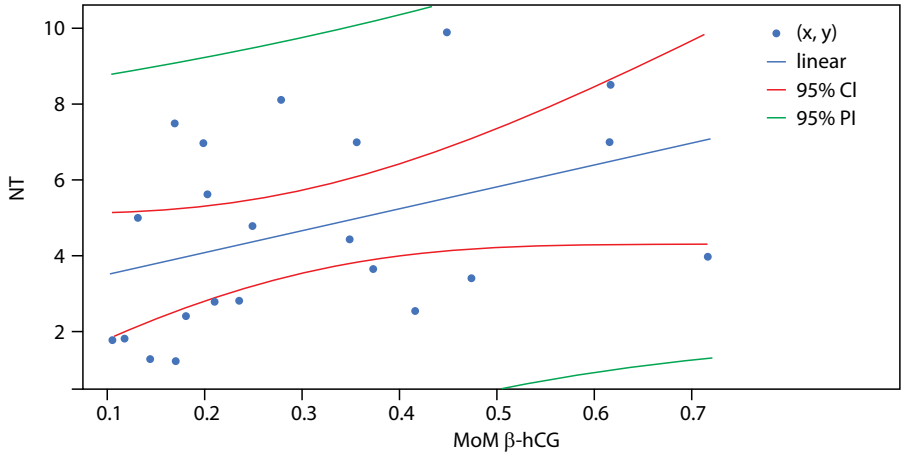

Figure 7. Correlation analysis between free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) and fetal nuchal translucency (NT) in a group with prenatally diagnosed fetal trisomy 18

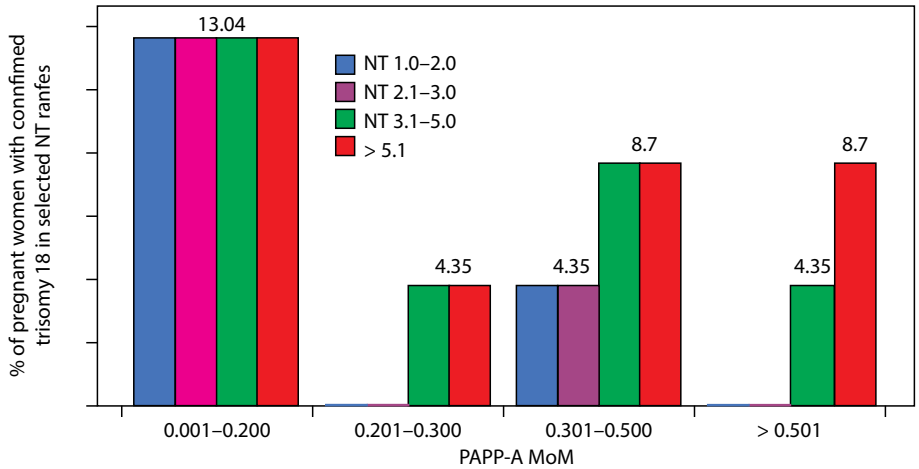

Figure 8. Nuchal translucency (NT) analysis in selected associated plasma protein A (PAPP-A) multiples of median (MoM) ranges in the group of pregnant women with diagnosed fetal trisomy 18

value of biochemical parameters in the analyzed range. However, in the PAPP-A MoM 0.201-0.300 range, all fetuses had a widening of nuchal translucency (> 3.1 mm). It was also observed that in the >0.501 PAPP-A MoM range (normal values), 13% of fetuses with trisomy 18 had a NT widening, which was the only test parameter that determined an increased risk of trisomy 18 (Fig. 8).

In the group of pregnant women with fetal trisomy 18 and with β-hCG MoM values < 0.200, nuchal transparency < 3.1 mm was observed in 26.1% of cases, and only 13% of fetuses had widening of the NT, which may indicate higher diagnostic value of biochemical parameters within this range. In the 0.201–0.500 MoM range, the majority (34.8%) of fetuses had widened NT. In contrast, in the > 0.500 β-hCG MoM range (normal values) in 13% of fetuses, a widening of the nuchal translucency was observed, which (similarly to PAPP-A) was the only test parameter determined the increased risk of trisomy 18 (Fig. 9). This indicates that the β-hCG MoM and PAPP-A MoM values are independent of the nuchal translucency values, but instead complement each other. It is also worth noting that the lower the MoM values of biochemical parameters PAPP-A and β-hCG, the lower the impact of neck translucency on the risk assessment of fetal trisomy 18. The analysis of median ranges of PAPP-A and free β-hCG demonstrates that none of the analyzed parameters is sensitive enough to give an unambiguous answer about the possibility of trisomy 18 occurrence.

DISCUSSION

Non-invasive, first-trimester prenatal screening encompassing a combination of maternal serum biochemistry assays (β-hCG and PAPP-A) and ultrasound-assessed NT enables accurate identification of approximately 90% of chromosomal abnormalities with 5% false positive results [5, 6, 8, 9, 20]. The components of the first trimester testing do not always correlate with each other in accordance with

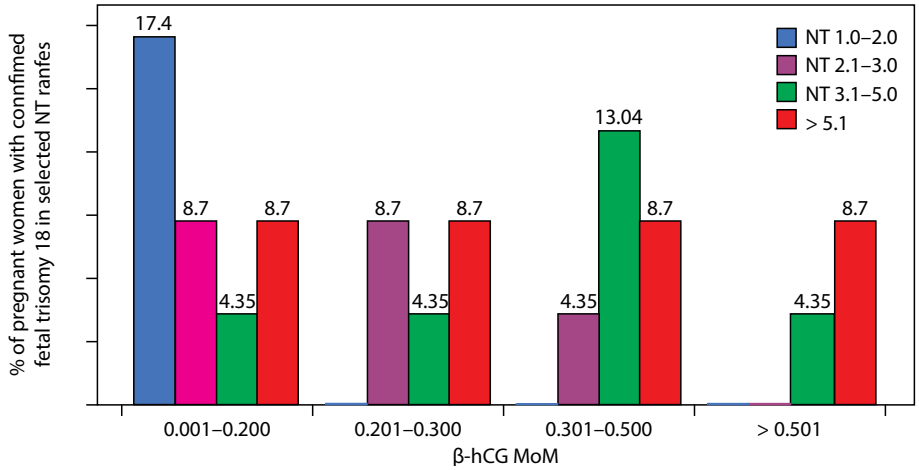

Figure 9. Nuchal translucency (NT) analysis in selected free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) ranges in the group of pregnant women with diagnosed fetal trisomy 18

accepted standards, but ultimately the test result indicates an increased risk of fetal malformation. The analysis showed that the mean β-hCG MoM and PAPP-A MoM in the group of pregnant women with fetal trisomy 18 did not differ significantly from the reports of other authors [7–20].

The distribution of trisomy 18 in particular β-hCG MoM and PAPP-A MoM ranges was analyzed. The study confirmed that the majority of cases of prenatally diagnosed trisomy 18 met the criteria for each of the examined parameters, 61% for < 0.300 PAPP-A and β-hCG MoM. The analysis also showed the diagnostic value of β-hCG and PAPP-A parameters in the < 0.200 MoM range being independent of the fetal nuchal translucency widening in the group of patients with fetal trisomy 18. However, it is worth noting that between the group of pregnant women with normal fetal karyotype results (40%) and one with trisomy 18 (35%) there were no differences in PAPP-A MoM and β-hCG MoM values within 0.200–0.500 MoM range. This not only indicates does not only indicate a small low diagnostic value of biochemical parameters in aforementioned PAPP-A and β-hCG MoM range, but emphasizes the role of the quality of testing. The method of storing blood intended for testing blood storage conditions prior to analysis and the quality of the devices the type of laboratory equipment for determining these parameters using to establish small differences of PAPP-A and β-hCG levels in these groups, may be necessary for the calculation of the final risk of fetal trisomy 18. This particularly applies to the concentration of β-hCG, which incorrect storage results in an increase in its levels, diminishing the diagnostic value of this parameter in the final risk assessment. The analysis of biochemical parameters in this range clearly showed that an additional ultrasound assessment of nuchal translucency is necessary for the correct assessment of the risk of trisomy 18 occurrence in the first prenatal examination.

The correlation of the examined biochemical parameters and measurement of nuchal translucency showed that the characteristic decrease in PAPP-A MoM and β-hCG MoM < 0.3 and widening of NT occurred only in 22% of pregnant women, similarly to reports of other authors [7–9, 15]. In 22% of cases of prenatally diagnosed trisomy 18, there was no widening of nuchal translucency, therefore only the biochemical parameters confirmed the effectiveness of the first trimester testing [20]. On the other hand, in 13% of cases the results of biochemical tests were normal, and only the widening of nuchal translucency decided about an increased risk in the test calculation. All cases with prenatally confirmed fetal trisomy 18 demonstrated an increased risk in the test. This confirms that the principle of the screening test is to select a group with an increased risk for Edwards syndrome occurrence [5].

CONCLUSIONS

Of the biochemical parameters analyzed in the study, maternal β-hCG MoM was found to change parallelly to fetal NT widening in case of trisomy 18 diagnosis. Taking into account the ranges of PAPP-A and β-hCG MoM values used in obstetric practice for the aneuploidy risk assessment, maternal β-hCG and PAPP-A MoM results presented less then 0.200 might be used independently of NT widening in fetus for trisomy 18 risk evaluation.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRIC

Ginekologia Polska
2020, vol. 91, no. 12, 755–763

ISSN 0017–0011

DOI 10.5603/GP.a2020.0130

COVID-19 infection in symptomatic pregnant women at the midpoint of the pandemic in Spain: a retrospective analysis

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ABSTRACT

Objectives: Determine the strengths and weakness of a symptomatic screening for COVID-19 in pregnant women. Analyze the clinical presentation, management, and outcomes. Design: Descriptive retrospective observational study. Setting: Mancha-Centro Hospital (Spain).

Material and methods: Population: Symptomatic pregnant women with confirmed diagnosis of COVID-19. Between the 12th of March and 17th of April 2020, all the symptomatic pregnancies were screened with diagnostic test for SARS-CoV-2. Data collection was done by reviewing the medical records and telephone interviews. Main outcome measures: Clinical characteristics, management, treatment, and obstetric and neonatal outcomes.

Results: Twenty patients with positive COVID-19 diagnostic test out of thirty-four suspected. The most common symptoms were fever (70%), cough (65%) and myalgia (35%). A unique symptom of presentation in 20% of cases. COVID-19 pneumonia was diagnosed in 30% by chest X-ray and one case had pulmonary embolism associated diagnosed by CT-Scan. Thromboprophylaxis was indicated in 16 out of 20 patients. Eight women finished their pregnancy during the observation period. Type of birth: 25% natural birth, 12.5% assisted vaginal delivery and 62.5% caesarean section. We had three severe cases, two of them with intensive care support. All neonates had negative test for COVID-19 infection.

Conclusions: We recommend universal screening of all pregnant woman for COVID-19 during the pandemic because of the limits of the symptomatic screening seen in this studio and the ratio of asymptomatic pregnancies with positive test for COVID-19 recently published.

Key words: COVID-19; pregnancy; screening; outcomes; symptoms; delivery

Ginekologia Polska 2020; 91, 12: 755–763

INTRODUCTION

The World Health Organization (WHO) designates COVID-19 as a new disease caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. This infection was defined as a pandemic the 11th of March of 2020 because of the alarming levels of spread and severity of the disease [2].

Spain is second in the world in number of infections with around 223,000 [3]. It is the first country in mortality with a ratio of 48.21 deaths per 100,000 population [3]. That means a lethality of the virus is 10.2%, reviewing the data collected until the 25th of April [3].

Our center, Hospital Mancha Centro, is one of the fourteen placed in a region of Spain called Castilla La Mancha. This area is one of the ¨hot spots¨ of the pandemic in Spain. With a mortality ratio of 174 deaths per 100,000 population, it represents 11 of each 100 deaths of COVID-19 in Spain, numbers found similar in the most devastated areas worldwide. Our hospital has one of the highest ratio of hospitalizations per population in Spain reaching the maximum of 4.34 hospitalized patients per 1,000 population the past 4th of April [4].

The management of the obstetric population during the pandemic is a big challenge because of the limited specific information (based in case series reports about pregnancy and COVID-19 [5–8]), the need for hospital monitoring during pregnancy and the necessary incomes for labour with the risk of infection that implies access to the hospital during the pandemic.

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What remains to be clear are the clinical characteristics of the obstetric population infected by SARS-CoV-2, the real risks of vertical transmission, course of medical treatment, obstetric management, the balance of risk/benefit of maternal breastfeeding and quarantine appliance to mother-newborn [9–15]. To clarify this gap, we present our descriptive study analyzing the clinical presentation, management and obstetric — neonates outcomes of symptomatic pregnant woman with confirmed positive test for COVID-19.

MATERIAL AND METHODS

Study design and population

This article presents a descriptive retrospective observational study on a non-probabilistic sample of symptomatic pregnant women with confirmed diagnosis of COVID-19 infection seen at a secondary hospital between the 12th of March and the 17th of April 2020.

The inclusion criteria were all pregnancies with a confirmed COVID-19 infection diagnosed by qualitative serological positive antibody test and/or detection of real-time reverse transcription polymerase chain reaction (RT-PCR) from a nasopharyngeal exudate sample. All patients were selected, in various pregnancy trimesters or puerperal period, for carrying out the diagnostic tests if they presented any of the following symptoms compatible with the SARS-CoV-2 infection: fever over 37.8ºC, cough, dyspnea, dysgeusia, anosmia, headache or myalgia. There were no exclusion criteriums.

Positive cases were closely monitored (by phone or in consultation) following the new protocol designed in the obstetrics department of the Mancha-Centro Hospital. All the complementary studies done (complete blood count, biochemistry, C-reactive protein, D-dimer, chest X-ray or CT scan) and the specific treatment measures were prescribed according to the clinical needs of each patient. Antenatal corticoids treatment for fetal lung maturation were indicated without changing our habitual protocol of preterm pregnancy up until 34 weeks. Thromboprophylaxis treatment with low molecular weight (LMW) heparin was indicated following the recommendations of the International Society of Thrombosis and Hemostasis [16] and the Spanish Society of Gynecology and Obstetrics [17].

Birth and puerperium of the positive patients was done in a specific isolated delivery room by an obstetric team formed by a midwife and a gynecologist, with personal protective equipment (PPE). The newborns were separated from the mothers after birth and studied by nasopharyngeal exudate sample, analyzed for SARS-CoV-2 by RT-PCR. If the companion of the pregnant women, studied by diagnostic test, was negative for COVID-19, they had the possibility to be with their newborn during the isolated hospital admission. Breastfeeding was discouraged until a negative result of the maternal RT-PCR. To maintain the stimulation during mother-child quarantine, breast pump extraction was performed, and breast milk was discarded.

Data collection

The information for the study was collected in two steps: review of the medical records and by telephone interview. Through the clinical history we reassessed the dates of birth and pregnancy, gestational age at birth, laboratory testing, needs of intensive care or hospitalization, treatment for the COVID-19 infection, complications (during pregnancy or birth and puerperium), type of anesthesia used during labor, method of birth, weight of the newborn, Apgar score, type of neonatal reanimation, skin to skin contact, type of cord clamping and lactation. All of the information was recorded in a personalized data form for each patient. After the first reviewing step, the clinical data was re-evaluated by telephone interview, making an overhaul of the items of the clinical history, and finishing with a re-examination of the compliance with treatment and recommendations given at discharge.

The starting date of COVID-19 infection was considered when the patient reported the beginning of the symptoms. The pneumonia diagnostic criteriums were based in radiologic findings and its severity defined by the recommendations of the Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) [18].

The severe cases follow at least one of the next clinical criteriums: severe respiratory distress, respiratory failure requiring mechanical ventilation or shock and organ failure requiring intensive care.

Statistical analysis

Continuous variables were expressed as means with their standard deviation (SD). For the quantitative variables we defined their absolute and relative frequencies. All the statistical analysis is descriptive and has been carried out using the statistical software SPSS v24.0.

No funding supports this study.

RESULTS

The total number of pregnant women with COVID-19 (confirmed by laboratory tests) was 20 out of 34 suspected cases with compatible clinic. The medium age of the patients was 34.9 years (SD = 5.00). Among all the infected pregnancies, 55% (11/20) were in their third trimester of gestation and 40% (8/20) gave birth during the study period.

Clinical features

The most common symptoms were fever over 37.8ºC present in 70% (14/20), cough in 65% (13/20) and myalgia in 35% (7/20). We studied the proportion of cases with

multiple symptoms compared to patients with one isolated symptom and we found that 20% (4/20) of them had one unique symptom (fever 2/20 and myalgia 2/20).

On the date of diagnosis of COVID-19 infection, the media of the laboratory tests were: Lymphocytes 1.66 10^3/µL (SD = 0.68), D-dimer 2.04 µg/mL (SD = 3.36) and C-Reactive Protein (CRP) 5.10 mg/dL (SD = 7.64).

The analysis of the relationship between symptoms and complementary studies showed that in the pregnancies with dyspnea the 66.7% (2/3) presented a radiologic diagnosis of COVID-19 pneumonia. When we compared the laboratory tests with each symptom, we found that the lowest value of lymphocytes with a media of 1.00 (SD = 0.10) was in the dyspnea patients while the highest values of D-dimer and CRP were in the pregnancies with myalgia with a media of 5.56 (SD = 6.03) and 9.80 (SD = 12.22) respectively.

All the clinical characteristics and their relationship with the complementary studies are shown in Table 1.

Regarding the transmission of the disease, 50% (10/20) of the pregnancies reported an unknown origin, 35% (7/20) had contact with a symptomatic person and 15% (3/20) assured that the risk of infection was inside the hospital.

The diagnosis was stablished by qualitative serologic tests in 12 of the 15 cases and by RT-PCR in 11 of the 13 patients in which they were done. In 10 patients we made chest X-rays, finding 60% (6/10) radiologic images compatible with COVID-19 pneumonia (Tab. 2., Fig. 1).

Hospital admissions were 40% (8/20) of the patients and related to labour or maternal disease. Most of the pregnancies 70% (14/20) hadn´t had any pregestational pathology. As remarkable risks, two patients with obesity (body mass index over 30 Kg/m^2) and another two smokers. Also, two patients had preeclampsia as a relevant pregnancy disease.

Prophylaxis of thromboembolic events with LMW heparin was prescribed in 80% (16/20) of the patients.

Obstetric results and newborn outcomes

Eight women, positive for COVID-19, finished their pregnancy during the observation period. Two of them before 34 weeks of gestation, three between 34–37 weeks and another three on term. From them, 87.5% (7/8) presented normal prenatal growth and morphological ultrasound. We found only one case of intrauterine growth restriction associated with severe preeclampsia.

Regarding the type of delivery, 25% (2/8) had a natural birth, 12.5% (1/8) an assisted vaginal birth and 62.5% (5/8) finished on cesarean section. There weren´t any cases of induction of labour on maternal benefit.

Five patients of the total of births were classified as non-severe COVID-19 infection. In this group we observed two natural and one assisted vaginal birth, all of whom used

Table 1. COVID-19 symptomatology in pregnant women and correlation with imaging and laboratory tests

Variables	Total	Temperature > 37.8°C	Cough	Dyspnea	Dysgeusia	Anosmia	Headache	Myalgia
		% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
		70.0 (14)	65.0 (13)	15.0 (3)	20.0 (4)	30.0 (6)	25.0 (5)	35.0 (7)
Number of combined symptoms	Total % (n)	n	n	n	n	n	n	n
1	20.0 (4)	2	0	0	0	0	0	2
2	25.0 (5)	3	4	0	1	2	0	0
3	40.0 (8)	6	6	2	1	2	3	4
4	5.0 (1)	1	1	0	0	0	1	1
5	10.0 (2)	2	2	1	2	2	1	0
Chest X-ray	Total % (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
None	50.0 (10)	50.0 (7)	53.8 (7)	0.0 (0)	25.0 (1)	50.0 (3)	80.0 (4)	71.4 (5)
No signs of COVID	20.0 (4)	21.4 (3)	30.8 (4)	33.3 (1)	50.0 (2)	33.3 (2)	0.0 (0)	0.0 (0)
COVID pneumonia	30.0 (6)	28.6 (4)	15.4 (2)	66.7 (2)	25.0 (1)	16.7 (1)	20.0 (1)	28.6 (2)
	Total Mean (DE)	Mean (DE)	Mean (DE)	Mean (DE)	Mean (DE)	Mean (DE)	Mean (DE)	Mean (DE)
Lymphopenia ^3/µL	1.66 (0.68)	1.59 (0.69)	1.67 (0.69)	1.00 (0.10)*	1.93 (0.72)	1.90 (0.56)	1.92 (0.79)	1.67 (0.98)
D- Dimer µg/mL	2.04 (3.36)	2.18 (3.91)	2.42 (4.11)	0.65 (0.58)	0.77 (0.68)	0.77 (0.68)	0.35 (0.01)	5.56 (6.03)*
C Reactive Protein (CRP) mg/dL	5.10 (7.64)	6.87 (8.71)	6.51 (9.38)	6.53 (5.69)	0.60 (0.37)	0.60 (0.37)	4.80 (5.37)	9.80 (12.22)*

*Higher value of analytic result according to each symptom

Table 2. Diagnosis, treatment and clinical evolution for all the pregnancies and severe cases

	Total % (n = 20)	Puerperium (n = 8)	Severe cases (n = 3)
Variables	% (n)	% (n)	% (n)
Age Media (DE)	34.9 (4.90)	35.3 (5.97)	36.0 (5.57)
Infectious transmission			
Unknown	50.0 (10)	62.5 (5)	0.0 (0)
Symptomatic family	35.0 (7)	12.5 (1)	33.3 (1)
In-Hospital	15.0 (3)	25.0 (2)	66.7 (2)
Diagnosis			
Rapid test positive	80.0 (12/15)	75.0 (6/8)	100 (3/3)
RT PCR positive	85.0 (11/13)	50.0 (4/8)	N.A. (0/0)
Pregestational morbidity	40.0 (8)	37.5 (3)	33.3 (1)
Gestational morbidity	30.0 (6)	62.5 (5)	66.7 (2)
Hospitalization	40.0 (8)	100.0 (8)	100 (3)
Intensive care	10.0 (2)	25.0 (2)	66.7 (2)
Respiratory distress on labor	5.0 (1)	12.5 (1)	33.3 (1)
Chest X-Ray			
None	50.0 (10)	0.0 (0)	0.0 (0)
No signs of COVID-19	20.0 (4)	25.0 (2)	0.0 (0)
COVID-19 pneumonia	30.0 (6)	75.0 (6)	100 (3)
Treatment			
Symptomatic	15.0 (3)	0.0 (0)	0 (0)
Hydroxychloroquine	30.0 (6)	75.0 (6)	100 (3)
Antibiotics	25.0 (5)	62.5 (5)	66.7 (2)
Oxygen support	15.0 (3)	20.0 (2)	66.7 (2)
Lopinavir/ritonavir	15.0 (3)	20.0 (2)	66.7 (2)
Interferon	10.0 (2)	20.0 (2)	66.7 (2)
LMWH	80.0 (16)	87.5 (6)	100 (3)
Lymphopenia 3/µL Media (DE)	1.66 (0.68)	1.30 (0.67)	0.80 (0.36)
D-dimer µg/ml Media (DE)	2.04 (3.36)	2.58 (4.07)	4.73 (6.76)
CRP mg/dl Media (DE)	5.10 (7.64)	6.96 (9.07)	15.63 (10.25)

N.A. — not accomplished

epidural anesthesia and presented normal oxygen saturation with values over 95% in pulse oximetry during labour. In this non-severe group, we didn´t find any episodes of intrapartum desaturation that indicated cesarean section and internal fetal heart monitoring was avoided. The indication of the two cesarean sections were an obstructed labour and a programmed cesarean section on maternal request for denied vaginal birth after cesarean.

The three rest of cesarean sections were done in severe cases of COVID-19 infection which we present below.

The first case was a pregnant woman of 28 weeks with severe pneumonia upon admission and had a negative response to medical treatment. She needed invasive mechanical ventilation because of an oxygen saturation less than 90%. At that point we completed her pregnancy by cesarean section with general anesthesia and posterior intensive care admission (Fig. 1).

The second case was an asymptomatic pregnancy of 34 weeks with preterm rupture of membranes. A cesarean section was indicated because of breech presentation and done with spine anesthesia. During her puerperium she started with a rapid onset of severe respiratory distress with mild symptoms of COVID-19 infection. Without other thrombotic risk factors more than the cesarean section, she

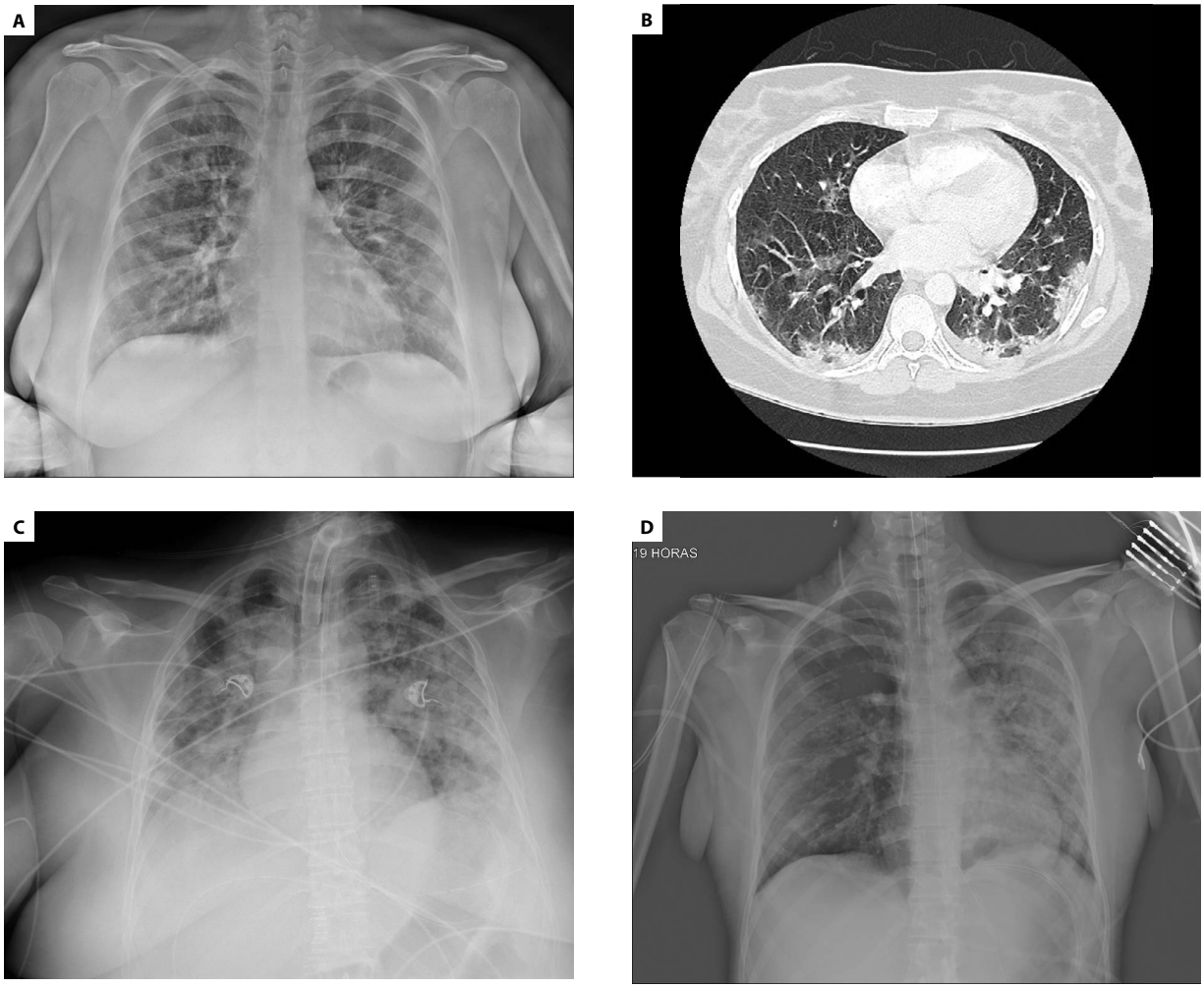

Figure 1. Radiologic findings of severe cases; (**A–B**) 29 years. 34 + 3 weeks. Pulmonary embolism and pneumonia at the third day of puerperium; (**C**) 47 years. 29 weeks. Severe preeclampsia. COVID-19 pneumonia. Intensive care; (**D**) 32 years. 29 weeks. COVID-19 pneumonia. Intensive care

had a pulmonary embolism combined with COVID-19 pneumonia diagnosed on CT scan (Fig. 1).

The third case was a high-risk pregnancy by in vitro fertilization with egg donation of an obese woman at 47 years of age. At 28 weeks of pregnancy she needed admission for hypertensive medical control and was diagnosed of severe preeclampsia. During her hospital stay she started with a combination of urologic infection and respiratory symptoms being diagnosed with a SARS-CoV-2 respiratory infection and urological sepsis. At 29 weeks she finished via cesarean section with general anesthesia on maternal benefit because of a poor response to medical treatment with bad clinical and analytic evolution and she was taken to the intensive care unit (Fig. 1). On the 5th day of puerperium, she started with an early multiorgan failure with a massive postpartum bleeding, so an abdominal hysterectomy was done.

All the severe COVID-19 infection patients were managed with prophylactic heparin treatment, hydroxychloroquine and lopinavir/ritonavir. The pregnancies less than 34 weeks also received antibiotic treatment, oxygen support, interferon and antenatal corticosteroids for fetal lung maturation.

The severe group presented a higher degree of analytic changes with the next medias: D-dimer 4.73 µg/mL, CRP 15.63 mg/dL and lymphocytes 0.8 10^3/µL.

After birth, 87.5% (7/8) of the cases had an early cord clamping. One case of skin to skin contact was allowed because the infection wasn't known until the 3rd day after birth.

All patients with a vaginal birth were accompanied by a relative with contact restrictions and PPE. All the non-severe cases were discharged without any puerperal complications on the first 24 h postpartum.

About the neonatal outcomes we founded a medium weigh of 2560.8 g (SD = 980.9), medium Apgar score at first minute of 8.25 (SD = 1.39) and at fifth minute of 9.38 (SD = 0.92). About the type of reanimation of the newborn was type I in 12.5% (1/8) and type III in 37.5% (3/8) and no reanimation in 50% (4/8). Breastfeeding was mainly maternal

63.5% (5/8), one being directly from the maternal breast and the remaining four stimulated by breast pump until a negative COVID-19 result. All the neonates were accompanied by an asymptomatic relative with negative result of RT-PCR COVID-19. No clinical or serologic evidence of vertical transmission was noticed, and no neonatal deaths were reported.

The most relevant details of the obstetric and newborn results are resumed in the Table 3.

Table 3. Obstetric and neonatal outcomes; n = 8

Variables	Total % (n)
Gestational age	
< 34 weeks	25.0 (2)
34–37 weeks	37.5 (3)
> 37 weeks	37.5 (3)
Induction of labor	12.5 (1)
Intrapartum fever	12.5 (1)
Oxygen saturation on labor	
> 95%	75.0 (6)
90-95%	12.5 (1)
< 90%	12.5 (1)
Type of anesthesia	
Spinal	25.0 (2)
Epidural	50.0 (4)
General	25.0 (2)
Type of birth	
Eutocic delivery	25.0 (2)
Assisted vaginal delivery	12.5 (1)
Caesarean section	62.5 (5)
Puerperium	
Pneumonia	75.0 (6)
Pulmonary embolism	12.5 (1)
Postpartum hemorrhage	25.0 (2)
Newborn weigh Media (DE)	2680.8 (980.9)
Early cord clamping	87.5 (7)
Skin to skin contact	12.5 (1)
Apgar minute 1 Media (DE)	8.25 (1.39)
Apgar minute 5 Media (DE)	9.38 (0.92)
Type of neonatal reanimation	
No reanimation	50.0 (4)
Type I	12.5 (1)
Type II	0 (0)
Type III	37.5 (3)
Type of breastfeeding	
Artificial	37.5 (3)
Maternal with breast pump until negative	25.0 (2)
Direct maternal	12.5 (1)
Direct and breast pump	25.0 (2)

DISCUSSION

Main findings

Our study demonstrates a relevant percentage of 20% (4/20) of pregnancies with just one symptom at the time of diagnosis. We observed a 60% (6/10) of radiologic findings of COVID-19 pneumonia in those patients studied with chest X-ray.

During labour, two cesarean sections were done to improve maternal oxygenation in two patients with severe clinical state. The remaining 75% (6/8) didn´t have any respiratory distress.

Thromboprophylaxis treatment with (LMW) heparin was indicated in 80% (16/20) of the pregnancies regardless of the symptoms and D-dimer. However, we report a case of pulmonary thromboembolism during puerperium on a COVID-19 pneumonia.

The main recommendations about newborn management as early cord clamping and mother–neonate isolation where done in 87.5% (7/8) cases. All neonates were accompanied by an asymptomatic relative with negative result of RT-PCR COVID-19.

The postpartum period went without complications in 62.5% (5/8) so the mother could have an early hospital discharge in the first 24 hours.

Strengths and limitations

This study is one of the first case series reports published from Spain about the pandemic COVID-19 in pregnant women with symptoms and positive RT-PCR. Our hospital is part of a region in Spain, called Castilla La Mancha, with one of the highest mortality rates (113 deaths per 100,000 population). It was one of the most affected areas worldwide just surpassed by Madrid (Spain), Lombardy (Italy) and New York (USA) with 118, 126 and 120 deaths per 100,000 each respectively, on the 25th of April [19].

Currently, there are few completed studies published with the recompilation of symptoms, information about clinical exposure, details about the pregnancy and postpartum treatment as well as the obstetrical complications and critical care of the severe cases. We have detailed the type of quarantine, anesthesia, oxygen saturation during labour and breastfeeding of the newborns.

It must be taken into account that this study has excluded all pregnant woman with symptoms and compatible radiological diagnose of COVID-19 pneumonia without

confirmation via positive test for SARS-CoV-2. Consequence of the limitations of availability and diagnostic capacity of the tests for COVID-19 in Spain.

Thromboprophylaxis in pregnant women during the pandemic has been one of the main challenges of the treatment. During the period of the study, different views and protocols had been published and that is why just 16 out of 20 patients of our cohort had received LMW heparin.

Interpretation

The optimal management of the pregnant women during the COVID-19 pandemic has been a big challenge for our obstetric department. At the beginning of the pandemic we needed to decide the suspicious symptoms in pregnant patients and the type of diagnostic tests that apply to them. After that, design the specific pathways of isolation for the maternal and neonatal care, doing staff training on the use of the PPE. Additionally, we have developed a hotline for the patients to report their symptoms before their arrival to the hospital, following the recommendations of the Center for Disease Control and prevention (CDC) [20].

The need for implementation of a universal screening for all the pregnant women admitted to the labour room has been one of the main discussions of management. In hospitals where it has been done, the prevalence of COVID-19 in asymptomatic patients published is 13.5% [21] and 13% [22]. In our department, during the period of the study, we have conducted a symptomatic screening excluding from our result ten patients with compatible symptoms but negative RT-PCR. We should take into account that we were limited by the local availability of the test for COVID-19 and their diagnostic accuracy. In our hospital each nasopharyngeal test was done with the kit GeneXPert SARS-CoV-2, giving the results of sensitivity and specificity of 35% and 96%. We also used an IgM/IgG rapid detection test. This kind of test could be criticized for an initial screening test because of the window period [23] but in our study, as it was used in symptomatic patients, it was useful. As a result of these factors, we decided to change since the last 20th of April to a universal screening of all the pregnancies in our obstetric department.

About the symptoms analyzed, most of our pregnancies had fever and cough as their more common symptoms, like the results of Guan et al. and Wang et al. [24, 25]. We have considered anosmia and ageusia as compatible symptoms for COVID-19 [26–28], reporting in our series 4/20 pregnancies with ageusia and 6/20 with anosmia.

Thromboprophylaxis on the pregnant women with COVID-19 is recommended during pregnancy and puerperium with LMW heparin, even though the studies are limited and presently unknown if the prophylactic or therapeutic anticoagulation has any impact in the result of the infection [15]. Even though the level of D-dimer could be normally higher during pregnancy, we consider COVID-19 infection and a higher level of D-dimer as the main thrombotic factors. They are related to a higher risk of venous thromboembolism, severity and mortality of the disease [25, 29, 30]. In our study the severe cases had a D-dimer media of 4.73 µg/mL (SD = 6.76) and a case of pulmonary embolism has been diagnosed although the treatment with 40 mg of heparin each 24 hours.

The severity and mortality of the disease had been related to cardiovascular disease, diabetes, hypertensive disorders and obesity (BMI ≥ 30) [31, 32]. In pregnant women with COVID-19, we should consider the impact on risk of hyperglycemias, weight gain and preeclampsia. Nowadays, there are no studies proving the relationship in pregnant women, but it is logical to suppose that those risk factors will worsen the pregnancies prognosis. The published studies report similar morbidity to our series [5, 6, 8, 33] and the reports of Breslin et al. [5] involving two maternal intensive care admissions of mothers with high BMI (> 35) leads one to question if COVID-19 increases the risk of severe morbidity in high risk pregnancies.

We consider that the correct management of these high-risk pregnancies is the key and should be done by established protocols and with preventive measures like the prophylaxis with aspirin [10].

CONCLUSIONS

The symptomatic screening for COVID-19 on pregnant women is limited by two main factors: the percentage of pregnancies with just one symptom at hospitalization, in our cohort a 20%, and by the local availability and reliability of the diagnostic test during the pandemic. Combining this information with the results reported in the literature of asymptomatic pregnancies with positive diagnostic test for COVID-19 and the implications of the misdiagnosing, we recommend to start routine testing for SARS-CoV-2 in obstetric patients at their hospitalization, regardless of their symptoms or the reason for admission.

The symptoms and severity of the infection among pregnant women is normally between mild and moderate, so we consider appropriate the vaginal birth excepting those patients with bad clinical response or respiratory distress during labour.

We recommend doing an big effort on the information for patients and a share decision making about the mother–newborn isolation in positive cases of COVID-19 since the fear can lead a pregnant mother to minimize or even deny the suspicious symptoms.

Conflict of interests

The authors declare no competing interests. Completed disclosure of interest forms are available to view online as supporting information.

Contribution to authorship

EOM and MPG conceived the study question. MPG and RHP collected the data. AHM performed the statistical analyses. EOM, MPG and RHP drafted the first version of the manuscript and it was revised and approved by APP and ERR. AHM wrote the abstract.

Details of ethics approval

Local IRB (Institutional Review Board) was consulted, being this article was exempt from the need for approval because of the non-interventional, observational study and the sanitary emergency. This work has also the approval of the medical director of our center and each pregnant woman was informed about the study and gave their consent to use their clinical information. The ethics principles have been respected. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgments

We would like to mention the disposition and help of Jesús Villajos, our external epidemiologist consultant. Also, we would like to thank Mercedes & John Hussey Pailos for the external review of this text, your help has been a key in the final review of the article. A very loving and special thanks to Dr. Pola for her example of courage and her ability to overcome adversity. We wish to thank the health providers for their wisdom, compassion and courage demonstrated in these dramatic days. A special mention to all who have left this world without being able to say goodbye as they deserved as a result of this pandemic.

Funding

No funding.

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VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 764–768

ISSN 0017–0011

DOI 10.5603/GP.a2020.0131

Expression and significance of Parkinson disease protein 7 in placental, serum and umbilical cord blood in preeclampsia

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ABSTRACT

Objectives: To study the role of changes in the expression of human Parkinson's disease protein 7 (PARK7/DJ-1) in preeclampsia.

Material and methods: We selected 120 gravidas, including 60 cases of severe preeclampsia group and control group, and divided into early onset preeclampsia group (< 34 weeks), late onset preeclampsia group (≥ 34 weeks) and control group according to the onset of pregnancy. The expression level of DJ-1 was detected by ELISA. The expression level of DJ-1 in placenta tissue of gravidas was detected by Western-blot and RT-PCR.

Results: The level of DJ-1 in serum and cord blood of preeclampsia group was higher than that of control group. The relative level of DJ-1 protein and DJ-1 mRNA in placenta tissue of preeclampsia group was higher than that of control group.

Conclusions: The expression level of DJ-1 in serum, umbilical cord blood and placenta tissue increased in preeclampsia patients, suggesting that DJ-1 may take part in the pathophysiology process of preeclampsia.

Key words: DJ-1; preeclampsia; placenta; cord blood; oxidative stress

Ginekologia Polska 2020; 91, 12: 764–768

INTRODUCTION

Preeclampsia (PE) is a specific disease in pregnancy. It is the main cause of the incidence and death of gravidas and neonates [1]. The global morbidity is about 3% to 5% [2], and about 18% of pregnancy related deaths are caused by preeclampsia [3]. However the pathophysiological mechanisms of PE remain poorly known. Zhou et al. [4] showed that excessive oxidative stress leads to uterine spiral artery remodeling and superficial placental implantation, which can lead to the onset of preeclampsia. DJ-1, also known as PARK 7, is a multifunctional protein implicated in familial Parkinsonism, Oxidative stress is the main function of DJ-1. Kwon et al. [5] found DJ-1 in placenta of patients with preeclampsia is widespread and high expression, and mainly localized in trophoblast cells, which is closely related with the pathogenesis of preeclampsia, that may be involved in the pathogenesis of DJ-1 in preeclampsia. Thus, the aim of this study was to determine the difference of DJ-1 expression in serum, umbilical cord blood and placenta of women with normal and preeclamptic pregnancies.

Preeclampsia (PE) is a specific disease in pregnancy. It is the main cause of the incidence and death of gravidas and neonates. The global morbidity is about 3% to 5%, and about 18% of pregnancy related deaths are caused by preeclampsia. However, the changes of preeclampsia are complex and uncertain. The etiology and pathogenesis are not clear. In recent years, the study found that excessive oxidative stress causes uterine spiral arteries remolding and placental shallow implantation, which can lead to the onset of preeclampsia. Parkinson disease protein 7 (PARK7/DJ-1) is a member of the ThiJ/PfpI/DJ-1 superfamily, antioxidant stress is the main function of DJ-1. Kwon HS, etc., found that DJ-1 is widely distributed and highly expressed in placental tissues of preeclampsia, and is mainly located in the chorionic trophoblast cells closely related to preeclampsia, and that DJ-1 may participate in pathogenic process of preeclampsia. In this study, we detected the changes in expression of DJ-1 in gravidas' serum, umbilical cord blood and placenta tissues, and investigation the role of it in the pathogenesis of preeclampsia.

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Table 1. Subject clinical characteristics (mean ± SD)

	Early-onset preeclampsia	Late-onset preeclampsia	Early control group	Late control group
Age [years]	32.7 ± 5.3	30.2 ± 5.2	29.7 ± 4.2	30.9 ± 3.6
Gestational age [weeks]	32.6 ± 2.9	38.0 ± 1.8	31.4 ± 3.0	38.9 ± 1.1
SBP [mm Hg]	165.4 ± 15.4	162.3 ± 16.8	115.0 ± 9.5	117.5 ± 9.8
DBP [mm Hg]	104.5 ± 10.7	100.3 ± 9.4	70.2 ± 6.7	74.1 ± 6.9
MAP [mm Hg]	124.8 ± 11.1	121.0 ± 10.9	85.1 ± 6.2	88.6 ± 6.8
Pre-pregnancy BMI [kg/m^2]	22.4 ± 4.1	22.4 ± 4.1	20.6 ± 3.1	22.0 ± 2.3
Pregnancy BMI [kg/m^2]	28.3 ± 4.3	28.8 ± 3.8	26.4 ± 2.8	28.0 ± 2.7

BMI — body mass index; DBP — diastolic blood pressure; SBP — systolic blood pressure; MAP — mean arterial pressure

MATERIAL AND METHODS

Patients

Sixty women with severe preeclampsia and 60 normal pregnant women were enrolled. They were admitted to Fujian Provincial Maternity and Children Hospital, affiliated hospital of Fujian Medical University from November 2015 to March 2017 for delivery. They were divided into four groups: early-onset group, late-onset group, early control group and late control group. PE was defined as hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mm Hg after 20 weeks' gestation) and proteinuria (≥ 300 mg in a 24 h urine collection or one dipstick measurement of ≥ 1+) [6]. The clinical situations are summarized in Table 1. Informed consent was signed by each participant, and the study was approved by the ethics committee of Fujian Medical University.

Specimen collection and enzyme-linked immunosorbent assay

The specimen collocation was similar to Yan et al. [7]. Blood samples were collected from the peripheral veins of all the women. Umbilical cord blood was collected from the umbilical vein immediately after delivery. Placental tissue was collected from the root of the umbilical cord immediately after cesarean section. The DJ-1 ELISA Kit was purchased from XiTang (Shanghai, China). The measurement was performed following the manual.

Western blot

Protein from the placental tissue was transferred to PVDF membranes and incubated with monoclonal rabbit anti-GAPDH (1:1500; Abcam, UK) antibodies overnight at 4°C, then followed by incubation with HRP-conjugated secondary antibodies. After extensive washing, proteins of interest were detected by enhanced chemiluminescence system (ECL, Thermo Scientific, UK) and quantified by densitometry using Quantity One.

RT-PCR

Total RNA was extracted from placental tissue with a TRIzol reagent kit (Invitrogen, CA, USA). DJ-1 mRNAs and the internal standard (glyceraldehydes 3-phosphate dehydrogenase (GAPDH)) expressions were quantified by real-time polymerase chain reaction (PCR). Primer sequence was as follows, sense 5′-GTAGCCGTGATGTGGTCATTT-3′, antisense 5′-CTGTGCGCCCAGATTACCT-3′. PCR assays were performed following the manual. The results were normalized to GAPDH expression levels.

Statistical analysis

Statistical analysis was performed using one-way ANOVA with Levene test and unpaired t-test using SPSS18.0. Pearson correlation analysis was used to analyze the correlations. $P < 0.05$ was considered as significant.

RESULTS

Maternal serum DJ-1

As shown in Figure 1A, maternal serum DJ-1 was 1.6040 ± 0.465 ng/L in the early-onset preeclampsia group and 1.2810 ± 0.419 ng/L in the late-onset preeclampsia group. Preeclampsia DJ-1 was significantly higher compared to 1.0698 ± 0.333 ng/L in the early control group and 1.0625 ± 0.329 ng/L in the late control group ($p < 0.05$). between the early-onset preeclampsia group and the late-onset preeclampsia group was significantly different.

Umbilical cord blood DJ-1

As shown in Figure 1B, the DJ-1 umbilical cord blood level in the early-onset preeclampsia group is significantly different compared to the late-onset preeclampsia group. Both the early-onset preeclampsia group (1.3928 ± 1.036 ng/L) and the late-onset preeclampsia group (1.4544 ± 0.695 ng/L) had significantly higher mean DJ-1 compared with the late control group (0.9764 ± 0.390 ng/L).

Protein Expression of DJ-1 in placental tissue

As shown in Figure 2, the DJ-1 expression in placenta was significantly higher in the early-onset preeclampsia group (1.15 ± 0.26), and late-onset preeclampsia group (1.50 ± 0.45), compared to late control (0.82 ± 0.47; $p < 0.05$).

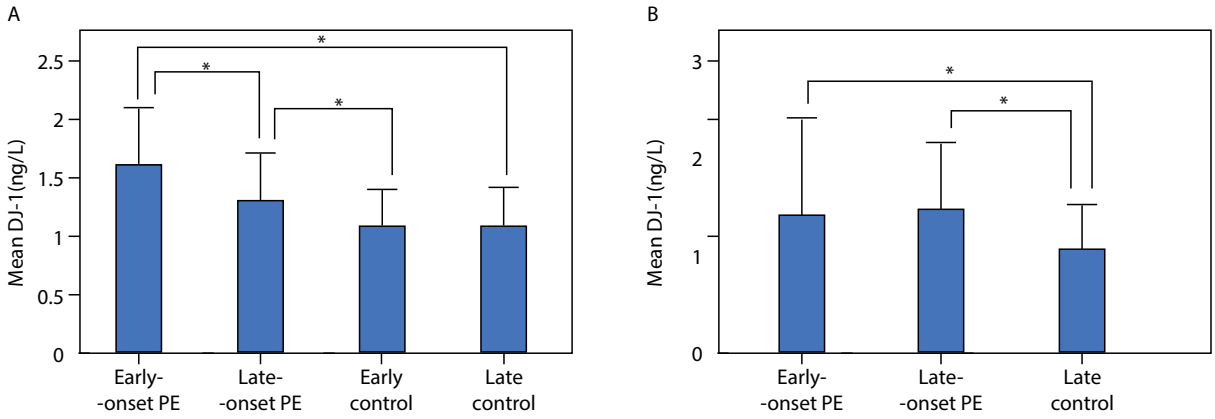

Figure 1. Mean DJ-1 in (**A**) maternal serum and (**B**) umbilical cord blood. PE, preeclampsia

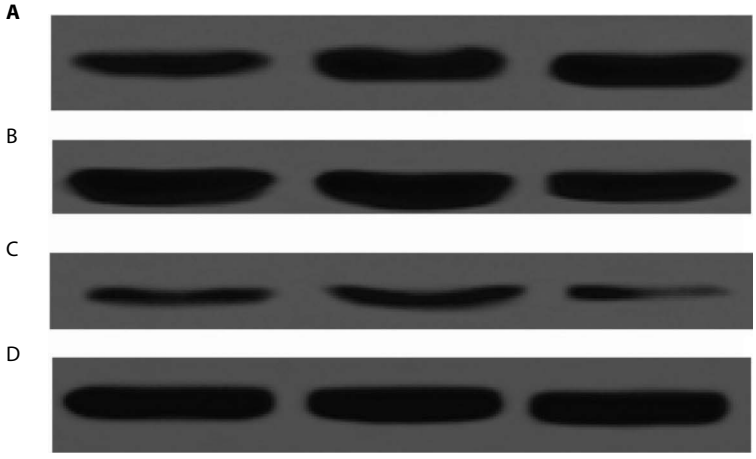

Figure 2. Expression of DJ-1 in placenta tissue for (**A**) early-onset preeclampsia, (**B**) late-onset preeclampsia, (**C**) the late control group and (**D**) β-actin

MRNA expression of DJ-1 in placental tissue

As shown in Figure 3, the mRNA expression of DJ-1 in placenta was significantly higher in the early-onset preeclampsia group (3.315 ± 1.08), and late-onset preeclampsia group (3.608 ± 1.87), compared to late control (1.5463 ± 0.409).

Correlation analysis

The umbilical cord blood DJ-1 in the early-onset preeclampsia group and late-onset preeclampsia group correlated negatively with Neonatal birth weight ($r = -0.448$ and $r = -0.648$, $p < 0.05$). There is no significant correlation between the control groups (Fig. 4)

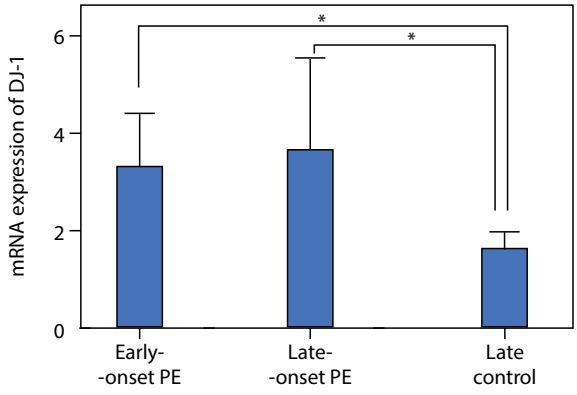

Figure 3. DJ-1 mRNA expression in human placentas from normal and preeclamptic pregnancies. In reverse transcriptase-polymerase chain reaction (RT-PCR), mRNA of DJ-1 was significantly increased in preeclamptic placenta ($p < 0.05$)

DISCUSSION

Preeclampsia and oxidative stress

Oxidative stress reflects an imbalance between the overproduction and incorporation of free radicals and the dynamic ability of a biosystem to detoxify reactive intermediates [8]. Once this balance is broken, the body's ROS production increases, excessive accumulation of ROS will cause oxidation reactions, resulting in destruction of cell structure and function, and participate in the occurrence of disease [9]. The oxidation and antioxidant system exists in a relatively balanced state within the normal pregnancy mother [10]. Numerous studies have described elevated

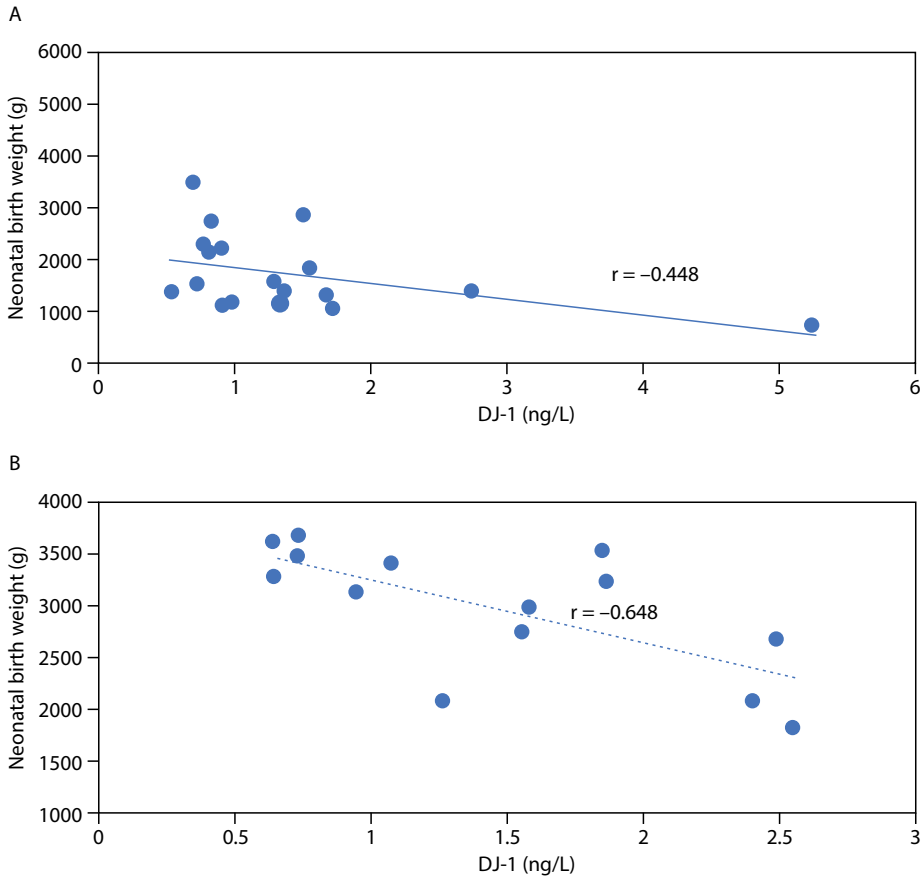

Figure 4. Umbilical cord blood DJ-1 vs Neonatal birth weight in (**A**) early onset and (**B**) late-onset preeclampsia

markers of oxidative stress in placental tissue from women with preeclampsia [11]. Ding Det al. [12] confirmed placental trophoblast cells mitochondria increased ROS production in preeclampsia, indicating that excessive ROS injury to the mitochondrial DNA of placenta trophoblast cells so as to involve in the pathogenesis of diseases. The possible role of late in pregnancy, the placenta of patients with preeclampsia decreased blood perfusion, maternal compensatory mechanisms lead to reperfusion injury induced by maternal placenta, produce excess ROS oxidative stress caused by injury [13]. Excessive oxidative stress may lead to extravillous trophoblast (EVT) inhibited proliferation, invasion and apoptosis decreased, the uterine spiral artery recasting failure and shallow placental implantation, promote the pathogenesis of preeclampsia [14].

DJ-1 and preeclampsia

DJ-1 was first proposed as a mitochondrial dependent oncogene, and recent studies have demonstrated that DJ-1 is a multifunctional protein that is ubiquitously expressed in more than 20 tissues of the human body. Antioxidant stress is the main function of DJ-1. Normally DJ-1 is highly conserved during biological evolution. In oxidative stress, DJ-1 can oxidize itself to protect cells against ROS induced apoptosis, and the isoelectric point of DJ-1 is reduced from 6.2 to 5.8 [15], DJ-1 is thought to be involved in oxidative stress. The main mechanisms of DJ-1 oxidative stress are: 1) DJ-1 enhances the antioxidant capacity by stabilizing and inhibiting nuclear factor erythroid 2-related factor(Nrf2)proteins Keap1 and the subsequent ubiquitination of Nrf2; 2) DJ-1 can change the oxidative stress related enzyme activities involved in oxidative stress [16]; 3) DJ-1 in order to remove ROS, which is oxidative stress products, will be oxidized by itself, thus losing normal resistance to apoptosis. Although the pathogenesis of preeclampsia has not yet been determined, with the deepening of research for many years, scholars at home and abroad have put forward some reasonable and scientific theories about its etiology and pathogenesis, among which oxidative stress is an important theory.

Kwon HS et al Study on the expression of DJ-1 in preeclampsia placenta tissue and localization. The results showed that preeclampsia trophoblast cells on the positive staining of DJ-1 protein increased significantly compared with the control group, and the high expression of DJ-1 in trophoblast cells. The research also confirmed in placental tissue of preeclampsia on DJ-1 mRNA than the normal group. The incidence of DJ-1 increased the expression of thought in preeclampsia, mainly on placental trophoblastic cells,

caused by the oxidative stress injury induced by placental dysfunction [6]. The relative expression level of DJ-1 based on Western-blot and RT-PCR was detected in the placenta, the levels were increased compared with the control group the expression of DJ-1 in the placenta of preeclampsia group, and the difference is statistically significant. And the results are consistent. But the specific mechanism of DJ-1 in preeclampsia remains to be explored.

Correlation between umbilical cord blood DJ-1 expression and neonatal birth weight

The intrauterine growth and development of fetus can be judged by the birth weight of newborns. To some extent, low birth weight reflects the severity of fetal growth restriction. The results of the study showed that preeclampsia group neonatal body mass was significantly better than the control group were decreased significantly, the correlation analysis found that umbilical cord blood DJ-1 expression level and neonatal birth weight was negatively correlated, the expression level of DJ-1 in umbilical cord blood and fetal growth and development are closely related. A possible mechanism for the patients with preeclampsia due to uterine spiral artery remodeling abnormal trophoblast shallow invasion to ischemia, hypoxia, anti-angiogenesis and angiogenic factors in the body to lose balance. This imbalance is not conducive to angiogenesis leads to widespread endothelial dysfunction, the trophoblast cells around in a relatively hypoxic state, thus the generation of oxidative stress, the effects of nutrient transport to the fetus, fetal growth restriction [17].

CONCLUSIONS

The study found that patients with preeclampsia, the expression level of DJ-1 in placental tissues were significantly higher than those of normal pregnant women, and studies have found that DJ-1 is mainly expressed in closely related to the pathogenesis of preeclampsia trophoblast cells, that increased expression of DJ-1 and oxidative stress may be involved in the pathogenesis of preeclampsia. The elevated DJ-1 level may be one of the important factors to preeclampsia. The study also found that preeclampsia umbilical cord blood DJ-1 levels were significantly increased, and there is significant correlation with neonatal body mass, human umbilical cord blood DJ-1 can affect fetal growth and development status. Through the study of DJ-1 in the pathogenesis of preeclampsia, contribute to a more in-depth understanding of eclampsia the mechanism for the prevention and treatment of disease, to develop new ideas.

Funding

National Health and Family Planning Commission Science Foundation(2019-WJ-04)
Fujian Science and Technology Project(2018Y0005)
Fujian Provincial Health Technology Project(2017-CX-11)
Key Clinical Specialty Discipline Construction of Fujian ,P.R.C ([2015] no. 593)

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VIA MEDICA

REVIEW PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 769–772

ISSN 0017–0011

DOI 10.5603/GP.2020.0116

How mother's obesity may affect the pregnancy and offspring

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ABSTRACT

One of the main reasons for the epidemic of obesity, which has already influenced the economic condition of health system worldwide, is our modern lifestyle having an unbalanced calorie intake and insufficient physical activity. Maternal-fetal nourishment and metabolism are the mechanisms of fetal programming of obesity-adiposity and non-communicable diseases that have been most extensively investigated. A mother's obesity is related to adverse outcomes for both mother and baby. Maternal overnutrition is also associated with a higher risk of gestational diabetes, preterm birth, large-for-gestational-age babies, fetal defects, congenital anomalies, and perinatal death. Women with obesity should be encouraged to reduce their body mass index (BMI) prior to pregnancy, and to limit weight gain during pregnancy. Obstetric ultrasound imaging in pregnant women is negatively affected by abdominal adipose tissue, having an adverse influence on congenital anomaly detection rates and the estimation of fetal weight.

Key words: obesity; body mass index; BMI; gestational diabetes mellitus; GDM; pregnancy; glucose intolerance; folates; vitamin B; homocysteine

Ginekologia Polska 2020; 91, 12: 769–772

INTRODUCTION

Obesity is a global health issue that may relate to metabolic syndrome as well as insulin resistance, type 2 diabetes, hypertension, dyslipidemia, nonalcoholic fatty liver disease, renal failure, and cardiovascular disease. Obesity is widespread globally and represents one of the most challenging public health problems due to its associated morbidity, mortality, and health care costs. The growing incidence of obesity, one of the causes of preventable death, can been seen in both developed and developing nations in recent years [1]. Obesity among women of reproductive age is already widespread worldwide [2]. According to WHO's Body mass index (BMI) classifications, overweight is defined as 25–29.9 kg/m^2, class I obesity is defined as 30.0–34.9 kg/m^2, class II obesity as 35.0–39.9 kg/m^2, and class III obesity as > 39.9 kg/m^2.

Obesity during pregnancy also increases the chances of spontaneous and recurrent miscarriages, nonoptimal ultrasound screening for fetal abnormalities, congenital heart and neural tube defects, wound infections, maternal thromboembolic and anaesthesia complications, depression, breastfeeding problems, gestational hypertensive disorders, gestational diabetes, preterm birth, and large for gestational age at birth [2]. The mechanisms of these outcomes include changes in maternal glucose and hormone levels, altered gene expression, and fetal epigenetic modification. Gestational diabetes mellitus (GDM) is a common disorder that influences approximately 7–14% of pregnancies. GDM is defined as a state of glucose intolerance and hyperglycemia with onset during pregnancy [3]. The increasing global incidence of type 2 diabetes mellitus (T2DM), combined with increases in childhood and adolescent obesity, is a significant public health concern. Maternal hyperglycemia is proven to be a risk factor for overweight or obese offspring [4].

Adipose tissue as an active endocrine organ, when produced in excess, may dysregulate metabolic, vascular, and inflammatory pathways in organs during pregnancy, affecting obstetric outcomes. Although the direct cause of obesity is usually the person's lifestyle, it is more significant to consider how the varied individual risks of obesity are promoted by obesogenic environments [5].

FETAL PROGRAMMING

By its effect on the intrauterine fetal environment, maternal overnutrition has a significant and life-long impact on the health of the child and on offspring obesity. Maternal nutritional status, unbalanced metabolism, infection, stress, and environmental conditions all have a vital influence on fetal growth and programming. Hence, each of the

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periconceptional, '*in utero*,' and postnatal periods are crucial in programming the next generation's medical condition. Transgenerational transmission may commence '*in utero*', and as a result of epigenetic changes, may result in long-lasting or temporal changes in metabolic programming [6, 7], which may in turn lead to adverse health problems in adult life [8]. Such can be a result of fetal exposure to hyperglycemia, hyperinsulinemia, hyperlipidemia, or inflammatory cytokines. Pregnant women with obesity have a greater risk of many maternal and perinatal complications, and the risk probability elevates with each increased degree of maternal obesity [9]. Excessive gestational weight gain in women who are already overweight or obese, accounts for one-quarter of pregnancy complications (*e.g.*, gestational hypertension, preeclampsia, gestational diabetes, and preterm birth) and almost one-third of large for gestational age children [10].

VITAMIN B12, FOLATES, HOMOCYSTEINE

The micronutrients vitamin B12 (B12) and folate are vital for the synthesis of DNA, protein, and lipids, and as such are associated with the fetal programming of obesity [11]. An essential part of this process is the conversion of homocysteine (Hcy) to methionine, for which B12 and folate are primary cofactors. Moreover, the mitochondrial conversion of methylmalonyl-CoA to succinyl-CoA depends on B12 as a coenzyme. When the enzyme is absent, accumulation of the former compound stops fatty acid oxidation because of promoting lipogenesis. Therefore, some scientists claim that low levels of B12 may be connected to adipose tissue dysfunction and complications linked with obesity [12]. Decreased levels of B12 during pregnancy influence maternal obesity, insulin resistance in the mother and fetus, and neonate lipid profiles [12, 13]. Research from India and UK indicates that vitamin B12 and folate concentrations during pregnancy are associated with obesity and insulin resistance in mother and offspring [12, 14]. It has also been proved that B12 deficiency during pregnancy is widespread, especially in women with obesity, is individually connected with GDM, and may be partly responsible for macrosomia [15]. According to some American studies, high folate consumption is associated with worsening clinical symptoms of vitamin B12 deficiency. Summing up, scientists conclude that vitamin B12 and folate should be considered together in assessing their influence on metabolic syndrome factors such as insulin resistance in obese patients [16]. Since there have been many studies on maternal consumption of carbohydrates and fats and their connection with developing GDM, it has been suggested that group B vitamins may be involved in the origination of glucose intolerance [17].

Homocysteine (Hcy) is an intermediate molecule of methionine metabolism whose concentrations are increased in the presence of folate and vitamin B12 deficiencies. Increased homocysteine levels are associated with insulin resistance. It has been speculated that elevated homocysteine concentrations impair the endothelial function in skeletal muscles, adipose tissue, and the liver, thus reducing insulin delivery to these insulin-sensitive organs [18]. It has been proven that both acute and chronic exposures to homocysteine results in detrimental effects on b-cell metabolism and insulin secretion [19].

OBESITY AND GESTATIONAL DIABETES MELLITUS

Obesity is closely correlated with the metabolic complication gestational diabetes mellitus (GDM) that may arise from changes in glucose regulation. In offspring, there is an association between increased BMI during childhood and adolescence and exposure to type 2 diabetes mellitus (T2DM) during pregnancy and gestational diabetes mellitus (GDM) [20, 21]. The HAPO Study showed there was a continuous correlation between maternal glucose levels during pregnancy and newborn adiposity outcomes [22]. According to the HAPO-FUS study, children exposed to increased levels of glucose *in utero* are more likely to develop childhood adiposity, including becoming overweight or obese. Glucose levels lower than those that are diagnostic of diabetes are associated with higher childhood adiposity; and this may have implications for long-term metabolic health [23]. Exposure to maternal gestational diabetes mellitus (GDM) during intrauterine life leads to increased fetal growth. In the PREOBE study, children born to obese mothers had significantly higher birth weights and waist circumferences while infants born of mothers with GDM had higher waist/height indices compared with the control group [24]. Some other studies have found that the intrauterine effects of GDM on offspring BMI are impaired when data is adjusted for pre-pregnancy maternal BMI [4, 25]. A few studies have reported that GDM is associated with higher levels of abdominal fat [26–28], suggesting that intrauterine exposure to diabetes may increase the risk for visceral adiposity, which is, in turn, linked to adverse health complications, including type 2 diabetes and cardiovascular disease [29].

OBESITY AND PREGNANCY COMPLICATIONS

Women with obesity are not only at increased risk for developing diabetes, preeclampsia, preterm delivery, thromboembolic disease, and macrosomia, and experiencing stillbirth, but also have an increased risk for labour induction failure. Moreover, obesity is also associated with both elective and emergency caesarean delivery, a risk that increases in correlation with increased maternal weight [30]. Goetzinger et al. [31], examined the extremes of abnormal fetal growth and found no connection with maternal BMI. According to Kritzer et al. [32], the percentage error of

estimated fetal weight (5.0–7.1%) was significantly lower than previously reported (16–20%). Obesity may also impair the visualization of fetal anatomy, with poor image quality making clinical interpretation difficult or impossible. Despite, technological advances, ultrasound imaging of obese patients remains challenging due to the adverse effects of adipose tissue on the penetration of sound waves [33]. Maternal obesity, even in the absence of gestational diabetes, is a potential risk factor for conditions such as neural tube defects, congenital heart defects, anorectal atresia, hydrocephaly, hypospadias, and limb reduction defects. Aksoy et al. [34], found a significantly higher percentage error higher on the BMI scale, and reported a greater risk of nonoptimal visualization when BMI (kg/m^2) was above the 90th percentile. Thus, patients with a BMI higher on the scale had higher failure rates than those with a lower BMI. In contrast to the above, Field et al. [35], and Farrell et al. [36], found that the accuracy of clinical and sonographic estimated fetal weight (EFW) measurements was not affected by increasing maternal obesity. In the study by Dashe et al. [37], it was shown that visualizing fetal anatomic structures is limited by increased maternal BMI during the standard second-trimester ultrasound examination. The finding that maternal obesity reduces the quality of prenatal, obstetric ultrasonography has also been described by other researchers [38, 39].

OBESITY IN PREGNANCY AND FETAL MACROSOMIA

Fetal macrosomia is defined as a birth weight of > 4,000 g and is associated with delivery complications such as maternal birth canal trauma, severe postpartum bleeding, shoulder dystocia, brachial plexus injury, fractures, and stillbirth due to perinatal asphyxia. The classification differs from a large-for-date newborn who is ≥ 90th or ≥ 95th centile for gestational age. Macrosomia occurs in up to 10% of deliveries and can be caused by genetic factors, maternal obesity or diabetes, or a fetal medical condition that accelerates fetal growth [40]. Nowadays, most screening strategies are based on cross-sectional evaluation of fetal size (abdominal circumference [AC] or fetal weight) during the third trimester. An accurate identification of excessive fetal weight leads to induction of labour for suspected fetal macrosomia and results in fewer birth complications without increasing cesarean section and instrumental delivery rates. There are studies about serial ultrasound measurement for early detection of LGA and macrosomia, but with inconsistent results [41]. There is an open-ended discussion in the literature regarding which sonographic EFW formula best evaluates fetal macrosomia. In 2010, Hart et al. [42], recommended a new formula for weight estimation of the macrosomic fetus based on fetal biometry and maternal weight, that improved macrosomia detection rates. Some other researchers have used AFI to predict fetal macrosomia [43]; while others proved that a head circumference/AC ratio measuring < 0.95th percentile had the highest sensitivity (39.4%) and specificity (89.4%) followed by a fetal AC measuring > 90th percentile [44].

CONCLUSION

In conclusion, pregnancy outcomes are influenced by pre-pregnancy BMI. Maintaining a healthy pre-pregnancy BMI reduces the risks of delivering preterm, large for gestational age, and macrosomic infants. It is crucial to limit adverse maternal and fetal outcomes caused by maternal obesity. To prevent the growing number of pregnancies affected by GDM, particularly in high-risk groups, there is a need for obesity-prevention strategies that begin early in life. If early pregnancy B12 status is individually predictive of incident GDM, such findings could potentially offer simple interventions to improve the metabolic conditions of pregnant women and their children. With GDM on the rise, there is an urgent need for more cost-effective strategies to reduce the burden of maternal and offspring consequences. Women with obesity should be encouraged to lose weight before they get pregnant, and to limit their weight gain during pregnancy.

Conflict of interest

The authors declare no conflicts of interest.

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VIA MEDICA

REVIEW PAPER / OBSTETRICS

Ginekologia Polska
2020, vol. 91, no. 12, 773–776

ISSN 0017–0011

DOI 10.5603/GP.a2020.0133

Biomarkers of angiogenesis in twin gestations and the risk of preeclampsia — review of the current literature

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ABSTRACT

Twin pregnancy is one of the key risk factors for the development of preeclampsia.

Soluble fms-like tyrosine kinase-1, placental growth factor, and soluble endoglin are molecules involved in the process of angiogenesis with a proven role in the pathogenesis of preeclampsia. The aim of the review was to summarize available data on maternal serum levels of the biomarkers of angiogenesis and their usefulness in predicting preeclampsia in twin pregnancies. Most of available data suggest biomarkers concentrations differ between singleton and twin gestation and are related to chorionicity of twin pregnancy. Several algorithms including biomarkers of angiogenesis in prediction of PE in twin pregnancy are available and seem promising, however more large prospective surveys are necessary to assess their usefulness in general clinic use.

Key words: placental growth factor; serum soluble fms-like tyrosine kinase-1; endoglin; preeclampsia; twin pregnancy

Ginekologia Polska 2020; 91, 12: 773–776

INTRODUCTION

Pre-eclampsia is one of the most serious pregnancy complications and occurs in approximately 5% of pregnant women [1]. According to the Polish Society of Gynecologists and Obstetricians, pre-eclampsia (PE) is diagnosed in case of hypertension which develops after 20 gestational weeks accompanied by proteinuria, acute kidney injury, hepatic, hematological or neurological complications, or fetal risks [2]. Currently, two hypotheses are linked to PE etiology: impaired implantation and abnormalities in the immune response of the pregnant woman to the implanted fertilized egg. Abnormal trophoblast implantation and the lack of adequate conversion of the spiral arteries of the uterus lead to trophoblast cell hypoxia and the resultant reperfusion, which is associated with the formation of free radicals and disrupted secretion of angiogenic factors. They include placental growth factor (PlGF), one of the elements of the family of endothelial growth factors (VEGF) which are responsible for angiogenesis. It is secreted in high concentrations by the cyto- and syncytiotrophoblast [3]. Its level increases with the development of gestation reaching the peak around 30 weeks of gestation. Subsequently, the secretion decreases [3]. Soluble fms-like tyrosine kinase-1 (sFlt-1) is a molecule which binds circulating vascular growth factors (PlGF and VEGF) making them biologically inactive. Intragestationally, sFlt-1 concentration remains constant until approximately 32 weeks, and then it increases [4, 5]. Endoglin (Eng) is a transmembrane glycoprotein which serves as a TGF beta receptor. Its high expression occurs on the surface of decidual cells and the trophoblast [6]. Eng participates in nitrogen oxide metabolism. Therefore, it also influences angiogenesis and vascular function. The concentration of soluble Eng (sEng) correlates with the occurrence of hypertension and proteinuria during pregnancy [7]. Reduced PlGF concentrations and increased sFlt-1 and sEng, which have anti-angiogenic properties, contribute to the development of PE in singleton pregnancies.

In patients with twin gestations PE is more common than in single gestations [8]. According to Laine et al. [9], a cohort study conducted in over 16 thousand of twin gestations showed that the risk of PE was over 3-fold higher in twin than single pregnancies. The reason for such a correlation may be linked to the secretion of different amounts of pro- and anti-angiogenic factors by the placenta in single and multiple pregnancies and the difference between placental weight. Published literature includes reports on the correlation between PE occurrence and the type of chorionicity of a twin gestation. However, published data are contradictory. Most studies demonstrated that PE was more common in patients with dichorionic rather than monochorionic gestations

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[10–12]. However, other authors reported a higher frequency of PE in women with a monochorionic pregnancy [13] or the lack of correlation with chorionicity [14–16]. Moreover, the etiology of the above-mentioned correlations is unknown. It may be due to the differences in the secretion of pro- and anti-angiogenic factors by the placenta in mono- and dichorionic gestations. The present paper includes a review of available literature concerning PlGF, sFlt-1 and sEng secretion in mono- and dichorionic twin gestations.

DIFFERENCES IN THE SECRETION OF ANGIOGENIC FACTORS IN SINGLE AND TWIN GESTATIONS

Placental weight is markedly different between single and twin gestations. According to Bdolah et al. [17] the average weight of the placenta is 716 g in a single gestation, while in a twin gestation it is considerably larger and weighs on average 1246 g ($p < 0.001$). The biomarkers of angiogenesis are released by trophoblast cells, so it may be assumed that their concentration in the sera of patients with single and twin pregnancies are different. The majority of studies published worldwide showed that those hypothetical differences are present. Faupel-Badger et al. [18] investigated the concentration of sFlt-1 in the sera of patients with single and twin pregnancies at 10, 18, 26, and 35 weeks of gestation and perinatally. They demonstrated that sFlt-1 concentrations were higher in twin pregnancies compared to single pregnancies in each of the analyzed time intervals. Similar results were also published by other authors [17–24]. Observations regarding PlGF secretion in single and twin gestations are also consistent. Numerous authors reported higher PlGF concentrations in women with twin gestations compared to single gestations [19–26]. Faupel-Badger et al. [18] observed higher PlGF concentrations in twin gestations than in single gestations in each analyzed interval of gestations except week 35 in which the correlation was inverse. Sanchez et al. analyzed the levels of biomarkers of angiogenesis in single and multiple gestations over the 1st trimester. They reported higher sFlt-1 and PlGF concentrations in twin pregnancies [20]. Maynard et al. [24] analyzed a group of patients with a high risk of PE development between 22 and 36 gestational weeks. In the study group sFlt-1 concentrations were higher and increased more rapidly in twin gestations than in single gestations, while PlGF concentrations were higher than in single gestations, but they decreased more rapidly. Studies concerning sEng presented contradictory results. Three studies have been published so far. They compared sEng concentrations in the sera of patients with single and twin pregnancies. According to Jeyabalan et al. and Faupel-Badger et al. sEng was secreted in higher amounts in patients with twin pregnancies, while Sánchez et al. did not observe such differences [18–20].

DIFFERENCES IN THE SECRETION OF ANGIOGENIC FACTORS IN MONO- AN DICHORIONIC TWIN GESTATIONS

No unambiguous data have been published in professional literature available worldwide to confirm whether PlGF, sFlt-1 and sEng secretion was dependent on the chorionicity in twin gestations. Cowans and Spencer [27] analyzed PlGF concentrations in 440 dichorionic, 116 monochorionic and in 607 single pregnancies in the 1st trimester. Higher PlGF concentrations were noted in 41% of dichorionic gestations and in 16% of monochorionic gestations compared to single gestations. Francisco et al. [26] also reported a higher PlGF concentration in the serum of women with dichorionic twin gestations compared to monochorionic and single gestations in the 1st trimester. Faupel-Badger et al. [18] determined sFlt-1 and sEng concentrations in subsequent measurements during pregnancy. The authors noted higher sFlt-1 and sEng concentrations in monochorionic compared to dichorionic gestations, also after correlations were made according to gestational age. Conversely, contradictory results have also been published in professional literature worldwide. Sanchez et al. [20] and Svirsky et al. [25] reported no differences in the secretion of PlGF, sFlt-1 and sEng between mono- an dichorionic gestations.

DIFFERENCES IN THE SECRETION OF ANGIOGENIC FACTORS IN TWIN GESTATIONS COMPLICATED AND UNCOMPLICATED WITH PE

In 2016 Tsiakkas et al. [28] reported on the distribution of PlGF concentrations at weeks 12, 22, 32 and 36 of single gestations complicated and uncomplicated with PE. Having examined over 40 thousand pregnant women the authors concluded that lower PlGF concentrations were strongly correlated with PE development. In the same year, the results of PROGNOSIS study were published. It was a prospective observational cohort study conducted in over 1000 women whose sFlt-1:PlGF ratio was determined between 24 and 36 weeks of gestation in order to assess its usefulness in the prediction of PE occurrence. The study showed a high negative predictive value of sFlt-1:PlGF ratio < 38 for PE development over the following 7 days (99.3%) and a 66% sensitivity for the ratio > 38 for the development of PE over the following four weeks [29]. Those findings facilitated the development of sensitive methods of PE prediction in single pregnancies.

However, professional literature available worldwide includes no such explicit results as regards the correlation of the biomarkers of angiogenesis with the risk of PE in twin gestations. Most published reports showed that sFlt-1 concentrations in twin gestations complicated by PE were higher than in gestations uncomplicated by PE [19–22, 30]. Moreover, lower PlGF concentrations were observed in

gestations with PE [19, 21, 22, 25, 26, 31, 32]. The differences between both biomarkers were confirmed in all trimesters of gestation. According to Dröge et al. an increased sFlt-1:PlGF ratio is associated with a higher risk of PE in twin gestations. The authors reported higher sFlt-1 and lower PlGF concentrations in twin pregnancies with PE. sFlt-1:PlGF ratio was as high in twin gestations with PE as in single gestations complicated by PE [22]. Independent teams of Powers et al. and Metz et al. demonstrated an analogous correlation between sFlt-1+sEng:PlGF index and the development of PE in twin gestations [19, 31]. Jeyabalan et al. [19] also noted significantly higher sEng concentrations between 31 and 35 gestational weeks in women who developed PE. Dröge et al. also analyzed the correlation between PE severity and the concentrations of angiogenesis biomarkers in twin gestations. The authors observed a significantly higher sFlt-1 and lower PlGF concentration both in mild and severe pre-eclampsia compared to uncomplicated gestations [22].

The analysis of professional literature also showed contradictory results which revealed no differences between the concentrations of angiogenesis biomarkers in twin gestations complicated and uncomplicated with PE. According to Sanchez et al. [20] sFlt-1 concentrations were similar in the sera of women with PE and uncomplicated ones. Furthermore, Saleh et al. [23] noted no significant differences regarding PlGF and sFlt-1 secretion in pregnancies complicated and uncomplicated by PE. However, their study included only 21 women.

Basing on the observed differences, a number of authors assessed the usefulness of those biomarkers in the models of PE prediction in twin gestations. Rana et al. [33] developed an algorithm to estimate the risk of PE-related complications in twin gestations for the following 2 weeks. The algorithm is based on gestational age, the highest measurement of blood pressure, the presence of proteinuria and the value of sFlt-1:PlGF ratio. The area under the curve (AUC) reached 0.93 with the use of all the above elements. In 86% of women whose sFlt-1:PlGF ratio reached or exceeded 85, the delivery occurred within two weeks following the measurement. However, various authors disagreed as regards the cut-off value of sFlt-1:PlGF ratio for twin gestations. According to Dröge et al. [22] the optimal cut-off value for PE prediction in twin gestations was ≥ 53. Saleh et al. [23] verified the usefulness of ≤ 38 ratio to rule out short-term risk of PE development in twin gestations. However, 5 out of 13 women with the ratio of > 38 and 4 out of 8 women with the ratio of ≤ 38 developed PE. The authors concluded that the value of the ratio of ≤ 38 was not useful in twin gestations. Predictive algorithms of the 1st trimester of pregnancy seem to be more effective. Boucoiran et al. [21] demonstrated a high effectiveness of PE prediction in the 1st trimester of a twin gestation with the use of PlGF concentrations (AUC 0.81, 10% of false positive results). Francisco et al. developed a PE predictive model comprising maternal factors, mean blood pressure, the pulsatility index of uterine arteries and PlGF concentration in the 1st trimester of pregnancy. The detection rate of PE resulting in delivery prior to 32 weeks of gestation was 100% (AUC 0.94), while before 37 weeks of gestation it was 99% (AUC 0.82). However, the percentage of false positive results was high (75%) [26]. The algorithm is available online: https://fetalmedicine.org.

The presented options of algorithms to predict PE seem promising. However, it is still impossible to assess their clinical usefulness. It is necessary to conduct a large prospective study and include such factors as twin gestation chorionicity or PE severity. The inclusion of such factors is necessary to improve the algorithms before they are recommended for general use. The development of an effective algorithm would contribute to the improvement of the perinatal care of women with twin gestations and the improvement of the perinatal results of twin gestations.

SUMMARY

Twin pregnancy is one of the risk factors for development of PE. Soluble fms-like tyrosine kinase-1, placental growth factor, and soluble endoglin are biomarkers involved in the process of angiogenesis with a proven role in the pathogenesis of PE. Most of available data suggest their concentrations differ between singleton and twin gestation and between monochorionic and dichorionic twin pregnancy. Several algorithms including biomarkers of angiogenesis in prediction of PE in twin pregnancy are available and seem promising, however more large prospective surveys are necessary to assess their usefulness in general clinic use.

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CLINICAL VIGNETTE

Ginekologia Polska
2020, vol. 91, no. 12, 777–778

ISSN 0017–0011

DOI 10.5603/GP.a2020.0128

Umbilical artery aneurysm

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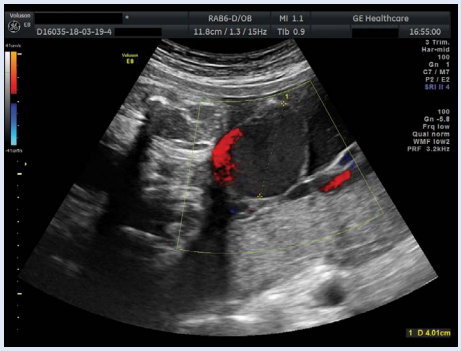
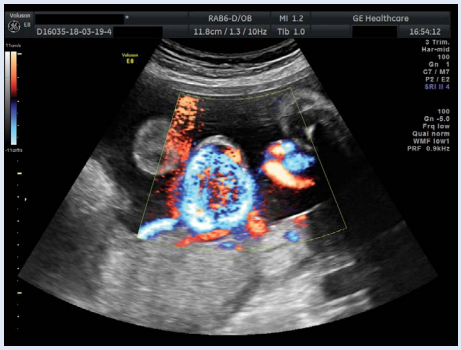
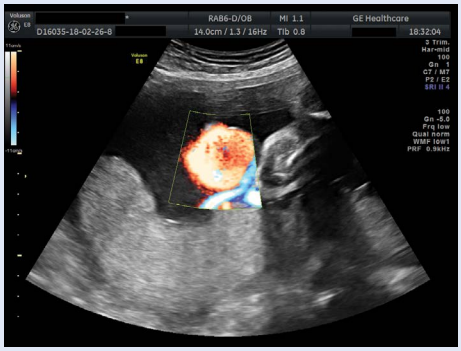
Ginekologia Polska 2020; 91, 12: 777–778

A 37-year-old pregnant woman (GII PII) had a routine ultrasound examination in the third trimester, at 30 weeks of gestation. Previous ultrasound tests, both in the first and the second trimester, showed no abnormalities. The patient remained under the supervision of a gynecologist (first visit at 5 weeks of gestation). The first pregnancy resulted in the delivery of a healthy child. The current pregnancy was complicated by diet-treated gestational diabetes (G1). Assessment of the glycemic profile revealed no abnormalities.







An ultrasound examination revealed a single, male fetus (gestational age consistent with 30 weeks of pregnancy) with an estimated weight of about 1700 g and normal amount of the amniotic fluid (MVP/AFI). An anechoic cyst (40 × 35 mm) in communication with the umbilical artery was visualized near the umbilical cord attachment. Doppler examination showed turbulent flow in the dilated vessel. Fetal vascular flows, both arterial and venous, showed no abnormalities. No other fetal structural defects were found.

The next ultrasound examination was scheduled for and performed at 34 weeks of pregnancy and showed no signs of chronic hypoxia or fetal growth restrictions. The pregnant woman was referred to a pregnancy pathology ward for intensive supervision. The welfare of the fetus was monitored throughout the entire period by serial ultrasound and cardiotocography. At 37 weeks of pregnancy, cesarean section was performed electively (born: male, live, full-term, weight: 3050 g, length: 53 cm). Assessment of the postnatal state: Apgar 10 at 1, 3, 5 and 10 min after delivery, without visible dysmorphic features.

Histopathological examination of the placenta confirmed the three-vessel umbilical cord with an eccentric attachment. In the area of the umbilical cord attachment, an aneurysm-dilated vessel and the adjacent pseudocyst, probably due to the degeneration of Wharton's jelly, were found.

Until now (at 3 and 6 months of life), the psychophysical development of the child remains normal.

Umbilical artery aneurysm (UAA) is the least common anomaly of the umbilical vessels caused by abnormal structure of the umbilical artery wall. Wharton's jelly has a protective role in formatting the UAA, even in the case of significant thinning of the vessel wall. To the best of our knowledge, there were 17 cases of UUA published, out of which only 6 led to the delivery of a healthy newborn. UAA is correlated with a high risk of aneuploidy and intrauterine deaths. UAAs are most often located at the placental umbilical cord attachment, where the branching of the vessels causes a loss of the protection provided by Wharton's jelly. In the 17 described cases, as many as 11 umbilical artery aneurysms were located at the umbilical cord placental insertion. It often coexists with the Wharton jelly degenerative pseudocyst, which has also been described by other authors. This seems to confirm the aforementioned protective effect of Wharton jelly on the umbilical vessels [1–3].

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According to the literature, 63% of UAA cases are associated with a single umbilical artery. It may be the result of a compensatory enlargement of a single umbilical artery, with an increase in the cardiac output of the developing fetus. It also explains why all cases were diagnosed after 21 weeks of pregnancy [1, 2].

There were 4 cases of UAA in fetuses with chromosome 18 trisomy. This may be associated with abnormal placental vasculature in this aneuploidy. UAA is also more common in male fetuses (60%) [1–5].

An aneurysm of the dilated umbilical artery can cause intrauterine asphyxia, disseminated intravascular coagulation (DIC) and fetal anemia. As mentioned above, only one-third of cases ended up in the delivery of a healthy child. Other cases were associated with intrauterine fetal demise or death shortly after delivery. This pathology is the result of clot formation in the lumen of the vessel, an expanding aneurysm can compress the umbilical vein leading to fetal anemization or an abnormal, thin wall of the vessel is also prone to rupture, which may result in perivascular hematoma. The risk of rupture is highest during delivery; therefore, a cesarean section should be the suggested mode of delivery [1–5].

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CLINICAL VIGNETTE

Ginekologia Polska
2020, vol. 91, no. 12, 779–780

ISSN 0017–0011

DOI 10.5603/GP.a2020.0129

Placental mesenchymal dysplasia and hepatic cyst

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ABSTRACT

Placental mesenchymal dysplasia (PMD) is a rare benign vascular anomaly of the placenta. It can be misdiagnosed as a molar pregnancy resulting in unnecessary termination of pregnancy.

A 30-year-old woman was referred to our hospital at 18 gestational weeks due to suspicion of molar pregnancy. The ultrasound showed a bulky placenta with multiple cysts. Oligohydramnion and fetal hypoechogenic cystic area without doppler flow were diagnosed at 23 weeks. The baby was operated on after delivery, and an 80 mm multifocal cyst originating from the right lobe of the liver was removed. The placenta demonstrated swelling stem villi with enlarged vessels and increased interstitial cells without trophoblast proliferation. PMD and fetal hepatic cyst can coexist; however, the relationship between those conditions remains to be elucidated. PMD is associated with adverse pregnancy outcomes but also with a good prognosis.

Key words: placental mesenchymal dysplasia; hepatic cyst

Ginekologia Polska 2020; 91, 12: 779–780

INTRODUCTION

Placental mesenchymal dysplasia (PMD) is a benign placental, vascular anomaly characterized on ultrasonography by placentomegaly and grape-like vesicles. The incidence of PMD reaches 0.02% [1].

CASE REPORT

A 30-year old primipara was referred to hospital at 18 weeks of gestation due to suspicion of molar pregnancy. We diagnosed complete placenta praevia with excessive vascular flow behind the placental bed. The placenta was enlarged, changed by the areas of multiple cystic echoes without vascular flow (Fig. 1 and 2). The ultrasound revealed a normal fetus. The amniotic fluid was diminished. Imaging features raised the possibility of triploidy and partial molar pregnancy. Amniocentesis revealed normal fetal karyotype (46 XX). Polymerase chain reaction analysis of the amniotic fluid for TORCH microorganisms was negative. The patient's blood test revealed an elevated level of AFP. The HCG concentration was normal. The next follow-up at 23 weeks revealed an enlarged placental width with multiple cystic areas and highly vascular retroplacental surface, suggestive of accreta. Additionally, hypoechogenic cystic area of 33 × 21 mm without any doppler flow was diagnosed in the fetal abdominal cavity. The amount of amniotic fluid was still diminished. At 28 weeks of gestation, the placenta continued to be large and hydropic in ultrasound scanning. The fetal growth curves were normal but the hypoechogenic cyst enlarged to 73 × 40 mm, changed into multilocules still without any doppler flow. It was located mostly on the right side next to the liver and right kidney (Fig. 3). At 29 weeks 1-day of gestation, the mother presented with pre-term premature rupture of membranes (PROM). Due to pathological CTG, an emergency caesarean delivery was performed. The infant weighed 1320 g (74 percentile) with Apgar scores of 5 and 6 at 1 and 5 min, respectively. The gasometry from the umbilical artery excluded fetal acidosis. Intraoperatively, the placenta was normally adherent to the uterus. Gross examination of the placenta showed enlarged stem

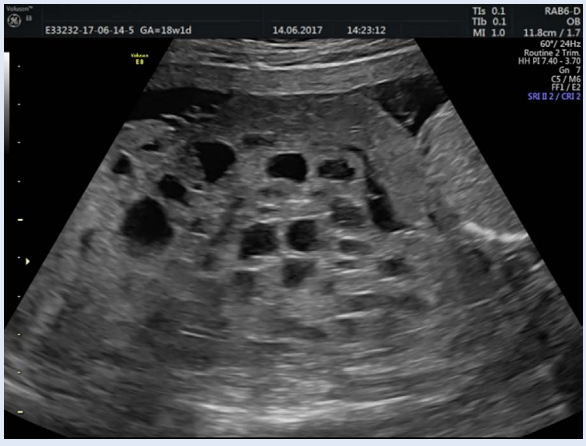

Figure 1. Enlarged placenta with the areas of multiple cystic echoes

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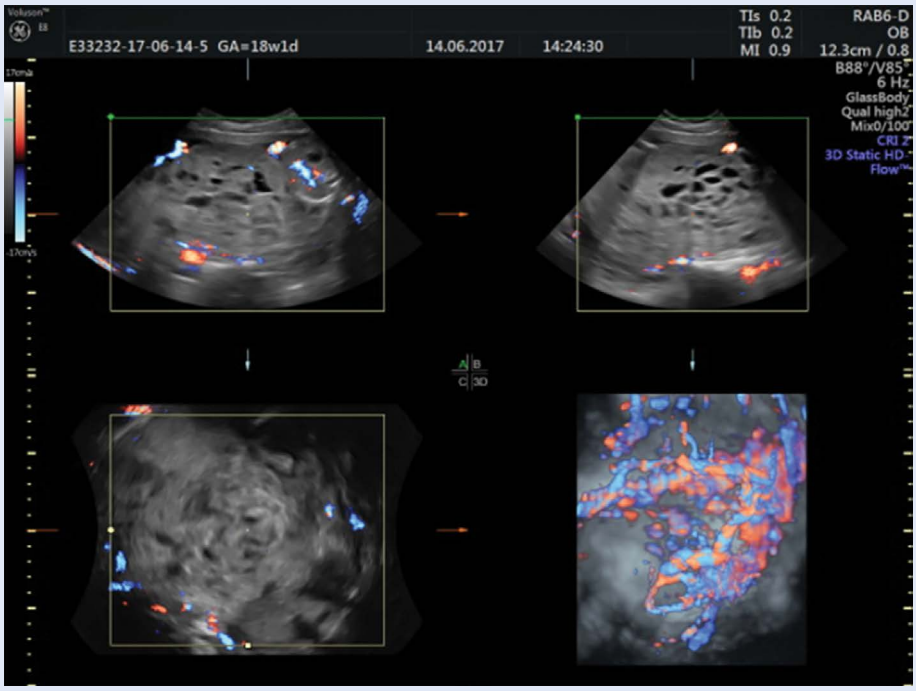

Figure 2. Enlarged placenta with the areas of multiple cystic echoes and excessive vascular flow behind the placental bed

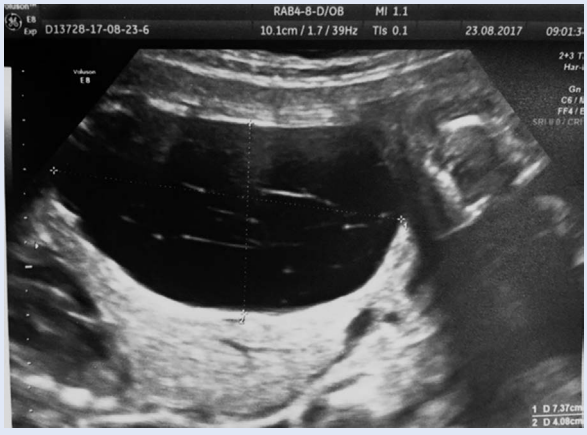

Figure 3. Hypoechogenic cyst in fetal abdominal cavity

villi. The placenta weighed 500 g what was around 90th percentile for that age of pregnancy [2]. Microscopic sections showed villi with thick dysplastic vessels surrounded by a myxoid edematous stroma with cistern formation. The findings were consistent with PMD. The neonate did not present with any macroscopic abnormalities at the time of delivery except enlarged abdomen circumference. In postpartum abdominal ultrasound, an 80 mm multifocal cyst was confirmed. The neonate was operated on day 4 post-delivery. The cyst appeared to have the origin in the right lobe of the liver. The outcome of the histopathological examination was the simple cyst of the liver. The baby was discharged at seven weeks after caesarean section in a good condition. The mother recovered normally and was discharged home on day 4 of postpartum.

DISCUSSION

In literature cases with PMD revealed cystic placentas in 80%, enlarged in 50% and dilated chorionic vessels in 16% [3]. Increased hCG level was found in 38% and AFP in 70% of cases [3]. The most common complication was preterm delivery (52%), PROM reached 17%. Intrauterine growth restriction (IUGR) occurred in 33%. Genetic abnormalities were found in 28%, Beckwith-Wiedemann syndrome was the most common, reaching 23% [4]. Neonatal hepatic tumors were diagnosed in 17% of PMD, mostly hepatic mesenchymal tumors [3]. Intrauterine fetal death was reported in 13% of cases, but 9% of patients had normal pregnancy outcomes [3]. It is important to distinguish PMD from a partial mole because incorrect diagnosis may result in pregnancy termination. PMD is associated with adverse pregnancy outcomes but also with a good prognosis.

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