CEOG Clinical and Experimental Obstetrics & Gynecology

Analysis of the cytokine profile (IL-2, IL-4, IL-6, IL-10, IL-17, IFN - γ and TNF-α) on threatened miscarriage

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Summary

Objectives: The purpose of our study was to analyze the cytokine profile in threatened miscarriage, defined as vaginal bleeding and uterine contractions with a closed cervix, occurring before the end of week 22 of gestation. *Materials and methods:* The study included 46 women hospitalized at the Obstetrics & Gynecology Clinical Hospital. Group 1 comprised of 14 patients diagnosed with threatened miscarriage, prior to pharmacological treatment. Group 2 comprised of 20 patients with identical diagnoses. The patients were administered drotaverine hydrochloride (3x40 mg) and progesterone transvaginally (2x100 mg). Group 3 constituted the control group, comprising of 12 women in normally-progressing pregnancy, before completing week 22 of gestation. *Results:* The present analysis was focused on the cytokine profile of the women included in the study, but no considerable changes in cytokine concentration were observed. The results of determining the IFN-γ and TNF-α, IL-2, IL-4, IL-6, IL- 10, IL-17, and CRP levels in all groups proved to be comparable; moreover, no considerable differences were found in the CRP level or vaginal flora. *Conclusions:* The present authors failed to identify any significant differences in the cytokine profile between patients in normally-progressing pregnancy and patients diagnosed with threatened miscarriage. This finding needs to be confirmed in a study in a larger group of pregnant women.

Key words: Threatened miscarriage, Prenatal care, Infections, Physiology of reproduction.

Introduction

Threatened miscarriage, defined as vaginal bleeding and uterine contractions with a closed cervix, occurring before the end of week 22 of gestation [1, 2], is one of the most frequently diagnosed obstetric complications and concerns of up to 15% of all identified pregnancies. Miscarriage can also occur at a very early stage, during which pregnancy can only be identified through measuring the β-hCG concentration, a phenomenon referred to as biochemical pregnancy. The etiopathogenesis of miscarriage is multi-faceted and is not yet fully understood. The most frequent causes include chromosomal aberrations, improper development of the embryo, and faulty implantation or infection [3, 4]. Abnormal implantation can result, *inter alia*, from immunological disorders or luteal deficiency.

Pregnancy is an extremely interesting condition in terms of immunology. The developing fetus has its own set of antigens, 50% of which are foreign to the mother's body, so the existence of a mechanism ensuring immunological tolerance to these antigens appears rather obvious. On the other hand, there must be efficient systems of defence against infections for both the mother and the embryo, without which pregnancy continuation would not be possible. This specific adaptation takes place at many levels, first of all resulting from the presence of a chorion with a reduced expression of the major histocompatibility complex (MHC) antigens. A well-balanced Th1/Th2-type cellular response is also essential; moreover, the effects of blocking antibodies, active cellular immunoregulation at the deciduous membrane level, and the immunosuppressive influence of progesterone on the immunological system also come into play [5].

Progesterone appears crucial for pregnancy continuation, as it contributes to the decidualization of the endometrium, enabling the implantation process, as well as inhibiting the mother's immunological response to fetal antibodies, and is responsible for preventing uterine contractions [6, 7]. A number of studies conducted on animal models have confirmed the major influence of progesterone, through the progesterone-induced blocking factor (PIBF), on pregnancy continuation. The PIBF isolated from the blood of pregnant mice which received progesterone has been found to pro-

Revised manuscript accepted for publication March 14, 2017

tect their fetuses against anti-progesterone-induced reabsorption or high activity of natural killer (NK) cells [8]. The purpose of this study was to analyze the cytokine profile in threatened miscarriage.

Materials and Methods

Patient enrolment methods and ways of obtaining the research material and its storage had been previously approved by the Bioethics Committee at the Poznan University of Medical Sciences (specifically approved only for this study on February 5, 2009, Resolution No. 78/2009). The patients gave their written obtained consent for this study. The consent procedure was approved by Ethics committees.

The study included 46 women hospitalized at the Obstetrics & Gynecology Clinical Hospital in Poznań, in the 2011–2013 period. Group 1 comprised of 14 patients diagnosed with threatened miscarriage, prior to pharmacological treatment. The diagnostic criteria of threatened miscarriage included a normally-developing embryo or fetus confirmed through ultrasonography examinations, the occurrence of vaginal bleeding/spotting, and contraction-like abdominal and/or low back pain. Group 2 comprised 20 patients with identical diagnoses. The patients were administered drotaverine hydrochloride (3x40 mg) and progesterone transvaginally (2x100 mg). Group 3 constituted the control group, comprising 12 women in normally-progressing pregnancy, before completing week 22 of gestation.

The patients had not been previously treated for infertility. Laboratory tests did not demonstrate any coagulation abnormalities. The degree of cleanliness (biocenosis) of the vagina was stage 3 in all groups. The study was conducted using laboratory assays. Blood samples were obtained on admission to hospital, in the morning, on an empty stomach. The level of CRP and progesterone were measured immediately in the hospital laboratory. Blood for other tests (IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, and TNF-α) was centrifuged and frozen at -20°C. The levels of IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, and TNF-α in the serum were determined by ELISA (double measurements) to verify the measurement error. Concentrations were determined in pg/ml, as mean ± standard deviation (SD) and median (Me).

The statistical analysis for the three groups of variables was checked using the Kruskal-Wallis One Way Analysis of Variance on Ranks. The significance level assumed for all of the tests was $p \leq 0.05$. Statistical calculations were made using the STATISTICA software.

Results

The women from all the study groups were comparable in terms of the week of gestation during the study and the level of progesterone, which enabled further statistical analysis. Group 1 included two miscarriages (one within seven days), and group 2 also included two miscarriages (one within seven days). In the control group there was one miscarriage within seven days. No treatment group included a miscarriage in 48 hours of hospitalization. Statistically significant correlations were not observed concerning the time of delivery and the body mass at birth (Table 1). The present analysis was focused on the cytokine profile of the women included in the study, but no considerable changes in cytokine concentration were observed. The results of determining the IFN-γ and TNF-α, IL-2, IL-4, IL-6, IL-10, IL-17, and CRP levels in all groups proved to be comparable (Tables 2-5); moreover, no considerable differences were found in the CRP level or vaginal flora.

Discussion

The immunology of pregnancy appears an extremely interesting and complex phenomenon. The surface of the fetal-maternal junction displays special features, connecting two apparently opposite functions. On the one hand, it protects a fetus that is susceptible to pathogenic infections and, on the other hand, it shows immunotolerance to fetal tissues that are foreign in terms of antigens. Such a subtle balance requires the cooperation of a number of mechanisms [5], with the Th1/T2 immune response equilibrium being one of them. Many researchers have drawn attention to the reduced ratio in the number of Th1/Th2 lymphocytes in a normally-progressing pregnancy [9, 10], at the same time attributing the pregnancy-protection role to the Th2-type response. Such a correlation seems to be supported not only through direct measurements of the lymphocyte sub-population, but also through the cytokines they produce. Both the increased pro-inflammatory interleukins concentration and the decreased anti-inflammatory interleukins concentration appear to exert an adverse impact on pregnancy in its early stages [11, 12]. Nonetheless, Groen *et al.* failed to prove such a clear-cut interdependence [13]. The purpose of the study carried out by Galazios *et al.* was to investigate the role of interleukin-6 and interleukin-8 in the spontaneous abortion of the first and second trimester of pregnancy. Their data suggest that IL-6 and IL-8 might be crucial factors which take part in the defensive reaction of maternal organization during the second trimester of pregnancy. No significant differences between women with first trimester abortions and those with no previous history of abortions were found [14]. Interleukin-6, IL-10, and IL-13 were measured by Paradisi *et al.* in the serum of women with threatened abortion, women with missed abortion, normal pregnant women, and normal non-pregnant women present in the study control groups. Serum concentrations of the selected T-helper 2-type cytokines showed no significant differences between women with threatened abortion and those of normal pregnant and non-pregnant women, whereas the data showed significantly lower values in women with missed abortion [15]. In turn, Calleja-Agius *et al.* noted that the monocyte expression of TNF-α and circulating levels of TNF-α, IFN-γ, IL-10, and IL-6 were significantly lower, whereas circulating levels of TNF-α/IL-10, IFN-γ/IL-10, and the TNF-α/IL-6 ratios were significantly higher in women with threatened miscarriage who subsequently miscarried, compared to women with a normal outcome [16].

The efficiency of miscarriage prevention with the use of gestagens raises controversies. Although progesterone has been widely recognized as a factor contributing to the sus-

Table 1. — *Study group characteristics.*

Group	Gestational age (weeks)		Progesterone (ng/ml)		Delivery age (weeks)		Mass (g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	13.71	4.25	57.93	42.04	38.33	1.58	3221.11	335.76
2	12.45	4.32	47.25	31.88	36.57	6.09	3230	550.11
III	11.67	4.64	37.46	11.92	38.8	1.23	3235	396.41
p	0.486	0.834	0.45	0.995				

**The level of statistical significance was assumed at p < 0.05, Kruskal-Wallis One Way Analysis of Variance on Ranks.*

Table 2. — *IL-6 and IL-10 levels in patients diagnosed with threatened miscarriage (group 1 - medications excluded, group 2 - medications included), and in the control group.*

Group	IL-6 (fg/ml)			IL-10 (fg/ml)		
	Mean	SD	CI of mean	Mean	SD	CI of mean
1	550.68	508.91	293.83	318.6	384.85	222.2
2	1221.41	1742.54	839.88	159.71	113.39	54.65
3	743.51	744.09	472.77	543.56	1385.26	880.16
p	0.936			0.459		

**The level of statistical significance was assumed at p < 0.05, Kruskal-Wallis One Way Analysis of Variance on Ranks.*

Table 3. — *IL-17 and CRP levels in patients diagnosed with threatened miscarriage (group 1 - medications excluded, Group II - medications included), and in the control group.*

Group	IL-17 (fg/ml)			CRP (mg/l)		
	Mean	SD	CI of mean	Mean	SD	CI of mean
1	43.29	74.73	43.15	6.04	5.72	3.30
2	231.71	610.11	294.07	11.03	18.20	8.52
3	392.22	1128.43	716.97	3.10	1.88	1.26
p	0.626			0.615		

**The level of statistical significance was assumed at p < 0.05, Kruskal-Wallis One Way Analysis of Variance on Ranks.*

Table 4. — *IL-2 and IL-4 levels in patients diagnosed with threatened miscarriage (Group 1 - medications excluded, Group 2 - medications included), and in the control group.*

Group	IL-2 (fg/ml)			IL-4 (fg/ml)		
	Mean	SD	CI of mean	Mean	SD	C.I. of Mean
1	57.88	216.55	125.03	108.75	69.3	40.02
2	178.77	506.45	244.1	207.42	475.93	229.39
3	1130.89	3917.52	2489.07	153.77	234.33	148.89
p	0.501			0.88		

**The level of statistical significance was assumed at p<0.05, Kruskal-Wallis One Way Analysis of Variance on Ranks.*

Table 5. — *TNF-α i IFN-γ levels in patients diagnosed with threatened miscarriage (Group 1 - medications excluded, Group 2 - medications included), and in the control group.*

Group	TNF-α (fg/ml)			IFN-γ (fg/ml)		
	Mean	SD	CI of mean	Mean	SD	CI of mean
I	57.69	159.64	92.17	41.32	105.99	61.2
II	102.88	295.5	142.43	158.2	517.61	249.48
III	53.51	185.35	117.77	1256.47	4352.55	2765.48
p	0.398			0.701		

**The level of statistical significance was assumed at p<0.05, Kruskal-Wallis One Way Analysis of Variance on Ranks.*

taining of an early pregnancy, luteal deficiency is a relatively rare cause of miscarriage. Therefore, there are numerous studies proving the positive influence of progesterone products, such as the meta-analysis conducted by Carp [7] showing a 26% increase in the percentage of live births among women treated with progesterone products. A similar correlation was also argued by Wahabi *et al.* [17]. However, there is also a multitude of reports on the lack of correlation between the gestagen treatment of threatened miscarriage and the actual obstetric outcomes. In the study by Coomarasama *et al.* [18], no significantly higher rate of live births was observed among women with recurrent miscarriage problems of unknown etiology, treated with progesterone products. Yassaee *et al.* [19] showed that the use of vaginal globules containing progesterone could lead to a decreased miscarriage rate, but the results were not statistically significant. The present study also failed to prove a significant increase in live births and obstetric outcomes expressed through the week of pregnancy and weight at birth. The lack of such correlation might have stemmed from an insufficiently numerous group of patients diagnosed with threatened miscarriage, this drawback pertaining to many studies focusing on this issue, as was pointed out by the meta-analysis authors Wahabi *et al.* [9].

The present authors did not observe any significant differences in the cytokine profile between patients in normally-progressing pregnancy and patients diagnosed with threatened miscarriage. The comparison of the relevant cytokine concentrations in the blood serum of the mother and fetus, as well as in the amniotic fluid and vaginal secretions, is also worth investigating. Such a study would provide a deeper insight into the maternal-fetal characteristics from the immunological perspective.

Conclusion

The present authors failed to identify any significant differences in the cytokine profile between patients in normally-progressing pregnancy and patients diagnosed with threatened miscarriage. This finding needs to be confirmed in a study on a larger group of pregnant women.

Acknowledgement

The study was financed with the funds for education in 2010–2014 as research project N N407194537, entitled: "Preterm birth as a growing social concern. The analysis of the immunological symptoms and the progesterone-induced blocking factor (PIBF) in patients at risk of miscarriage or premature labour".

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