RESEARCH STUDIES

Predictive role of cytokines level in the amniotic fluid and maternal blood in the possible development of gestational hypertension

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Abstract

Pregnancy induced hypertension, which includes both gestational hypertension (GH) and preeclampsia, is the most common medical disorder and remains one of the major causes of maternal and fetal death. The pathogenesis of GH is still unknown, yet immunologic and inflammatory causes may play an important role in the pathophysiology of this disease. The purpose of this study is to investigate the midtrimester IL-6, IL-8, IL-10, IL-12, TNF-α, SDF-1a and VEGF in the amniotic fluid (AF) and maternal blood regarding the above as being predictive for the gestational hypertension outcome. In the group of 128 pregnant women over 35 years old the six ones have developed GH. Our study results demonstrate that GH is associated with a strong increase of IL-6, IL-12 in maternal blood and a significant decrease of serum IL-10. Although AF level of both IL-6 and IL-10 has been significantly lower in GH subjects. In conclusion this study shows the relationship between the midtrimester IL-6, IL-12 and IL-10 concentrations in AF and maternal serum and GH outcome.

Key words: gestational hypertension, pregnancy after 35 years old, amniotic fluid, maternal serum, cytokines.

Прогностическая роль уровня цитокинов амниотической жидкости плода и крови матери в вероятности развития гестационной гипертензии

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Реферат

Артериальная гипертензия при беременности, включающая в себя гестационную гипертензию и преэклампсию, является наиболее частым из осложнений беременности и остаётся одной из основных причин материнской смертности и гибели плода. Патогенез гестационной гипертензии до сих пор полностью не изучен, однако иммунологические и воспалительные факторы могут играть важную роль в патофизиологии этого заболевания. Целью данного исследования является изучение в период второго триместра беременности соотношения концентраций IL-6, IL-8, IL-10, IL-12, TNF-α, SDF-1a и VEGF в амниотической жидкости плода и материнской сыворотке крови и их связь с последующим развитием гестационной гипертензии. В группе из 128 беременных в возрасте старше 35 лет, в шести случаях выявлено развитие гестационной гипертензии. Полученные результаты определили, что наличие гестационной гипертензии связано с существенным увеличением концентрации сывороточного IL-6 и IL-12 и одновременным снижением IL-10 в крови матери, при том, что в амниотической жидкости выявлено существенное снижение как IL-10, так и IL-6. В заключении данное исследование доказывает связь между уровнями IL-6, IL-10 и IL-12 в амниотической жидкости плода и крови матери, выявленными во втором триместре беременности, и последующим развитием гестационной гипертензии.

Ключевые слова: гестационная гипертензия, беременные старше 35 лет, амниотическая жидкость, цитокины.а

Introduction

Later childbearing has become a widespread phenomenon in the developed countries in the last three decades [1]. In our country the proportion of 35-year-old women and older ones in prenatal medical and obstetrical service institutions has also increased significantly in the recent years. By the evidence of the National Center of Statistics during the last decade the rate of birth for women over 35 years old increased from 5.7% in 2000 to 7.2% in 2011 [2].

The medical risks of the pregnancy for women over 35 years old are related to the ageing of body and reproductive system that includes an increasing risk of a fetal loss, such chromosome anomalies as a Down syndrome, a multiple pregnancy, diabetes, a placenta previa, a placental abruption, Caesarean birth, a preterm labour and a low birth weight, but the leading causes of maternal and neonatal morbidity and mortality worldwide still remains preeclampsia, which may occur due to the pregnancy induced hypertension (PIH) [3, 4, 5]. High blood pressure during the pregnancy increases the resistance of blood vessels, which can hinder blood flow in different organ systems including the uterus, liver, kidneys, brain and placenta. That is why a placental abruption sometimes may occur as a result of gestational hypertension what can lead to the fetal problems including an intrauterine

growth restriction and a stillbirth. In this case the preceding diagnosis of GH is of major importance to prevent the possible complications during the labour. The early detection of pregnancy complications such as GH and preeclampsia would allow the maximum time for initiation of treatment and might help prevent some complications of the disease. In practice, the clinical symptoms of the disease often appear too late to allow a worthwhile intervention. Commonly used tests, those that reveal the increase of C-reactive protein, leukocyte subsets and concentrations of pro-calcitonin are nonspecific and, certainly, unreliable for a true identification.

Many studies have investigated the role of cytokines in the pathogenesis of the essential hypertension. The experimental evidence shows that the rennin angiotensin system (RAS) and the sympathetic nervous system interact with such pro-inflammatory cytokines as IL-6 and TNF-α and stimulate chemokine monocyte chemoattractant protein-1 and nuclear factor-κB. Simultaneously, pro-inflammatory cytokines increase the production of reactive oxygen species; they also affect a vascular function and endothelium-derived factors involved in blood pressure regulation [6, 7, 8, 9]. Several investigations with the usage of amniotic fluid samples suggest the role of pro-inflammatory cytokines as biomarkers for pathological gestations and, especially, for intrauterine infection, but the findings concerning a predictive role of cytokines for PIH and preeclampsia relating to the age of the mother remain inconclusive [10, 11].

In the Republic of Moldova, according to the Guidelines for Perinatology, pregnant women after 35 years old belong to the risk group and require an additional monitoring, which includes specific tests for detecting congenital malformations [12]. The cultivation of amniotic fluid is still considered a gold standard for the identification of certain kinds of birth defects and is done between the 15th and 20th weeks of pregnancy [13].

The aim of this study is to find a correlation between the pregnancy with GH outcome and concentrations of immune mediators IL-6, IL-8, IL-10, IL-12, TNF-a, VEGF and SDF-1a in fetal amniotic fluid and maternal blood collected during the second trimester of pregnancy from women after 35 years old.

Material and methods

Subjects

A clinical trial has been performed in the National Center of Reproductive Health and Genetics in the Republic of Moldova and has included the pregnant women after 35 years old, who have been subjected to an invasive prenatal diagnostics during the years of 2011 and 2012. According to the pregnancy outcome, from a cohort of 128 women 6 patients have been included in the group with GH and 17 women with uncomplicated normal pregnancies have been included in a comparison group. All the subjects have been normotensive before 20 weeks' gestation and in all the cases the hypertension has been lowered in the postpartum period.

The maternal age has ranged from 35 to 40 years old (36.8 ± 0.59) in the control group and from 35 to 38 years old (36.2 ± 0.54) in the hypertensive group. The mean arterial pressure (MAP) has been calculated as diastolic pressure plus one third of the pulse pressure.

The study has been approved by the Medical Ethics Committee, State University of Medecine and Pharmacy "Nicolae Testemitsanu". An informed consent has been obtained from each subject at the beginning of the study.

Amniocentesis and blood samples

The intervention has been performed transabdomenally aseptically under the ultrasound guidance, 20 ml of amniotic fluid has been extracted first for the further diagnostic tests according to the cytogenetical screening, subsequently 10 ml of amniotic fluid has been removed and divided into 2 ml aliquots and stored at -48°C until being analyzed.

The venous blood has been collected after the amniocentesis procedure and drawn into the specimen tubes for serum extractions and tubes containing EDTA as anticoagulant for plasma extractions. The plasma and serum have been prepared by centrifugation, aliquoted and stored at -48° C until being analyzed.

Assay of serum and amniotic fluid cytokines concentrations

The IL-6, IL-8, IL-10, IL-12, TNF-α, VEGF and SDF-1a concentrations have been measured by specific quantitatively affordable enzyme linked immunosorbent assay (ELISA) kits (PeproTech Inc., Minneapolis, USA.) according to the manufacturer's instructions. The assays have been carried out in flat-bottomed 96-well immunoplates (MaxiSorp, Nunc, Wiesbaden, Germany). The amniotic fluid and serum have been measured using BioTek's PowerWave HT microplate spectrophotometer. A standard curve has been made in parallel to each assay and the results have been converted into pg/mL.

Statistical analysis

The statistical analysis of the data has been performed by two-tailed Student's t-test and ANOVA test. The differences have been considered significant for $p \leq 0.05$ and highly significant for $p \leq 0.01$. The data are presented as a mean value ± SEM.

Results

In a cohort of 128 pregnant women over 35 years old examined in the present study, the six ones have developed the gestational hypertension. In all the cases the hypertension has occurred after 32 weeks of gestation and has not been complicated by proteinuria.

There have not been differences between the two study groups regarding MAP baseline values before the time of 20 weeks of gestation (88.5 ± 2.05 mm Hg in GH group vs. 89.4 ± 1.59 mm Hg in control group; $p = 0.72$). On the other hand, at the 32-35th weeks of gestation a statistically significant increase of MAP ($p < 0.001$) has been noted in GH group (111.1 ± 0.82 mm Hg) while there have not been considerable changes in the control subjects (90.0 ± 1.51 mm Hg) (fig. 1).

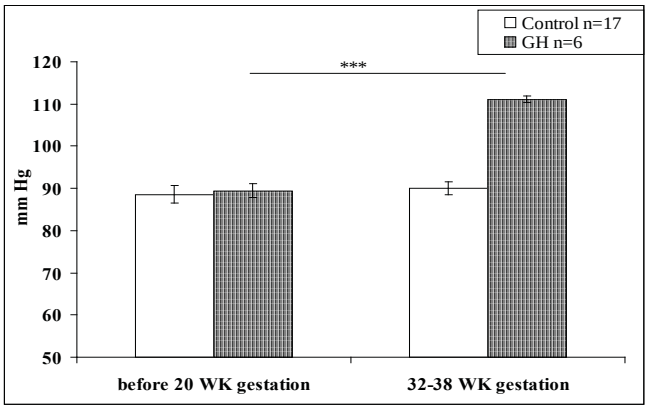

Fig. 1. The mean arterial pressure (MAP) during pregnancy. All the data are expressed as a mean value ± SEM. *** p < 0.001.

a clearly different from the rest of this group (223-1585 pg/ml) IL-10 level (2098 pg/ml) and by excluding it from the calculations a statistically significant difference between the groups (p = 0.04) has been obtained. Nonetheless, there has been no difference between IL-12 levels in the AF in the both groups, and the average value has been approximately equal.

TNF-α has been detected in the maternal serum only in a few samples taken from the both investigated groups (the data is not shown). In the AF samples the TNF-α concentrations have been low and approximately similar varied in the both groups (p = 0.247).

The concentrations of other investigated cytokines – IL-8, VEGF and SDF-1a – have not been different both in the maternal blood and the AF in the GH group subjects and control ones (tab. 1).

Table 1

Concentrations of IL-6, IL-8, IL-10, IL-12, TNF-α, SDF-1α and VEGF measured in the second trimester maternal blood and amniotic fluid samples obtainied from the unaffected women and women who developed gestational hypertension later

Immune mediators	The average pg/mL concentration range					
	In amniotic fluid		p	In maternal serum		p
	GH (n = 6)	Control (n = 17)		GH (n = 6)	Control (n = 17)	
IL-6	1701.67 ± 199.6	2378.22 ± 235.66	0.042	1957.00 ± 314.17	1055.18 ± 81.14	0.034
IL-8	2886.33 ± 471.76	2718.71 ± 315.35	0.77	371.17 ± 55.06	332.35 ± 25.06	0.54
IL-10	934.00 ± 97.48	1776.71 ± 253.31	0.006	260.00 ± 11.16	430.82 ± 63.51	0.017
IL-12	1850.00 ± 195.29	1898.00 ± 115.46	0.837	1414.17 ± 290.58	709.82 ± 126.73	0.062
SDF-1α	1473.67 ± 111.71	1442.71 ± 72.59	0.821	629.83 ± 101.01	522.76 ± 43.66	0.363
VEGF	2342.33 ± 109.26	2107.65 ± 492.98	0.648	404.33 ± 44.22	356.58 ± 40.98	0.442
TNF-α	55.81 ± 28.48	18.14 ± 1.63	0.247	-	-	-

All data are expressed as a mean value ± SEM.

IL-6, IL-8, IL-10, IL-12, TNF-α, SDF-1a and VEGF have all been found in the AF and mothers' blood in the second trimester of pregnancy.

The significant positive correlation has been between pro-inflammatory IL-6 in the blood collected from the pregnant women who have developed the GH (p < 0.05) and the control group. On the other side, in the AF the concentrations of IL-6 have showed an opposite trend. There the levels of IL-6 in AF from GH group have been significantly lower (p < 0.05) in comparison to the normal pregnancy group.

The concentrations of anti-inflammatory IL-10 also show a positive correlation between GH and the development of pregnancy. The blood levels of IL-10 in the GH group have been significantly lower (p = 0.017) than in the control group. Similarly, there has been a significant decrease of IL-10 in the AF collected from the subjects with GH (p < 0.01).

The serum levels of IL-12 in the pregnancy complicated by GH have been elevated approximately twice as much in comparison with the control group, however there has been no statistical difference between these groups (p = 0.06). One woman from uncomplicated pregnancy group has displayed

Discussion

The pathogenesis of GH is still unknown, yet immunologic and inflammatory factors may play an important role in the pathophysiology of this disease. IL-6 and other pro-inflammatory cytokines are the important components of immune response and according to our findings appear to be involved in the early cellular disorders that underlie GH. The measurement of immune mediators in the period of pregnancy has been the method to diagnose an intrauterine infection; in our case we have considered that this measurement can be correlated with the early identification of GH.

Our results demonstrate that GH is associated with the significant increase of IL-6 in blood in the second trimester of pregnancy when the blood pressure is unchanged. This finding is consistent with some previous reports by Munno et al. [14], Teran et al. [15], Afshari et al. [16], who have found the increase of serum IL-6 in women with pre-eclampsia and suggested a potential role of this inflammatory cytokine in the etiology of preeclampsia. In our study nobody have had proteinuria which is the main component for the pathogenesis of preeclampsia. It is already well de-

fined that IL-6 can increase the permeability of endothelial cells, reduce the prostacyclin synthesis by inhibiting the cyclooxygenase enzyme, increase the tromboxane A_2 to prostacyclin ratio and also stimulate a platelet-derived growth factor [16]. Also, IL-6 can stimulate the expression of RAS components and leads to the increase of angiotensin II level which has a dominant role in the pathogenesis of hypertension [8, 9]. At the same time it has been demonstrated that angiotensin II stimulates the release of IL-6, and angiotensin type I receptor antagonist lowers aortic mRNA expression of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 as well as plasma levels of IL-6 and IL-1β. Also, the effects of IL-6 on vascular contractile mechanisms have been shown, the fact that supports the hypothesis that IL-6 can play its role in the hypertensive actions of angiotensin II [17, 18, 19]. Furthermore, IL-6 can induce secreting of auto antibodies that activate angiotensin II type I receptor (AT1-AA) [20], what supports the hypothesis that IL-6 can play an important role in the development of PIH.

Our results on IL-6 in the AF are consistent with those from the previous studies that show the relationship between PIH and the concentrations of IL-6. Silver et al. [21] has found that the low levels of IL-6 correlate with small-for-gestational-age fetuses and preeclampsia, whereas its elevated levels correlate with preterm labour and delivery. Different observations show the elevated mildtrimester levels of IL-6 in the AF in the pregnancies with subsequent preeclampsia, but it is not clear if there has been any maternal infection; yet the maternal infection is a potent stimulus for IL-6 production.

IL-10 is a particularly intriguing cytokine as it is characterized as pleiotropic and having a dual immunologic function. The previous studies have evaluated the concentrations of circulating IL-10 in pregnant women with PIH and preeclampsia; however, the results remain controversial [22, 23]. Our finding of a significant decrease of IL-10 in the AF and maternal serum in the GH group compared with the control group suggests that this cytokine is involved in the pathogenesis of GH. It is suggested that IL-10 has a protective effect on the fetal-placental unit because it inhibits the secretion of such inflammatory cytokines as IL-6 and TNFα [23]. According to the above authors, IL-10 reconstitution prevents the onset of preeclampsia-associated features in both in vivo and in vitro models of preeclampsia.

We have observed a strong tendency towards the higher levels of IL-12 in the blood of the GH group subjects compared with the unaffected pregnancy subjects. Our results comply with the similar findings that have found that serum IL-12 concentrations are elevated in women with both severe preeclampsia and HELLP syndrome. The authors in the modern literature suggest that the regulation of the IL-12 production and metabolism are abnormal in women with preeclampsia and HELLP syndrome what, perhaps, contributes to the immunologic alterations characteristic of these disorders [24].

In conclusion the research group demonstrates the relationship between the midtrimester IL-6, IL-12 and IL-10 concentrations in the AF and maternal serum and GH outcome. We report the dependence between the elevated maternal level of IL-6 and IL-12 and the decreasing concentration of IL-10 and the subsequent development of GH, mostly after 32 weeks of gestation. We also provide the evidence of the association between the decreased levels of IL-6, IL-10 in the AF and GH in women aged over 35. Further studies are required to produce more consistent results that can benefit the field of obstetrics.

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