CLINICAL SIGNIFICANCE OF LYMPHOCYTE-PLATELET ADHESION, CYTOKINES AND ENDOTHELIAL DYSFUNCTION IN THE DEVELOPMENT OF GESTATIONAL HYPERTENSION

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ABSTRACT

The aim of our study was to determine the significance of lymphocyte-platelet adhesion (LPA), interleukins, transforming necrosis factor - α (TNF- α), and endothelial dysfunction in the development of gestational hypertension (GH). The study included 139 pregnant women aged between 17 and 27 years (21.3 ± 4.22 years). GH was diagnosed in 119 women after 20 weeks of pregnancy. 20 patients (control group) were with physiological course of pregnancy. The distribution of patients by groups was carried out according to the level of blood pressure (BP) in accordance with ICD-10 (Geneva, WHO, 2002). The survey was conducted at the moment of detection pregnancy from 7 to 10 weeks and in dynamics of I, II and III trimesters of gestation. In the dynamics of gestation, were studied the number of desquamated endothelial cells circulating in the systemic circulation (CECs), nitrates levels, the adhesion of platelets by estimation their ability to form co-aggregates with lymphocytes by determining the percentage of lymphocytes aggregates with thrombocytes (lymphocyte-platelet plugs), the concentrations of IL- β and TNF- α by immunoenzymatic method. Results of the study showed that in pregnant women at risk of hypertensive disorders, especially after 20-22 weeks of gestation and later, lymphocytes ability to platelets adhesion is rose, the concentrations of pro-inflammatory cytokines and NO level are increased. The direct relationship between DBP high level with degree of LPA, CECs, NO, IL- β and TNF- α cytokines at gestation period of 20-22 weeks indicates their importance in the pathogenesis of hypertensive disorders in pregnant women.

UDC CODE & KEYWORDS

■ UDC: 618.3+616.12-008.331.1:611-018.74+616-008.1 ■ Gestational hypertension ■ Lymphocyte-platelet adhesion ■ Interleukins ■ Transforming necrosis factor - α ■ Endothelial dysfunction

INTRODUCTION

Hypertension developing during gestation is a cause of pre-eclampsia which leads to poor perinatal and postnatal outcomes for both mother and fetus (Sidorova et al., 2008; Shalina et al., 2010; Makarov et al., 2011). Functional systems, structures of cell membranes, activity of the hemostatic system, as well as the endothelial state and its secretory function play the special role in this process (Sidorova et al., 2008; Strizhakov et al., 2011). As Sidorova et al. (2008) and Makarov et al. (2012) report, the realization of defence mechanisms because of damaged blood vessels at the level of the whole organism is accompanied by increased activity of platelet adhesion to lymphocytes, which was called the phenomenon of lymphocyte-platelet adhesion. Changes in the state of vascular endothelium, platelet adhesion to lymphocytes and features of mechanisms in the systemic circulation of mother are poor studied.

Objective: To determine the significance of lymphocyte-platelet adhesion (LPA), interleukins, transforming necrosis factor - α (TNF- α), and endothelial dysfunction in the development of gestational hypertension (GH).

Materials and Methods

The study included 139 pregnant women aged between 17 and 27 years (21.3 ± 4.22 years). GH was diagnosed in 119 women after 20 weeks of pregnancy. 20 patients (control group) were with physiological course of pregnancy. Exclusion criteria for the study were: (1) chronic hypertension, (2) somatic diseases (coronary heart disease, hypertension, diabetes, renal and hepatic pathologies). The distribution of patients by groups was carried out according to the level of blood pressure (BP) in accordance with ICD-10 (Geneva, WHO, 2002). The 1st group consisted of 39 women, whose systolic BP (SBP) increased up to 140 mm Hg and diastolic BP (DBP) rose to 90 mm Hg after 20 weeks of pregnancy. The 2nd group included 47 women with SBP over 140 to 160 mm Hg and DBP greater than 90 to 100 mm Hg. In the 3rd group were 33 patients with SBP more than 160 mm Hg and DBP greater than 100 mm Hg. In women of control group at this time of observation SBP was mean 105.8 ± 3.26 mm Hg and DBP was mean 68.7 ± 2.84 mm Hg.

The survey was conducted at the moment of detection pregnancy from 7 to 10 weeks and in dynamics of I, II and III trimesters of gestation. GH was verified at absence of distinct clinical symptoms that characteristic for arterial hypertension, and negative kidney samples: protein content in urine less than 0.002 g/l, glomerular filtration rate - 155.8 ml/min, the normal levels of creatinine and urea. In the dynamics of gestation, the number of desquamated endothelial cells circulating in the systemic circulation (CECs) was counted by Aladovec (1978) method in the modification by Petrishev and Vlasov (2003). Nitrates levels were determined by Metelskaya and Gumanova method (2005)¹⁰. The adhesion of platelets was assessed by their ability to form co-aggregates with lymphocytes as described by Vitkovskiy et al. (1999) by determining the percentage of lymphocytes aggregates with thrombocytes (lymphocyte-platelet plugs). The concentrations of IL- β and TNF- α were measured by immunoenzymatic method ELISA on computerized immunoenzymatic analyzer (IEA-AT-858 LTD, China) with reagents of "Cytokine" company (St. Petersburg, Russia).

Statistical and regression analysis was performed using Statistica 6.0 for Windows. Student's t-test and Pearson's correlation coefficient (r) was assessed. Significant differences were considered at $P \leq 0.05$.

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Results and Discussion

We have found that pregnant women with GH that developed in terms of 7-10 weeks of gestation had no damage of the endothelial cells and increase in their number in blood, in comparison with control (Table). Along with this, in 5 (10.8%) patients of the 2nd group and in 8 (24.2%) patients of the 3rd group, the number of CECs exceeded the average levels. With increasing gestational age to 22 weeks the number of CECs in women of the 1st, 2nd and 3rd groups increased, respectively, in 1.5, 1.9 and 2.2 times ($P<0.01$ and $P<0.001$), reaching a maximum at term of more than 22 weeks. Thus, the number of desquamated CECs was over the control values in 1.6, 2.0 and 2.6 times, respectively.

Table 1: Parameters of lymphocyte-platelet adhesion (LPA), cytokines of endothelial dysfunction and blood pressure level in pregnant women with gestational hypertension, $M\pm m$

Groups	NO, mkmol/L	IL-1 β , pg/ml	TNF- α pg/ml	EECs, 10 ⁴ /L	LPA, %	SBP/DBP, Hg mm
1 st group, n=39						
from 7 till 10 weeks	18.9 $\pm$ 0.84	5.9 $\pm$ 0.194	3.5 $\pm$ 0.133	3.7 $\pm$ 0.144	13.6 $\pm$ 0.598	108.4 $\pm$ 3.59/65.7 $\pm$ 2.61
from 20 till 22 weeks	23.7 $\pm$ 0.96*	8.6 $\pm$ 0.346*	5.3 $\pm$ 0.152*	5.2 $\pm$ 0.187*	15.9 $\pm$ 0.593*	123.4 $\pm$ 4.26/75.5 $\pm$ 3.21
>22 weeks	28.9 $\pm$ 1.21*	11.2 $\pm$ 0.462*	9.1 $\pm$ 0.382	6.1 $\pm$ 0.195*	17.1 $\pm$ 0.647*	138.9 $\pm$ 5.68/81.7 $\pm$ 4.09
2 nd group, n=47						
from 7 till 10 weeks	19.3 $\pm$ 0.77	6.1 $\pm$ 0.189	3.3 $\pm$ 0.139	3.9 $\pm$ 0.148	13.8 $\pm$ 0.483	106.2 $\pm$ 4.11/68.4 $\pm$ 2.76
from 20 till 22 weeks	26.4 $\pm$ 1.09*	10.9 $\pm$ 0.375*	5.9 $\pm$ 0.206*	6.5 $\pm$ 0.187*	17.6 $\pm$ 0.598*	116.8 $\pm$ 2.41/88.2 $\pm$ 2.93*
>22 weeks	31.2 $\pm$ 1.17*	14.8 $\pm$ 0.577*	12.8 $\pm$ 0.422*	7.9 $\pm$ 0.324*	19.3 $\pm$ 0.572*	168.9 $\pm$ 6.59/98.5 $\pm$ 4.26*
3 rd group, n=33						
from 7 till 10 weeks	20.2 $\pm$ 0.89	6.1 $\pm$ 0.268	3.6 $\pm$ 0.142	4.1 $\pm$ 0.156	14.2 $\pm$ 0.511	105.3 $\pm$ 3.59/67.6 $\pm$ 2.57
from 20 till 22 weeks	34.5 $\pm$ 1.19*	12.3 $\pm$ 0.467*	6.8 $\pm$ 0.266*	7.7 $\pm$ 0.397*	19.1 $\pm$ 0.543*	120.3 $\pm$ 3.87/93.7 $\pm$ 2.21*
>22 weeks	42.3 $\pm$ 1.57*	16.8 $\pm$ 0.485*	15.6 $\pm$ 0.685*	10.3 $\pm$ 0.402*	21.6 $\pm$ 0.798*	172.4 $\pm$ 4.11/118.5 $\pm$ 3.42*
Control group, n=20						
from 7 till 10 weeks	18.1 $\pm$ 0.71	5.8 $\pm$ 0.168	3.3 $\pm$ 0.145	3.6 $\pm$ 0.133	13.5 $\pm$ 0.318	105.8 $\pm$ 3.26/68.7 $\pm$ 2.84
from 20 till 22 weeks	20.3 $\pm$ 0.85	6.2 $\pm$ 0.174	3.5 $\pm$ 0.122	3.5 $\pm$ 0.104	13.3 $\pm$ 0.545	107.4 $\pm$ 3.49/69.1 $\pm$ 3.16*
>22 weeks	22.4 $\pm$ 0.74	6.6 $\pm$ 0.251	3.8 $\pm$ 0.159	3.9 $\pm$ 0.144	12.9 $\pm$ 0.503	110.5 $\pm$ 3.62/68.2 $\pm$ 3.56*

Note: * - $P<0.05$ to control

Source: Author

As Petrishchev and Vlasov (2003) write, increase in the number of CECs circulating in blood is a highly specific marker of endothelial dysfunction (ED). NO expression due to initiation of inducible form of NO-synthase (iNOS) in response to depression of activity of the basal level of endothelial NO-synthase (eNOS) can contribute to vascular wall damage (Markov, 2005; Manukhina et al., 2007). At the same time, at 7-10 weeks of gestation, in pregnant women of main group NO level in the systemic circulation was within the upper limit of control. In 5 (10.6%) and 8 (24.2%) patients of the 2nd and 3rd groups, respectively, were found elevated CECs and NO levels. After 20-22 weeks of gestation, changes in NO-system were exacerbated: in the 1st group these indices were 1.2-fold ($P<0.05$) and 1.3-fold ($P<0.01$) higher control levels; in the 2nd group - 1.3-fold ($P<0.01$) and 1.4-fold ($P<0.001$); in the 3rd group - 1.7-fold and 1.9-fold ($P<0.001$), respectively. Evidently, increase of NO level with increasing of gestational age in pregnant women with GH is due to peculiarities of restructuring of the membrane structures of endothelial cells as a consequence of exposure of environmental factors (Sukhikh et al., 2008; Shestopalov et al., 2009). This influences on the repair processes in the vascular endothelium of pregnant women with predisposition to GH. Since NO is involved in the implementation of various pathophysiological processes, including cell cooperation, NO expression in pregnant women prone to GH can enhance the aggregation of blood cells. Abakumov et al. (2005), Markov (2005) and Pokrovsky et al. (2005) established that NO expression stimulates formation of tissue factor of activation of coagulation hemostasis. According to Sukhikh et al. (2008), NO overexpression has the ability to support vasospasm in microcirculation of the body of pregnant women for a long time. As a result, tissue hypoxia is exacerbated; area of possible development of DE at the system and polyorgan levels is expanded (Oliveira, 2005). Deceleration of blood flow in organs and tissues against vasospasm and thrombosis reduces potential of shift that, subsequently, reduces eNOS activity and initiates iNOS activity and NO formation (Salpov et al., 2006).

There was found a strong direct relationship between the amount of CECs and NO level in blood of pregnant women of main group ($r=0.77$; $P<0.01$). Hence, damage of the endothelium leads to NO increase, inducing, thereby, processes of endothelial damage and increasing CECs in blood stream. Nikitina et al. (2007) believed that pro-inflammatory cytokines that stimulate iNOS formation in neutrophils, macrophages, endothelial cells and vascular smooth muscle cells contribute to this process. We confirm this fact in our research, which has found increase of NO and CECs levels in the circulating blood. At the same time, in patients of the 1st, 2nd and 3rd groups in terms of 7-10 weeks of gestation these indices were within the control values. After 20-22 weeks, IL-1 β and TNF- α contents in the 1st group were higher in 1.4 and 1.5 times ($P<0.01$), in the 2nd group – in 1.8 and 1.7 times ($P<0.001$), in the 3rd group – in 2.0 and 1.9 times ($P<0.001$), respectively, in comparison with control. Activation of macrophages, which are capable to produce active pro-inflammatory cytokines, has an important place in the initiation of IL-1 β and TNF- α (Sidorova et al., 2010). The impact of the latter on the endothelium exacerbates endothelial damage, thereby, increasing the number of CECs and NO level in blood, activating processes of aggregation, adhesion and hemostasis. We have established a strong direct relationship between CECs in pregnant women after 20-22 weeks of gestation and IL-1 β and TNF- α levels ($r=0.81$ and $r=0.86$; $P<0.001$).

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Thus, damage of the endothelium and other tissues during pregnancy is the triggering factor of ED and cytokine production. In this case, there was a strong relationship between the concentrations of IL- β , TNF- α and NO in blood of pregnant women predisposed to GH ($r=0.81$ and $r=0.90$; $P<0.001$, respectively). Previous studies in vitro by Makarov et al. (2012) showed that NO expression may be one of the mechanisms of the phenomenon of leukocyte aggression. Probably, NO expression leads to stimulation of cells of the immune system and facilitates the production of the studied pro-inflammatory cytokines. Increase of the concentrations of IL- β and TNF- α leads to activation of the hemostatic system, consequently, reducing the potential of shift, stimulating iNOS activity and, as a result, increasing NO level. Our studies are consistent with previous findings. After 7-10 weeks of gestation, all pregnant women of main group marked increase of adhesiveness of platelets to lymphocytes, which was within the upper limit of control. After 20-22 weeks of gestation, lymphocyte-platelet plugs increased in the 1st, 2nd and 3rd groups in 1.2 ($P<0.05$), 1.3 ($P<0.01$) and 1.4 ($P<0.001$) times, respectively, with trend to increase significantly at later terms. Studies by Ostanin et al. (2010) showed that T-helper cells (CD4+) were able to spontaneous rosette formation with intact platelets. In this case, IL- β and TNF- α in vitro stimulates co-aggregation of helper-induced cells with platelets and induces it from natural killers (CD16+). At the same time, Gazieva and Chistyakova (2009) found that IL-4, IL-10, IFN- γ , IL- β and TNF- α , which do not have much influence on lymphocyte-laminar contacts, are inhibitors of LPA. According to Makarov et al. (2012), the LPA phenomenon plays the important role in the development of protective and reparative processes. Platelets were established to promote migration of lymphocytes and their fixation on the surface of damaged vascular wall that allows them to withstand the shear force of blood flow (Oliveira, 2005; Ostanin et al., 2010). Platelets release a number of anti-inflammatory and growth factors. Damage of the vascular endothelium by aggression factors and hypoxia hampers expression of most of the known adhesion molecules (Alexandrova, 2007). As a result, cell migration and cooperation in certain areas of fixation of the vascular wall are disrupted. In this regard, platelet functions are enhanced. Platelets provide contact of lymphocytes and collagen fibers, partly compensate missing antigen-preventing function, helps to promote lymphocytes deeper the damaged part of the vascular system (Salpov et al., 2006; Starodubtseva et al., 2013).

Because of disturbance of the processes of lymphocyte contact with collagen fibers, the reaction of stabilization in the lesions is decreased, vascular permeability is increased, hemostasis on the lesion site is developed, the system of immune response is initiated, as well as the conditions for angiogenesis and tissue proliferation are appeared.

In pregnant women with predisposition to GH we found a direct strong relationship between adhesive ability of lymphocytes, blood platelets and CECs in the circulating blood ($r=0.88$; $P<0.001$). Therefore, high concentration of pro-inflammatory cytokines, between which and the percentage of lymphocyte-plate co-aggregates was marked a strong direct relationship ($r=0.83-0.88$; $P<0.01$) as well, is believed to be the starting mechanism of these processes in the development of GH. Earlier established fact of in vitro LPA amplification by IL- β and TNF- α by Ostanin et al. (2010) and Pinegin et al. (2009) confirms our findings. Gazieva and Chistyakova (2009) reported that lymphocytes in interaction with antigens enhance production of IL- β and TNF- α by immunocompetent cells in the mechanisms of protecting reaction, while NO expression increases the percentage of LPA (Sidorova et al., 2010). In pregnant women with a tendency to the development of GH after 20-22 weeks of gestation we revealed a strong direct relationship between adhesion ability of lymphocytes, blood platelets and NO concentration ($r=0.85$; $P<0.001$). At the same time, a clear direct relationship between high percentage of LPA and DBP levels after 20-22 weeks of gestation was found ($r=0.89$; $P<0.001$). In the period of 7-10 weeks, this relationship was absent ($r=0.18$; $P<0.005$).

Thus, autoimmune processes in the endothelium of maternal blood vessels may be one of possible mechanisms of hypertensive disorders in pregnant women.

Conclusion

In pregnant women at risk of hypertensive disorders, especially after 20-22 weeks of gestation and later, lymphocytes ability to platelets adhesion is rose, the concentrations of pro-inflammatory cytokines and NO level are increased.

The direct relationship between DBP high level with degree of LPA, CECs, NO, IL- β and TNF- α cytokines at gestation period of 20-22 weeks indicates their importance in the pathogenesis of hypertensive disorders in pregnant women.

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