The role of mast cells in the materno-fetal interface in pre-eclampsia

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Abstract
Density of MCs were counted in samples of placenta and uteri taken from 21 women with PE and 12 with normal pregnancy. Utero-Placental index (U/PI) was calculated as a ratio between the density of MCs in the uterus and placenta. Results obtained in PE women were compared to normal subjects. Relationship between the MCs density and duration of PE, predomination of symptom and eclampsia occurrence were assessed. Results: 1) There were no differences in the MCs density in placentas and uteri of PE women as compared to healthy, although the U/PI in PE women was higher (31.24 vs. 10.03). 2) There was a higher MCs density in placentas of PE women who suffered for more than 5 weeks, compared to PE women suffering less (0.85 vs. 0.25). 3) MCs density was higher in placentas of women in whom the hypertension was the predominant symptom, compared to patients with predominating proteinuria (0.81 vs. 0.14), although the U/PI was higher in women with proteinuria (51.1 vs. 9.48). 4) MCs density in placentas of PE women with a history of eclampsia was lower (0.16 vs. 0.75) and the U/PI was higher (61.1 vs. 8.15) than in PE women without eclampsia. Conclusions: 1) MCs density in placentas of PE women is higher if the disease lasts long but the course is not very severe. In women with a short duration of the disease and the rapid course, the placental MCs density is lower compared to women with normal pregnancy. 2) Higher U/PI in PE women than in healthy patients, and higher ratio in eclamptic women than in preeclamptic patients may be an expression of increased migration of maternal MCs as the inflammatory response and the standby for induction of the labour.

Key words: mast cell, pregnancy, preeclampsia

Introduction

Despite extensive research, etiological factors and pathophysiological mechanisms the determination of the development of pre-eclampsia (PE) still remains unclear. Since the association of the disease and the pregnancy is indisputable and it is known that the fetal and trophoblast antigens differ from maternal ones, it seems clear to search for the origin of the disease in abnormal response of maternal immune system to fetal antigens.

It is believed that the failure of recognition of paternal antigens by maternal immune system causes faulty, incomplete expression of non-classical antigen HLA-G on the surface of extravillous trophoblast. That causes the abnormal trophoblast migration into the spiral arteries, where in normal pregnancy trophoblast is supposed to replace the endothelium and muscle membrane of spiral arteries. Trophoblast invasion in the arteries is too shallow and transformation of the spiral arteries is inadequate [1, 2]. As a result, the reactivity of unchanged arteries to maternal pressor agents is excessive and maternal blood pressure tends to increase, what impairs the placental perfusion.

The reduced blood flow through spiral arteries causes a defect of endothelium and an activation of the coagulation system, with the predomination of the thromboxane A2 production over the PGI-1, as well as impaired output of NO and increased production of endothelin. These factors secondarily exacerbate the arterial spasm and cause progressive damage to the endothelial cells what can be regarded as a type of inflammatory response [3].

Currently, much attention is paid to the role of mast cells (MCs) in physiological and pathological processes that occur during pregnancy. Both MCs and their products are present in large amount in the uterus and placenta [3, 4]. MCs progenitors are derived from CD34 + stem cells in the bone marrow. Then they migrate into the tissues, where they become mature. The maturation process is regulated by many factors, the most important
are: SCF (stem cell factor), IL-3, estrogens and progesterone [4, 5]. During the whole pregnancy chemokines are produced in the decidua and the placenta. As the uterus is growing to accommodate the fetus, smooth muscles of uterus extend and chemokines are released. It causes an enhanced migration of MCs progenitors to uterus and their maturation.

The best known product of MCs is histamine, which affects the myometrium fibers by H1 receptor. Furthermore, MCs secrete serotonin, heparin, tryptase, chymase, TNF-alpha, prostaglandins F2alpha, II-4, II-6, II-8, and leukotrienes. MCs are also a source of factors that promote angiogenesis, among them the best known Vascular Endothelial Growth Factor (VEGF), transforming growth factor beta (TGFbeta) and basic fibroblast growth factor (bFGF) and many others [1, 4, 6].

Normal pregnancy depends on many complex processes. These processes allow the proper implantation of the embryo, the sufficient development of the vascular network in uterus and placenta providing good conditions for the fetus’ development during pregnancy, and then are responsible for initiating the delivery and maintenance of normal contraction of uterus during the labor. Due to the ability of producing a number of substances that modulate the environment of uterus and placenta, at all these stages MCs may participate as regulatory factors. It is believed that MCs may play an important role in many pathological states of pregnancy, including PE.

**Objective**

The objective is to evaluate the amount of mast cells in placentas and uteri of women with pregnancy complicated by PE compared to normal pregnancy.

**Materials and methods**

Patients were hospitalized at the Department of Obstetrics and Gynecology of Polish Mother’s Memorial Hospital Research Institute in Łódź.

Group I (study group)

– pregnant women with PE,  n = 21,

Group II (comparative group)

– women with normal pregnancy, n = 12.

All patients delivered by elective caesarean section, none of them had previous labor action. The indications for cesarean section in the study group were: severe (or rapidly deteriorating) condition of the patient related to PE or the deterioration of the fetus’ well-being. In the group of healthy women cesarean section was performed due to breech position of the fetus, very thin uterine scar after previous cesarean section and due to non-obstetric indications.

Patients were recruited to group I according to the generally accepted criteria for PE-diagnosis (blood pressure > 140/90 diagnosed after 20 weeks and proteinuria > 0.3 g per day). This group was divided into two subgroups:

- Ia – patients whose predominant symptom was hypertension, high or hardly amenable to treatment (mean blood pressure > 160/110), with proteinuria 0.3-3 g/per day.
- Ib – patients whose predominant symptom was proteinuria > 3 g/per day, while the mean blood pressure was between 140/90 and 159/109.

During the caesarean section in each patient after the delivery of the fetus and placenta, a tiny excision of uterus wall (from the place previously lying under the placenta) was taken. Each excision contained both the endometrium and the myometrium. When the operation was completed, a fragment of the mother’s surface of placenta was taken (from the central point of placental surface, under the place of umbilical cord attachment).

**Preparation of the formulations.** Tissue pieces were fixed in 15% formalin and 95% alcohol solution, Bouin’s, Zenker’s, Regaud’s or Helly’s fluids and then embedded in paraffin. Paraffin blocks were sliced with microtome and stained in pyanacyjanyl erythrosine solution. After the second change of xylene, the sections were embedded in balsam under a cover. Mast cells were recognized as crimson, oval-shaped or elongated structures.

**Morphometric analysis.** Histological preparations were analyzed using the computer image analysis system consisting of a computer Authentic ADM with AMD Duron TM and graphics processor, combined with a video camera Sony SSC-DC58AP and light microscope Carl Zeiss Jenamed 2. The formulations were analyzed in 200-fold magnification. Microscope image was collected by a video camera generating an analog output. After processing by the analog-to-digital converter, a digital signal was transmitted to the morphometric analysis. Tests were carried out for the blank, with no clinical data.

A number of MCs spotted in placental and uteri samples of each patient were evaluated. The average number of MCs per 1 square millimeter of the surface of each sample was calculated (density of MCs). Then, the density of MCs in uterus was divided by the density of MCs in placenta (U/P index). We treated this index as a factor that characterizes the relationship between maternal and fetal mast cells response to PE.
Then, there was assessed the relationship between
the MCs density in both tissues, U/P ratio and such
factors as:
1) duration of PE,
2) predomination of symptom (hypertension or proteinuria)
3) eclampsia occurrence (or symptoms immediately preceding the attack)

Statistical analysis was performed (after checking the normality of distribution and evaluation of variance)
using Student’s t-test or the Cochran-Cox test at a level of significance of 0.05.

Results
The data characterizing both groups are presented in Table 1.

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<thead>
<tr>
<th>Table 1. Characteristics of the patients</th>
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<tr>
<td><strong>Group I – PE women</strong>&lt;br&gt;<strong>N = 21</strong>&lt;br&gt;</td>
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<tr>
<td><strong>mean value</strong></td>
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<tr>
<td>Age</td>
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<td>Week of delivery</td>
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<td>Newborn’s weight</td>
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<th>Table 2. MCs density in both groups (mean number of MCs per square millimeter of tissue)</th>
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<td><strong>Group I – PE women</strong>&lt;br&gt;<strong>N = 21</strong>&lt;br&gt;</td>
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<tr>
<td><strong>MC’s density</strong></td>
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<tr>
<td>Placenta</td>
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<td>Uterus</td>
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<td>U/P index</td>
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<th>Table 3. Relationship between MCs density and the duration of PE</th>
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<td><strong>Lasting &gt; 5 weeks</strong>&lt;br&gt;<strong>n = 9</strong>&lt;br&gt;</td>
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<tr>
<td><strong>MCs density</strong></td>
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<tr>
<td>Placenta</td>
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<th>Table 4. Relationship between MCs density and the predominant clinical symptom</th>
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<td><strong>Predominant hypertension</strong>&lt;br&gt;<strong>n = 10</strong>&lt;br&gt;</td>
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<tr>
<td><strong>MCs density</strong></td>
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<td>Placenta</td>
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<th>Table 5. Relationship between MCs density and the eclampsia occurrence</th>
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<td><strong>Patients with eclampsia</strong>&lt;br&gt;or upcoming eclampsia&lt;br&gt;</td>
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<tr>
<td><strong>MCs density</strong></td>
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<tr>
<td>Placenta</td>
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<td>Uterus</td>
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<td>U/P index</td>
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There were no differences in the MCs density in samples taken from placentas and uteri of PE women (treating the group as a whole) as compared to healthy pregnant women, although the U/P ratio in women with PE was higher than in healthy women (31.24 vs. 10.03 \( p = 0.043 \)) – Table 2.

It was investigated whether the duration of the disease influenced MCs density in placenta and uterus – Table 3. In 9 women symptoms of PE lasted for more than 5 weeks, during that time the patients were successfully treated. The other 12 patients required the termination of pregnancy in shorter time because of the deteriorating mother’s or fetus’ condition. There was a higher MCs density in placenta samples of PE women who suffered from this disease more than 5 weeks compared to women with PE who suffered from it than 5 weeks (0.85 vs. 0.25 MC/mm², \( p = 0.0037 \)), while the U/P index was higher in patients with a shorter duration of the disease, although there was no statistical significance.

Assessing the MCs density in placentas according to the dominant clinical symptom it was proved that MCs density was higher in women suffering from severe hypertension than in patients where a high grade proteinuria was observed. (0.81 vs. 0.14 MC/mm², \( p = 0.0119 \)). However, the U/P index was higher in women with dominant proteinuria (51.1 to 9.48, \( p = 0.05 \)) – Table 4.

Among the PE patients, a subgroup of pregnant women with particularly fast and dynamically expanding course of this disease was separated. This subgroup included women with the history of eclampsia or with initial symptoms of upcoming eclampsia (blurred vision, headache, disturbed awareness, epigastric pain, coagulation disorders, HELLP syndrome). In this group the interval between the onset of the disease and labor was short, because the drug therapy in these patients was particularly unsatisfactory, which was the reason of premature, immediate delivery by caesarean section. The MCs density in placentas of these women was lower (0.16 vs. 0.75 MC/mm², \( p = 0.006 \)), whereas the U/P index was higher in women with milder course of the disease (61.1 vs. 8.15, \( p = 0.049 \)) – Table 5.

**Discussion**

Pathological changes detected by many researchers in the organisms of pregnant women with PE are regarded to be similar to those perceived in inflammatory reaction [3, 8-10].

The crucial role of MCs is to participate in the expansion of inflammation, the most common form of response of the immune system to foreign stimuli. This response is facilitated by vasodilatation as a result of MCs degranulation and histamine release. Other substances produced in MCs (serotonin, heparin, cytokines) are also involved in the development of inflammatory process. Therefore, it seems that the role of MCs in the pathogenesis of PE is significant and the mechanism of action complicated.

Szewczyk et al. showed that the density of MCs in placentas of women with PE was higher than in normal pregnancy, which was also confirmed by others [1, 10]. The author indicates that histamine secreted by MCs, acting via H2 receptor, increases the production of VEGF, the most important factor stimulating the angiogenesis. Moreover, MCs are a source of many other substances which exhibit the above effect (bFGF, TGF-beta, IL-8 and TNFalpha). This suggests that the increased amount of MCs in PE pregnant women is a compensating effect for disorders in the development of a vascular network in placenta and for a chronic vasocstriction of maternal spiral arteries in the course of PE [1]. The role of MCs in angiogenesis is also highlighted by other authors [11, 12].

In our study, considering the group of women with PE as a whole, we did not find higher density of MCs in placentas compared to healthy pregnant women. However, in PE women in whom the disease course was long (more than five weeks), we noticed higher MCs density in placentas compared with those PE women, whose disease lasted shorter. Similarly, a higher density of MCs was observed in placentas of patients with hypertension as the predominant symptom than in the patients with prevailing proteinuria. Furthermore, we noticed that placentas of women with a particularly fast and rapidly increasing symptoms demonstrated lower MCs density than other PE patients, and even lower than healthy subjects. Therefore, it appears that the length of the disease affects the development of fetal compensatory mechanisms.

Perhaps the divergence between our results and the results obtained by Szewczyk et al. can be explained by the different selection of PE groups. In the above research the average gestational age of delivery of PE women is not given. Basing on the given data – the average newborn’s birth weight 3293 g – we presume that most of those patients must have delivered at full-term, so the PE process must have proceeded relatively long and probably could have been treated effectively. In our PE group, the mean gestational age of delivery was 34.1 weeks, and the birth weight of a newborn was 2072 g.
and apoptosis. The role of fetal MCs in the pathogenesis of PE is unclear. Many studies have shown that histamine causes vasoconstriction of placental vessels, which can potentially increase fetal hypoxia [13-15]. At the same time, the significant role of histamine in promoting angiogenesis exerted via the extracellular receptors H1 and H2 can be counteracted by excessive stimulation of the intracellular receptor Hic, which inhibits this process [12, 16]. The dual role of endogenous histamine in the angiogenesis has been enhanced by Norrby [12].

MCs have the ability to produce iNOS, which can affect metabolic processes in surrounding cells. Nitric oxide produced by endothelium of small vessels in appropriate quantities regulates vascular tension and thus maintains normal blood pressure. The excessive production of iNOS by macrophages and MCs may have a highly destructive effect. Szewczyk et al. proved that although in placentas of PE women there was noticed higher density of MCs the production of iNOS by these cells was reduced. That suggests that there is a blockage in iNOS production by placental MCs in pregnancy complicated by PE [16].

It is believed that, apart from the still poorly known etiologic mechanisms, PE is associated with the damage of endothelial cells caused by the passage of the excessive amounts of trophoblast microparticles into the maternal circulatory system. The amount of these microparticles is too abundant and maternal macrophages and monocytes are not able to pick them and destroy [7, 8]. This process occurs due to the increased apoptosis of trophoblast cells in PE and shedding necrotic debris into mother’s circulation which induces an inflammatory response of macrophages [8, 17, 18]. Pyszak et al. showed in in vitro cultured trophoblast cells that histamine produced by MCs may reduce the dexamethasone-induced apoptosis acting via H1 receptor [6]. This could affect the size of trophoblast proliferation, differentiation and apoptosis.

Chymase released by MCs can influence the development of PE by regulating the vascular tone and permeability [4]. Vascular tone is dependent mainly on the endothelin production. Although the main source of ET-1 are vascular endothelial cells, the other isoforms of endothelins have been isolated in a variety of cells, including the uterus and placenta. Saijo et al. reported that the newly discovered endothelin 1, composed of 31 amino acids [ET-1 (1-31)], plays a role in the pathogenesis of PE by exerting a stronger effect on the myometrium contraction in women with PE compared with healthy ones. However, the shrinking of umbilical arteries caused by ET-1 is weaker in PE women than in healthy ones [19].

Mitani reported that in the myometrium of PE women both the number of MCs and the production of [ET-1 (1-31)] is increased which, according to the author, suggests the overproduction of endothelin and its involvement in the pathogenesis of this disease [20].

It is believed that MCs play a significant role in the induction of uterine contractions, including the induction of labor. The main products of these cells: histamine, serotonin and TNFalpha cause myometrial contraction proved in in vitro studies. Another product, PgF2alpha, supports the contractions of uterus by inducing weak and frequent myometrial cell shrinking, which are important in the process of cervical ripening [4, 21, 22].

In our study the ratio between the uteri and placental MCs density (U/P index) seemed to be of interest. We found that it was higher in women with PE in comparison to the controls (31.24 vs. 10.03, p = 0.043). The value of this ratio was also higher in patients whose predominant symptom was proteinuria versus in women with predominating hypertension (51.1 vs. 9.48, p = 0.05). The same relationship also appeared in case of PE women with a history of eclampsia (or signs of upcoming attack) compared to women without this condition (61.1 vs. 8.15, p = 0.049).

We suggest that the high concentration of MCs in the uterus in women with PE, particularly of the most turbulent, rapid and life-threatening form of this disease - eclampsia, may be a defensive reaction of the woman’s organism and a natural expression of the tendency to labor induction to remove trophoblast tissue, the cause of the disease.

A number of mediators and cytokines produced by MCs are involved in the development of the PE. MCs products can affect the blood vessels contributing to the origin of hypertension through increased production of endothelin. On the other hand, in PE the vasodilatation effect on maternal vessels induced by histamine is impaired [23].

However, their ability to induce the migration of neutrophils and monocytes (cells removing the necrotic trophoblast debris from the maternal circulation) seems to have a positive impact on the reduction of this harmful process. Although it has been shown that histamine narrows veins of the placenta and the umbilical cord,
MCs are involved in the angiogenesis promotion occurring in ischemic placenta. It may be regarded as a defensive mechanism of the fetus.

The effect of increasing uterine contractility caused by a lot of MCs mediators can be considered as a tendency to remove fetal/trophoblast tissues from the organism of PE women. This – which is commonly known – may be also a reason for the increased incidence of preterm births in PE patients.

Conclusions

1) MCs density in placentas of women with preeclampsia is higher if the disease lasts more than several weeks and the course of the disease is not very severe. In women with a short duration of the disease and a rapid course, the placental MCs density is lower compared to women with normal pregnancy.

2) Higher U/P index in preeclamptic women than in healthy patients, and also higher index in eclamptic women than in preeclamptic patients may be an expression of increased migration of maternal MCs as the inflammatory response and the standby for induction of the labour.

References


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