Preeclampsia (PE) is known as one of the complex medical and social challenges for modern obstetrics and gynecology due to the high occurrence of morbidty, serious complications, and mortality. The incidence of preeclampsia in the structure of complications, which may develop over gestation, ranges from 6% to 12% in healthy pregnant women, and from 20% to 40% in pregnant women with extragenital diseases or conditions. In the global structure of maternal mortality over the past 10 years, PE has been consistently ranking the thirds position [1]. Obesity is considered as one of the key risk factors for PE; the incidence of obesity among pregnant women makes up 15-38% [2]. Though, much has been done in investigating PE and obesity, the ultimate mechanisms of the pathogenesis of the separate conditions as well as the comorbidity between PE and obesity are still remaining unclear.

Endothelial dysfunction (ED) and low-intensity systemic inflammation are the main pathogenetic mechanisms of both PE and obesity. Endothelial dysfunction manifests by the reduced production of endothelial nitric oxide (NO), which runs down endothelium-dependent vasodilatation, the increase in oxidative stress, known as an initiating factor of endothelial damage with the further development of characteristic PE symptoms [3]. Chronic vascular affection is characterized by the damage to endothelial cells along the vascular wall that is manifested by the presence of circulating endothelial microparticles (CEM). It has been proven that CD32+CD40+ CEM serve as a marker of endothelial dysfunction, and changes in their content represent the ED progression in pathological conditions or the possibility of the endothelial recovery [4]. Circulating endothelial microparticles have been found out to stimulate inflammatory processes in the vascular wall by activating the synthesis of such pro-inflammatory markers as IL-6, TNF-α, NF-κβ [5]. Previous reports have demonstrated the level of CD31+/CD42+/CD62+/CD105+ CEM is probably higher in women with PE, but so far no data have been found referring the CEM level in pregnant women with PE and obesity that may contribute much to early ED detection under these circumstances [6].

Vascular endothelial growth factor (VEGF), a marker representing the vascular endothelium condition, plays an important role in the PE pathogenesis. VEGF is known to stimulate the production of a potent vasodilator NO associated with gestational vasodilatation typical for normal pregnancy. It has recently been shown that the C rs3025039 allele polymorphism of the VEGF-A gene is associated with the PE development in pregnant women [7]. Moreover, the studies on VEGF-gene knockout mice have demonstrated the deterioration of VEGF signalling pathways in the kidney results in clinical manifestations of preeclampsia such as the deterioration of VEGF signalling pathways in the kidney results in clinical manifestations of preeclampsia such as
The aim of this study was to investigate the serum levels of soluble CD32+CD40+ circulating endothelial microparticles (CEM) in pregnant women with PE and class II-III obesity compared to pregnant women with PE and normal body weight. The study included 122 pregnant women in the third trimester of pregnancy, divided into six groups: Group 1 consisted of pregnant women with PE and class I obesity; Group 2 consisted of pregnant women with PE and class II-III obesity; Group 3 consisted of pregnant women with PE and normal body weight; Group 4 consisted of pregnant women with PE and class I obesity; Group 5 consisted of pregnant women with PE and class II-III obesity; and Group 6 consisted of pregnant women with PE and normal body weight.

The expression level of circulating endothelial microparticles CD32+CD40+ in venous blood was measured by flow cytometry using murine anti-human monoclonal CD40 antibodies conjugated to FITC (BD Pharmingen, USA) by the flow cytometer “EPIX XL-MCL” (Beckman Coulter, USA). Murine IgG labelled with fluorescent dyes were used as control. Data for calculating the absolute number of particles, taking into account the dilution during the measurement, were represented in the form of A×10^7/l.

The serum VEGF concentration was assessed by enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Vector-Best, Russia).

Statistical analysis of the findings obtained was performed using the program “Statistica 7.0” (StatSoft Inc., USA) by methods of descriptive statistics with the calculation of quantitative indicators as average sample values (M) and mean error (m) in the study groups. The statistical significance of the differences was determined by the Student’s t-test. The difference value of p<0.05 was considered statistically significant.

Materials and Methods

Our study included 122 pregnant women in the third trimester who received antenatal care at maternity welfare clinics and obstetric departments of Poltava city for 2018 – 2019. They were divided into 6 groups: the 1st group (control) consisted of women with uneventful uncomplicated course of pregnancy and physiological body weight index (BMI) 18.5–24.9 kg/m^2 (n=20); the 2nd group included pregnant women with class I obesity (n=15); the 3rd group included pregnant women with class II–III obesity (n=15); the 4th group was made up of pregnant women with PE and physiological body weight (n=8); the 5th group consisted of pregnant women with PE and class I obesity (n=14); the 6th group included pregnant women with PE and class II–III obesity (n=14). We studied the serum VEGF concentration and performed the count of CD32+CD40+ circulating endothelial microparticles. The study design was approved by the Commission on Bioethics, Ukrainian Medical Stamotological Academy (Minutes № 170, 24.01.2019). Written consents were given by all study participants.

The class of obesity in pregnant women was calculated by the height-weight parameters with the BMI calculation, taking into account the gestational age and age of women, according to the table developed by N.S. Lutsenko [11]. Inclusion criteria were the following: monosiesis, women in the third trimester of gestation without severe extragenital diseases or conditions.

The expression level of circulating endothelial microparticles CD32+CD40+ in venous blood was measured by flow cytometry using murine anti-human monoclonal CD40 antibodies conjugated to FITC (BD Pharmingen, USA) and PE murine anti-human monoclonal CD32 antibodies (BD Pharmingen, USA) by the flow cytometer “EPIX XL-MCL” (Beckman Coulter, USA). Murine IgG labelled with fluorescent dyes were used as control. Data for calculating the absolute number of particles, taking into account the dilution during the measurement, were represented in the form of A×10^7/l.

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Results

Analysis of the expression level of CD32+CD40+ CEM showed (Fig. 1A) that in the group of the pregnant women with class I obesity the indicators tend to increase in 2.5 times in contrast to the group of the pregnant women with uncomplicated pregnancy and physiological body weight (3.29±0.83×10^7/l vs. 1.33±0.54×10^7/l, p<0.05). However, in the pregnant women with class II–III obesity, the level of CD32+CD40+ CEM was probably 6.6 times higher than in the groups of women with normal pregnancy and physiological body weight (8.86±1.48×10^7/l vs. 1.33±0.54×10^7/l, p<0.05). We found the level of CD32+CD40+ CEM in the group of the pregnant women with class II–III obesity was probably in 2.7 times higher than in the group of the pregnant women with class I obesity (8.86±1.48×10^7/l vs. 1.33±0.54×10^7/l, p<0.005).

The level of CD32+CD40+ CEM (Fig. 1B) in the group of the pregnant women with PE and concomitant class I obesity was probably higher than in the pregnant women with PE and physiological body weight (9.76±2.66×10^7/l vs. 7.64±1.26×10^7/l, p<0.05). Similarly, in the pregnant women with PE and class II–III obesity, the expression level of CD32+CD40+ CEM is probably 1.7 times higher than in the group of the pregnant women with PE and physiological body weight (13.13±0.55×10^7/l vs. 7.64±1.26×10^7/l, p<0.002). No significant difference was observed between the groups of the pregnant women with PE and class I obesity, and the pregnant women with PE and class II–III obesity (9.76±2.66×10^7/l vs. 13.13±0.55×10^7/l).

Analysis of the expression level of CD32+CD40+ CEM in the blood serum of the studied groups showed that this marker in the pregnant women with class I obesity is probably lower than in the pregnant women diagnosed as having PE and concomitant class I obesity (3.29±0.83×10^7/l vs. 9.76±2.66×10^7/l, p<0.05). The expression level of CD32+CD40+ CEM in the pregnant women with class II–III obesity is probably lower than that in the pregnant women with PE and class II–III obesity (8.86±1.48×10^7/l vs. 13.13±0.55×10^7/l, p<0.05).
Thus, the results obtained show that the expression level of CD32\(^+\)CD40\(^+\)CEM gradually grows in the pregnant women along with the increase in obesity when compared with the women who have normal uncomplicated pregnancy and physiological body weight. A similar tendency in the elevation of the expression level of CD32\(^+\)CD40\(^+\)CEM and the increasing level of obesity was observed in the pregnant women with confirmed diagnosis of PE.

The assessment of the serum VEGF concentration in the pregnant women reveals (Fig. 2A) that the group with class I obesity has the values, which are probably lower in contrast to the group of women with physiological body weight (234.7±15.4 pg/ml vs. 876.1±101.9 pg/ml, p<0.001). Similarly, in the pregnant women with class II–III obesity, the level of VEGF concentration is probably 6.2 times lower than in the group of the women with uncomplicated pregnancy and physiological body weight (141.1±10.8 pg/ml vs. 876.1±101.9 pg/ml, p<0.001). The level of VEGF concentration in the group of the pregnant women with class II–III obesity is probably 1.7 times lower than the relevant indicators in the group with class I obesity (141.1±10.8 pg/ml vs. 234.7±15.4 pg/ml, p<0.0001).

The level of VEGF concentration (Fig. 1B) in the group with PE and class I obesity is probably 1.8 times lower than in pregnant women with PE and physiological body weight (92.9±5.5 pg/ml vs. 167.6±10.3 pg/ml, p<0.005), whereas the level of VEGF concentration in the pregnant women with PE and concomitant class II–III obesity is probably 2.2 times lower than in the group of the pregnant women with PE and physiological body weight (73.6±11.1 pg/ml vs. 167.6±10.3 pg/ml, p<0.0001). We have observed that the level of this growth factor is probably 1.3 times lower in the group of pregnant women with PE and class II–III obesity than the relevant indicator in the group of pregnant
women with PE and class I obesity (73.6±11.1 pg/ml vs. 92.9±5.5 pg/ml, p<0.05).

The analysis of the serum VEGF concentration has demonstrated the level of this marker in the pregnant women with class I obesity is probably higher than in the pregnant women with PE and class I obesity (234.7±15.4 pg/ml vs. 92.9±5.5 pg/ml, p<0.001). The level of VEGF concentration in the pregnant women with class II-III obesity is probably higher than in the pregnant women with PE and concomitant class II-III obesity (141.1±10.8 pg/ml vs. 73.6±11.1 pg/ml, p<0.001).

Hence, the data showed a significant decrease in the level of VEGF concentration in the pregnant women against the background of increasing obesity in comparison with the women with physiological body weight. Similarly, in pre-eclampsia, the VEGF concentration decreases significantly with increasing severity of obesity.

Vascularisation of the placenta plays a vital role in the development of a foetus and is crucial over the course of gestation. A number of factors are involved in the regulation of angiogenesis, including CEM, whose concentration reflects the state of the endothelium, and VEGF, which induces the synthesis of NO, a powerful vasodilator, necessary to maintain low vascular resistance of fetoplacental circulation [12].

**DISCUSSION**

The study has demonstrated that in gestation complicated with obesity, there is an increase in the level of the endothelial dysfunction marker CD32⁺CD40⁺CEM and a decrease of the angiogenic factor VEGF in the peripheral blood circulation. The increase in the level of CD32⁺CD40⁺CEM expression and the decrease in the level of the angiogenic factor VEGF in pregnant women with PE and concomitant obesity point out a more pronounced aggravating effect of obesity as one of the risk factors for pregnancy. At the same time, there is an increasing tendency to change both markers as the severity of obesity increases from class I to class II-III. The growth in CD32⁺CD40⁺CEM in obese pregnant women indicates a strong effect produced by mediators of adipose tissue inflammation and damaged placenta, namely IL-6, TNF-α and leptin on the structure of maternal endothelial cells through the induction of their apoptosis [13], increased CEM secretion and CD31⁺/CD42b⁺CEM circulation in the peripheral blood [14]. Activation of endothelial cells can contribute to both inflammatory reactions and vasoconstriction, leading to endothelium-dependent vasodilatation, intense vasoconstriction, and others. In obese pregnant women, the increased expression of placental endothelial damage marker IL-1β is associated with increased maternal BMI [15]. We have found out a decrease in VEGF in obese pregnant women can result from either the VEGF blockade with soluble sVEGF form [16], or a decrease in the formation of complex interactions VEGF–Flt-1 in the placenta that causes a decrease in branching of placental angiogenesis in pregnancies complicated with obesity that results in the deterioration of intra-placental perfusion, followed by placental ischemia [17]. Some authors consider the increased VEGF and eNOS in the placental endothelium in obese women suggest the possible existence of a compensatory mechanism for remodelling in placental circulation associated with obesity [18].

Our data on elevated levels of CD32⁺CD40⁺CEM during gestation complicated with PE and obesity are consistent with findings in the literature [19], which showed that the level of CEM goes up with the progression of endothelial dysfunction severity and PE severity. It is known that PE is characterized by impaired development of placenta that is associated with inadequate trophoblast invasion and modelling of spiral arteries as a result of angiogenic, immune and inflammatory dysregulation at the interface between the maternal and foetal parts of the placenta. One of the main risk factors for PE occurrence in pregnant women is obesity, as adipose tissue is known as a powerful producer of inflammatory mediators that can contribute to the PE development. The elevation in the CD32⁺CD40⁺CEM we detected may indicate not only an increase in ED, but also an increase in the systemic inflammatory response involving pro-inflammatory cytokines IL-6 and TNF-α [11]. The decrease in VEGF concentration in pregnant women with PE and concomitant obesity is confirmed by data on the reduction of the level of free circulating VEGF [20] and the lowering in bioavailability of membrane-bound VEGF receptors in preeclampsia that is associated with systemic and placental angiogenic imbalance [21].

Thus, ED can be regarded as a potential contributor to the development of pathogenetic mechanisms in the development of preeclampsia that is mediated by the markers of inflammatory activation of endothelium CD32⁺CD40⁺CEM and VEGF, and obesity acts as an independent triggering factor for the development of preeclampsia and systemic inflammation and can also further to the development of metabolic imbalance, placental dysfunction and foetal distress.

Given the relevance of preeclampsia as a grave complication in modern obstetrics and the prevalence of obesity over the world, perplexity and various negative consequences for both mother and foetus, the further detailed study of the pathogenesis of preeclampsia is required to develop adequate etiopathogenetic treatment and elaborate the prevention program of this disease for pregnant women with concomitant obesity.

**CONCLUSIONS**

Our work has led us to conclude that:

1. Angiogenic factor VEGF in the serum of pregnant women with concomitant obesity over the third trimester naturally decreases with the increase in the level of obesity.
2. In pregnant women with preeclampsia, there is a significant decrease in the serum VEGF concentration, which progresses when combining preeclampsia and obesity.
3. An increased content of circulating endothelial microparticles CD32⁺CD40⁺ found out in the blood of patients with preeclampsia indicates severe endothelial damage.
4. The severity of endothelial dysfunction, which is manifested by the rise in the level of circulating endothelial microparticles CD32+CD40+, increases in direct proportion to the severity of obesity.

5. The count of circulating endothelial microparticles CD32+CD40+ and indicators of VEGF concentration in the blood can serve as reliable markers for determining the severity of endothelial dysfunction in pregnant women with concomitant obesity and preeclampsia.

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