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Original Article

Soluble urokinase-type plasminogen activator receptor (suPAR) and interleukin-6 levels in hyperemesis gravidarum

Raziye Desdicioglu [a,*], Melahat Yildirim [a], Gulcan Kocaoglu [b], Busra Demir Cendek [c], Gamze Avcioglu [d], Emre Erdem Tas [a], Ozlem Sengul [a], Ozcan Erel [d], Ayse Filiz Yavuz [a]

[a] *Department of Obstetrics and Gynecology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey*
[b] *Department of Obstetrics and Gynecology, Sanliurfa Mehmet Akif Inan Training and Research Hospital, Sanliurfa, Turkey*
[c] *Department of Obstetrics and Gynecology, Zubeyde Hanim Training and Research Hospital, Ankara, Turkey*
[d] *Department of Biochemistry, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey*

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Abstract

Background: The aim was to compare serum soluble urokinase-type plasminogen activator receptor (suPAR) levels as well as interleukin-6 levels (IL-6) in pregnant women with hyperemesis gravidarum (HG) and asymptomatic pregnant women.
Methods: Our study population consists of voluntary first trimester-pregnant women who applied to the outpatient clinic of the department of obstetrics and gynecology of Ankara Ataturk Training and Research Hospital. Between February and May 2016, 60 pregnant women were included in our prospective study. Serum suPAR and IL-6 levels were evaluated with the ELISA method. Twenty-nine pregnant women with HG and 31 asymptomatic pregnant women were included in the study.
Results: Serum suPAR level in the HG group was measured as 0.36 ± 0.56 ng/ml, whereas this level in the healthy pregnant control group was measured as 0.15 ± 0.15 ng/ml ($p < 0.05$). The interleukin-6 level in the HG group was 5.69 ± 2.16 pg/ml, whereas in the control group it was measured as 3.88 ± 0.28 pg/ml ($p < 0.05$).
Conclusion: Serum suPAR and IL-6 levels proved to be high in the HG group. It is likely that suPAR could play a role in the etiopathogenesis of hyperemesis gravidarum.

Keywords: Hyperemesis gravidarum; Interleukin-6; Soluble urokinase-type plasminogen activator receptor; suPAR

1. Introduction

Nausea − vomiting is a condition that affects 80% of pregnant women in their first trimester in particular. It continues throughout the gestational period in 10% of patients.[1] The condition of nausea−vomiting which is resistant to treatment and is accompanied by serious weight loss, disturbance in fluid-electrolyte balance, restraint in daily activities, and impairment in the general health condition is referred to as hyperemesis gravidarum (HG).

HG is seen in almost 1% of all gestations, and may also require hospitalization and advanced treatment methods.[2] The etiology of nausea−vomiting and HG that increases during the gestational period has not yet been clearly explained. Among the factors predisposing HG are ethnic origin, occupation, obesity, mother's age, history of HG in the previous gestation, history of infertility, and duration between gestations.[3] Hormonal changes, carbohydrate metabolism disorders, gastroenterological dismotility, genetic susceptibility, as well as immunological and inflammatory causes, are thought to be effective on the

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* Corresponding author. Dr. Raziye Desdicioglu, Department of Obstetrics and Gynecology, Faculty of Medicine, Ankara Yildirim Beyazit University, Bilkent Street, No: 1, Cankaya, Ankara 06800, Turkey.
E-mail address: raziyedesdicioglu@gmail.com (R. Desdicioglu).

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pathogenesis of HG.[3] Multiple gestations, trophoblastic diseases and some fetal anomalies may also be associated with HG.[3−5] However, no exact relationship has been proved between HG and any other single factor up till now. So long as its etiology and pathogenesis are not fully known, treatment for HG will continue to be empirical and suboptimal.

Soluble urokinase-type plasminogen activator receptor (suPAR) is the soluble form of urokinase-type plasminogen activator receptor (uPAR), which is formed secondary to the post-inflammatory-signal release.[6] The plasminogen activation system including suPAR is a significant factor in cell adhesion, migration and proliferation in cases with response to inflammation and infection.[7] These molecules are generally released from neutrophil, monocyte, macrophage and activated T cells, and serum levels reflect the active pathophysiological events at the cell surface.[8] Increased serum suPAR level is a marker of immune inflammatory activation.[8] In particular, suPAR levels that increased in sepsis in intensive care patients are shown to be associated with clinical prognosis and mortality.[9] It was determined that the increase in serum suPAR level in some patient populations indicated lipid and carbohydrate metabolism disorders as well as inflammation and immune response activation, and that suPAR proved to be a prognostic factor in patients with bacteremia in particular.[6,10] It was shown that suPAR level in healthy gestation during the 3rd trimester decreased in comparison to unhealthily pregnant women of the same age group.[11] It was also shown that suPAR level during the 3rd trimester increased in some complications regarding gestation (pre-eclampsia, asthma).[11,12]

Interleukin-6 is a cytokine with pluripotent activity, which is released from activated lymphocytes and macrophages.[13] The placenta was shown to be the major source of IL-6 and other cytokines.[14] Excessive production of these cytokines was associated with gestational complications involving pre-eclampsia, HG and missed abortion.[3,15,16]

There are several studies investigating the relationship between inflammatory markers and cellular immunity increase and the presence of HG and its severity during gestation.[3,17,18] Yet none of them is sufficient alone when it comes to diagnosis and clinical prognosis. Also, it was stated that the diagnostic and prognostic value of suPAR levels, though showing a positive correlation with other proinflammatory markers, proved to be higher than other proinflammatory markers like interleukin 6 and TNF alpha and that it was not affected by the circadian rhythm.[6,10,19,20]

In our study, it was planned that this new marker and IL-6 serum levels be compared in pregnant women with HG and in a control group of the same gestational week with no nausea−vomiting problem during 1st trimester. It was also planned to investigate whether or not serum suPAR and IL-6 levels showed any correlation with the clinical severity of HG.

2. Methods

Our study population consisted of voluntary first trimester-pregnant women who applied to the outpatient clinic of the department of obstetrics and gynecology of Ataturk Training and Research Hospital. A written informed consent form was received from all the participants. Approval was also received from the ethical committee of Ataturk Training and Research Hospital. Our study was planned to be conducted prospectively. In our study, singleton pregnant women with less than 14 weeks gestation with weight loss and ketonuria $\geq 2+$ as well as nausea and vomiting were included in the HG group. Normal singleton pregnant women less than 14 weeks without nausea and vomiting were included in the control group. Pregnant women with systemic diseases such as asthma and diabetes as well as urinary tract infections, hyperthyroidism, GI-induced nausea and vomiting were excluded from the study. For the exclude any infection in the pregnant women in the HG and control group, physical examination, temperature measurements, white cell counts and urine tests were used. Patients positive for infection markers were excluded from the study. Pregnant women with infection treatment for any system within the previous 1 month were excluded from the study.

Twenty-nine patients with HG and 31 control patients were included in the study. The patients' age, body mass index (BMI), gestational age, number of pregnancies and births were recorded. The values of weight at the start of pregnancy and the values of weight loss of patients in the study group were recorded. The number of vomiting episodes per day was questioned.

Urine analysis, complete blood count and fasting biochemical tests were completed for all pregnant women. After the venous blood samples taken from the patients were centrifuged, serum samples of patients were stored at $-80\ ^{\circ}C$ in a deep freezer for measurement of suPAR and IL-6 levels. For suPAR assays, a micro ELISA reactive (Receptor (PLAUR/uPAR) ELISA Kit, Hangzhou Eastbiopharm Co. Ltd. Hangzhou, PRC) and a micro plate reader (Biotek ELx 800, BioTek Instrumentations, Inc, Winooski, VT, USA) were used.

IL-6 levels were measured by using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with Human IL-6 Platinum Elisa kit (Affymetrix Ebioscience).TSH levels were measured by using by Automated Chemiluminescence Systems (Bayer, NY, USA).

The Mann−Whitney U test was used for evaluation of variables without normal distribution. The significance level was considered as $p < 0.05$. Both the relationship between serum suPAR, IL-6 and thyroid stimulating hormone (TSH) levels and the relationship between clinical markers of the patients and these parameters were assessed with correlation analysis.

3. Results

In our study, the mean age of the patients in the HG group was 27.10 ± 5.7 and the mean age of the control patients was 27.35 ± 5.1 years. There was no significant difference between the two groups in terms of mean age ($p > 0.05$). While there was no significant difference between the two groups in terms of the number of pregnancies, gestational age and baseline BMI, the number of vomiting episodes and weight loss were significantly higher in the HG group ($p < 0.05$).The demographic data are summarized in Table 1.

There was no significant difference between the two groups in terms of serum white blood cell (WBC), hemoglobin (Hb), hematocrit (Hct), and platelet (Plt) counts. Serum suPAR level was found to be significantly higher in the HG group compared to the control group (0.15 ± 0.15 ng/ml in the control group; 0.36 ± 0.56 ng/ml in the HG group; $p < 0.05$). Serum TSH level was found to be significantly lower in the HG group compared to the control group (2.01 ± 1.49 uIU/mL in the control group; 1.13 ± 1.29 uIU/mL in the HG group) and it was seen that this difference was significant ($p = 0.02$). IL-6 levels in the HG group were found to be higher than the control group ($p < 0.05$). Laboratory data are summarized in Table 2. In the correlation analysis, there was no correlation between serum suPAR level and gestational age, the number of vomiting episodes per day, urine ketone levels, and maternal weight loss. There was a positive correlation between IL-6 level and serum TSH level. There was no correlation between suPAR level and TSH. There was no correlation between IL-6 and suPAR level.

4. Discussion

HG is a clinical condition characterized by fluid and electrolyte changes, dehydration and metabolic changes and its etiology is unknown. In our study, first trimester suPAR and IL-6 levels were shown to be significantly increased in pregnant women with HG compared to the control group. This study is the first study investigating the relationship between suPAR levels and HG.

SuPAR is a marker released from the membrane-dependent plasminogen activator and is associated with immune system activation. It has been shown to be associated with many conditions such as infectious, inflammatory diseases and cancer.[6,10,21,22] Moreover, in past studies it was seen that suPAR was not affected by serum freeze-thaw procedures in contrast to circadian rhythms and other inflammatory markers.[8,23] This makes suPAR a highly reliable marker in the diagnosis and progression of diseases. There is no study investigating the relationship between serum suPAR level and HG, but third trimester serum suPAR levels of patients with preeclampsia were significantly higher in studies conducted during pregnancy.[12] First trimester serum suPAR levels were not associated with preeclampsia developing in a subsequent pregnancy.[12] In a study, it was found that serum suPAR levels were higher in pregnant women with asthma compared to pregnant women without asthma but the difference was not significant. In the same study, serum suPAR levels were found to be lower in healthy pregnant women compared to a healthy non-pregnant control group in the same age group.[11] In our study, serum suPAR level was found to be significantly higher in pregnant women diagnosed with HG in the first trimester compared to the control group. There are other studies evaluating the relationship between immune mediators and HG. Adenosine deaminase (ADA) activity has been shown to be significantly increased in patients with HG.[24] In a study investigating the relationship between HG and CRP and Vaspin – released from visceral fat and considered a proinflammatory marker – the levels of the two markers were found to be significantly higher in the HG group.[17] Moreover, tumor necrosis factor alpha (TNF-α) levels were found to be increased in HG.[25] The result of our study is consistent with these results and it also shows the effectiveness of immune mechanisms. It was not affected by circadian rhythms and serum freeze-thaw procedures and this feature makes its use advantageous in clinical practice. Unlike our results, in a study evaluating levels of neopterin, which is a mediator associated with cellular immunity, there was no significant difference between patients with HG and control group in terms of levels of neopterin.[26] This different result may be related to the selection criteria for patients in the HG group.

It was shown that macrophage-derived cytokines such as IL-6 are found in the human placenta.[14] Probably these cytokines regulate the development and function of the trophoblast. In the early weeks of pregnancy, increased extrathymic T cells and natural killer in pregnant women with HG suggests that cytokine activation may be involved in the pathogenesis of the disease. There are studies examining the relationship between IL-6 and HG. Among these studies, while some studies found that IL-6 level was increased in HG group with no significant difference, some studies found that it was lower in HG group compared to control group.[3,16,26] In our study, IL-6 level was significantly higher in the HG group. It was considered that IL-6 production is stimulated by trophoblast-derived IL-1 and this increase also raises hCG production via IL-6 receptors.[27,28] The level of hCG was higher, similar and lower in patients with HG compared to control patients.[29–31] This may explain how the IL-6 level was different in the studies. In our study, the level of hCG could not be evaluated.

Table 1
Demographic features of groups.

Parameters	Control (n = 31)	HG (n = 29)
Age (years)	27.35 ± 5.18	27.10 ± 5.77
BMI (kg/m^2)	24.64 ± 4.87	24.69 ± 3.86
Gravida	2.16 ± 1.03	2.10 ± 1.29
Pregnancy week	9.68 ± 2.52	9.93 ± 2.56
Loss of weight (kg)	0.13 ± 0.49	3.00 ± 1.90*
Vomiting episodes (per day)	0.10 ± 0.39	5.17 ± 3.41*

HG: Hyperemesis gravidarum.
*$p < 0.05$.

Table 2
Comparison of parameters.

Parameters	Control (n = 31)	HG (n = 29)
IL-6 (pg/ml)	3.88 ± 0.28	5.69 ± 2.16*
suPAR (ng/ml)	0.15 ± 0.15	0.36 ± 0.56*
TSH (μIU/ml)	2.01 ± 1.49	1.13 ± 1.29*
WBC (K/mm^3)	8964.67 ± 2382.70	11,490 ± 17339
Hemoglobin (gr/dl)	12.30 ± 1.02	12.70 ± 0.90
Hematocrit (%)	36.90 ± 2.33	38.05 ± 2.51
Platelet count (K/mm^3)	258.87 ± 53.4	249.59 ± 50.06

IL-6: Interleukin-6, suPAR: Soluble Urokinase – type Plasminogen Activator Receptor, TSH: Thyroid stimulating hormone, WBC: White blood cell.
*$p < 0.05$.

Serum TSH levels were found to be significantly lower in the HG group. Transient hyperthyroidism was detected in approximately 60% of the patients with HG.[32] The increased level of hCG in circulation binds to the TSH receptor and so it stimulates the thyroid gland. Therefore, it was considered that TSH levels are temporarily suppressed in the first trimester of pregnancy. Especially in HG, TSH suppression is more obvious in patients with high levels of hCG.[25] Our findings are consistent with results of studies on this subject. In our study, free T3 and T4 levels in all patients could not be evaluated. While IL-6 level was significantly correlated with TSH in our study, there was no correlation between suPAR level and TSH. There was no correlation between IL-6 and suPAR level.

Based on these results, the difference in suPAR levels in the HG group can be considered to be associated with the etiopathogenesis of HG.

Sometimes steroids are used in pregnant women with treatment-resistant nausea and vomiting and cause dramatic improvement. This suggests that the immune system and the inflammatory response may be active in the etiopathogenesis of HG.[16]

The limitations of our study are that it contains a small number of patients and is a cross-sectional study. Moreover, only the first trimester suPAR and IL-6 levels of the patients were examined. In future studies, second trimester levels should also be evaluated in the serum of a larger number of patients.

Starvation arises due to nausea and vomiting in patients with HG. The immune functions which are normally suppressed in the fasting state are active in pregnant women with HG. This suggests that immune mechanisms may be effective in the pathogenesis of the disease.[25] This study is the first study investigating the relationship between suPAR levels and HG and the increased level of suPAR in the HG group suggests that cellular immune mechanisms are important in the pathogenesis of HG. In the future, the presence of treatment methods to prevent overstimulation of the immune system in pregnant women would enable reduction of the impact of this disease which negatively affects the daily life of pregnant women and involves an extra financial burden (labor loss, financial losses because of hospitalizations, etc.) on the state. In this sense, our study is extremely significant in terms of illuminating the ethiopathogenesis of HG. SuPAR is more reliable because it is not affected by some factors that affect other markers[6,10,19,20] and this makes it advantageous to use for diagnostic purposes.

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