Serum Levels of B Lymphocyte Activating Factor (BAFF) and a Proliferation-Inducing Ligand (APRIL) in Patients with Preeclampsia

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Citation

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Abstract

Objectives: To elucidate the role of BAFF and APRIL in pathogenesis of preeclampsia.

Materials and Methods: This was a case-controlled study being performed at Zeinabie and Hafez Hospitals of Shiraz University of Medical Sciences. We included 135 subjects in 3 study groups including 45 patients with preeclampsia (group 1), 45 healthy pregnant individuals (group 2) and 45 healthy non-pregnant individuals (group 3). Serum levels of BAFF and APRIL were measured and compared between groups.

Result: The mean age of the participants was 27.8 ± 6.4 years. There was not any significant difference between three study groups regarding the baseline characteristics. Serum levels of APRIL were significantly higher in those patients with preeclampsia compared to healthy pregnant women (7.46 ± 5.41 vs. 3.59 ± 2.61 pg/dL; p=0.009). In the same way, serum levels of APRIL was significantly higher in those with preeclampsia compared to healthy non-pregnant individuals (7.46 ± 5.41 vs. 4.16 ± 3.15 pg/dL; p=0.034). There was not any significant difference between three study groups regarding the serum levels of BAFF.

Conclusion: Our findings demonstrate that serum levels of APRIL are higher in those with preeclampsia compared to both normal pregnant and non-pregnant individuals. However serum levels of BAFF are not different between preeclamptic patients and normal individuals. Thus it can be concluded that APRIL is likely one of the factors responsible for immunological disturbances leading to development of preeclampsia.

INTRODUCTION

Preeclampsia is one of the most common complications of pregnancy with estimated prevalence of 6% to 8% in all pregnancies. It is associated with poor pregnancy outcome and several pregnancy complications including fetal growth restriction, premature birth, and infant and maternal morbidity and death [1]. Endothelial and/or vascular dysfunction in the early pregnancy leads to hypertension in the late pregnancy [2] associated with several clinical symptoms including edema, and proteinuria [3].

Although several studies have investigate the pathogenesis of preeclampsia, its exact underlying mechanisms is yet to be identified. Recent studies have provide evidences that preeclampsia is a result of activation of both innate and adaptive immune system leading to excessive and exaggerated systemic inflammatory response [4,5]. It was shown by Saito et al. [6] that patients with preeclampsia suffer from predominance of Th-1 immunity secondary to lack of Th2 skewness characteristic for healthy pregnancy. They found that peripheral blood percentage of Th2 decreases significantly in those with preeclampsia compared to normal pregnant women and thus the ratios of Th1/Th2 increase significantly in the third trimester of pregnancy [6]. Furthermore they found that peripheral production of interleukin (IL)-2, interferon (IFN)-g and tumor necrosis factor (TNF)-a by peripheral blood mononuclear cells (PBMCs) are increased in those with preeclampsia compared to healthy pregnant women. The serum levels of these proinflammatory cytokines positively correlate with the systolic blood pressure and the concentration of Th1 cytokines [7]. In another recent study, Szarka et al. [8] found that patients with preeclampsia suffer from increased IL-2/IL-4 and IFN-gamma/IL-4 ratios suggestive of a shift

toward Th1-type immunity in addition to increased circulating levels of the pro-inflammatory cytokines including IL-6, tumor necrosis factor (TNF)-alpha, the chemokines IL-8, IP-10 and monocyte chemotactic protein (MCP)-1, as well as the adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in comparison to healthy pregnant women leading to a proinflammatory systemic environment. They also observed a positive correlation between serum concentrations of interferon-gamma-inducible protein (IP)-10, MCP-1, ICAM-1 and VCAM-1 with blood pressure values, renal and liver function parameters, C-reactive protein (CRP), malondialdehyde, von Willebrand factor antigen and fibronectin levels [8].

Previous studies have demonstrated that the immune regulations during the pregnancy is modulated by the TNF superfamily [9-11] being responsible for placental cell growth, cell death, cell migration and hormone production [12,13]. Death-inducing TNF superfamily ligands genes have already been detected in placental trophoblast cells [14]. It was also shown by Phillips et al. [14] that trophoblast cells also include some nonapoptosis- inducing ligands of this superfamily (APRIL, BAFF and CD30L/CD153). The maternal decidual cells are also responsible for providing the embryo with nutrients and oxygen during the invasive phase of the implantation before the placenta matures. They also serve as an immunologic barrier between two distinct immune systems of mother and the fetus [15,16]. Up to know the expression of TNF superfamily members in maternal decidual cells has not been investigated.

BAFF is known as B cell-activating factor belonging to the TNF family, also called B lymphocyte stimulator (BLyS) TNF- and Apop-releated leukocyte expressed ligand 1 (TALL-1), TNF homologue that activates apoptosis, nuclear factor (NF)-□B, and c-Jun NH2-terminal kinase (THANK), and zTNF4 [17,18]. The BAFF is an important immune-modulator by the fact that its overexpression or deficiency is associated with the development of autoimmune diseases [19]. BAFF binds to two other receptors including transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI) and B cell maturation antigen (BCMA) also recognizing another TNF-like ligand: c). BAFF/APRIL is important immune modulator ligand. Previous studies have shown that TNF-□ as well as proinflammatory cytokines, chemokines and adhesion molecules play an important role in the pathogenesis of preeclampsia [6-8]. However the role of BAFF and APRIL in this filed has not been investigated till now. Thus the main objective of this study was to determine the serum levels of BAFF and APRIL in patients with preeclampsia and to compare them to those with normal pregnancies in same gestational ages.

MATERIAL AND METHOD

Patients

This was a prospective case-controlled study being performed in Zeinabieh and Hafez hospitals, both tertiary healthcare centers affiliated with Shiraz University of Medical Sciences, during an 18-months period from August 2009 to February 2011. In this study we included 3 study groups including 45 patients with preeclampsia (group 1), 45 healthy pregnant individuals with uncomplicated pregnancy (group 2) and 45 healthy non-pregnant individuals (group 3). All the patients and the healthy individuals were matched regarding the age. Group 1 and 2 were matched regarding the gestational age.

International Society of the Study of Hypertension in Pregnancy (ISSHP) criteria was used for diagnosis of the preeclampsia [17]. Accordingly preeclampsia was defined as sustained pregnancy-induced hypertension accompanied with proteinuria. The hypertension was defined as blood pressure higher than 140/90mmHg being measured in supine position in two distinct episodes with an interval of at least 6 hours. Those with a sustained 15-mmHg or 30-mmHg rise in the diastolic or systolic blood pressure (respectively) from the first trimester of the pregnancy were also diagnosed as hypertension. The proteinuria was defined as ≥30mg/dL protein concentration (or 1+ on a urine dipstick) in the two or more random spot urine sample being collected with an interval of at least 4 hours apart. Severe preeclampsia was defined as blood pressure values higher than 160/110mmHg accompanied by more than 500mg proteinuria in a 24 hour period or 3+ or 4+ proteinuria on a dipstick in random spot urine sample. Those with blood pressure ≥ 160/110mmHg with epigastic pain, diplopia, headache, oliguria, seizures, thrombocytopenia, pulmonary edema, rise in liver enzymes and creatinine and fetal growth restriction were also categorized as severe preeclampsia. All the women with preeclampsia recruited for the study were in the third trimester of pregnancy and were all of Iranian nationality.

None of the participants were smokers and none of them had cardiovascular, metabolic, neoplastic, gastroduodenal, or

inflammatory diseases. Patients with multiple gestation, gestational diabetes mellitus, chronic hypertension, underlying neoplasm, connective tissue disease, inflammatory or infective disorders, infectious diseases recognized in pregnancy, and heart failure were excluded from the study. We also excluded those patients with history of treatment with aspirin, warfarin, lipid-lowering or antihypertensive drugs, nonsteroidal anti-inflammatory drugs, or antibiotics. Complication of pregnancy including premature rupture of membrane, polyhydramnios, olighydramnios, chorioamnionitis, other medical conditions and sings of active labor were also excluded from the study. The women selected as controls had no signs of gestational complications or fetal distress and all gave birth to healthy neonates of appropriate size for gestational age. Non-pregnant individuals did not had any other medical conditions and referred to gynecologic clinics of these two centers from routine check-ups. The study protocol was approved by the institutional review board (IRB) of Shiraz University of Medical Sciences and the approval of the Ethics Committee was achieved before beginning of the study. All the participants' parents gave their informed written consent.

Study Protocol

All the patients were selected from those referring to gynecology and obstetrics and gynecology clinics of Zeinabieh and Hafez hospitals. All the recruited patients were interviewed and examined and their demographic information including sex, age, parity, gravidity and number of live births or abortions were recorded by means of a questionnaire. After explaining the advantages and risks of the study to the patients clearly, a 4cc blood sample was obtained by venipuncture from the cubital vein. Soluble BAFF was quantitated in serum diluted 1:2 by ELISA using a commercial kit (Pooyesh Teb Fars, Iran). APRIL was determined in the same serum, using an ELISA kit (Pooyesh Teb Fars, Iran). The intra-assay and interassay coefficients of variation (CV) were <6% for all assays performed. Serum levels of BAFF and APRIL were compared between groups.

Statistical analysis

Based on 95% confident intervals and an 80% power to detect a significant difference for BAFF and APRIL level at level of 5%, 40 subjects were needed for each group. In order to compensate for non-evaluable subjects, we included 45 subjects in each study group. All statistical analyses were performed with the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). The serum levels of BAFF and APRIL were compared using one-way analysis of variance (ANOVA). For comparison between groups, Bonferroni was used as a post hoc test. For those parametric data without normal distribution Kruskal-Wallis and Mann-Whitney U test was used. Data were reported as means ± SD for 95% CI. A two-sided $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 135 subjects were included in three distinct study groups: 45 patients with preeclampsia (group 1), 45 healthy pregnant individuals with uncomplicated pregnancies (group 2) and 45 healthy subjects who were not pregnant (group 3). The mean age of the Participants were 27.8 ± 6.4 (range 16-43) years. There was not any significant difference between 3 study groups regarding age (p=0.993).

The gestational age of those who were pregnant (group 1 and 2) was found to be 32.7 ± 3.5 (27-40) weeks. The gestational age did not differ significantly between the pregnant individuals (32.87 ± 3.7 vs. 32.67 ± 3.43; p=0.794) . In group 1, 24 (53.3%) patients were less than 34 weeks of gestation and 21 (46.7%) were more than 34 weeks of gestation. In the same way, of 25 healthy pregnant women, 25 (55.6%) were in first trimester and 20 (44.4%) were more than 34 weeks of gestation. There was not any difference between these two groups regarding the gestational age category (p=0.500).

Serum levels of BAFF and APRIL was found to be 0.51 ± 1.25 (0.01 – 14.3) pg/mL and 5.07 ± 1.4 (0.05–78.3) pg/mL in all the participants. There was not any significant difference between three study groups regarding the serum levels of BAFF. However serum levels of APRIL was significantly different between 3 study groups (p=0.018) (Table 1). Serum levels of APRIL were significantly higher in those patients with preeclampsia compared to healthy pregnant women (7.46 ± 5.41 vs. 3.59 ± 2.61 pg/dL; p=0.009). In the same way, serum levels of APRIL was significantly higher in those with preeclampsia compared to healthy non-pregnant individuals (7.46 ± 5.41 vs. 4.16 ± 3.15 pg/dL; p=0.034). Of the patients with preeclampsia, 21 (46.7%) had mild preeclampsia and 24 (53.3%) had severe preeclampsia. The serum levels of BAFF (0.58 ± 0.5 vs. 0.35 ± 0.21 pg/dL; p=0.088) and APRIL (11.2 ± 22.4 vs. 4.36 ± 4.26 pg/dL; p=0.339) did not differ significantly between those with severe and mild preeclampsia.

Table 1

Serum levels of BAFF and APRIL between 3 study groups

	Patients with preeclampsia (Group 1)	healthy pregnant individuals (Group 2)	healthy non-pregnant individuals (Group 3)	p-value
Age (years)	27.8 ± 6.8	27.7 ± 6.5	27.9 ± 6.1	0.993
Parity (Number)	1.9 ± 1.2	1.7 ± 0.9	1.7 ± 0.8	0.650
Gestational age (Weeks)	32.8 ± 3.7	32.6 ± 3.4	–	0.794
BAFF (pg/mL)	0.46 ± 0.44	0.71 ± 0.21	0.32 ± 0.25	0.085
APRIL (pg/mL)	7.46 ± 5.41	3.59 ± 2.61	4.16 ± 3.15	0.018

DISCUSSION

Preeclampsia, the most common complication of pregnancy, is accompanied by maternal and fetal morbidity and mortality. Endothelial and vascular dysfunction is responsible for the pathogenesis of the disease. However the exact pathomechanism of the disease is yet to be identified. It is known that the disease is a result of several environmental and genetic factors including race, age and genetic background [6-8].

Recent studies have provide evidences that preeclampsia is a result of activation of both innate and adaptive immune systems leading to excessive and exaggerated systemic inflammatory response [4,5]. Previous studies have shown that BAFF induces transcription of several downstream genes including cytokine IL-10 and some other inflammatory factors [17]. IL-10 is believed to be a potent immunosuppressor because it suppresses the cytokine production and inhibits Th1 functions [18]. It has been previously shown that the functions of Th2 are beneficial to the pregnancy while the Th1 is involved in harmful immune responses. Thus suppressing Th1 will result is alleviated pregnancy outcome. Th2 related cytokines including IL-4, IL-10, transforming growth factor-β (TGF-β) are almost necessary for fetal acceptance and for inhibiting unwanted immune responses toward the fetus. The proinflammatory cytokines produced by Th2 including interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) cause inflammation at the maternal-fetal interface resulting in endothelial dysfunction and consequently hypertension [6-8].

We performed this study in order to elucidate the role of BAFF and APRIL in the pathogenesis of preeclampsia. In this regards we compared the serum levels of BAFF and APRIL in three distinct groups of subjects including those with preeclampsia, healthy pregnant subjects and healthy non-pregnant individuals. We found that patients with preeclampsia have significantly higher levels of APRIL compared to both normal pregnant and non-pregnant individuals suggestive of its role in pathogenesis of the preeclampsia. Serum levels of BAFF and APRIL did not correlate with the severity of the disease. We also did not find any significant difference regarding the serum levels of BAFF between those with preeclampsia and healthy pregnant and non-pregnant subjects. These findings accept the hypothesis that immunologic disturbances through APRIL are responsible for developing preeclampsia.

BAFF and APRIL expression has been shown to be cell type-specific as well as gestation-dependent [19]. Philips et al. [20] showed that APRIL protein was prominent on the microvilli of syncytiotrophoblast and in the cytoplasm of both syncytiotrophoblast and villous cytotrophoblast of first trimester placentas but was essentially undetectable in term placentas. However, BAFF was prominent in villous cytotrophoblast cells but was weak in syncytiotrophoblast. Both APRIL and BAFF has been shown to be more abundant in early pregnancy compared to late pregnancy. All these evidences suggest that placental BAFF and APRIL assist the mother immune system in producing high quantities of delivery required antibodies into the fetus by targeting lymphocyte precursors in mothers in a paracrine manner.

It can be concluded that increased levels of APRIL in patients with preeclampsia which was observed in our study can be considered the first step in developing the disease. In the other words, as APRIL plays an important role in regulation of placental cell viability, its dysregulation will result in placental cell dysfunction leading to preeclampsia.

We note some limitations to our study. First, we measured the serum levels of BAFF and APRIL in those with preeclampsia and healthy pregnant and non-pregnant subjects. Future studies using different methods including reverse transcriptase-polymerase chain reaction (RT-PCR), Western blotting and immunohistochemical assays for detecting the expression of BAFF and APRIL in pregnancy products including the placenta, trophoblast and deciduas also serum level of BAFF and APRIL is strictly recommended. Second, our study population was small and this may has resulted in calculation bias. Future studies with larger study population will shed light on the role of these molecules in pathogenesis of preeclampsia. However to the best of our knowledge, this is the first study, evaluating the

role of these two TNF superfamily members in pathogenesis of preeclampsia.

In conclusion, our findings demonstrate that serum levels of APRIL are higher in those with preeclampsia compared to both normal pregnant and non-pregnant individuals. However serum levels of BAFF are not different between preeclamptic patients and normal individuals. Thus it can be concluded that APRIL is likely one of the factors responsible for immunological disturbances leading to development of preeclampsia. Cohort studies are recommended to evaluate the correlation between baseline BAFF and APRIL levels at pregnancy and occurrence of preeclampsia. We also recommend using anti-APRIL mAb in research of preeclampsia in animal models.

References

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