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Serum Levels of Soluble Fas and Fas Ligand in Iranian Women with Pre-eclampsia

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Abstract

Background: The precise responsible mechanism of pre-eclampsia remains controversial however, recent data suggest a main role of the abnormal activation of the adaptive immune system and Apoptosis. In this study, we have measured serum levels of Fas/Fasl as two important members of extrinsic apoptotic pathway in patient with pre-eclampsia.

Methods: 207 participants including 99 pre-eclampsia patients and 108 age and sex-matched normal pregnant women were involved in the case-control study. Plasma sample from each participant was collected and stored at –20℃ until batch processing.

Serum levels of Fas and Fas ligand were measured by ELISA for each participant including 99 pre-eclampsia patients and 108 normal pregnant women. Following a test of statistical normality, nonparametric data were analyzed by Mann-Whitney.

Results: sFas levels in case group was significantly higher than controls; 584 (397-892) pg/ml in cases opposed to 341 (213-602) pg/ml in controls (p value< 0.01). sFasL in pre-eclampsia women was a little lower than controls; 255 (173-318) pg/ml and in case group compared to 265.5 (184-381.5) pg/ml in controls.

Conclusions: We have found the increased levels of sFas in patients with pre-eclampsia in compare with the healthy pregnant women. It seems that abnormality in sFAS is related with pre-eclampsia.

Keywords: Pre-eclampsia, Pregnancy, sFAS, sFASL, Apoptosis.

Introduction

The Fas and FasL exist in soluble form (sFas/sFasL) and membrane form (mFas/mFasL). Membrane forms induce apoptosis of activated lymphocytes in immune privilege tissues such as placenta. However, the role of soluble form of these proteins is still debated (9-11). In normal pregnancy, the Th1 subtype which recognize paternal antigens, undergo apoptosis through expressing Fas and interact with the FasL (expressed on trophoblast) and Th2 subtype increased to prohibit of inflammation. This balance of Th1/Th2 effects on the differential ability of Th1 and Th2 cells to express Fas ligand and to undergo activation-induced cell death (AICD) (12-14). However, in pre-eclampsia, it has been shown that the Th1

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cells were increased and subsequently the inflammation and trophoblast destruction accrued. It seems that abnormal levels or expression of sFas/sFasL by inhibitory role for membrane form would change the Th1/Th2 ratio balance and pregnancy proceed, and subsequently lead to pre-eclampsia (15-19).

To date, conflicting observations have been reported regarding changes in the levels of soluble form of Fas/FasL among different populations (20, 21), which might be due to the patient's race or the complex and unknown role of Fas/Fasl in preeclampsia. In addition to controversial reports, our observation in the study of polymorphism of Fas and FasL in Pre-eclampsia (5) promoted us to do more research in the field of sFas/FasL by analysis the variation of sFas/sFasl levels in pre-eclampsia in a larger sample size.

Materials and Methods

Statement of Ethics

The Ethics Committee of Mashhad University of Medical Sciences approved the study. Informed consent was obtained from all patients and controls.

Patients

207 pregnant women with age range 18-35 years were included in this study (Mashhad, Iran). The participating women were classified into two groups: the first group included 99 pre-eclampsia patients and the second group included 108 age and sex-matched normal pregnant women. Pre-eclampsia was diagnosed by the attending physician on the basis of a blood pressure of 140/90 mm-Hg or more after the 20th weeks of gestation and proteinuria≥ 300 mg/24h in the collected urine sample. The gestational age was≥ 25 weeks at the time of sample collection. Patients with history of inflammation and hypertension before 20th week of pregnancy and also, patients with essential hypertension were excluded. The Ethics Committee of Mashhad University of Medical Sciences approved the study. Informed consent was obtained from all patients and controls.

Collection of Samples and Laboratory Methods

Blood samples were collected and centrifuged within 30 minutes from collection at 500 G for 20 minutes. The resultant supernatant plasma was collected and stored at –20℃ until batch processing by ELISA for sFas (sFas; R&D System, Cat: DY326, Abingdon, UK) and sFas ligand (sFasL; Abcam, USA). Lower limited of detection for sFas were 20 pg/mL and 44 pg/mL for sFasL. The intra and inter-assay coefficient of variation was < 5% and < 10%, respectively, for all assays.

Statistical analysis

Following a test of statistical normality, nonparametric data were analyzed by Mann-Whitney. Data were expressed as median (interquartile range, IQR).

A p-value of < 0.05 was considered statistically significant

Results

Our study included 99 pre-eclampsia case patients and 108 control subjects. Baseline characteristics of the controls and cases are shown in Table 1. The average age of patients was 28.20 ± 6.06 year, ranged from 18 to 36 years. Healthy controls were aged 18 to 35 years with mean age of 27.15 ± 6.31. There was no statistically significant difference in mean maternal age or parity distribution between groups.

As shown in table 2, sFas and sFasL were measured in 99 women with pre-eclampsia and 108 healthy pregnant women. sFas levels in case group was significantly higher than controls; 584 (397-892) pg/ml in cases opposed to 341 (213-602) pg/ml in controls (p value<0.01). sFasL in pre-eclampsia women were slightly lower than controls; 255 (173-318) pg/ml and in case group compared to 265.5 (184-381.5) pg/ml in controls.

Discussion

We measured serum levels of Fas and sFasL in pre-eclampsia patient and normal pregnancy and found significantly difference in sFas levels between groups. The levels of sFas were noticeably higher in cases (p value<0.01), and the levels of sFasL were lower in case group but not significantly (p= 0.18). To date, inconsistent reports were published about levels of sFas/sFal in patient with pre-eclampsia. Karthikeyan et al reported higher, but not significant, levels of sFas

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Table 1. Clinical information [a]

Character	Pre-eclampsia	Controls subject
Maternal age (years)	28.20 ± 6.06	27.15 ± 6.31
Systolic blood pressure (mmHg)	154.31 ± 16	111.60 ± 11.38
Diastolic blood pressure (mmHg)	97.41 ± 9.46	68.42 ± 8.83
Proteinuria (mg/24 hours)	938.67 ± 1288	45 ± 17.3
Parity	**Primiparity: 51.9%** **Multiparity: 48.1%**	**45.7%** **54.3%**

a: Values are presented as mean ± SD or N (%).
There was not statically significant difference in maternal age between patients with respect to the control subjects (p_{value}= 0.17 and p_{value}= 0.68, respectively).

Table 2. The median values and interquartile ranges of sFas and sFasL in case and control groups.

	Pre-eclampsia (99)	Controls (108)	p value
sFas (pg/ml)	584 (495)	341 (389)	<0.01
sFasL (pg/ml)	255 (145)	265.5 (197.5)	0.18

Data presented as Median (IQR). P value by Mann-Whitney test and p< .05 was considered significant.

in pregnant women with hypertension in compared with normal pregnancy (15), and although, Koenig et al did not observe significant difference between cases and controls, among case group the levels of sFas in serum was higher than umbilical cord blood. In the other study, Darmochwal-Kolarz, Oleszczuk and Wang et al reported significant elevated levels of sFas in pregnancy complicated with pre-eclampsia in compared with normal pregnancy (16, 17, 19, 22). The precise function of soluble form of sFas remained unelucidated but the probably role of sFas is inhibit of the membrane form to induce apoptosis by fill the FasL (23). In result of such function, apoptosis in Fas expressed cells, same as Th1 important in immune balance in pregnancy- been decreased (12, 24, 25). As remarked in introduction, in pre-eclampsia, the elevated population of Th1 cell responsible for secret the inflammatory cytokines were seen (26). Elevated levels of sFas can the responsible mechanism for rise of the Th1 subtype in pre-eclampsia. It's reported that in normal pregnancy the levels of sFas in first trimester month was decreased and in continue rise up slowly until end of pregnancy (27), so elevated levels of sFas can response the imbalance of immune system in pregnancy complicated by pre-eclampsia. Our results about sFas levels were consistent with this hypothesis but the results of same mentioned studies in pre-eclampsia, were controversial.

We found slightly but not statically significant decreased amount of sFasL in pre-eclampsia patients in compared with controls. Limited number but controversial and inconsistent studies have been reported in this field in patient with pre-eclampsia. Wang et al, and kunts et al, have found higher levels of FasL expression in patient with pre-eclampsia in compare with normal pregnant women (16, 22). Also, Joyce et al, have reported elevated FasL expressing in leukocytes of Pre-eclampsia patients and decreased levels of sFasl (16). In consistent with our finding, Karthikeyan et al, have demonstrated lower sFasL levels in pregnant women with hypertension than healthy pregnant and non-pregnant women (15).

The role of sFasL is quite controversial, with two concepts, the first concept; sFas and sFasL inhibit the function of membrane form and apoptosis, and the second one; sFas/sFasL's ability to induce apoptosis in human cell line but not as strongly as the membrane form does (11, 23, 28). Interestingly, in first trimester of pregnancy, trophoblast cell secrets sFasL to induce apoptosis in T cells in maternal-fetal interference (29). So, insufficient levels of sFasL in pregnancy may responsible for decreased apoptosis in Th1 subtype, immune imbalance

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and in subsequent, Pre-eclampsia. However, we found no significant difference between sFasL levels in patients with preeclampsia and controls. In this study, we concluded that in preeclampsia, sFas levels change but not significantly, and sFas increase significantly.

Our results are not entirely consistent with other reports that may be derived from the effect of the patient's race, gestational age in different populations, and sample size. To better understanding of pre-eclampsia mechanism, a comprehensive study seems necessary, considering different aspects of immune system simultaneously, such as the percentage of each subtype of T cell and specially Th1/Th2 balance, evaluating other apoptosis related molecules, T cell phenotype and expression of inhibitory molecules (such as Fas/FasL).

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