

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

Elham Masoumi^{#1,2}, Asad Mirzaei^{#3,4}, Haniyeh Ghaffari-Nazari⁵,
Sahar Tahaghoghi-Hajghorbani^{6,7}, Seyed Amir Jalali^{*8}, Jalil Tavakkol-Afshari^{*5}

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°C 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

1: Department of Immunology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

2: Research Committee, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

3: Parasitology department, paramedical school, Ilam University of Medical Science, Ilam, Iran.

4: Zoonotic Diseases Research Center, Ilam University of Medical Sciences, Ilam, Iran.

5: Immunogenetic and Tissue Culture Department, Immunology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

6: Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

7: Department of Immunology, School of Medicine, Mazandaran University of Medical Science, Sari, Iran.

8: Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

First two authors equally contributed to this work.

*Corresponding authors: Seyed Amir Jalali; Tel: +98 9128890956; E-mail: jalalia@sbmu.ac.ir & Jalil Tavakkol-Afshari; Tel: +98 9151134220; E-mail: tavakkolja@mums.ac.ir.

Received: 7 Jul, 2020; Accepted: 19 Jul, 2020

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 \geq 300 mg/24h in the collected urine sample. The gestational age was \geq 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°C 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

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 ($p_{\text{value}} = 0.17$ and $p_{\text{value}} = 0.68$, 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 secretes 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

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

References

1. Ciarmela P, Boschi S, Bloise E, Marozio L, Benedetto C, Castellucci M, et al. Polymorphisms of FAS and FAS ligand genes in preeclamptic women. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2010;148(2):144-146.
2. DiFederico E, Genbacev O, Fisher SJ. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *Am J Pathol*. 1999;155(1):293-301.
3. Moffett A, Hiby S. How does the maternal immune system contribute to the development of pre-eclampsia? *Placenta*. 2007;28:S51-S56.
4. Kwak-Kim J, Chung-Bang H, Ng S, Ntrivalas E, Mangubat C, Beaman K, et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Human Reproduction*. 2003;18(4):767-773.
5. Masoumi E, Tavakkol-Afshari J, Nikpoor AR, Ghaffari-Nazari H, Tahaghoghi-hajghorbani S, Jalali SA. Relationship between Fas and Fas Ligand gene polymorphisms and pre-eclampsia. *J Obstet Gynaecol Res*. 2016;42(10):1272-1278.
6. Roh C-R, Lee J-W, Kang B-H, Yang S-H, Kim B-G, Bae D-S, et al. Differential expressions of Fas and Fas ligand in human placenta. *J Korean Med Sci*. 2002;17(2):213-216.
7. Mor G, Gutierrez LS, Eliza M, Kahyaoglu F, Arici A. Fas-fas ligand system-induced apoptosis in human placenta and gestational trophoblastic disease. *Am J Reprod Immunol*. 1998;40(2):89-94.
8. Jalali SA, Shandiz FH, Afshari JT, Ghochan MDT, Nikpoor AR, Mohammadi M. Status of FAS

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).

Acknowledgements

This research was financially supported by a grant from the research council of Mashhad University of Medical Sciences in Iran (No. 910497). The authors declare that there is no conflict of interest.

- and FAS Ligand Gene Polymorphisms in Patients with Breast Cancer in Northeastern IRAN. *Reports of biochemistry & molecular biology*. 2018;7(1):23-29.
9. Nagata S. Fas and Fas ligand: a death factor and its receptor. *Advances in immunology*. 1994;57:135-144.
10. Uckan D, Steele A, Wang B, Chamizo W, Koutsonikolis A, Gilbert-Barness E, et al. Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. *Mol Hum Reprod*. 1997;3(8):655-62.
11. Li N-L, Nie H, Yu Q-W, Zhang J-Y, Ma A-L, Shen B-H, et al. Role of soluble Fas ligand in autoimmune diseases. *World J Gastroenterol*. 2004;10(21):3151-3156.
12. Ramsdell F, Seaman MS, Miller RE, Picha KS, Kennedy MK, Lynch DH. Differential ability of Th1 and Th2 T cells to express Fas ligand and to undergo activation-induced cell death. *Int Immunol*. 1994;6(10):1545-53.
13. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon?. *Immunol today*. 1993;14(7):353-6.
14. Mellor AL, Munn DH. Immunology at the maternal-fetal interface: lessons for T cell tolerance and suppression. *Annu Rev Immunol*. 2000;18(1):367-91.
15. Karthikeyan VJ, Lip GY, Baghdadi S, Lane DA, Beevers DG, Blann AD. Soluble Fas and Fas ligand in pregnancy: influence of hypertension. *Angiology*. 2012;63(1):35-8.

16. Kuntz TB, Christensen RD, Stegner J, Duff P, Koenig JM. Fas and Fas ligand expression in maternal blood and in umbilical cord blood in preeclampsia. *Pediatr Res.* 2001;50(6):743-9.
17. Darmochwal-Kolarz D, Leszczynska-Gorzela B, Rolinski J, Oleszczuk J. The expression and concentrations of Fas/APO-1 (CD95) antigen in patients with severe pre-eclampsia. *J Reprod Immunol.* 2001;49(2):153-64.
18. Darmochwal-Kolarz D, Leszczynska-Gorzela B, Rolinski J, Oleszczuk J. T helper 1-and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. *European Journal of Obstetrics & Gynecology and Reproductive Biology.* 1999;86(2):165-170.
19. Laskowska M, Laskowska K, Leszczyńska-Gorzela B, Oleszczuk J. Evaluation of the maternal and umbilical vein serum sFas/sFasL system in pregnancies complicated by preeclampsia with intrauterine growth retardation. *European Journal of Obstetrics & Gynecology and Reproductive Biology.* 2006;126(2):155-159.
20. Hsu C-D, Harirah H, Basherra H, Mor G. Serum soluble Fas levels in preeclampsia. *Obstetrics & Gynecology.* 2001;97(4):530-532.
21. Bayram M, Taskaya A, Bagriacik EU, Ilhan MN, Yaman M. The effect of maternal serum sFAS/sFASL system on etiopathogenesis of preeclampsia and severe preeclampsia. *J Matern Fetal Neonatal Med.* 2012.
22. Hu W, Wang Z, Dong M, Wang H. Expression of Fas and FasL in serum and placenta of preeclamptic pregnancy and its significance. *Zhejiang da xue xue bao Yi xue ban.* 2005;34(6):499-502.
23. Enjoji M, Yamaguchi K, Nakashima M, Ohta S, Kotoh K, Fukushima M, et al. Serum levels of soluble molecules associated with evasion of immune surveillance: a study in biliary disease. *Liver International.* 2004;24(4):330-334.
24. Zhang X, Brunner T, Carter L, Dutton RW, Rogers P, Bradley L, et al. Unequal death in T helper cell (Th) 1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J Exp Med.* 1997;185(10):1837-49.
25. Musiał K, Zwolińska D. Matrix metalloproteinases and soluble Fas/FasL system as novel regulators of apoptosis in children and young adults on chronic dialysis. *Apoptosis.* 2011;16(7):653-9.
26. Saito S, Sakai M. Th1/Th2 balance in preeclampsia. *J Reprod Immunol.* 2003;59(2):161-73.
27. Hoshimoto K, Hayashi M, Ohkura T. Plasma levels of soluble Fas during normal pregnancy. *Gynecol Obstet Invest.* 2001;51(2):96-8.
28. Kim S, Kim JY, Lee TH, Suk K, Cha H-S, Koh E-M, et al. Soluble Fas ligand-susceptible" memory" cells in mice but not in human: potential role of soluble Fas ligand in deletion of auto-reactive cells. *Autoimmunity.* 2002;35(1):15-20.
29. Abrahams VM, Straszewski-Chavez SL, Guller S, Mor G. First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. *Mol Hum Reprod.* 2004;10(1):55-63.