

High sensitivity C-reactive protein in preeclamptic (PE) women

Dr. Sahar B. Aziz

M.B.,Ch.,B., FICMS (Clinical Biochemistry)

Lecturer, Dep. of Biochemistry- College of Medicine-

University of Mosul- Iraq

Abstract

Background:

Preeclampsia is an exacerbation of generalized inflammatory response, physiologically present in the third trimester of pregnancy.

Aim: To evaluate TNF- α and hs-CRP measurement in the context of preeclampsia.

Patients and methods:

A case control study design was performed. The study included (25) non pregnant women (group1), (25) normotensive pregnant women (group 2) and (25) pregnant women complicated by preeclampsia of singleton gestation in the third trimester (group 3) who were attending AL- Batool teaching hospital and AL- Hadba antenatal clinic in Mosul city during a period of 6- months from 1st November 2012 to 1st May 2013. For urinary protein determination of proteinuria by reagent strip was done.

Serum hs-CRP and TNF- α levels were determined using enzyme linked immunosorbent method.

Results:

The results obtained revealed a highly significant increase in circulating hs-CRP levels in the last trimester of pregnancy compared to non pregnant women with $p < 0.0001$, and a significant increase in serum TNF- α and hs-CRP levels were also found among preeclamptic women with $p < 0.001$ compared to normotensive pregnant women.

While, TNF- α showed a significant positive correlation with hs-CRP in preeclamptic women ($r = 0.47$, $p < 0.001$).

Conclusion:

Data of the present study show that both hs-CRP and pro-inflammatory cytokines are present in higher concentrations in women with preeclampsia, which highlight the importance of measuring hs-CRP, TNF- α in preeclamptic women which may contribute to the pathophysiology of this pregnancy disorders.

Keywords: Preeclampsia, Normal pregnancy, Tumor necrosis factor α , Pro inflammatory state, hs-CRP.

Introduction

Preeclampsia develops in 5-7% of human pregnancies. It is characterized by an elevated

blood pressure and proteinuria which develops after 20th weeks of gestation¹. It is a complication of pregnancy constituting a major cause of maternal and fetal morbidity and

High sensitivity C-reactive protein in preeclamptic (PE) women

mortality¹. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia remain unclear¹. However several etiologies have been implicated in the development of preeclampsia including abnormal trophoblast invasion of uterine blood vessels, immunological intolerance between fetoplacental and maternal tissues, maladaptation to the cardiovascular changes, dietary deficiencies and genetic abnormalities². Moreover endothelial cell dysfunction and inflammation are considered to play a major role in the pathophysiology of preeclampsia^{3,4}. The etiology of endothelial dysfunction in preeclampsia is not known, but it has been postulated to be part of an exaggerated maternal inflammatory response to pregnancy⁵. Activated circulating leukocytes^{6,7} increased production of reactive oxygen species⁸ and increased release of inflammatory cytokines, such as Tumor necrosis factor α (TNF- α) and Interleukin-6 (IL-6)^{9,10} as well as abnormal activation of the clotting system¹¹ in women with preeclampsia compared with normotensive women, supports this hypothesis. In this study, TNF- α and hs-CRP were measured in pregnant women with preeclampsia because the possible role of these markers of inflammation in the pathophysiology of preeclampsia^{12,13}. TNF- α is a proinflammatory cytokine derived from macrophages, lymphocytes, vascular endothelial cells, trophoblasts and Hofbauer cells in the placenta; it induces functional alterations in endothelial cells³. TNF- α up regulates endothelial expression of platelet derived growth factor, endothelin-1 and plasminogen activator inhibitor-1, all of which are associated with vasoconstriction and are found to be elevated in preeclampsia³. TNF- α also has been shown to cause microvascular protein leakage and hypertriglyceridemia which are associated with preeclampsia³. C-reactive protein (CRP) is an acute phase reactant produced by the liver in response to the pro-inflammatory cytokines interleukin (IL-6) and tumor necrosis factor (TNF)¹⁴. CRP is a sensitive marker of inflammatory activity in the body. CRP level

increases during inflammatory response to tissue injury or infection¹⁵. It has been shown that CRP is elevated in women with PE^{12,14}. Determination of hs-CRP has been suggested to be more sensitive than conventional measurement of CRP and provides better sensitivity in confirmation of inflammation¹⁶. It can be used as an early marker of low grade inflammation and further help in detecting pathophysiological process early in pregnancy, so as to predict adverse pregnancy outcome and try preventive therapies well in time¹⁴.

Patients and Methods

This study enrolled between 1st Nov.2012 – 1st May 2013, in AL- Batool Teaching Hospital and AL- Hadba antenatal clinic in Mosul city, Iraq. Fifty pregnant women (25- preeclamptic and 25 normal pregnant) with 3rd trimester gestational aged were involved in this study . The control group involved non- pregnant, normotensive apparently healthy women (n=25).

The study sample were divided in to (3)groups:

Group 1 (control group -C) was represented by (25) patients selected according to the following inclusion criteria : healthy non pregnant women during the reproductive period.

Group 2: (Normal pregnant women - NP) included 25 pregnant women with normal pregnancy according to the following inclusion criteria : normotensive pregnant women during the entire period of pregnancy, third trimester of pregnancy , single fetus pregnancy.

Group 3: (preeclamptic women-PE) included 25 pregnant women selected according to the following inclusion criteria: pregnant patients with preeclampsia (matching the diagnostic criteria of the international society for the study of hypertension in pregnancy (ISSHP): two blood pressure reading of $>$ or $=140/90$ mm Hg took at least with +1 protein uria on dip stick analysis¹⁷). For the diagnosis of preeclampsia at the third trimester of pregnancy, single fetus.

High sensitivity C-reactive protein in preeclamptic (PE) women

Exclusion criteria: Which were used for cases and control were: gestational or chronic hypertension, preexisting medical conditions such as inflammatory diseases (systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, etc.), acute inflammatory diseases (tonsillitis, urinary tract infections, etc.) cardiovascular disease, diabetes mellitus and renal disease.

The subjects of all groups were interviewed and general information was taken, parity, gravity, gestational age, previous history of hypertension, previous history of diabetes mellitus, drug history, and history of smoking.

The following measurements were taking including: age in years, blood pressure in (mmHg), body weight (kg), height (cm), the body mass index (BMI) was calculated according to the equation:

$$\text{BMI} = \text{weight (kg)} / \text{height (m)}^2 \cdot 18$$

Methods:

Five ml of venous blood was collected in a plain tube from each individual by after an over night fast of 12 hours. The tubes are placed in a water bath at 37°C for 15 minutes for blood clotting to occur. Serum samples were obtained by centrifugation of blood at 3000 RPM for 10 minutes the resultant serum was used for the determinations of TNF- α , and hs-CRP by enzyme-linked immunosorbent assay. The test kits used for determination of serum hs-CRP was manufactured by Monobind Inc. (USA), while TNF test kit was manufactured by Assay Max, (USA). For the urinary protein, 2-5 ml of random voided urine was collected, for qualitative determination of protein urea by reagent strips. Standard statistical methods for the analysis of data were used to determine the mean, standard deviation (SD), ANOVA test, Tukey's pairwise test, in addition to Pearson correlation. The statistical results were considered significant at $P \leq 0.05$ 19.

Before sample collection all participants were informed about the research, and were agreed to participate in this study.

Results

The demographic profile of the subjects are shown in table 1.

The biochemical parameters of the subjects are shown in table (2).

A significant elevation in hs,CRP, TF- α levels (according to ANOVA and Tukey's tests) were observed in normal pregnant and preeclamptic women, (Table 3). Among the groups for each parameters, many with different letters horizontally have significant differences at $P \leq 0.05$ using Tukey's test.

Using Pearson correlation test, a significant positive correlation between above parameters were found. Fig (1,2).

Discussion

Preeclampsia is a disease of pregnancy associated with endothelial cell damage and endothelial cell activation. There is an increasing evidence that preeclampsia is a systemic inflammatory disease⁴. Studies have shown that markers of inflammation have an active role in preeclampsia¹².

The present study demonstrated an elevated level of TNF- α and hs-CRP in the maternal plasma of preeclamptic patients as compared with normotensive pregnant women with the same gestational age ($p < 0.001$, table 3) or with a control group ($p < 0.0001$, table 3).

Similar results were reported by Teran E et al (2001)¹², who showed that the level of TNF- α and hs-CRP were significantly higher in preeclampsia ($p < 0.001$) as compared with controls and normal pregnancy group. Furthermore; in 2011 Molvarec et al.¹⁹, showed a significantly elevated level of TNF- α and hs-CRP ($p < 0.005$) in a preeclamptic group as compared to healthy non-pregnant women or to healthy normotensive pregnant women.

The increased levels of TNF- α for women with preeclampsia may be as a result of the inadequate trophoblast invasion and placental hypoxia^{20,21}. It has been hypothesized that placental hypoxia amplifies the release of inflammatory stimuli into the maternal

High sensitivity C-reactive protein in preeclamptic (PE) women

circulation 4. Another potential source of TNF- α in preeclampsia is activated maternal leukocytes. As monocytes/macrophages are generally the main reservoir of proinflammatory cytokines and are the first cells to be activated in non-specific immune responses, these can be good candidates for excessive TNF- α synthesis in preeclampsia²⁰. Elevated serum TNF- α level in preeclamptic female could explain increase blood pressure as TNF- α has an inhibiting effect on endothelial nitric oxide and stimulatory action on endothelin-1 and prostaglandins³. Blockade of endothelial nitric oxide generation causes constriction of resistance vessels, hypertension, altered platelet reactivity, and adhesion of leukocytes to the endothelium, this, endothelial dysfunction likely triggers leukocyte activation and a sequence of events that leads to further endothelial damage¹². Verccrysse L, et al 1998 showed that TNF- α can induce endothelial dysfunction and injury to ultrastructure of placenta and umbilical vascular endothelium. This injury may play a role in the pathogenesis of pregnancy induced hypertension²². The mechanism underlying altered endothelial function in preeclampsia is likely associated with the increase in the concentration of CRP, and TNF- α that observed in the present study. CRP is produced by the liver and the production is stimulated by the inflammatory cytokines including TNF- α ¹⁴, indeed, the present study show significant positive correlation between serum TNF- α and hs-CRP level ($r=0.47$, $p<0.001$), Fig.1. CRP is a sensitive marker of tissue damage and inflammation plays an important role in elucidating the inflammatory response characteristics of preeclampsia²³. CRP acts as a scavenger and is responsible for the clearance of membranes and nuclear antigens²⁴. Hwang HS et al (2007) showed that hs-CRP could be used as a severity marker in women with severe preeclampsia²⁸. Moreover, In 2013 Farzadnia M et al found that in severe preeclampsia group the hs-CRP levels were significantly higher than that in mild preeclamptic and normotensive groups. Also Sonal S and Poornima S found (2013) that the

hs-CRP level were higher in severe preeclampsia group as compared to mild preeclamptic and normotensive groups on the basis of blood pressure, proteinuria and pathological edema³⁰.

Also, according to the results of the present study, the last trimester of normal pregnancy seems to be a controlled state of systemic inflammation (2,4) as expressed by the significantly elevated serum hs-CRP levels as compared to non-pregnant woman (table (2), but this elevation is still significantly less than the that seen in preeclamptic pregnancy, these result were in agreement with Molvarec et al (2011)¹⁹ and Teran et al. (2001)¹². Although an increase in circulating TNF- α level is generally found during the course of pregnancy, this does not reach statistical significance, Elenkov et al. (2001)³¹, found no significant difference between normal pregnant and healthy control group. In contrast to our result Anim-Nyame et al (2003)³², Teran et al. (2001)¹², found a significant difference between normotensive pregnant group and healthy control group, The differences between the results might be at least partly explained by the different methods as well as by the reduced plasma half-life of this cytokine²⁰.

Finally; the present study was undertaken in women with established preeclampsia and it is not possible to determine whether the increase in C-reactive protein and cytokines was a cause or a consequence of the disease. To test the hypothesis that inflammation is a major risk factor for preeclampsia, it would be necessary to undertake a longitudinal study of C-reactive protein and cytokine levels from early pregnancy before the onset of preeclampsia.

Conclusion

1. Serum hs-CRP and TNF- α concentrations were significantly increased in the last trimester of gestation in preeclamptic women compared to normotensive pregnant women and the control group and play a role in pathogenesis of preeclampsia.

High sensitivity C-reactive protein in preeclamptic (PE) women

2. The positive and significant correlation of hs-CRP with TNF- α makes this marker of inflammation a potential marker of the severity of the preeclamptic syndrome.
3. Preeclampsia is an exacerbation of a generalized inflammatory response, physiologically present in the last trimester of pregnancy.

References

- Ambreen A A, and Melinda H, Preeclampsia: Systemic Endothelial Damage Leading to Increased Activation of the Blood Coagulation Cascade. *Journal of Biotech Research*.2012; 4:26-43.
- Stekking E, Zandstra M, Peeters LL, Spaandern ME. Early-onset preeclampsia and the prevalence of postpartum metabolic syndrome. *Obstet Gynaecol*. (2009); 114 (5): 1076-84.
- Sazina M, Rajiv G and Imam B. Serum tumor necrosis factor- α in preeclampsia. *Indian J Physiol Pharmacol* 2005; 49 (2): 236-240.
- Fatemeh M, Fatemeh R, and Amir H K. Association of Maternal Serum C-Reactive Protein Levels with Severity of Preeclampsia *Acta Medica Iranica*. (2009); Vol. 47, No. 4.
- Redman et al., Preeclampsia: An excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol*. 1999; 180:499-506.
- Haeger M et al., Complement, Neutrophil, and macrophage activation in women with severe preeclampsia and the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *Obstet Gynecol* (1992); 79: 19-26.
- P. Von Dadelszen et al., Maternal peripheral blood leukocytes in normal and preeclamptic pregnancies. *Br J Obstet Gynecol*. 1999; 106: 576-581.
- Walsh SW, Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. *Semin Reprod Endocrinol*. (1998); 16: 93-104.
- Williams MA et al., Maternal second trimester serum tumor necrosis factor-alpha-soluble receptor p55 (sTNFp55) and subsequent risk of preeclampsia. *Am J Epidemiol*.1999; 149: 323-329.
- Sacks GP et al., Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol*.1998; 79: 80-86.
- Perry KG and Martin JN, Abnormal homeostasis and coagulopathy in preeclampsia and eclampsia. *Clin Obstet Gynecol*. (1992); 35: 338-350.
- Teran E, Escudero C, Moya W, Flores M, Vallance P, Lopez-Jaramillo P. Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with preeclampsia. *Int J Gynecol Obstet* 2001; 75: 243-249.
- Sharma A, Satyam A, Sharma JB. Leptin, IL-10 and inflammatory markers (TNF-alpha, IL-6 and IL-8) in pre-eclamptic, normotensive pregnant and healthy non-pregnant women. *Am J Reprod Immunol* (2007); 58: 21-30.
- Archana J D, Sangeeta D, Kanchan M, Satish K. Role of Early Second Trimester High Sensitivity C-Reactive Protein for Prediction of Adverse Pregnancy Outcome. *JK SCIENCE* (2011); 13 (3): 141-144
- Braekke K, Holthe MR, Harsem NK, Fagerhol MK, Staff AC. Calprotectin, a marker of inflammation, is elevated in the maternal but not in the fetal circulation in preeclampsia. *Am J Obstet Gynecol* (2005); 193 (1): 227-33.
- Yu H, Rifai N. High-sensitivity C-reactive protein and atherosclerosis: from theory to therapy. *Clin Biochem* (2000); 33: 601-610.
- Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy*. (2001); 20(1): IX-XIV.

High sensitivity C-reactive protein in preeclamptic (PE) women

- McDonald JH (2009). Hand Book of Biological Statistics, 2nd ed. Sparky House, Baltimore, Maryland, U.S.A; pp 17-20
- Molvarec A, András S, Szilvia W, Gabriella B, István K, Zoltán P and János R. Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia. *Reproductive Biology and Endocrinology* (2011); 9:124-1-9
- Dan M, Nicolae C, Ligia D, Septimiu C, Raluca B. Implication of Tumor Necrosis Factor - Alpha in Preeclampsia. *Applied Medical Informatics*, (2008); Vol. 23, No. 3-4, pp: 11-18.
- Merih B, Mehmet S, M. Baran C, Emin U, Melek Y, Fusun C. Maternal Inflammatory Response in Severe Preeclamptic and Preeclamptic Pregnancies. *J Clin Gynecol Obstet.* (2012); 1(2-3): 40-45.
- Vercrysse L, Hanes SM, Johnson PM, Keith JC, Van Assche FA. Immunolocalisation of Tumor Necrosis Factor - alpha (TNF-alpha) in the placental bed of normotensive and hypertensive human pregnancies. *Placenta* (1998); 19(4): 231-239.
- Ustun y, Engin-ustun Y, Kamaci M. Association of fibrinogen and CRP with severity of preeclampsia. *Eur J Obstet Gynaecol Biol.* (2005); 121(2): 154-8.
- Nanda K, Sadanand G, Muralidhara Krishna C S, K L Mahadevappa, C-Reactive protein as a predictive factor of preeclampsia. *Int J Biol Med Res.* (2012); 3(1): 1307-1310.
- Kumru S, Godekmerdan A, Kutlu S, Ozcan Z. Correlation of maternal serum high-sensitive C-reactive protein levels with biochemical and clinical parameters in preeclampsia. *Eur J Obstet Gynecol Reprod Biol.* (2006); 124(2): 164-7.
- Irfan I, Ilhamjaya P, Agustina, Uleng B, Maisuri T, Rosdiana N, Agussalim B. The Level of C-Reactive Protein (hs-CRP) in Preeclamptic Pregnancy. *JST Kesehatan*, (2011); Vol.1 No.3: 296-300.
- ACOG Committee on Obstetric Practice. ACOG practicebulletin. Diagnosis and management of preeclampsia and eclampsia. *Num v9er 33*, January 2002. American College of Obstetricians and Gynecologists. *Int J Gynaecol Obstet.* (2002); 77(1): 67-75.
- Hwang HS, Kwon JY, Kim MA, Park YW, Kim YH. Maternal serum highly sensitive C-reactive protein in normal pregnancy and pre-eclampsia. *Int J Gynaecol Obstet.* (2007); 98:105-109.
- Farzadnia I M, Hossein A, Maliheh H, Hamid R. A Comparative Study of Serum Level of Vascular Cell Adhesion Molecule-1 (sVCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1) and High Sensitive C - reactive protein (hs-CRP) in Normal and Pre-eclamptic Pregnancies. *Iran J Basic Med Sci*, Jan 2013. Vol. 16, No. 1,
- Sonal S and Poornima S, Maternal Serum High Sensitive C-reactive Protein in Non-gestation and Preeclamptic Gestation. *Biomedical & Pharmacology Journal.* (2013); Vol. 6(1), 107-110.
- Elenkov I, Ronald L. Wilder, Vladimir K, Amrey A, Mariana A, Scott F, Marianna C, Keith S, and George P. II-12, TNF- α and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab*, October 2001; 86(10): 4933-4938.
- Anim-Nyame N, Gamble J, Sooranna S.R, Johnson MR, Steer PJ. Microvascular permeability is related to circulating levels of tumour necrosis factor-alpha in pre-eclampsia. *Cardiovascular Research* (2003); 58: 162-169.

High sensitivity C-reactive protein in preeclamptic (PE) women

Table (1): Demographic profile of study subjects

Parameters	Group 1 (n=25)		Group 2 (n=25)		Group 3 (n=25)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Age (years)	25.48 ± 5.75	16-35	25.26 ± 4.18	16-33	27.96 ± 4.45	20-36
Gestational age (week)	Non-pregnant		34.23 ± 3.16	28-39	35.42 ± 2.45	28-39
BMI (Kg/m ²)	23.2 ± 2.17	19.9-28.7	27.26 ± 3.1	19.3-30.8	32.34 ± 4.2	19.3-36.2

Table (2): Biochemical parameters showed the following results

Parameters	Group 1 (n=25)		Group 2 (n=25)		Group 3 (n=25)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
hs,CRP (mg/L)	4.63 ± 2.76	0.4-9.1	7.05 ± 3.18	1.9 - 12.3	9.51 ± 2.57	5.3-14.5
TF-α (pg/ml)	18.76 ± 14.73	8-80	24.11 ± 17.91	3.9-94	67.12 ± 26.1	32-136

Table (3): Comparison of the hs,CRP , TF-α among the student groups

Parameters	Group 1	Group 2	Group 3	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	
hs,CRP (mg/L)	4.63 ± 2.76 ^a	7.05 ± 3.18 ^c	9.51 ± 2.57 ^{bc}	0.0001
TNF-α (pg/ml)	18.76 ± 14.73 ^a	24.11 ± ^b 17.91	67.12 ± 26.1 ^b _c	0.0001

One way ANOVA test with Tuk'ys pairwise comparison was applied

Among the group for each parameter, means with different letters horizontally have significant difference at p≤0.05.

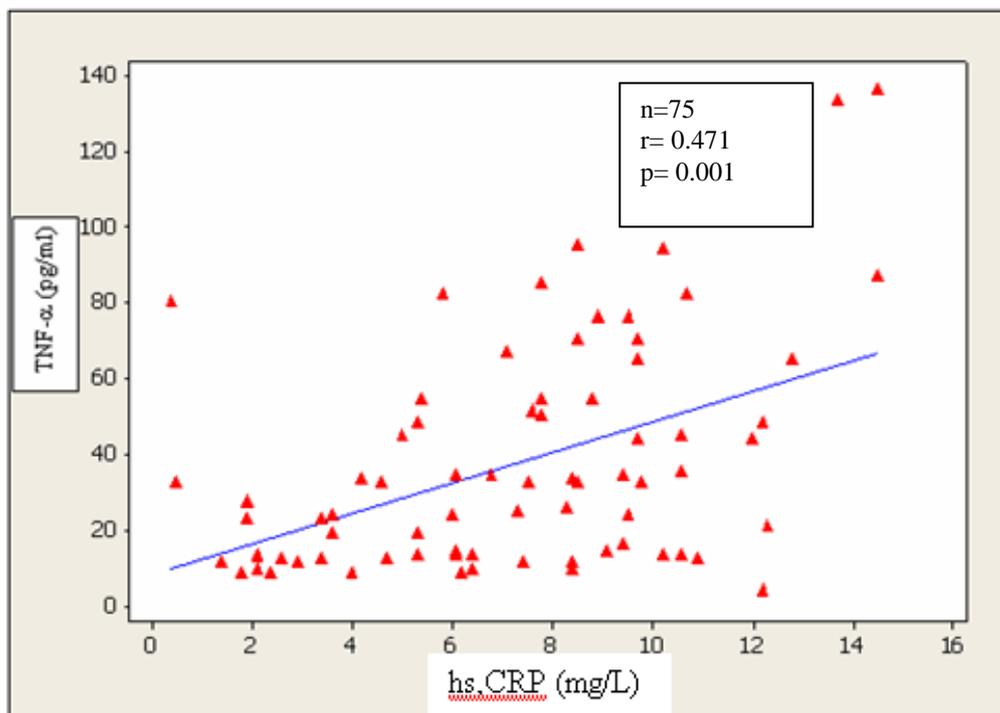


Figure (1): Correlations between hs-CRP and TNF- α for all sampled women (n=75).

