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

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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

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 preeclampsia3,4. 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 leukocytes6,7 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 preeclampsia12,13. 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 PE12,14. 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 leaset with +1 protein uria on dip stick analysis17). For the diagnosis of preeclampsia at the third trimester of pregnancy, single fetus.

Exclusion criteria: Which were used for cases and control were: gestational or chronic hypertension, preexisting medical conditions such as inflammatory diseases (systemic lupus erythematous, 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 :

BMI = weight (kg) / height (m2).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, Tuky'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 Tuky's tests)) were observed in normal pregnant and preecclamptic women, (Table 3). Among the groups for each parameters, many with different letters horizontally have significant differences at $P \leq 0.05$ using Tuky'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 hypoxia20,21.It has been hypothesized that placental hypoxia amplifies the release of inflammatory stimuli into the maternal

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-alpha14, 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.

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.

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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}	$24.11 \pm^{b} 17.91$	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 $p \leq 0.05$.

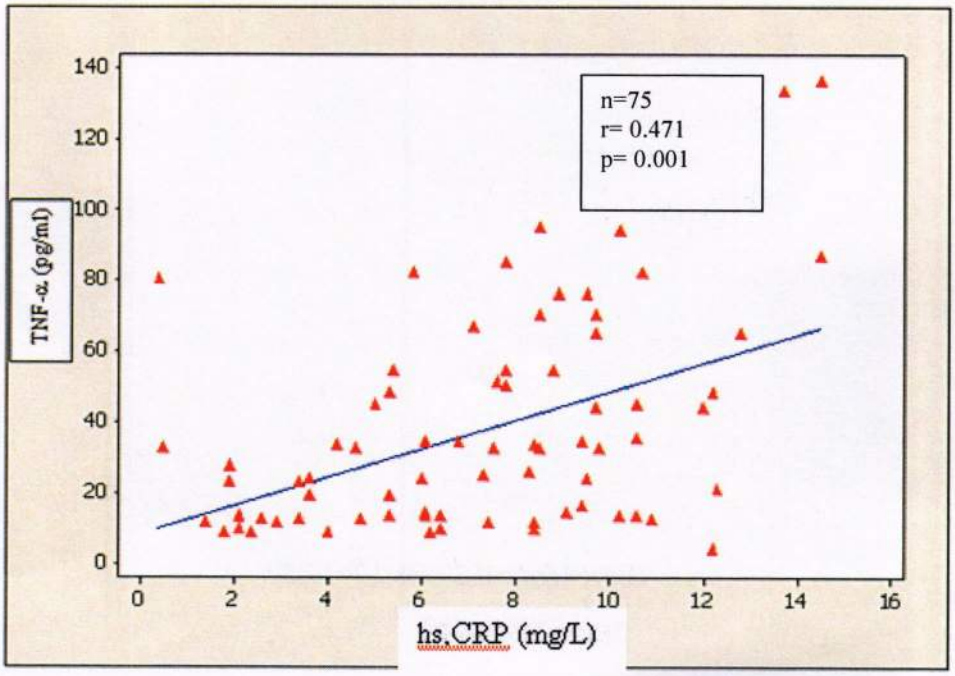
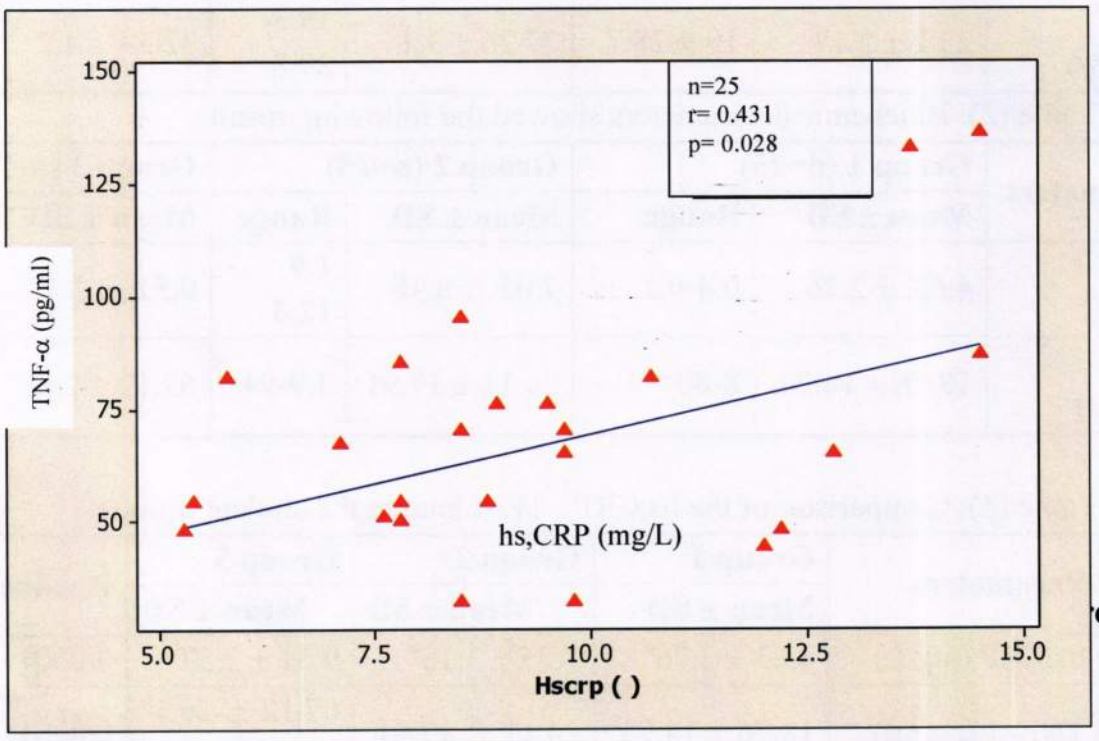


Figure (1): Correlations between hs-CRP and TNF-α for all sampled women (n=75).



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