Inflammation rather than Oxidative Stress is a Better Marker for Preeclampsia

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Abstract

Background: Preeclampsia is a leading cause of maternal morbidity and mortality, characterised by hypertension and significant proteinuria with or without oedema occurring from late second trimester to third trimester of gestation in a previously normotensive, non-proteinuric woman. The condition is associated with an inflammatory response as well as oxidative stress. This study is therefore designed to assess the relationship between inflammatory and oxidative stress markers in severe preeclampsia, and to determine which is more associated with severe preeclampsia.

Methods: We recruited 60 pregnant women in their third trimester into this study, grouped as 30 women with severe preeclampsia and 30 normotensive, non-proteinuric women. Serum highsensitivity C-reactive protein and total antioxidant status as well as urinary protein levels were determined.

Results: It was observed that the systolic blood pressure, diastolic blood pressure and serum HsCRP were significantly elevated in case subjects when compared with control subjects while the mean serum level of TAS was significantly reduced in case subjects when compared with controls. HsCRP also had a significant positive correlation with SBP, DBP and proteinuria while TAS had a significant negative correlation with HsCRP, SBP, DBP and proteinuria. Using the ROC curve, it was also observed that HsCRP has a greater area under curve than TAS.

Conclusion: It could therefore be concluded that HsCRP is a better marker for severe preeclampsia than TAS and its measurement is useful in the management of preeclampsia.

ABBREVIATIONS

HsCRP: High Sensitivity C-Reactive Protein; TAS: Total Antioxidant Status; SDP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ROC: Receiver Operating Characteristic; BUHREC: Babcock University Health Research Ethics Committee; SPSS: Statistical Package for Social Sciences; IL: Interleukin; TNF-α: Tumor Necrosis Factor Alpha; ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species; SD: Standard Deviation.

INTRODUCTION

Pre-eclampsia is a pregnancy-specific disorder, affecting virtually every organ system. It is characterised by a host of abnormalities resulting in vascular endothelial damage and subsequent vasospasm, leading to the development of hypertension, significant proteinuria, with or without oedema occurring after 20 weeks of gestation in a previously normotensive non-proteinuric woman [1,2]. It is multifactorial and forms an integral part of the continuum of hypertensive disorders of pregnancy [3]. Preeclampsia has been reported to have a worldwide prevalence of 3% - 10% of all pregnancies [4], although a more recent study observed a prevalence of 1.2% [5]. Preeclampsia is a leading cause of maternal morbidity and mortality. It was reported to have contributed up to 46.3% and 43% of maternal deaths in Kano and Jigawa States of Nigeria respectively [6].

In normal pregnancy there is a systemic inflammatory response [7], and consequently, an acute phase response which is exacerbated in preeclampsia; and might play a role...
in its pathogenesis [8,9]. In preeclampsia, the inflammatory response is evidenced through endothelial dysfunction [8,10], persistent leukocyte and platelet activation [11,12] and elevated inflammatory cytokines: interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [13-16]. Consequently, there is increased production of C-reactive protein (CRP), an acute phase protein, by the liver in response to placental pro-inflammatory cytokines - IL-6 and TNF-α, emphasizing the role of CRP as an inflammatory marker [17]. Furthermore, this leads to oxidative stress through depletion of antioxidants.

Oxidative stress is an imbalance in the production and the elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as their clearance by defensive antioxidants [18]. Although toxic, oxidative stress might be beneficial through its role in intracellular killing [19], and is also important for placental development in pregnancy [20]. There are a wide range of studies implicating oxidative stress in the pathophysiology of preeclampsia because it damages the maternal vascular endothelium, therefore compromising its important function [21-30]. Similarly, various studies explained inflammatory responses in preeclampsia as shown by endothelial dysfunction [9,10]; persistent leukocyte and platelet activation [11,12]; and elevated inflammatory cytokines: interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) [13-16]. This study is therefore designed to assess the relationship between inflammation and oxidative stress in severe preeclampsia, and to determine which of them serves a better marker for severe preeclampsia.

MATERIALS AND METHODS

A total of sixty (60) pregnant women in their third trimester, recruited from the Antenatal clinic and Antenatal Ward of Lagos Island Maternity Hospital, Lagos State participated in this study. Thirty (30) of them (case subjects) were diagnosed of severe preeclampsia using the cut off blood pressure of 160/110mmHg and proteinuria of 3+ according to The National High Blood Pressure Education Program working Group on High Blood Pressure in Pregnancy. The remaining 30 were normotensive, non-proteinuric women who served as controls. Informed consent was obtained from each participant and ethical clearance was obtained from Babcock University Health Research Ethics Committee (BUHREC). Pregnant women with diabetes mellitus, chronic hypertension, renal disease, HIV, syphilis, HBsAg, HCV, urinary tract infection, smokers, alcohol consumers and those who did not give consent were excluded.

Demographic information on medical history, smoking habit and alcohol use were obtained using a semi-structured questionnaire. The blood pressure was measured with the participant in supine position using mercury sphygmomanometer with appropriate cuff. Systolic and diastolic blood pressure was measured at the Korotkoff phase 1 and 5 respectively. Five (5) mL of venous blood was collected aseptically through venupuncture and dispensed into plain bottles, allowed to clot, centrifuged, and separated within one (1) hour of collection to extract the serum. The serum was stored at -20°C until the time of analysis for hsCRP, and TAS.

HsCRP was analysed based on the principle of solid-phase enzyme-linked immunosorbent assay (ELISA) using a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule immobilised on the microtitre wells with kits supplied by Calbiotech Inc. (California, USA). Ten microlitre (10μL) of CRP standards, diluted samples and controls (1:100) were added to appropriate wells. One hundred microlitre (100μL) of CRP Enzyme Conjugate Reagent was added to each well and they were thoroughly mixed and then incubated for 60 minutes at room temperature after which the wells were ‘washed’. One hundred microlitre (100μL) of TMB solution was added into each well and then incubated at room temperature for 15 minutes after which 50 μL of Stop Solution was added to each well to stop the reaction. Absorbance was read at 450nm with a microtiter well reader. The grades of standard were used to plot a curve of absorbance against concentration for the calculation of hsCRP concentration.

Randox kit [Total Antioxidant Status (TAS) RANDOX Laboratories Ltd, UK] was used to determine serum TAS. The method is based on the suppression of the blue green colour of 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation by antioxidants.

Also, 5ml of random mid-stream urine (MSU) was collected into universal bottle for semi-quantitative analysis of urine protein using Rapid Labs dipstick strips (Rapid Labs Ltd, Essex UK). The corresponding value for 3+ proteinuria was 3.0g/L.

All data were analysed using SPSS version 17.0 and values were expressed as mean ± standard deviation (SD) for case subjects and controls. Student’s t-test was used to compare the difference between the means of the two groups, while Receiver Operating Characteristic (ROC) curve was used to determine the diagnostic properties of both HsCRP and TAS while Pearson’s correlation was used to study the association between variables. P-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Results

Table (1) shows the anthropometric and biochemical parameters of participants. The systolic blood pressure (SBP), diastolic blood pressure (DBP) and serum HsCRP were significantly elevated in case subjects when compared with control subjects while the mean serum level of TAS was significantly reduced in case subjects when compared with controls.

In our case subjects, HsCRP had a significant positive correlation with SBP, DBP and proteinuria while TAS had a significant negative correlation with HsCRP, SBP, DBP and proteinuria (Table 2).

The diagnostic properties of HsCRP and TAS were determined using ROC curve shown in Figure (1).

Discussion

Preeclampsia has been regarded as one of the leading causes of maternal mortality and morbidity in the world occurring in 4-5% of pregnant women [31]. Elevated blood pressure and proteinuria are vital criteria in the diagnosis of preeclampsia. In the present study, both SBP and DBP were elevated in subjects with severe preeclampsia, and the corresponding value for 3+ proteinuria was 3.0g/L.
Table 1: Anthropometric and biochemical parameters in severe preeclampsia and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Severe preeclampsia n = 30</th>
<th>Control n=30</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.7 ± 4.96</td>
<td>29.1 ± 5.22</td>
<td>0.743</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>159.9 ± 13.74</td>
<td>116.2 ± 7.56</td>
<td>0.000*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>100.1 ± 9.51</td>
<td>77.1 ± 3.94</td>
<td>0.000*</td>
</tr>
<tr>
<td>HsCRP (mg/L)</td>
<td>9.86 ± 2.05</td>
<td>6.12 ± 4.31</td>
<td>0.000*</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td>1.37 ± 0.042</td>
<td>2.37 ± 0.88</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*Significant at P<0.01, results are reported as mean ± standard deviation;

Abbreviations: SBP: Systolic Blood Pressure; DBP: Diastolic blood pressure; HsCRP: High Sensitive C-Reactive Protein; TAS: Total Antioxidant Status.

Table 2: Correlations among HsCRP, TAS and co-founding parameters of preeclampsia in women with severe preeclampsia.

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>TAS</th>
<th>Proteinuria</th>
<th>HsCRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.864*</td>
<td>-0.537*</td>
<td>0.354</td>
<td>0.444*</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td>-0.474*</td>
<td>0.323</td>
<td>0.413*</td>
</tr>
<tr>
<td>TAS</td>
<td>-0.537*</td>
<td></td>
<td>-0.578*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.354</td>
<td>0.323</td>
<td>-0.578*</td>
<td></td>
<td>0.458*</td>
</tr>
<tr>
<td>HsCRP</td>
<td>0.444*</td>
<td>0.413*</td>
<td>-0.541*</td>
<td>0.458*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at P< 0.01 (two-tailed);

Abbreviations: SBP: Systolic Blood Pressure; DBP: Diastolic blood pressure; HsCRP: High Sensitive C-Reactive Protein; TAS: Total Antioxidant Status.

Figure 1 ROC Curve for HsCRP and TAS in Preeclampsia.

with preeclampsia when compared to controls. Elevated blood pressure in preeclampsia could be attributed to preponderance of vasoconstrictors over vasodilators. Our observation of elevated serum HsCRP in women with severe preeclampsia compared with control subjects supports the report of Bargale et al. [32]. This observation could be due to exaggerated maternal endothelial activation which results into inflammatory changes in endothelial function when compared to normal pregnancy, and consequent clinical manifestation of hypertension and proteinuria [33].

Maxwell et al. [34], recommended the determination of serum TAS rather than monitoring the response to a single antioxidant, as this helps to give a representation of mixed antioxidant response. Therefore, in this study, serum TAS was measured and it was observed that the serum level of TAS was significantly reduced in subjects with preeclampsia when compared with control. The observed reduced serum TAS in women with severe preeclampsia when compared with the control subjects is in agreement with the studies of Kaur et al. [35], and Boutet et al. [36], that reported decreased serum antioxidant concentrations...
in maternal circulation and placenta of women with preeclampsia. This observation could be attributed to abnormal placenta and the resultant placental hypoxia has been implicated as the major cause of preeclampsia. Placenta hypoxia leads to oxidative stress and this becomes more pronounced as pregnancy progresses. Marked oxidative stress within the placenta further aggravates vascular dysfunction in the placenta which in turn gives rise to inflammatory apoptosis and structural damage [33-38].

Aydin et al. [23], and Freeman et al. [39], reported that oxidative stress and inflammatory response are related to endothelial dysfunction in preeclampsia. This is also supported by the observed significant positive correlation between HsCRP and indicators of preeclampsia (SBP, DBP and proteinuria). However, there was a significant negative correlation between TAS and HsCRP, SBP, DBP, proteinuria.

Furthermore, the observed area under the ROC curve was 0.64 for HsCRP and 0.35 for TAS. Thus, suggesting that HsCRP is better than TAS for the diagnosis of severe preeclampsia and it is comparable to the existing indicators for the diagnosis of severe preeclampsia and might be useful in monitoring preeclampsia.

CONCLUSION

It could be concluded that there is an association between oxidative stress and inflammatory response in the aetiology of preeclampsia. Furthermore, maternal serum level of HsCRP is significantly associated with severe preeclampsia and serum HsCRP was found to be a better predictor than TAS for the severity of the disease.

ACKNOWLEDGEMENTS

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REFERENCES

27. Rusterholz C, Hahn S, Holzgreve W. Role of placentally produced


