

Original Research Article

The Role of Serum Ck-Nac, Ldh and Crp in Early Prediction of Severity of Hypertension and Hypertensive Complications in Pregnancy

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Abstract

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Gestational hypertension (GH) is characterized most often by new-onset elevations of blood pressure (BP) after 20 weeks of gestation in the absence of accompanying proteinuria and can lead to hypertensive complications in pregnancy most commonly pre-eclampsia and eclampsia. Despite being a major contributor of maternal and perinatal morbidity and mortality, the cause of this entity is unclear. This study was designed to evaluate the role of serum creatine kinase-N-Acetyl-Cystein (CK-NAC), lactate dehydrogenase (LDH) and C-reactive protein (CRP) in early prediction of severity of hypertension and hypertensive complications in pregnancy. A total of 300 subjects were recruited for this study. These comprised 150 (43 mild, 58 moderate and 49 severe) hypertensive and 150 age-matched normotensive individuals. The mean values of CK-NAC, LDH and CRP were significantly higher ($P < 0.05$) in mild (185.0 ± 71.5 , 220.8 ± 52.1 and 12.6 ± 9.8), moderate (194.3 ± 84.3 , 226.4 ± 52.7 and 18.7 ± 8.9) and severe hypertensive subjects (187.9 ± 67.3 , 232.2 ± 60.5 and 25.9 ± 10.9 respectively) when compared with the control (101.6 ± 27.7 , 201.7 ± 28.2 and 5.5 ± 2.1). Also, the serum level of CRP was significantly elevated ($P < 0.05$) as the severity of gestational hypertension progressed from mild (12.6 ± 9.8) to moderate (18.7 ± 8.9) and severe cases (25.9 ± 10.9). The mean value of CK-NAC correlated positively with LDH ($R = 0.194$, $P = 0.033$) and CRP ($R = 0.173$, $P = 0.041$). Therefore, our results suggest that increased serum CK-NAC, LDH and CRP levels are associated with gestational hypertension, with CRP level showing more consistent association with the progression of gestational hypertension.

Keywords: Creatine kinase-N-Acetyl-Cystein, C-reactive protein, Hypertension, Lactate dehydrogenase, Pregnancy

INTRODUCTION

Gestational hypertension (GH), a pregnancy induced hypertension (PIH) characterized by a blood pressure of at least 140/90 mmHg of onset or first recognition in a previously normotensive woman and with proteinuria ≤ 0.3 gm in 24 hours urine collection (World Health Organization, 2014). Being a multi-systemic disorder, GH

can affect so many systems in the body if not properly managed and can lead to pre-eclampsia, impaired liver functions, kidney impairment, pulmonary oedema, fetal growth restriction, placental abruption, premature delivery and maternal and perinatal mortality (Olatinwo et al., 2009). Despite being the leading cause of maternal death

and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of GH have not been fully elucidated. However, several factors have been postulated as contributory mechanisms to the rise in blood pressure during pregnancy. These factors include among others, an expansion in total plasma volume of up to 40% and increase in the synthesis of thyroid hormones (Omole and Ashimi, 2008; Olatinwo et al., 2009). Also, the relevance of endothelial dysfunction and elevated levels of inflammatory markers in predicting cardiovascular risk is gaining increasing recognition (Ridker et al., 2000) and in that respect assessment of CK, LDH and CRP may be of good value in predicating the hypertensive complications in pregnancy.

Concentrations of creatine kinase-N-Acetyl-Cystein (CK-NAC) in serum are often increased in patients with musculoskeletal and endothelial disorders (Qublan et al., 2005; Ghosh et al., 2011), but evidence for any change in gestational hypertension is lacking. The serum CK-NAC activity in healthy individuals depends on age, race, lean body mass and physical activity (Saha et al., 2009) and thus has since become an important clinical marker for muscle damage.

Lactate dehydrogenase (LDH), an intracellular enzyme is often measured to evaluate the presence of cellular damage and according to Qublan *et al;* (2005) serum LDH is a useful predictor of foetal outcome. Also, concentrations of LDH in serum are often increased in patients with cellular damage, but its importance in gestational hypertension is unknown.

C - reactive protein (CRP) is an acute phase protein which is synthesized in the liver. It is present in trace amount in normal healthy person and rise significantly following injury and inflammation (Ghosh et al., 2011).

In view of their clinical importance, there is therefore the need to carry out a study on these biochemical parameters in hypertensive pregnant women. This could help in the diagnostic, therapeutic and monitoring purposes and therefore contribute to the reduction of maternal-fetal morbidity, and mortality. This present study aims to evaluate the role of serum CK-NAC, LDH and CRP in early prediction of severity of hypertension and hypertensive complications in pregnancy.

MATERIALS AND METHOD

Research Design

A total of 300 participants aged between 22 and 40 years were randomly selected for this study. The participants were made up of 150 (classified as mild (43), moderate (58) and severe (49) hypertensive pregnant women as

test subjects and 150 age-matched normotensive pregnant women as controls. The gestational age of each participant was established based on last menstrual period. The study was a cross sectional study designed to assess the levels of serum creatine kinase-N-Acetyl-Cystein (CK-NAC), lactate dehydrogenase (LDH) and C-reactive protein (CRP) in staged hypertensive pregnant women in Nnewi, Nigeria.

Study Site

This research work was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria.

Inclusion Criteria

Subjects with hypertension diagnosed after 20 weeks (2nd and 3rd trimesters) of gestation were used in the study. Also, apparently healthy age and trimester-matched normotensive pregnant women attending antenatal clinic were selected as control subjects.

Exclusion Criteria

Subjects with hypertension predating the index pregnancy, diabetes mellitus, and those with antenatal booking weight greater than 90 kg were excluded from the study. Also, all pregnant women with proteinuria \geq 0.3 gm were equally excluded from the study. Pregnant mothers with history of smoking and alcohol intake as well as those who refuse to consent were also excluded.

Ethical consideration

Ethical approval for this study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Informed written consent was obtained from the participants before the collection of data and blood samples.

Sampling Technique

Random sampling technique was used during sample collection. 5 ml of whole blood was collected using a plain specimen container. The serum obtained after centrifugation was stored at 2-8°C until analyzed. The biodata of all study participants were obtained using a structured interviewer administered pretested

Table 1. Demographic and anthropometric characteristics of the study participants (mean ± SD).

Parameters	Mild GH n = 43	Moderate GH n = 58	Severe GH n = 49	Control group n = 150	F-value	P-Value
Age (years)	28.3±5.1	26.8±4.1	27.7±4.8	26.9±4.4	1.340	0.262
BMI (kg/m ²)	26.2±3.4	26.6±3.3	27.1±3.5	25.9±3.3	2.941	0.108
Gestational age (weeks)	29.9±5.1	29.1±5.2	29.7±5.7	28.9±5.3	4.267	0.100
SBP (mmHg)	146.6±6.8	165.2±5.5	184.5±4.3	115.6±9.1	1319.6	0.05
DBP (mmHg)	89.9±6.4	103.2±3.1	112.7±3.2	68.5±3.5	2149.6	0.05

Table 1a. Multiple comparisons (Post Hoc) of SBP and DBP in mild, moderate and severe hypertension

Parameters	Groups	P-value (Post HOC)
SBP	MILD Vs MODERATE	0.05
	MILD Vs SEVERE	0.05
	MODERATE Vs SEVERE	0.05
	CONTROL Vs ALL GROUP	0.05
DBP	MILD Vs MODERATE	0.05
	MILD Vs SEVERE	0.05
	MODERATE Vs SEVERE	0.05
	CONTROL Vs ALL GROUP	0.05

questionnaire. Blood pressure of each participant was measured using Accoson mercury sphygmomanometer. Korotkoff's sound phases I and V were used to determine the systolic and diastolic blood pressures (SBPs and DBPs) respectively. Values above 140 and 90 mmHg for the SBP and DBP respectively were considered abnormal. Using the seventh Joint National Committee (JNC VII) criteria (National Institutes for Health, 2013), the hypertensive participants were further classified as mild (SBP 140-159, DBP 90-99 mmHg, n=43), moderate (SBP 160-179, DBP 100-109 mmHg, n=58) and severe (SBP >180, DBP >110mmHg, n=49)

Statistical analysis

The data generated were presented as mean ± standard deviation. Differences among groups were assessed with a one-way analysis of variance (ANOVA), while differences between groups were analyzed with student's T-test. Associations between continuous variables were described by Pearson's correlation coefficients. Significance was accepted at P<0.05. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), Version 20.0.

RESULTS

The demographic and anthropometric parametric analysis shows that the mean values of age in mild

(28.3±5.1 years), moderate (26.8±4.1 years) and severe hypertensive pregnant women (27.7±4.8 years) were not significantly different (P>0.05) when compared to the normotensive subjects (26.9±4.4 years) (P=0.262). There were also no significant differences (P>0.05) in the mean levels of body mass index in mild (26.2±3.4 m/kg²), moderate (26.6±3.3 m/kg²) and severe GH (27.1±3.5 m/kg²) compared to the control subjects (25.9±3.3 m/kg²); the gestational age in mild (29.9±5.1 weeks), moderate (29.1±5.2 weeks) and severe GH (29.7±5.7 weeks) when compared to the normotensive subjects (28.9±5.3 weeks).

However, the mean values of SBP and DBP in mild (146.6±6.8 and 89.9±6.4 mmHg), moderate (165.2±5.5 and 103.2±3.1 mmHg) and severe hypertensive pregnant subjects (184.5±4.3 and 112.7±3.2 mmHg) were significantly higher (P<0.05) compared to the control subjects (115.6±9.1 and 68.5±3.5 mmHg). Both SBP and DBP increases statistically (P<0.05) from mild to severe hypertension, However, the values for the control subjects are statistically lower than each of the hypertensive groups (Table 1a).

The mean levels of CK-NAC, LDH and CRP in mild (185.0±71.5_a, 220.8±71.5_a and 12.6±9.8_a), moderate (194.4±84.3_a, 226.4±52.7_a and 18.7±8.9_a) and severe gestational hypertension (187.9±67.3_a, 232.2±60.5_a and 25.9±10.9_a) were significantly higher (P<0.05) than the mean value of the control subjects (101.6±27.7, 201.7±28.2 and 5.5±2.1) respectively. Also, there were significant differences between the mean levels of CK-NAC in mild and moderate hypertensive pregnant women

Table 2. Serum levels (mean ± SD) of CK-NAC, LDH and CRP with mild, moderate and severe hypertension among the test subjects

Stages of GH Parameters	Mild n=43	Moderate n=58	Severe n=49	Control n=150	F-value	P-value
CK-NAC (U/L)	185.0±71.5 _{a,b}	194.3±84.3 _{a,b}	187.9±67.3 _a	101.6±27.7	60.037	0.05
LDH (IU/L)	220.8±52.1 _{a,c}	226.4±52.7 _a	232.2±60.5 _{a,c}	201.7±28.2	8.548	0.05
CRP (mg/L)	12.6±9.8 _{a,d}	18.7±8.9 _{a,d}	25.9±10.9 _{a,d}	5.5±2.1	172.5	0.05

Keys: a = significant difference between hypertensive stages and control, b = significant difference between mild and moderate GH, c = significant difference between mild and severe GH and d = significant difference across the hypertensive stages.

Table 3. Relationship between the serum levels of CK-NAC, LDH and CRP with mild, moderate and severe hypertension among the test subjects

Parameters	CK-NAC (U/L)	LDH (IU/L)	CRP (mg/L)
CK-NAC (U/L)	--	0.194 _a	0.173 _a
LDH (IU/L)	0.194 _a	--	0.201 _a
CRP (mg/L)	0.173 _a	0.201 _a	--

Key: a = mild positive significance (P <0.05).

(185.0 ±71.5_b and 194.3±84.3_b) and LDH in mild and severe gestational hypertension (220.8±52.1_c and 232.2±60.5_c), whereas the serum level of CRP showed significant increase (P<0.05) from mild (12.6±9.8_d) to moderate (18.7±8.9_d) and to severe gestational hypertension (25.9±10.9_d). In contrast, there were no significant differences (P>0.05) in mean values of CK-NAC in mild (185.0±71.5) and severe GH (187.9±67.3) and in moderate (194.3±84.3) and severe GH (187.9±67.3) respectively. Also, the mean value of LDH showed no significant differences between mild (220.8±52.1) and moderate (226.4±52.7) and between moderate (226.4±52.7) and severe GH (232.2±60.5). (Table 2)

There were significant positive correlations (P<0.05) between serum values of CK-NAC and LDH (R= 0.194_a), CK-NAC and CRP (R= 0.173_a), LDH and CRP (R= 0.201_a) respectively. (Table 3)

DISCUSSION

Hypertensive disorder in pregnancy is the most important public health problem in developing countries and one of the major risk factors for hypertensive complications in pregnancy such as pre-eclampsia, eclampsia and even cardiovascular diseases, and it has been reported that hypertension is in part an inflammatory and endothelial disorder and several workers have reported elevated

levels of CRP in hypertensive individuals but none has been done in relation to gestational hypertension. The main aim of the present study was to evaluate the association between blood pressure and serum CK-NAC, LDH and CRP levels across the mild, moderate and severe hypertensive categories.

The serum level of CRP was significantly elevated across the gestational hypertensive stages (mild, moderate and severe) and against the normotensive pregnant women also. Whereas CK-NAC level only showed a significantly increased mean value from mild to moderate hypertension and across the control group, while serum LDH level showed a significant elevation from mild to severe hypertension and across the control subjects. Therefore, only the serum level of CRP consistently increased as gestational hypertension advanced. This may infer that there is an underlying disease state that predisposes the hypertensive pregnant mothers to multiple organ dysfunctions as seen by the significant elevation of serum levels of CK-NAC, LDH and CRP in hypertensive pregnant women than the normotensive pregnant mothers. This finding indicates that endothelial dysfunction and inflammatory reaction may be associated with the progression of gestational hypertension. This is because the hyper-activity states of CK-NAC and LDH can cause increased arterial stiffness which is an important determinant of vascular endothelial dysfunction and changes in arterial wall elasticity (the major underlying cause of elevated blood pressure).

More so, CRP has been implicated in the reduced formation of vasodilators by endothelial cells which in turn promote vasoconstriction, leukocyte adhesion, platelet activation, oxidation and thrombosis. Therefore, the consistently progressive high serum levels of CRP from mild to moderate and severe gestational hypertension in conjunction with the significantly high levels of CK-NAC and LDH in the hypertensive pregnant women, may upregulate angiotensin receptors and enhance expression of plasminogen activator inhibitor-1 by endothelial cells. These changes could trigger hypertensive disorder in pregnancy and may promote hypertensive complications such as pre-eclampsia and eclampsia. This is substantiated by several studies which have shown that inflammatory markers such as CRP is an independent determinant of endothelium dependent vascular dysfunction among patient with coronary heart disease (CHD) (Ridker et al., 2000; Pauleto and Rattazzi, 2006) and this situation may also exist in patients with gestational hypertension. Our findings are in agreement to the ones reported by Sesso et al., (2003), Jian-jun, (2006), and Nanda et al., (2013).

In the light of these findings and from several other studies we hypothesize gestational hypertension per se may lead to multiple inflammatory stimuli at the vessel wall which in turn promote the production of a number of pro inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) as a defense against injurious factors. According to Shafi et al., (2000), inflammation further causes endothelial dysfunction, possibly by decreased capacity of the endothelium to generate vasodilatory factors, particularly nitric oxide (NO) which in turn raises blood pressure. This invariably could trigger the release of the endothelial markers like the CK-NAC and LDH as recorded in this study. This is substantiated by several studies which have shown inflammatory markers such as CRP as an independent determinant of endothelium dependent vascular dysfunction among patient with coronary heart disease (CHD) (Ridker et al., 2000; National Institutes for Health, 2013; Shafi et al., 2010) and this situation may also exist in patients with gestational hypertension. Our findings are in agreement to the one reported by Sesso et al., (2003) who also have shown a link between elevated CRP and increased risk of developing hypertension in a cohort study, including people with baseline blood pressure in pre-hypertensive range. Possible mechanisms for this association are oxidative stress and interaction with adhesion molecules, plasminogen activator inhibitor-1 and low density lipoprotein cholesterol (LDL-C) uptake (King et al., 2004).

The findings of this study, therefore suggests estimation of CK-NAC, LDH and most importantly CRP level(s) could serve as essential or potential tool(s) for early identification of individuals at risk for development of hypertensive complications of pregnancy including cardiovascular diseases, since both elevated CRP and progressive gestational hypertension are determinants of gestational hypertensive complications such as pre-eclampsia, eclampsia and even cardiovascular diseases. Thus, the finding of this study may provide a rationale for pharmacotherapy, in a broader subset of women with gestational hypertension.

CONCLUSION

In conclusion, our results suggest that increased serum CK-NAC, LDH and CRP levels are associated with gestational hypertension, with CRP level showing more consistent association with the progression of gestational hypertension. Thus serum CK-NAC, LDH and specifically CRP estimation can be an adjunct in the identification of hypertensive pregnant mothers at risk for development of hypertensive complications associated with pregnancy and including CVDs.

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