Original Article

Correlation of serum neutrophil gelatinase associated lipocalin with disease severity in hypertensive disorders of pregnancy

Rekha Sachan, ML Patel[1], Amrita Gaurav, Radheshyam Gangwar[2], Pushpalata Sachan[3]

Departments of Obstetrics and Gynaecology, [1]Medicine, and [3]Physiology, King George Medical University, [2]Department of Critical Care, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract

Background: Vascular endothelial dysfunction is considered central to the pathogenesis of hypertensive disorders of pregnancy (HDP). Serum level of neutrophil gelatinase-associated lipocalin (NGAL) is closely related to endothelial injury. The aim of this study was to examine the correlation of serum NGAL with disease severity in HDP.

Materials and Methods: This prospective case-control study was carried out for one year. After informed consent, ethical clearance, total 1,850 pregnant women were screened. Analysis was performed on 142 cases of HDP and 31 healthy controls. Quantitative measurement of serum NGAL levels was done by the enzyme linked immunosorbent assay (ELISA) technique, by using sandwich ELISA kit.

Results: Mean serum NGAL value in patients with oliguria was significantly higher when compared with non-oliguric patients ($P < 0.001$). Serum NGAL had a positive correlation with systolic blood pressure ($r \sim 0.5973$), diastolic blood pressure ($r \sim 0.6195$), blood urea ($r \sim 0.4392$), serum creatinine ($r \sim 0.6112$), serum uric acid ($r \sim 0.3878$). Sensitivity and specificity of serum NGAL using a cut-off value of 545 pg/ml, for the diagnosis of HDP, was 97.89% and 93.55% respectively, using 95% confidence interval.

Conclusion: Between the two groups, we found that serum NGAL had a positive correlation with disease severity and better sensitivity and specificity in the evaluation of HDP.

Key Words: Eclampsia, hypertensive disorders of pregnancy, preeclampsia, serum neutrophil gelatinase-associated lipocalin

Address for correspondence:
Dr. Rekha Sachan, Professor Department of Obstetrics and Gynaecology, King George Medical University, Lucknow, Uttar Pradesh, C-28, Sec-J Aliganj, Lucknow - 226 024, Uttar Pradesh, India. E-mail: drrekhasachan@gmail.com
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INTRODUCTION

Hypertensive disorders of pregnancy (HDP) is a major disease, which seriously endangers the safety of mother and fetus during pregnancy. Hypertensive disorders remain one of the largest single cause of maternal and fetal morbidity and mortality, accounting for maternal death in developed countries, Latin America

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and Africa as 16.1%, 25.7%, 9.1% respectively and 18% of fetal death.[1,2] HDP are found in 10% of first pregnancies and 6-8% of all pregnancies.[3–5] In India, incidence of eclampsia is 1 in 115 deliveries (0.87%), the maternal mortality rate is 7.8%[6] and 8.3% due to preeclampsia.[7]

The pathogenesis of HDP is not completely clear. It is a multifactorial and multichannel disease and its central pathogenesis is the systemic activation and injury of maternal endothelial cells, which bring out a variety of clinical symptoms such as raised blood pressure (BP), proteinuria, systemic inflammatory response and accumulation of antiangiogenic factors, which seems to cause the disease by depriving the glomerular endothelium of essential growth factors.[8] Vascular endothelial dysfunction is considered central to the pathogenesis of HDP. Serum level of neutrophil gelatinase-associated lipocalin (NGAL) is closely related to endothelial injury. It is responsible for the decrease in glomerular filtration rate (GFR) primarily through reduction in ultra-filtration as opposed to diminished plasma flow.[9] Hence, we assume that if there is a way to assess the endothelial damage and determine its extent of development, it is possible to give a reasonable prevention in HDP.

Human NGAL belongs to the lipocalin family of proteins, consists of single disulfide bridged polypeptide chains of 178 amino acid residues with a calculated molecular mass of 25 kDa.[10] Serum NGAL is a novel biomarker and is detectable very early after kidney injury. It is up-regulated by renal distal tubular cells within minutes after ischemic-perfusion injury and secreted in urine and serum.[11,12] Plasma concentration of NGAL increases 3-fold within 2 h of acute kidney injury. After ischemic injury, NGAL levels in kidney tissue rises by 10-fold within 3 h.[13] Very few studies have been conducted on serum NGAL in human pregnancy, but the characteristics of this molecule, which increases in hypertensive pregnant women with renal damage and inflammation and is correlated with angiogenic factors, suggest that it might be involved in HDP, particularly in preeclampsia and eclampsia, in which all these factors are present. Studies speculated that NGAL may be related to the pathogenesis of preeclampsia and eclampsia and increase in NGAL level in blood during the second trimester of pregnancy is a sensitive indicator of occurrence of preeclampsia.[14,15]

The aim of the study was to assess the diagnostic accuracy of serum NGAL in preeclampsia and eclampsia and correlation of serum NGAL levels with severity of disease in preeclampsia and eclampsia.

MATERIALS AND METHODS

This prospective case-control study was carried out over a period of 1 year, at Department of Obstetrics and Gynecology, in collaboration with Department of Internal Medicine, King George Medical University, Lucknow, Uttar Pradesh, India. Ethical clearance was obtained from Institutional Ethic Committee of the University. A total of 1,850 pregnant women were screened over a period of 1 year, who signed written consent, 149 of whom diagnosed with HDP and enrolled in our study, seven were lost to follow-up. Analysis was therefore carried out on 142 cases. Subgroups were defined as per National High BP Education Program Working Group (2000),[8] mild preeclampsia was seen in 65, severe preeclampsia in 32 and eclampsia in 45 cases. The women enrolled had a gestational age of 20-40 weeks. 31 healthy pregnant non-hypertensive women were taken as controls. This control group was well-matched with the cases for maternal age, pre-pregnancy body mass index and gestational age. Patients with pre-existing renal disease, hypertension, diabetes mellitus, gestational hypertension, active urinary tract infection and refusal to cooperate with the study were excluded, data were collected and analyzed.

"Pregnancy induced hypertension" was defined as BP ≥140/90 mm of Hg on two occasions at least 6 h apart, in women who are normotensive in pre-pregnancy phase. "Mild preeclampsia" was defined as BP ≥140/90 mmHg, but less than 160/110 mmHg with proteinuria ≥300 mg/24 h. "Severe preeclampsia" was defined as the presence of BP ≥160/110 mmHg with urinary protein excretion of ≥2.0 g/24 h or any of these, oliguria or <400 ml urine/24 h, visual disturbances, serum creatinine ≥1.2mg/dl, platelets less than 100,000/mm^3, microangiopathic hemolysis. "Eclampsia" was defined as the occurrence of new onset grand mal seizure in a patient of preeclampsia. "Hemolysis Elevated Liver Enzymes and Low Platelet Counts" (HELLP) syndrome was defined as elevation of liver enzymes (aspartate aminotransferase more than 70 IU/L), hemolysis (lactate dehydrogenase [LDH], more than 600 IU/L) and low platelet counts (≤100,000/mm^3). "Proteinuria" was defined as 24 h urinary protein excretion of ≥300 mg.

Measurement of BP was performed 4 hourly in patients of preeclampsia and eclampsia, in the right arm supine position and Korotkoff V sounds were taken for measurement of diastolic blood pressure (DBP). BP was measured by mercury sphygmomanometer. Assessment of edema was performed clinically, on general examination applying thumb pressure over the medial malleolus for 30 s.

Moreover, 5 ml of venous blood was obtained in plain vial from the cases and controls. Collected blood samples were stored at 4°C temperature. Centrifugation was carried out at 6,000 rpm. Samples were frozen at –20°C until assayed. Quantitative measurement of serum NGAL levels was carried out by the enzyme linked immunosorbent assay (ELISA) technique, by using sandwich ELISA kit (Uscn Life Science Inc., China). The investigations were carried out in the accredited lab, serum creatinine estimation was carried out by Jaffess method, sulfosalicylic acid test used for urinary protein estimation and uric acid estimation was carried out by nephalometry.

Demographic characteristics, gestational age, BP on admission, biochemical parameters (including complete hematology, biochemistry and 24 h urinary protein) were recorded. The renal manifestations (proteinuria, hyperuricemia and acute renal failure) were recorded in the individual patient. Strict BP and labor monitoring was done. Antihypertensive drugs were given. For mild preeclampsia, tablet methyldopa was given. Methyldopa is a centrally acting and belongs to category B drug, which is safe for the mother and fetus. Labetalol was given in cases of severe preeclampsia and eclampsia, which is Food and Drug Administration category C drug. Magnesium sulfate Pritchard regimen was given for control of convulsion in eclampsia along with fluid replacement and intensive monitoring was done. All patients were followed-up to 6 weeks postpartum.

The categorical data was described as *n* (%) whereas continuous variables as mean ± standard deviation. The association between two or more categorical variables was tested by χ^2 statistics by using appropriate correction. Prior to carrying out any test on continuous data, the normalcy of data was tested. Two-sample *t*-test was used to see the difference between the mean of two different groups, if data was normally distributed. If data was not found to be normally distributed, Mann-Whitney test used to test the level of significance between two values. For more than one- group, one-way analysis of variance (one-way ANOVA) was used to test the difference among more than two groups in case of normally distributed data. Bonferroni correction was applied to the level of significance in order to avoid family error. If the data was not normally distributed, Kruskal-Wallis test was used in place of one-way ANOVA. Diagnostic evaluation for preeclampsia was performed. Area under curve (AUC) was calculated and receiver operating characteristic (ROC) curve was drawn. Arbitrary cut-off values for these were calculated. Data were analyzed using the statistical software package, STATA 11.2 (College Station, TX, US). A difference between the two values was considered to be significant if $P \leq 0.05$.

RESULTS

Analysis was carried out on 142 cases, included 65 cases of mild preeclampsia (45.8%), 32 cases of severe preeclampsia (22.5%), 45 cases of eclampsia (31.7%) and 31 controls. Table 1 show that the maximum number of cases was in the age range of 25-35 years, of these 76.92% in mild preeclampsia group, 59.38% in severe preeclampsia and 57.76% in eclampsia group.

Majority of women in the controls (87.10%) belonged to middle socioeconomic status. Maximum cases in mild preeclampsia (67.69%) group and severe preeclampsia (71.88%) group belonged to middle class. Whereas, the majority of patients in eclampsia group (73.33%), belonged to low socio-economic status (based on Kuppu Swamy's socio-economic status scale updated 2012). In cases and controls mean period of gestational age almost equal ($P = 0.076$).

Nearly, 53.52% cases were primigravida and 46.48% cases were multigravida, whereas in controls, 41.94% were primigravida. Two patients in mild preeclampsia group had a history of a similar disease in the past, in which one patient had a history of antepartum eclampsia and one patient had a history of preeclampsia and one patient in severe preeclampsia group had a history of preeclampsia in a previous pregnancy.

Mean systolic blood pressure (SBP) among controls was 117.84 ± 4.7 mmHg, in mild preeclampsia group 145.32 ± 9.7 mmHg, severe preeclampsia group 159.88 ± 8.3mmHg and in eclampsia group 156.89 ± 9.5 mmHg. The difference was statistically significant among study and control groups ($P < 0.0001$) and mean diastolic BP in controls was 77.42 ± 6.8 mmHg, in mild preeclampsia group 93.72 ± 5.2 mmHg, severe preeclampsia 103.25 ± 7.6 mmHg and in eclampsia group was 101.20 ± 7.9 mmHg. The difference was statistically significant among cases and control groups ($P < 0.0001$).

Biochemical investigation showed significant differences in the levels of all parameters among the study and control groups [Table 2].

After analysis within the groups (controls, mild preeclampsia, severe preeclampsia and eclampsia), it was observed that mean serum NGAL levels constantly in increasing order with the severity of disease, the maximum value of mean serum NGAL was found in

Table 1: Demographic profile

Groups	Control	Mild preeclampsia	Severe preeclampsia	Eclampsia	*P* value
Number	31	65	32	45	
Age groups (%) (years)					
<25	9 (29.03)	15 (23.08)	12 (37.50)	19 (42.22)	0.136
25-35	22 (70.97)	50 (76.92)	19 (59.38)	26 (57.78)	
>35	0 (0.00)	0 (0.00)	1 (3.13)	0 (0.00)	
Gravida (%)					
1	41.94 (13)	50.77 (33)	53.13 (17)	57.78 (26)	0.548
2	32.26 (10)	26.13 (17)	18.75 (6)	24.44 (11)	
3	16.13 (5)	13.85 (9)	12.50 (4)	11.11 (5)	
4	6.45 (2)	1.54 (1)	9.38 (3)	2.22 (1)	
5 or more	3.23 (1)	7.79 (5)	6.25 (2)	4.44 (2)	
Mean period of gestation	35.86±2.0	35.80±4.0	35.80±3.6	35.43±3.4	0.076
Socio-economic status (%)					
Low	12.90 (4)	32.31 (21)	28.13 (9)	73.33 (33)	0.0001
Medium	87.10 (27)	67.69 (44)	71.88 (23)	26.67 (12)	
Past history of hypertensive disease in pregnancy (%)					
Not present	100 (31)	96.92 (63)	96.88 (31)	100 (45)	
Present	0.00 (0)	3.08 (2)	3.13 (1)	0.00 (0)	
CNS (%)					
Normal	100 (31)	100 (65)	100(32)	57.78 (26)	0.000
Abnormal	0.00 (0)	0.00 (0)	0.00 (0)	42.22 (19)	
Hypertension (mmHg)					
Mean SBP	117.84±4.7	145.32±9.7	159.88±8.3	156.89±9.5	<0.0001
Mean DBP	77.42±6.8	93.72±5.2	103.25±7.6	101.20±7.9	<0.0001

CNS: Central nervous system abnormalities, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 2: Biochemical parameters

Investigation	Control (*N*=31)	Mild preeclampsia (*N*=65)	Severe preeclampsia (*N*=32)	Eclampsia (*N*=45)	*P* value
Hb (g/dl)	10.81±0.5	9.58±1.4	9.71±1.2	9.24±1.0	0.0059
Platelet count (lac/mm^3)	2.10±0.3	1.64±0.4	1.59±0.4	1.65±0.4	<0.001
Serum urea (mg/dl)	20.03±4.1	30.05±9.7	38.72±9.7	32.08±75	<0.001
Serum creatinine mg/dl	0.60±0.2	1.27±0.5	2.67±1.3	1.95±0.7	<0.001
Serum bilirubin (mg/dl)	0.49±0.3	1.31±0.8	1.89±1.2	1.86±0.8	<0.001
SALP (IU/L)	61.84±9.3	359.4±164.5	337.34±188	443.60±168.1	<0.001
SGPT (IU/L)	47.77±13.8	215.94±125.8	257.72±174.1	354.89±193.2	<0.001
SGOT (IU/L)	49.71±4.4	208.57±130.9	299.0±167.8	338.09±173.1	<0.001
Serum uric acid (mg/dl)	2.24±0.5	7.36±1.8	8.36±1.8	6.61±2.0	<0.001
Serum LDH (IU/L)	186.48±41.9	735.75±268.5	959.13±238.2	868.67±247.7	<0.001

Hb: Hemoglobin, SALP: Serum alkalyine phosphatase, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamate oxaloacetate transaminase, LDH: Lactate dehydrogenase

group of eclampsia and on comparison this difference was statistically significant ($P < 0.00001$) [Table 3]. Oliguria was found in 18.75% cases of severe preeclampsia and 20% cases of eclampsia group, the mean serum NGAL value in patients with oliguria was significantly higher when compared with non-oliguric patients ($P < 0.001$) [Table 4].

Mean serum NGAL level in controls without proteinuria was 294.61 ± 356.2 pg/ml, whereas in cases with proteinuria ≥300 mg/24 h, mean serum NGAL level was 2617.78 ± 2997.6 pg/ml and with proteinuria ≥2g/24 h, mean serum NGAL level was 8606.79 ± 5054.4 pg/ml. Maximum mean serum NGAL level 9526.42 ± 4494.3 pg/ml was observed with proteinuria ≥3-5g/24 h. This finding suggests that serum NGAL had a positive association with increasing proteinuria. The difference of mean serum NGAL between cases and controls was statistically significant ($P < 0.0001$) [Table 5].

The Spearman's rank correlation coefficient shows that serum NGAL had a positive correlation with SBP ($r \sim 0.5973$), DBP ($r \sim 0.6195$), serum urea ($r \sim 0.4392$), serum creatinine ($r \sim 0.6112$), serum uric acid ($r \sim 0.3878$) and it also had a positive correlation with serum bilirubin ($r \sim 0.4637$) and liver enzymes. Positive correlation coefficient of

these parameters with serum NGAL implies that serum NGAL increases with an increase in the above mentioned parameters. Serum NGAL has a negative correlation with birth weight of baby; mean serum NGAL increases with a decrease in birth weight of the baby indicate intrauterine growth retardation [Figure 1].

Sensitivity and specificity of serum NGAL in HDP was done using ROC curve and AUC was calculated. Sensitivity and specificity of serum NGAL using a cut-off value of 545 pg/ml, for the diagnosis of preeclampsia was found to be 97.89%and 93.55% respectively, using 95% confidence interval (CI). The positive predictive value was 98.58%, negative predictive value was 90.63%, AUC was 95.72% and accuracy index was 97.11%.

DISCUSSION

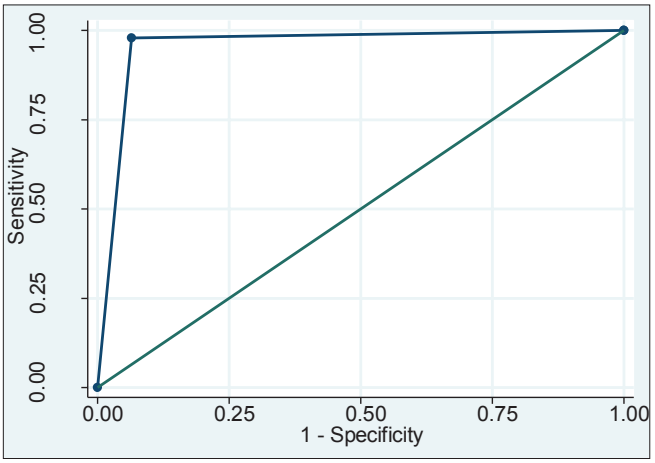
Preeclampsia is a syndrome characterized by hypertension, proteinuria and inflammation and a high concentration of antiangiogenic factors,[16] all conditions determining increased serum NGAL levels. Recent studies have demonstrated that serum NGAL levels were significantly increased at the end of the second trimester in women, who subsequently suffered preeclampsia as compared with the control group, suggesting that serum NGAL might be predictive for this syndrome.[14]

It has been suggested that a circulating increase of serum NGAL may be a consequence of a leukocyte-derived inflammatory activity and endothelial activation[17] and both these pathophysiological aspects are involved in HDP, particularly in preeclampsia. Therefore, it may be argued that after the second trimester, endothelial dysfunction and inflammatory activity, which may already be strongly advanced in women who will subsequently develop preeclampsia, can be the major responsible factors for increasing serum NGAL.

Table 3: Comparison of mean serum NGAL among different case groups

Groups	No. of patients (*n*=173) (%)	Serum NGAL pg/ml mean±SD
Control	31 (17.91)	294.61±356.2
Mild preeclampsia	65 (37.57)	3077.34±3227.4
Severe preeclampsia	32 (18.49)	9816.75±4814.6
Eclampsia	45 (26.01)	11372.80±2999.4

Test used Kruskal-Wallis χ^2 with ties=122.5966 with 3 difference $P<0.00001$.
NGAL: Neutrophil gelatinase-associated lipocalin, SD: Standard deviation



Recode of NGAL (S~)	Study	Control	Total
≥545	139	2	141
<545	3	29	32
Total	142	31	173

Diagnostic estimates
Statistics|Value (95% confidence interval)
————————+————————————
Sensitivity|97.89% (93.95, 99.56)
Specificity|93.55% (78.58, 99.21)
PosPred Val|98.58% (94.97, 99.83)
NegPred Val|90.63% (74.98, 98.02)
Area under ROC|95.72% (91.16, 100.00)
Accuracy Index|97.11% (93.38, 99.06)

Figure 1: Receiver operating characteristic curve

Table 4: Comparison of urine output among various case groups and controls

Urine output	Control (*N*=31)	Mild preeclampsia (*N*=65)	Severe preeclampsia (*N*=32)	Eclampsia (*N*=45)	Mean serum NGAL
>400 ml/24 h	100% (31)	100.00% (65)	81.25% (26)	80% (36)	5545.35±5457.6
<400 ml/24 h	0.00% (0)	0.00% (0)	18.7% (6)	20.0% (9)	10593.80±25200

Test used Mann-Whitney, $P=0.0010$. NGAL: Neutrophil gelatinase-associated lipocalin

Table 5: Distribution of patients in various groups on the basis of proteinuria

Urinary protein (in 24 h)	Control (*N*=31) (%)	Mild preeclampsia (*N*=65) (%)	Severe preeclampsia (*N*=32) (%)	Eclampsia (*N*=45) (%)	Mean serum NGAL (pg/ml)*
Nil	100 (31)	0.00	0.00 (0)	0.00 (0)	294.61±356.2
≥300 mg	0.00 (0)	58.46 (38)	0.00 (0)	2.22 (1)	2617.78±2997.6
≥2 g	0.00 (0)	41.54 (27)	46.88 (15)	57.78 (26)	8606.79±5054.4
3-5 g	0.00 (0)	0.00 (0)	53.13 (17)	46.00 (18)	9526.42±4494.3

Pearson χ^2 (12)=258.7052, $P<0.0001$. *Test used Kruskal-Wallis, $P<0.00001$ χ^2 with ties=101.6662 with 4 difference. NGAL: Neutrophil gelatinase-associated lipocalin

Analysis was carried out on 142 cases (mild preeclampsia 65, severe preeclampsia 32 and 45 eclampsia cases) and 31 controls. In this study, HDP was noted in 8.05%. HDP have been reported in other study 6-8%and may go as high as 20%.[18] Similarly, Mudaliar and Menon had reported incidence of preeclampsia in 7-9% pregnant women in an Indian study.[19] Prakash *et al*. reported preeclampsia in 5.8% pregnant women.[20] In the present study, out of 142 cases, 45.8%had mild preeclampsia, 22.5% had severe preeclampsia and 31.7% had eclampsia. Young age and primigravidity are proven risk factor for development of preeclampsia. Age wise distribution, in case groups was similar to controls, the mean age of controls was 27.19 ± 3.3 years, for mild preeclampsia 26.28 ± 2.7 years, 25.69 ± 2.7 years for severe preeclampsia and for eclampsia 25.20 ± 2.4 years (P = 0.136). Our observations were slightly differed from those reported by others (controls 28.8 ± 2.1 years, preeclampsia 28.52 ± 4.07 years) (P = 0.39).[14] In our study, increase in SBP and DBP was positively correlated with severity of disease, where maximum severity was found in the group of severe preeclampsia (P < 0.001). When SBP and DBP were correlated with the levels of serum NGAL, positive correlation was found (r = 0.5973 for SBP, r = 0.6195 for DBP). Similarly, few studies also reported a positive correlation of serum NGAL with the SBP (r = 0.51) and the DBP (r = 0.57) [14,21] [Table 6].

We estimated and compared serum NGAL among the three case groups (mild preeclampsia, severe preeclampsia, eclampsia) and controls. There was statistically significant difference in the mean serum NGAL levels among study groups and the controls (P < 0.001). The highest value of mean serum NGAL was found in the group of eclampsia (11372.80 ± 2999.4 pg/ml) followed by severe preeclampsia (9816.75 ± 4814.6 pg/ml) and mild preeclampsia (3077.34 ± 3227.4 pg/ml). These observations suggest that serum NGAL levels were increased with the severity of disease [Table 3]. Other author studied serum NGAL levels from the serum samples collected from 48 women who subsequently developed preeclampsia and 96 control women with uncomplicated pregnancies and compared them. They found that serum NGAL values were significantly raised in women who developed preeclampsia (P < 0.001) and also found that serum NGAL concentration throughout the whole pregnancy was different in the control group as compared to the preeclampsia group.[22] Similar findings were shown by Yan *et al*.[15]

Table 6: Correlation coefficient of NGAL levels with various clinical and biochemical parameters

Parameters	Correlation coefficient (r)
Systolic blood pressure	0.5973
Diastolic blood pressure	0.6195
Serum urea	0.4392
Serum creatinine	0.6112
Serum bilirubin	0.4637
SALP	0.4155
SGPT	0.5097
SGOT	0.5910
Serum uric acid	0.3878
Serum LDH	0.5995
Hemoglobin	−0.2084
Baby weight	−0.3114

SALP: Serum alkalyine phosphatase, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamate oxaloacetate transaminase, LDH: Lactate dehydrogenase, NGAL: Neutrophil gelatinase-associated lipocalin

After comparison with controls oliguria was found in 10.56% of patients with HDP. This was comparable to study carried out by various authors,[20] in which oliguria was found in 9.43% women with HDP.

Various studies suggest that measurement of serum uric acid is clinically useful in the evaluation of pregnant patients with hypertension, specifically after 20 week of pregnancy. Elevated serum uric acid in pregnancy is not only a valuable biomarker for preeclampsia it may also have a contributory role in the pathogenesis of maternal and fetal manifestation.[23,24] Some studies suggest that the degree of elevation correlates with the severity of maternal syndrome and fetal morbidity.[25,26] In the present study, serum uric acid levels were significantly raised in cases as compared with the controls (P < 0.001). The values of serum uric acid showed a positive correlation with mean serum NGAL level (r = 0.4716).

We had not only observed significantly increasing mean levels of serum urea and creatinine incases as compared to controls (P < 0.001), but also noticed that these levels constantly increasing with the severity of disease. Serum NGAL showed a positive correlation with serum urea (r = 0.4392) and serum creatinine (r = 0.6112). Therefore, we can say it might be a novel biomarker to predict the acute kidney injury in early stage and also useful in assessment of disease severity.[27]

In our study, most common manifestation was edema, a very common symptoms seen in 80% of normal pregnancies[18] and other manifestation was proteinuria, which might be due to glomerular involvement in the form of glomerular capillary endotheliosis, which results in reduced GFR and proteinuria. A systematic review reported that the correlation between proteinuria and severity of clinical disease was insufficiently reliable for clinical use.[28]

But our study indicate that proteinuria increases with the severity of disease ($P < 0.001$). Our finding suggests that serum NGAL had a positive association with an increasing proteinuria. The difference of mean serum NGAL between cases and controls was statistically significant ($P < 0.0001$) similarly reported by others.[14]

Mean serum LDH levels were significantly higher in case groups in comparison to controls ($P < 0.001$) and maximum value was observed in severe preeclampsia group (959.13 ± 235.2 IU/L), this signified association of serum LDH levels with severity of disease. Qublan *et al*. had studied serum LDH as a biomarker to predict adverse pregnancy outcome. They found that mean serum LDH level was highest in severe preeclampsia (774.91 ± 69.61 IU/L).[29] Similar association was reported by others.[30] Serum NGAL showed a positive correlation with serum LDH(r=0.5995), this suggest that serum NGAL could be a prognostic marker for HDP mainly severe preeclampsia and eclampsia.

Timely detection of HDP is important because intensive monitoring and administration of early treatment can be more selectively targeted to these women; thus, making timely intervention easier and cost-effective. Various studies suggested that the predictive value of serum uric acid is relatively poor for diagnosis and prognosis of preeclampsia.[31–33] Other study reported 87.7% sensitivity and 93.3% specificity; thus, they suggest that serum uric acid is a reliable predictor of preeclampsia,[34] and hence results are controversial. In our study, ROC curve was used to calculate, sensitivity and specificity of serum NGAL, using a cut-off value of 545 pg/ml, for the diagnosis of HDP. It was observed that 97.89% sensitivity and 93.55% specificity at 95% CI. The positive predictive value was 98.58% and negative predictive value was 90.63%, AUC was 95.72% and accuracy index was 97.11%. So our results slightly differed, to those obtained by other author, who had reported NGAL values for second trimester, an AUC of 0.87 (CI95%, 0.77-0.96) and in the third trimester, an AUC of 0.83 (CI95%, 0.72-0.94). They evaluated sensitivity and specificity of NGAL, at the optimal cut-off for both trimester 32.5ng/ml. They obtained 73.3%sensitivity and 96.7% specificity in the second trimester of pregnancy.[14]

Positive correlation between serum NGAL level and covariate such as systolic and DBP and proteinuria might be a consequence of endothelial dysfunction on which hypertension and proteinuria probably depend this might confirm the association between NGAL and renal dysfunction even if the tubular lesion is only present, which is a feature of preeclampsia syndrome.[35]

CONCLUSION

In this study, serum NGAL levels were evaluated in normal pregnant women and women suffering with HDP and correlated with severity of disease. Between the two groups, we found a positive correlation of serum NGAL with disease severity and better sensitivity and specificity in the evaluation of HDP as compared with previously reported studies.

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