Original Article

Comparison of serum neutrophil gelatinase associated lipocalin (NGAL) levels in pre-eclamptic and normotensive pregnant women

Shazmah Ahmad, Sumbla Ghaznavi, Nadia Rasheed, Muhammad Ikram Ullah

Department of Chemical Pathology, University of Health Sciences, Lahore, Pakistan and
Department of Clinical Laboratory Sciences, Jouf University, Saudi Arabia

Objective: To compare the serum neutrophil gelatinase associated lipocalin (NGAL) levels between pre-eclamptic (PE) and healthy pregnant women.

Methodology: Twenty-two pre-eclamptic and 20 normotensive pregnant women were enrolled in the study after taking written informed consent. Demographic data was recorded. Biochemical parameters like serum uric acid and serum creatinine levels were noted from patient record. Blood pressure (BP) was measured by a standard sphygmomanometer and spot urine sample was collected to detect urine protein by dipstick method. Blood samples were collected and serum levels of NGAL were determined by ELISA. Data analysis was carried out by using SPSS version 20.0.

Result: Out of 22 pre-eclampsia women, 64% had late pre-eclampsia and remaining presented with early disease. The degree of disease was severe in 55% patients and other presented with milder form of pre-eclampsia. The mean serum NGAL was 135.13±113.94 ng/ml in PE group and 132.10±53.36 ng/ml in control group (p=0.290). Serum uric acid and creatinine were high in PE group, indicating kidney injury in pre-eclamptic subjects.

Conclusion: Serum NGAL levels were not associated with pre-eclampsia disease although higher NGAL levels were found in patients which may indicates some inflammatory processes. Further studies on larger scale should be conducted to confirm the clinical findings for an increase of NGAL levels. (Rawal Med J 202;45:154-157).

Keywords: Pregnancy, Pre-eclampsia, serum NGAL.

INTRODUCTION

Pre-eclampsia (PE) is known to be caused by defective placental angiogenesis. It affects 3-5% of pregnancies and is traditionally diagnosed by the combined features of high blood pressure and proteinuria. As a result, ischemic conditions and hypoperfusion prevails, followed by widely spread anti-angiogenic factors causing systemic endothelial cell dysfunction, which enter into the maternal circulation.[1] New definitions also include maternal organ dysfunction, such as renal insufficiency, liver involvement, neurological or hematological complications, uteroplacental dysfunction, or fetal growth restriction.[2] Lipocalins are small secreted proteins that have different functions, but their common function is transport of small hydrophobic ligands such as steroids and lipids. Other functions include are cell aging, regulation, survival and differentiation.[3]

Physiological role of NGAL in renal injury is to reduce apoptosis and increase the normal proliferation of renal tubular cells and decrease renal cell injury.[4,5] However, the presence of other factors in defining growth/differentiation properties of NGAL, i.e. the extracellular kinases activation and binding with matrix metalloproteinase 9 (MMP-9), cannot be excluded.[4]

In previous studies, serum NGAL levels evaluated for all three trimesters of pregnancy and found high serum NGAL levels. High NGAL levels were found at the end of the second trimester in PE case as compared to the controls and it could be considered as a useful biomarker for PE.[6,7] In PE, considering the vascular endothelial dysfunction and renal dysfunction, it is observed NGAL production at the maternal-fetal interface.[8] Therefore, it can be suggested that the systemic endothelial injury related to PE can lead to the increased circulating NGAL levels.

Currently, there is no cost-effective, single and

reliable screening test for PE.[9] Lately, NGAL may be the proposed candidate diagnostic biomarker in early identification of PE.[10] Most of the studies in Pakistani population described the role NGAL with acute kidney injury (AKI) in different settings however, reports from Pakistan have not been presented for establishing the role of serum NGAL in PE. The objective of present study was to compare serum NGAL levels between pre-eclamptic and healthy pregnant women.

METHODOLOGY

This was a cross-sectional comparative study carried out in Department of Chemical Pathology, University of Health Sciences, Lahore. This study comprised of 22 women with PE and 20 normotensive pregnant. The inclusion of patients were done according to diagnostic criteria for PE which includes pregnant patients with hypertension (HTN) and proteinuria at or after 20 weeks, SBP 140 mm Hg or higher or DBP 90 mm Hg or higher at least 4 hours apart, after 20 weeks of gestation in a woman with previously normal BP and proteinuria: ≥1+ (30 mg/dl) on a urine dipstick test. The normotensive pregnant women had no proteinuria.

Patients were taken from the Gynecology and Obstetrics ward and control subjects were taken from outpatient department of Jinnah Hospital, Lahore. A written Informed consent was taken from all patients. The demographic data and the patient medical history were recorded. The measurement of blood pressure was carried out with standard protocol. The spot urine was collected from each participant and protein detection was performed by urine dipstick method (DIRUI Industrial Co., Ltd. Chanchun, Jilin, China). The color chart range was from negative to 4+ values.

Serum NGAL levels were determined by ELISA technique (Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Co., Ltd. Shanghai, China). NGAL values were taken from BIO-RAD-680 micro-plate ELISA reader. The absorbance of sample was measured at 450 nm. The intensity of the color and the concentration of the serum NGAL in samples were positively correlated.

Statistical Analysis: The data were analyzed by using SPSS version 20.0. For qualitative variables, frequencies and percentages were given. Data were checked for normal distribution by using Shapiro-Wilk test. Mann- Whitney U test was applied for non-normally distributed data. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Total forty two subjects including pre-eclamptic women with 30 weeks of gestation and controls were in this study. The mean age of control and patient group was not different statistically (p= 0.648). In group 1, 22 pregnant women were control and in group 2, 22 women were with PE. The onset of PE in patients showed that 36% cases with early PE, while 64% women presented with late PE. On the basis of disease status, 55% cases presented as severe PE (Table 1).

Table 1. Demographic data between control and pre-eclamptic women.

Qualitative variables	Disease status	Group 1 *n*=20	Group 2 *n=22*
Onset of PE	Early	Nil	8 (36%)
	Late	Nil	14 (64%)
Degree of PE	Mild	Nil	10 (45%)
	Severe	Nil	12 (55%)

Table 2. Comparison between control and pre-eclamptic women.

Variables	Groups	Mean ± SD	95% CI		*p*-value
			Lower Bound	Upper Bound	
Age (years)	Group 1	25.55 ± 3.05	24.1217	26.9783	*0.648
	Group 2	26.36 ± 3.86	24.6517	28.0756	
NGAL (ng/ml)	Group 1	132.10 ± 53.36	107.1448	157.0589	*0.290
	Group 2	135.13± 113.94	84.3974	186.3612	
Creatinine (mg/dl)	Group 1	0.67 ± 0.71			
	Group 2	0.84 ± 0.21			
Uric acid (mg/dl)	Group 1	3.17 ± 0.32			
	Group 2	6.53 ± 0.76			

*p-value ≥ 0.05: Non- significant.

The serum NGAL levels were 132.10±53.36 ng/ml and 135.13±113.94 ng/ml in group 1 and in group 2, respectively. No significant correlation of serum NGAL was observed between PE and normotensive pregnant women (p=0.290). Other biochemical parameters like serum creatinine and uric acid were also found statistically insignificant between the groups (Table 2).

DISCUSSION

Previous reports suggested that blood markers which are traditionally used for disease indications are relatively insensitive for diagnosis.[11] Serum NGAL was earlier considered the biomarker of AKI, but it has also been associated with PE without other pre-existing diseases in pregnant women.[5,12]

In present study, PE subjects were divided into early and late PE on the basis of disease onset and mild or severe PE according to the elevation of BP. Early onset presentation was seen in 36% and 64% women showed late onset PE. A study by Poon et al reported high frequency of late PE due to maternal risk factors for PE and categorized the PE due to the onset of disease among different races and inferred that Pakistani women were more likely to suffer with late presentation of the disease.[13] In our study, mild PE was present in 45% cases while 55% presented with severe PE. A study by Sachan et al reported 142 cases of PE and eclampsia, in which 45.8% were presented with mild PE and 22.5% cases documented as severe PE.[14] The possible reason for this difference may be in sample size, delayed in clinical facility for treatment and to follow the pregnancy which can identify and monitor the disease in its milder form.

Our study showed high serum NGAL concentrations in PE women as compared to normotensive pregnant women, however no statistical significant difference observed between groups (p= 0.290). Some previous studies did not find any association of serum and urine NGAL in patients and controls although the high serum NGAL levels were noted with severity of disease.[15,16] On the other hand, some studies documented the association of serum NGAL levels in normotensive and PE patients with a significant difference ($p<0.05$).[11,17] Differences in serum NGAL levels and lack of significant association probably might be due to small sample size and lacking in clinical confirmation of PE before sampling.

In present study, serum creatinine and serum uric acid were also considered to evaluate kidney injury in PE. These were not part of the study however the values were considered from the labs of the patients to see the likely presence of kidney injury in the two groups. The serum levels were higher in PE group than the control group, which is indicating damage to renal function, comparable to previous studies.[11,17] Serum creatinine and uric acid levels are routinely monitored in renal function, therefore they can be applied in assessment of disease severity and prediction for adverse outcome along with NGAL.[11,14,18]

Very little data is available for the involvement of NGAL in non-AKI diseases like pre-eclampsia. Increase in NGAL levels in PE may be due to the endothelial dysfunction which gives rise to oxidative stress, ischemic placenta and systemic inflammation during early trimesters. Development of hypertension and AKI in late trimesters may further enhance the secretion of NGAL. Consequently, serum NGAL levels may rise in PE patients and may be monitored for progression of PE and to assess the kidney functions.

CONCLUSION

Serum NGAL levels were not associated with pre-eclampsia although higher NGAL levels were found in patients may indicate some inflammatory process. In future, more studies with larger sample size should be conducted to confirm the clinical findings for an increase of NGAL levels in different races or ethnic groups.

Author Contributions:
Conception and design: Sumbla Ghaznavi
Collection and assembly of data: Shazmah Ahmad
Analysis and interpretation of the data: Nadia Rasheed
Drafting of the article: Nadia Rasheed, Muhammad Ikram Ullah
Critical revision of the article for important intellectual content: Sumbla Ghaznavi
Statistical expertise: Sumbla Ghaznavi, Muhammad Ikram Ullah
Final approval and guarantor of the article: Sumbla Ghaznavi
Corresponding author email: Nadia Rasheed: naddiya24@gmail.com
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