ORIGINAL RESEARCH PAPER

Volume-7 | Issue-6 | June-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SERUM LEVELS AND DISCRIMINATING ABILITIES OF LDH, 5'NT, AMINOTRANSFERASES IN MILD AND SEVERE PREECLAMPSIA

Biochemistry

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ABSTRACT

Introduction: Preeclampsia (PE) is a pregnancy specific multisystem disorder characterized by heterogeneous clinical and laboratory findings. There are no clinically useful screening tests to identify the women at risk.

Aims and Objectives: To investigate serum lactate dehydrogenase (LDH) activity in PE and to evaluate the relationship between changes in serum levels of LDH, 5'nucleotidase (5'NT), amino transferases, uric acid (UA) and urine proteins with the severity of PE and to correlate it to the pregnancy outcome.

Material and Methods: 60 women with PE and 30 women having normal uncomplicated pregnancies, matched for maternal age and gestational age were respectively recruited as cases and control groups. Serum levels of LDH, 5'NT, UA, urea, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) were estimated along with 24 hours urinary proteins. The data were analyzed using SPSS software version 17.0.

Results: There was a statistically significant increase in LDH, 5'NT, UA, ALT and AST in severe and mild PE compared to controls ($p<0.05$). These changes were more pronounced in severe PE as compared to that of mild PE cases ($p<0.05$). Among all the variables, the serum LDH and ALT showed better discriminating ability for PE and its severity.

Conclusion: LDH and ALT taken together have shown highest diagnostic efficiency in discriminating PE cases from non-PE controls and are useful in assessing disease severity, as well.

KEYWORDS

Preeclampsia, Lactate Dehydrogenase, Alanine Transaminase, Uric Acid, 5' Nucleotidase

Introduction

Preeclampsia (PE) is an idiopathic pregnancy specific multisystem disorder that complicates 5-8% of all pregnancies.[1,2] It is characterized by heterogeneous clinical and laboratory findings.[3] The clinical findings can manifest as maternal syndrome (hypertension, proteinuria and various other symptoms), or fetal syndrome (growth restriction) or both with subsequent increase in the perinatal and maternal morbidity and mortality.[3-5]

Despite of extensive research, the etiology of PE remains unknown. It is likely to be multifactorial in origin and begins to manifest early in pregnancy with adverse outcomes for both mother and the fetus. [3,5] There are no clinically useful screening tests to identify the women at risk of developing PE.[1] PE is diagnosed by elevated blood pressure and proteinuria and many biochemical markers such as lactate dehydrogenase (LDH), 5'nucleotidase (5'NT), uric acid (UA), amino transferases and serum creatinine have been known to be associated with its pathophysiology and severity.

High levels of LDH in association with PE has been reported in the literature [5-7], indicating cellular death and leakage of enzyme from the cell. Thus LDH can be considered as a useful marker of intravascular hemolysis in severe preeclampsia.[8] High levels of LDH, amino transferases and UA prompts aggressive and early intervention in the event of PE, and may help to minimize adverse maternal and perinatal events in the settings of severe PE.[1,6]

With this background, the present study was designed to investigate serum levels of LDH, 5'NT, aspartate transaminase (AST), alanine transaminase (ALT), UA in PE cases when compared to non-PE controls matched for age and gestational age. We also evaluated their associations with the severity of PE. In addition, the diagnostic accuracies of these biochemical variables have been studied for their utility in PE.

Material and methods

After obtaining Institutional ethics committee clearance, a total of 90 women were recruited from Govt. Maternity Hospital, Sultan bazaar, Hyderabad. Of which, 60 women diagnosed with PE were grouped as cases and the control group comprised of 30 age and gestational age matched non-PE women. The PE cases were further stratified into mild and severe PE groups of 30 cases each.

General examination was done for obtaining blood pressure, edema and weight gain status. 10ml of venous blood was collected in the early morning on fasting status under aseptic conditions into plain test tubes to obtain serum. The following parameters were analyzed; LDH (U/L), 5'NT (U/L), UA (mg/dl), ALT (U/L), AST (U/L), urea (mg/dl) and creatinine (mg/dl) in serum samples along with urinary proteins (mg/day). The LDH was assayed on the same day and the remaining serum was stored in deep freezer at -20°C for further use. The other analytes were estimated within 3 days. A 24 hrs urine specimen was collected into sterile bottle for urinary protein estimation. The following methodologies were used for the different parameters; blood urea (D.A.M Method), Serum creatinine (Modified Jaffe's Kinetic Method), 24 hrs urine protein (Turbidimetric method), LDH (Spectrophotometric method), 5'NT (Campbell colorimetric method), ALT and AST (Reitman and Frankel method).

The results obtained were normally distributed and are expressed as mean and standard deviation (SD) of various parameters in different study groups. Multiple comparisons analysis of variance (ANOVA) was used to assess the significance of difference of mean value of different parameters in between non-PE control, mild- and severe PE groups. F value was used to calculate the significance in between groups. The receiver operating characteristic curve (ROC) analysis was performed to report diagnostic accuracy measures for various study variables. Statistical analyses were performed using SPSS software version 17.0. A p-value of <0.05 was considered to be statistically significant.

Results

As presented in Table 1, the mean serum levels of LDH, 5'NT, UA, ALT, AST, urea, creatinine and urine proteins were significantly higher in severe PE compared to mild PE and controls. The mean serum LDH, 5'NT, UA, ALT and AST are significantly higher in mild preeclampsia compared to controls. But there was no statistically significant difference observed for urea, creatinine and urine proteins as shown in the Table 1 and Table 2.

Table 1: Mean and SD values of studied parameters across controls, mild- and severe preeclamptic groups

Parameters	Mild PE	Severe PE	Controls	F value	p-value
LDH (IU/L)	329.80± 83.05	598.63± 143.06	155.36± 40.80	154.58	<.001
5'Nucleotidase (IU/L)	9.40±3.54	19.59± 5.87	5.47± 1.91	94.31	<.001
Uric Acid (mg/dl)	5.17±0.97	6.93±1.05	3.1±0.60	139.00	<.001
ALT (IU/L)	24.81± 5.60	43.67± 13.00	13.58± 3.20	98.70	<.001
AST (IU/L)	19.89± 3.74	30.44± 8.94	12.53± 2.49	72.84	<.001
Urea (mg/dl)	24.03 ± 3.17	27.5 ± 7.62	22.86±2.76	6.89	<.002
Creatinine (mg/dl)	0.77 ± 0.11	0.95 ± 0.23	0.74 ± 0.11	14.779	<.001
Urine Protein (mg/day)	151.67 ± 80.95	644.43 ± 566.45	103.33 ± 20.4	24.613	<.001

Table 2: ANOVA results of between group comparisons for statistical significance

Variable	Controls vs. Mild PE	Controls vs. Severe PE	Mild PE vs. Severe PE
Serum LDH	< .001	< .001	< .001
Serum 5'Nucleotidase	<.002	< .001	< .001
Serum Uric Acid	< .001	< .001	< .001
Serum ALT	< .001	< .001	< .001
Serum AST	< .001	< .001	< .001
Blood Urea	< .669	< .003	< .033
Serum Creatinine	< .813	< .001	< .001
Urine Protein	< .852	< .001	< .001

To report maximum sensitivity, specificity, area under curve (AUC) and diagnostic efficiency values of various parameters in identifying PE, the best cut off values were obtained using ROC analysis. Best cut off values have been established by selecting a best point that provides greatest sum of sensitivity and specificity. The results of ROC analysis are shown in Table 3. Best cut off values for different parameters along with sensitivity, specificity and diagnostic efficiency values for total cases and severe PE compared to controls and mild PE are presented in Tables 3 and 4.

Table 3: The results of ROC analysis comparing between total PE cases and control groups

Parameter	Best Cut-Off	Sensitivity	Specificity	DiagnosticEfficiency	AUC	95% CI	p-value
LDH (IU/L)	250	95%	100%	97.78 %	0.997	.000 – 1.000	<.001
5'Nucleotidase (IU/L)	7.19	86.70 %	86.70 %	86.67 %	0.992	.849 – .987	<.001
Uric Acid (mg/dl)	4.15	93.30 %	100%	95.56 %	0.981	.952 – 1.000	<.001
ALT (IU/L)	19.5	98.30 %	96.70 %	97.78 %	0.997	.000 – 1.000	<.001
AST (IU/L)	16.5	90%	93.30 %	91.1 %	0.969	.935 – 1.000	<.001
Urea (mg/dl)	25.0	50%	83.30 %	61.1 %	0.678	.565 – .791	0.006
Creatinine (mg/dl)	0 .85	45%	80%	56.67 %	0.684	.572 –.797	0.004
Urine Protein (mg/day)	127.5	73.30 %	93.30 %	80 %	0.814	.725 – .903	<.001

AUC: Area under curve

As shown in Table 3, the serum LDH and ALT have shown highest diagnostic efficiency in discriminating PE cases from controls. UA, AST, 5'NT and urine proteins showed good diagnostic efficiency in discriminating cases from controls. Blood urea and serum creatinine exhibited poor diagnostic efficiency and they lack sensitivity. The AUC values showed that serum LDH and ALT are best discriminatory variables followed by UA, 5NT and AST in discriminating cases from controls.

Table 4: The results of ROC analysis comparing severe PE with mild PE group

Parameter	Best Cut-Off	Sensitivity	Specificity	DiagnosticEfficiency	AUC	95% CI	p-value
LDH (IU/L)	474.5	90 %	93 %	92 %	.973	.924 – 1.00	<.001
5'Nucleotidase (IU/L)	12.25	86.7 %	83.3%	85 %	.918	.849 - .987	<.001
Uric Acid (mg/dl)	6.35	76.7 %	93 %	85 %	.926	.863 - .988	<.001
ALT (IU/L)	28.5	86.7 %	90 %	88.3 %	.943	.888 - .998	<.001
AST (IU/L)	24.85	76.7 %	90 %	83.3 %	.855	.788 - .964	<.001
Urea (mg/dl)	25	63.3 %	63.3%	63.3 %	.663	.627 - .877	<.001
Creatinine (mg/dl)	0.85	63.3 %	73.7%	68.3 %	.769	.651 - .887	<.001
Urine Protein (mg/day)	267.5	86.7 %	90 %	88.3 %	.922	.885 - .998	<.001

As shown in Table 4, the serum LDH, ALT and urine proteins have shown highest diagnostic efficiency in discriminating severe PE from mild PE. Whereas, the 5'NT, UA and AST have shown good diagnostic efficiency in discriminating severe from mild PE. Blood urea and serum creatinine exhibited poor diagnostic efficiency and they lack sensitivity. AUC values showed that serum LDH, ALT, serum UA, urine proteins are best discriminatory in assessing the PE disease severity on comparison of severe PE to mild PE, followed by 5'NT and AST which exhibited good discriminatory power in assessing the severity.

Discussion

Early diagnosis of PE is important in the prevention of its onset.[9] The triad of high blood pressure, edema and albuminuria is neither specific nor sensitive enough, therefore the search is on for reliable marker. In the present study we evaluated different biochemical markers for this purpose.

We observed a statistically significant increase in serum LDH, 5'NT, UA, ALT, AST, urea, creatinine and urine protein levels in PE cases compared to controls. It has been reported that severe PE is frequently accompanied by hemolysis indicated by elevated LDH levels. Yoshio et al (2002) showed an increase in plasma 5'NT activity in preeclamptic women and correlated the increased activity with severity.[10] Many authors have shown increase in UA levels in PE.[11-14] The increase in aminotransferases in PE is due to the involvement of liver in pregnancy.[15, 3] Previous evidence shows an increased level of ALT and AST in PE.[3,12,13,21] The uremic condition in PE is ischaemic in origin.[9] Arterial constriction, glomerular endothelial swelling and intravascular fibrin deposition may lead to deterioration in renal function, resulting in increased urea and creatinine levels.[2,4,5] Proteinuria has been noted as a hallmark of PE which is due to dysfunctional endothelium.[10,11,12,13,14,19,23,25,28]

There was a statistically significant increase in LDH, 5'NT, UA, ALT and AST in severe and mild PE compared to controls and in severe PE compared to mild PE. In case of urea, creatinine and 24 hours urinary proteins, there was a statistically significant increase in severe PE compared to mild PE and controls. However there was no statistically significant increase in mild PE compared to controls. This finding suggests that disease severity may play an important role in the biochemical changes observed.

The current diagnostic criteria for PE are not as clear and there is no biochemical marker proposed to identify those women at risk for adverse pregnancy outcome. Thus there is an urgent need of biochemically good diagnostic markers. Angiogenic factors have

shown to be potential markers in this respect, however they are not available for routine laboratories.[1] In the present study, the diagnostic efficiency of different routine biochemical markers are evaluated using best cut off values and their discriminatory capacities for PE are represented as AUC values.

ALT and LDH have shown highest diagnostic efficiency in discriminating cases from controls whereas UA, AST, 5'NT and urine proteins showed good diagnostic efficiency. LDH has shown highest diagnostic efficiency in discriminating severe PE cases followed by 24 hrs urine proteins, whereas 5'NT, AST, ALT and UA showed good diagnostic efficiency. LDH, ALT and urine proteins have shown highest diagnostic efficiency in discriminating severe PE from mild PE, followed by 5'NT, UA and AST.

We assessed the diagnostic efficiency of combination of markers involving those which showed highest sensitivity with those with high specificity. We observed that LDH + UA, LDH + ALT, AST + UA, AST + ALT showed highest diagnostic efficiency in discriminating the severe PE patients from controls and mild preeclamptic patients. Combination of 5'NT + UA, 5'NT + ALT followed with good diagnostic efficiency.

PE remains to be a significant cause of maternal, perinatal death and complications. A healthy respect for this condition, coupled with aggressive and early intervention, may be able to minimize adverse maternal and perinatal events.[12] Our findings are in support of a previous study by Baha et al,[2] who also proposed a set of risk factors and their cut off values including LDH, aminotransferases, UA, creatinine and urinary proteins to discriminate the patients at high risk for significant maternal morbidity. In another study which is well in line with our findings showed a strongest predictive value for LDH, aminotransferases and uric UA.[12] In our study, the serum LDH, 5'NT, UA and ALT have shown highest diagnostic efficiency in assessing PE and its severity. The serum LDH and urine protein have shown maximum sensitivity. When the diagnostic efficiency of combination of markers were used for predicting highest sensitivity specificity, we observed that serum LDH + ALT showed highest diagnostic efficiency followed by LDH + 5'NT, LDH + uric acid, LDH + ALT, LDH + AST which showed good diagnostic efficiency in predicting PE.

Our study limitations have to be mentioned. Though the study showed fairly promising results on the diagnostic efficacy of routinely used biochemical markers, the case-control design of this present study may warrant further research with longitudinal/cohort studies to validate our results. As our results indicate that these biochemical markers are also useful in assessing the PE disease severity, large-scale studies with bigger sample sizes are needed in future.

Conclusion

In our study, the serum LDH, 5'NT, UA and ALT have shown highest diagnostic efficiency in assessing PE and its severity. Further, the combination of serum LDH + ALT showed highest diagnostic efficiency in discriminating preeclampsia. Thus, the combinatorial use of these routine biochemical parameters may be used for detection of preeclampsia.

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