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Original Article

Assessment of the Relationship Between Serum LDH And Ca 125 Values in Pre-Eclampsia Patients

Sarkar Biswajit[1], Ghosh Srabani[1], Pal Mrinal[2], Chandra Sangita[3], Bera Keya[4].
[1]Demonstrator, Dept of Biochemistry, Burdwan Medical College, Burdwan
[2]Associate Professor, Dept of Biochemistry, Burdwan Medical College, Burdwan
[3]Senior Resident, Dept of Biochemistry, Burdwan Medical College, Burdwan
[4]Professor & Head, Dept of Biochemistry, Burdwan Medical College, Burdwan

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ABSTRACT

Background:
Pre-eclampsia is one of the major causes of maternal and fetal morbidity and mortality. Early diagnosis of this disorder is essential for feto-maternal survival and a better outcome. However, laboratory diagnosis of this disorder is still not specific till now. The present study was undertaken to evaluate the role of serum LDH and CA 125 levels in pre-eclamptic mothers and to assess whether these two can be used as diagnostic markers of this disorder at an early stage.

Materials and Methods:
153 pre-eclampsia patients and an equal number of control mothers were included in this case-control study following the American College of Obstetricians (ACOG) guidelines. Serum LDH and CA 125 were measured in both case and control groups using spectrophotometric and CLIA principles respectively. Data obtained were analyzed for differences between the case and control groups and any correlation between the LDH and CA 125 values in the case group using Mann Whitney test and Spearman correlation test.

Results:
Data distribution in the present study followed a non-parametric pattern as evidenced by the Smirnov-Kolmogrov analysis. Both LDH and CA 125 values are found to be significantly higher in the case group (P<.001) as evident from the Mann-Whitney test (Median values 48 and 11 for CA 125, and 550 and 234 for LDH for the case and control groups respectively) with a P<.001. Spearmann's correlation analysis showed a significant positive correlation (R = 0.173, P = .033) between the two parameters.

Conclusion:
The present study helps to understand the relationship between the different pathogenetic processes of pre-eclampsia by measuring and monitoring the levels of serum LDH and CA 125 values using a timely pre-scheduled interval. This will help in both early detection of the severity of the disease and the timely management.

Introduction

Disorders related to pregnant mothers are particularly one of the gravest problems of society as these affect both the mother and her child simultaneously i.e these disorders affect both generations at one go. It is a major cause of feto-maternal morbidity and death with maternal mortality and fetal mortality of about 10-14% and 13-30% respectively [1-3]. According to the WHO reports, about 16% of the maternal deaths owe to PIH and 10,000 pregnant mothers die every year from it worldwide. It has been also noted that the incidence of PIH is higher in developing countries than the developed ones[4, 5]. In India, the incidence of pre-eclampsia among pregnant mothers is reported to be around 8-10%[6]. This serious disorder is included in the pregnancy-induced hypertensive (PIH) disorder group and is diagnosed with a systolic and diastolic blood pressure of more than 140 and 90 mm of Hg for at least 2 times with a difference of 4 hrs and/or proteinuria of > 300mg/24 hours in most of the patients.

*Correspondence:
Dr. Mrinal Pal
Associate Professor, Dept of Biochemistry, Burdwan Medical College, Burdwan, West Bengal
Email:mrinalpal77@rediffmail.com

The fetal outcomes of pre-eclamptic mothers are also accompanied by several adverse effects. With a 13% rate of perinatal death and a 20% lower birth weight rate, the complications of pre-eclamptic mothers are always much higher than their normal counterparts[7]. The mainstay of pre-eclampsia is endothelial changes in the placenta due to tissue necrosis, tissue hypoxia, and inflammation[8]. Due to the increased intracellular death, the LDH enzyme, a marker for anaerobic glycolysis, increases and so, raised values of this enzyme are found in blood[9-12]. Increased LDH levels have been reported in patients of pre-eclampsia in several earlier studies[13, 14].

CA 125, also known as MUC 16 is a glycoprotein belonging to transmembrane proteins. The degree and type of glycosylation of CA 125 depend on their host tissue and their roles are also different in different tissues[15, 16]. The most important and well-studied role of CA 125 is its involvement and increased synthesis in adenocarcinomas and ovarian cancers11. However, the presence of this cancer marker in the decidual tissues also marks its role in decidual invasion and placental function. Several studies have described an increase of tumor antigen in both mild and severe pre-eclampsia compared to normal pregnancy as well as gestational hypertension[17-21]. However, few studies are available now that depict any direct or inverse relationship between changes in this tumor marker and the LDH levels in the blood of pre-eclampsia patients. Based on this lacuna, we hypothesized that there is a direct association between these two important biochemical parameters in our study population and hence designed and planned the present study accordingly.

MATERIALS AND METHODS:

Study Design:

The present study was designed as a case-control study and was undertaken in the Dept of Biochemistry and Dept of Gynecology & Obstetrics of a tertiary care hospital in West Bengal. Cases were selected from the Out Patient Department (OPD) of the Dept of Gyne & Obstetrics following the method random serial sampling technique for a period of one year from May 2021 to April 2022. The study was undertaken following the Helsinki Declaration and ICMR guidelines for human studies and was initiated after obtaining the Institutional Ethics Committee (IEC) clearance.

Selection of Cases:

Inclusion criteria:

Pre-eclampsia was diagnosed and classified according to the American College of Obstetricians (ACOG) guidelines. After diagnosis, the cases were divided into severe and non-severe categories following ACOG guidelines. The maternal age group was between 25 to 30 years with gestational age between 30 to 32 weeks.

Exclusion criteria:

Patients with pre-gestational diabetes mellitus, renal disorders, obesity (BMI > 30), peripheral vascular diseases, cirrhosis of the liver, multiple gestations and symptomatic infectious diseases, chronic hypertensive disease, patients in labor and patients with any systemic, malignant, or endocrine disorders were excluded from the study.

Control population:

The control group was selected from the normal pregnant women of the same age group.

Determination of the sample size:

Considering the prevalence of pre-eclampsia as 8% in the Indian population, the power of study as 80% and a 95% confidence interval the sample size was calculated to be 153 for the present study. Same number of control subjects was included in the study.

Measurement of the study parameters:

i) *Assay of serum LDH*: Serum LDH was assayed using the spectrophotometric method. In principle, LDH catalyzed the reduction of pyruvate (present in the reagent) using NADH. A decreasing concentration of NADH is measured by the autoanalyzer at 340 nm. The rate of this decrease is multiplied with the factor provided and the rate of decrease of NADH is obtained. This rate is directly proportional to the LDH concentration in the test solution and is calibrated further to obtain the value of serum LDH in IU/L.

ii) *Assay of CA 125 in blood*:

CA 125 in blood was assayed using the principle of chemiluminescent immunoassay (CLIA). We used the CLIA autoanalyzer from Advia Centaur CP CA 125II for the assay of CA 125 in our study population.

iii) Quality control procedures:

All reagents were pre-validated and the coefficient of variation (CV) was monitored throughout the test procedures. Both of them were found to be lower than 6 throughout the whole process.

iv) Statistical procedures:

Data obtained were first tested for their normality distribution. Accordingly, the statistical methods were chosen to find out their mean differences and correlation values. All statistical analyses were performed using the SPSS software version 27 for Windows, IBM, USA. For all tests the statistical output was considered to be significant for a P value less than .05 for a 95% confidence interval.

RESULTS:

Following the inclusion and exclusion criteria 153 subjects were selected in the case and control group both. The data obtained from them showed a non-parametric distribution so we adopted the Mann-Whitney test for assessing the difference of study parameters between the case and control subjects. Similarly, we used the non-parametric correlation method using Spearman's correlation test to analyze the correlation between the study variables. The Box-Whisker plot illustrated the distribution of each parameter in both the case and control group.

The box whisker plot in Figure 1 shows the distribution of CA 125 values in the case and control group It is evident from this plot that the overall distribution of CA 125 is significantly lower in the control group compared to the case group.

Similarly, the second box whisker plot in Figure 2 shows the distribution of serum LDH values in the study population. From the plot, it is clear that the overall distribution of LDH is significantly lower in the control subjects compared to the case group.

As the data of the present study does not follow the normal distribution pattern as evident from the Smirnov Kolmogrov analysis (data not shown in the Table), we performed Mann Whitney test to compare the median values of the two groups (Table 1). Results show that the mean rank that signifies the group values and the median that signifies the central tendency was significantly higher (P <.001) for both CA-125 and LDH in the case group. Furthermore, a positive correlation score of .173 between the CA-125 and LDH levels in blood in the case group with a P value of .03 suggests that increases in their values are proportional to each other. The same result of the bivariate correlation analysis is more illustratively shown in Figure 3 using the scatter plot where the relationship between the discrete individual data from both parameters are shown, serum LDH on the horizontal axis and CA-125 on the vertical axis. The regression line passing through the scatter dots showed an upward trend with a correlation coefficient of 0.173 and a P value of .03 which signifies a direct and significant association between these two parameters.

Figure 1: Box Whisker analysis showing the distribution of median values of CA 125 values in the case and control population.

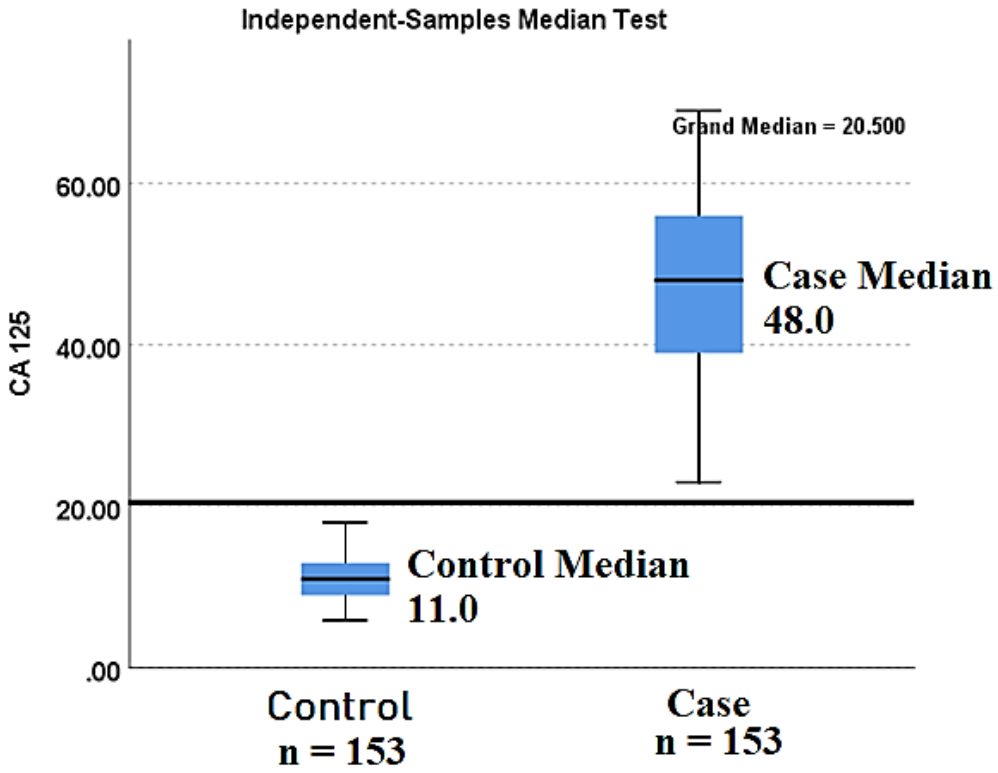


Figure 2 : Box Whisker analysis showing the distribution of median values of LDH values in the case and control population.

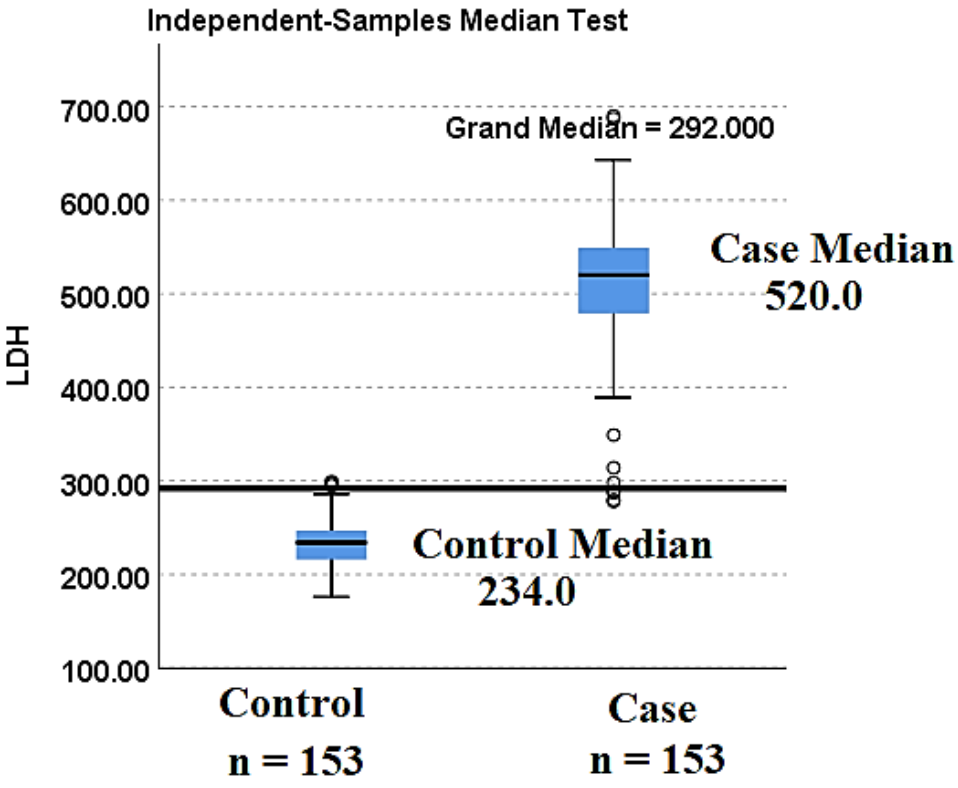


Table 1:Mann Whitney test showing distribution of the test parameters among the case and control population (N = 153 for both).

Test group	CA 125 Median value/Mean rank	LDH Median value/Mean rank	Asymp. Sig (P value)
Case (n = 153)	48.0/230	520/229.85	<.001*
Control (n = 153)	11.0/77	234/77.15	<.001*

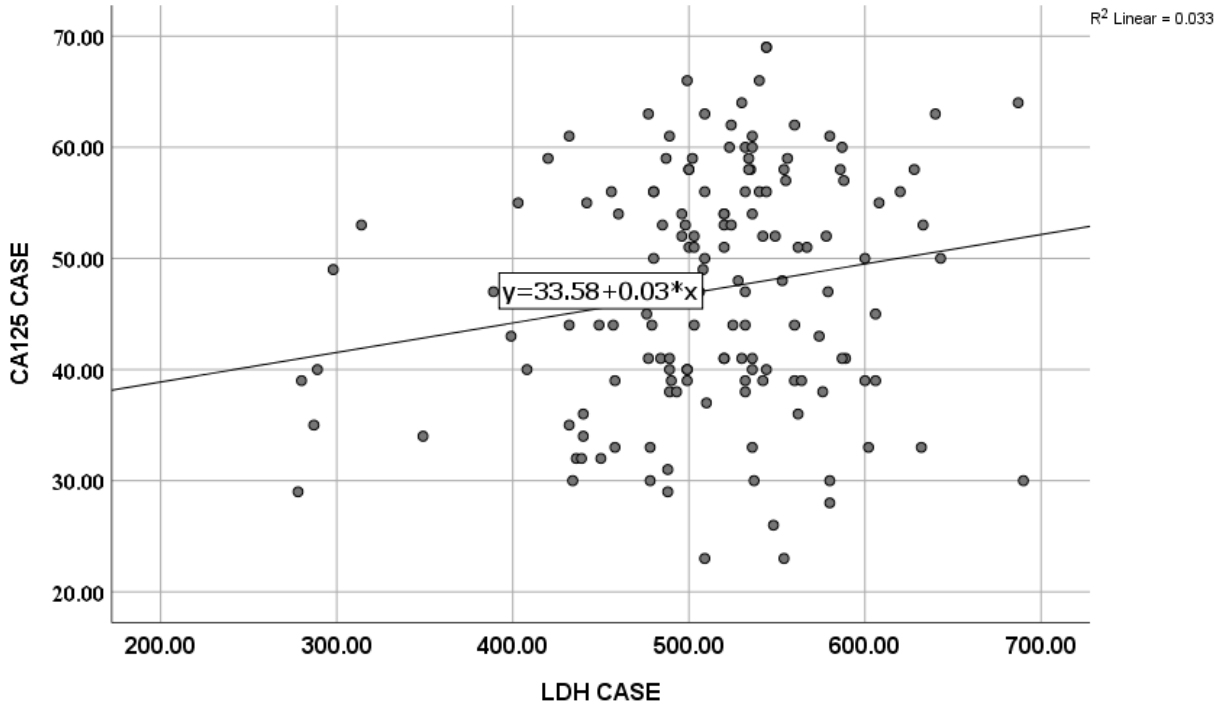
*P value considered significant for P <.05 at 95% confidence interval.

Table 2: Bivariate correlation analysis (Non-parametric Spearman correlation analysis) showing association between the CA 125 and LDH values in the case group.

Correlations				
			CA125 CASE	LDH CASE
Spearman's rho	CA125 CASE	Correlation Coefficient	1.000	.173*
		Sig. (2-tailed)	.	.033
		n	153	153
	LDH CASE	Correlation Coefficient	.173*	1.000
		Sig. (2-tailed)	.033	.
		n	153	153

*. Correlation is significant at the 0.05 level (2-tailed).

Figure 3: Scatter plot with trendline showing the strength of association between the case group's CA 125 and LDH values.



DISCUSSION:

In our present study, both LDH and CA-125 levels were found to be significantly higher in the pre-eclampsia patients compared to their normal counterparts. Although the exact pathogenesis of pre-eclampsia is still not fully elucidated, strong evidence have been reported for its being a hypertensive and multisystemic disorder with endothelial dysfunction leading to oxidative stress, cell hypoxia with autophagy and apoptosis induced abnormal cell death of the placental tissues [22, 23]. Pre-eclampsia also causes marked mitochondrial dysfunction leading to the generation of excessive reactive oxygen species (ROS) and insufficient production of ATP resulting in tissue hypoxia[24, 25]. All of these factors finally lead to an increased rate of cell necrosis, gross endothelial changes and hypertension: the hallmark pathogenesis of the pre-eclampsia [26, 27]. It is evident from previous studies that the increased destruction of the placental cells leads to higher levels of LDH in the blood[28]. Several studies already have described a significant increase of LDH values in pre-eclampsia patients. Reports have also indicated that the gene for LDH enzyme gets hyper-expressed in pre-eclampsia in comparison to the normal pregnancy[29, 30]. The results of our study corroborate with these findings. Overall distribution of the LDH in the case group was higher, as evident from the Box Whisker plot (Fig-1). The mean value of LDH was also higher in the case group (Table 1). The significant increases in both values convincingly suggest that serum LDH values raised significantly in our pre-eclampsia patients.

Similar to the LDH, the CA125 values were also found to be significantly higher in the case group as noted by the difference of the mean values as well as the whole dataset trend with those of the control subjects as stated in Table 1 and illustrated in the Box plot shown in the figure 2. Recent studies have also described an elevation of this tumour marker in pre-eclampsia[31, 32]. Several hypotheses have been proposed for this elevation in pre-eclampsia patients. Cebesoy et al (2009) and Karaman et al (2014) have proposed that the ascitic condition arising due to the hypoalbuminemia of pre-eclampsia are responsible for the increased levels of CA 125 in these patients whereas, Ozat et al (2010) suggested that the failure of proper invasion of the trophoblastic tissues in pre-eclampsia induces an inflammatory response leading to the rise of CA 125 values[33].

In the present study, we also found a direct correlation between the serum LDH values and CA 125 values in our pre-eclampsia patients as evident from the scatterplot shown in Figure 3. The trendline clearly shows that there is a direct association between the values of serum LDH and CA 125 values in our patient group which signifies that both of these markers are interlinked and directly increase with the severity of the disease. This direct association of both markers underscores their importance as prognostic markers that signify the course and prognosis of the disease and response to treatment. Our findings also signify that the process of tissue necrosis and hypoxia go side by side with the inflammatory episodes in the trophoblastic cells leading to hypoalbuminemia and ascites in pre-eclampsia and both are dependent on each other. Hence, the laboratory marker of tissue necrosis and hypoxia i.e. LDH signifies a contemporary increase in the laboratory marker of hypoalbuminemia and ascites i.e. the CA 125 and vice versa in the patients suffering from pre-eclampsia. Thus, our study not only helps in predicting the clinical outcome of the disease by estimating the LDH and CA 125 values but also the inter-relationship among the pathogenesis of tissue necrosis, hypoxia, inflammatory process and hypo-albuminemia in pre-eclampsia.

CONCLUSION:

In conclusion, the present study helps to understand the relationship between the different pathogenetic processes of pre-eclampsia by measuring and monitoring the levels of serum LDH and CA 125 values using a timely pre-scheduled interval. This will help in both early detection of the severity of the disease and their timely management.

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