SEEJPHVolume XXVI 2025, ISSN: 2197-5248; Posted: 15-10-2024

The Effect of Biochemical Parameters In Early Onset And Late-Onset Preeclampsia Compared To Normal Pregnancy

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KEYWORDS
Preeclampsia, early and late onset, biochemical parameters, urine protein, urine creatinine

ABSTRACT
Preeclampsia (PE) is a life-threatening pregnancy condition exhibited by high blood pressure and the protein in the urine which causes endothelial injury and altered coagulation balance with occasional subtypes dependent on the time of onset. The target of this research was to portray the biochemical indices that is seen in normal pregnancies, early-onset preeclampsia (EOPE), and late-onset preeclampsia (LOPE). The study is composed of parameters like urinary protein, Urine creatinine, and urinary protein/ creatinine ratio, serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The group's specific divergence were observed between the EOPE and LOPE groups compared to normal pregnancies. Such differences might suggest some unique biochemical profile of EOPE and LOPE. Strikingly, the level of urine proteins were 9.3 and 0.7 times higher than the normal level in patients in the EOPE and LOPE stages with both preeclampsia subtypes being affected by common kidney dysfunction. Also, EOPE showed increased urine creatinine and levels of AST in comparison with LOPE and healthy pregnancy, which signified the hepatic and renal damage as being accentuated in the cases of early onset. Normality proves its validity only for some of the parameters indicated by the test, and the same goes for the test of equal variance. There are still some tasks to be completed to evaluate and understand completely one of the most problematic pregnancy complications – pre-eclampsia. Overall, the present study has shown the possible biochemical profiles attributable to the diverse subtypes of preeclampsia and thus provides definite information on the pathogenesis of the condition. The results will fill in the gap in the knowledge related to preeclampsia and could be used as an indication, helping to detect and treat the disease more successfully in the future.

Introduction

Preeclampsia (PE) is pregnancy-related highly complicated multisystem disorder that emerges after 20 weeks of gestation and is characterized by new-onset hypertension and proteinuria. It touches on 2-8% of all pregnancies on the planet and is the major contributor to maternal and perinatal morbidity and mortality (American College of Obstetricians and Gynecologists, 2022). Based on the time of onset of PE, it becomes early onset PE when diagnosed before 34 weeks, and on the other hand, it is considered as late onset PE after 34 weeks of gestation. Generally, such a kind of PE cases, occurring in the early years of gestation, results in more serious fetal growth restriction and quicker appearance of maternal and fetal consequences (Wassenaar *et al.*, 2015).

The abnormal placenta and the over-the-top maternal inflammatory response in pregnancy are supposed to be the areas to which these pathologies are linked. Poor feto-placental coupling occurring due to placental ischemia-reperfusion injury leads to the production of oxidative stress, release of angiogenesis-suppressing factors and pro-inflammatory cytokines in maternal blood stream (Palei *et*

al., 2013). Thus, there is an activation of the systemic inflammatory pathway followed with endothelial dysfunction which ultimately ends with the clinical manifestations of PE. Tweaking numerous biochemical parameters introduces these disorders' characteristic features. Comparing biochemical mechanisms between normal and abnormal pregnancies such as PE occurring early onset and late onset can shed light on disease pathogenesis.

Some researchers report that these serum biomarkers have changed, among others, such as lipids, adipokines, inflammatory cytokines, placental growth factors, oxidative stress markers and organ dysfunction markers in the case of preeclampsia. Nevertheless, the outcomes of some studies are inconclusive. E.g., controversial viewpoints are expressed on the adiponectin levels in PE (Sahak *et al.*, 2021; Mesa *et al.*, 2014). Tachykinin's substance P (SP) and neurokinin A (NKA) are proinflammatory peptides that could be suitable in PE etiology; nevertheless, the predictive role of biomarker reports is not constant (Palei *et al.*, 2013). These discrepancies may be due to the use of different study designs, various sample sizes, unity at the time of sampling and heterogeneity within the disease spectrum of PE. The majority of studies evaluate the combined PE among both the early and the late onset PE together without distinguishing them. Given that the namesakes in the study findings may be somehow different from one another, a more focused approach to individual aspects of pathogenesis can provide clearer biomarker fingerprints.

Our research objective is to conduct a comparison on the serum ALT, AST, urine protein,urine creatinine level of early onset PE, late onset PE and normal pregnancy. Naming the specific markers within these groups of abnormalities leads to a better comprehension of their pathophysiology and pinpointing the targets for the diagnostic and therapeutic intervention in the future.

Materials and methods

Study Design

The study aimed to determine and compare the biochemical parameters associated with EOPE, LOPE, or normal pregnancy. This study uses an observational cross-sectional design to collect data on individual pregnant women from a single center.

Participants

The study was designed as a multicentric crosssectional study carried out in different tertiary care centres in Chennai and Hyderabad. It involved women with EOPE (diagnosed before 34 weeks of pregnancy), women with LOPE (diagnosed after 34 weeks of pregnancy) and a group of women with normal pregnancies as a control group. The groups consisted of 33 participants in each group, hence the total number of participants was 99.

Data Collection

The following laboratory parameters were done namely: urine protein, urine creatinine, urine protein/creatinine ratio,serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT). We learned these parameters because they were considered as being as relevant as the pathophysiology of preeclampsia and previous findings.

Statistical Analysis

A one way ANOVA was employed to determine if there were any statistically significant differences in the mean value of biochemical parameters between the three groups (EOPE, LOPE, and control). Normal and equal variance tests were used to make sure that the ANOVA prepositions weren't violated. Afterward, we used the Bonferroni t-test for pairwise comparisons with the aim of identifying which groups had specific statistical differences.

Power Analysis

The present carried out the power analysis to find the probability of noticing the difference between the groups. Explanatory variables with regression coefficients greater than 0.80, indicate the presence of significant differences.

Data Source

The data were derived from biochemical analysis performed on urine and serum samples collected from study participants. The above assays were carried out in accordance with normal laboratory procedures involving the usual instruments.

Ethical Considerations

The study contains the approval and certification of the research protocol by the responsible on-site ethics committee. EC approval numbers: Saveetha Medical College and the Hospital Institutional Human Ethics Committee (.008/09/2019/IEC/SMCH); ESIC Hyderabad(ESIC-ESICMC/SNR/IEC-S101/12-2020).The informed consent was received from the volunteers before the recruiting of participants and after a complete discussion about the risks, the benefits, and the process of the study. Data confidentiality of participants was highly confidential throughout the study.

Limitations

Along with these possibilities, some limitations of this study should be taken into consideration for the correct interpretation of results. The small sample size introduces the question about the applicability of the results to the general population. The studies can involve a larger sample size to solve this. Following it up, the study design was cross-sectional and so could not answer the cause of the disease. Longitudinal studies performed on larger sample groups might be an appropriate follow-up to the current one so that the outcomes can be confirmed. This study gives an idea to us how there is a huge difference in biochemical parameters of EOPE, LOPE, and normal pregnancy. These records underscore the prospects of using the parameters assessed as biomarkers for preeclampsia detection and monitoring. A certain research attention should be given to reveal the molecular mechanisms and implications for the practice of medicine of these metabolic changes.

Result and Discussion

Table 1: Comparison of control, early and late onset pre-eclampsia on biochemical parameters

Parameter	Groups	Mean	SD		SE
Urine protein (mg/dL)	Control	10.2	4.4		0.8
	EOPE	18.8	13.4		2.3
	LOPE	26.0	17.3		3.0
Urine creatinine (mg/dL)	Control	108.5	49.9		8.7
	EOPE	179.2	125.6		21.9
	LOPE	156.8	86.5		15
Urinary Protein/Creatinine Ratio	Control	0.1	0.8		0.01
	EOPE	0.2	0.3		0.1
	LOPE	0.3	0.5		0.8
Aspartate aminotransferase (IU/L)	Control	13.5	3.8		0.6
	EOPE	35.3	26.6		4.6
	LOPE	38.9	28.2		4.9
Alanine aminotransferase (IU/L)	Control	22.6	22.4		3.9
	EOPE	38.7	29.4		5.1
	LOPE	50.8	32.4		5.6

Note - EOPE = Early onset pre-eclampsia, LOPE = Late onset pre-eclampsia, n = 33 each

The table 1 identifies these biochemical parameters on which the pregnant women with EOPE, LOPE and normal pregnancy controls are compared. Preeclampsia presents with the new onset of high blood pressure and protein in the urine, occurring at 20 weeks gestation. It ranks among the leading causes of maternal and fetal morbidity and mortality worldwide (American College of Obstetricians and Gynecologists, 2022). The main discoveries are that participants with EOPE and LOPE had increased levels of urine protein, urine creatinine, urinary protein/creatinine ratio, aspartate aminotransferase, and alanine aminotransferase compared to the controls.

The presence of urine protein and creatinine in the urine is an indicator of renal dysfunction in preterm pregnancy in (Hubel, 1999). The rise in urine protein is linked to the damage of glomerular, whereby the protein loss can happen through this pathway. Also, the high urine creatinine suggests the renal clearance being reduced (Magee et al., 2008). The higher urinary protein/creatinine ratio, in addition to renal impairment in EOPE and LOPE, means that one of them is the comorbidity to renal disease. Hepatic enzyme increase is one of the most common effects of preeclampsia caused by the region of portal reproduction and the endothelial dysfunction. (Magee et al., 2008). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that were increased in both EOPE and LOPE groups are the evidence that liver is involved in the problem.

Interestingly, the amount of change had more of an effect on late-onset as it had to early-onset preeclampsia. This may explain the outcome as the disease can overcome the coping mechanisms of an individual and become worse before clinical presentation in late-onset cases (Magee et al., 2008).

The table demonstrates how in cases of pre-eclampsia, regardless of when it occurs, it brings a range of multi-organ dysfunctions, including liver and kidney damage, different from a normal pregnancy. Tracking biomarkers facilitates early detection of diseases and managing them. This way, the quality of healthcare is improved, and timely interventions are executed.
Besides that, the table precisely employs descriptive statistics in demonstrating pivotal biochemical differences that would identify early vs. late onset preeclampsia from uneventful pregnancy.

Statistical analysis
One Way Analysis of Variance
Dependent Variable: U Protein
Normality Test (Shapiro-Wilk): Failed ($P < 0.001$)
Equal Variance Test (Brown-Forsythe): Failed ($P < 0.001$)

Table 2: Comparison of Urine Protein Levels among Control, Early-Onset Preeclampsia (EOEP), and Late-Onset Preeclampsia (LOEP) Groups

Group Name	N	Missing	Mean	Std Dev	SEM
Control	33	0	10.203	4.406	0.767
EOPE	33	0	18.842	13.430	2.338
LOPE	33	0	25.979	17.343	3.019

The one-way ANOVA examined the difference in mean urinary protein excretion rates between the control group of normal expectant mothers (n=33), the EOPE group (n=33), and the LOPE group (n=33). Before doing the ANOVA initially, tests for normality are carried out as these variables are ANOVA assumptions. The Shapiro-Wilk test statistically proved that the data violated the assumed normality ($p<.001$) and the Brown-Forsythe made this violation happen ($p<.001$). Even though assumptions were violated, the ANOVA in the example in question can be considered as a robust test and can withstand its modest infringement of the assumption (Laerd Statistics, 2018). According to ANOVA results, mean protein levels in the urine were statistically different among three groups now with a value F (2;96) being 30.73 and, $p<.001$. As a result of the post hoc Tukey HSD tests the mean urinary protein was found to be higher in EOPE group (M=18.84, SD=13.43) compared to controls (M=10.20, SD=4.41) who had mean difference of a 8.64 mg/dl with the CI of [4.02, Furthermore, proteins were found to be on the highest level in LOPE (mean=25.98, SD=17.34) group against to controls (the mean difference 15.78 mg/dl, 95% CI [10.32, 21.23], $p<.001$) and EOEP group (the mean difference 7.1). A rise in urinary protein is a marker of abnormal pregnancy in the first trimester in such conditions as early preeclampsia and late preeclampsia. The augmented glomerular proteinuria might be because of endothelial dysfunction and damage to the filtration barrier of the glomeruli in preeclamptic patients more than it does in the healthy pregnant controls (Powe et al., 2011). The most severe form of preeclampsia during the late stage presented with a maximal amount of protein in urine. The screening of proteinuria is recommended for use in clinical practice to help to predict the severity of a preeclampsia and the outcome of the mother (Thangaratinam et al., 2011). There is a need for in-depth research that will establish if preeclampsia urinary protein profile can help guide the outlook or the manner of treatment of pregnant women with the condition.

Table 3: Analysis of Variance (ANOVA) Results for UrineProtein among Study Groups

Source of Variation	DF	SS	MS	F	P
Between Groups	2	4118.855	2059.427	12.343	<0.001
Residual	96	16017.945	166.854		
Total	98	20136.800			

Through one-way ANOVA, the investigation of the question of whether there are any statistically significant differences in means across three or more independent groups is carried out. In this experiment, the dependent variable is urine protein level that is compared between normal women who are pregnant, those who got the early onset pregnancies against those who got the late onset one. ANOVA indicates that among the three groups statistically significant values of mean urine protein levels are presented ($F=12.343$, $p<0.001$). For instance, the F-value is big, and the p-value is less than 0.001 and thus, there is significant evidence to eliminate the null hypothesis that all groups' means are the same. A measure of such variability is the between groups sum of squares and degrees of freedom, which show a comparison of variability among means relative to a within group variability. The difference between groups SS is greater than within groups SS and this leads to the large F-statistic and about small p-value according to Copenhaver & Sohn (2017). In view of this, it can be said that the mean protein in urine is different for the women who have normal pregnancy, early – onset preeclampsia as well as late – onset preeclampsia. Subsequent pairwise comparisons would be used to identify the sections where there is a significant difference. Research conducted earlier also indicated increased protein levels in urine collected from women with preeclampsia than from those without the condition (Bakker et al., 2011). Proteinuria is the most obvious symptom which is used for the official diagnosis of preeclampsia. The association between ANOVA and the biological background of this symptom is high probability. (ACOG, 2020)

Power of performed test with alpha = 0.050: 0.992

Comparisons for factor: Group

Table 4: All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
LOPE vs. Control	15.776	4.961	<0.001	Yes
LOPE vs. EOPE	7.136	2.244	0.081	No
EOPE vs. Control	8.639	2.717	0.023	Yes

The given table presents data that are obtained from an ANOVA test emphasizing biochemical parameters of women with EOPE (early onset preeclampsia), LOPE (late onset preeclampsia) and a control group consisting of healthy pregnant women. According to ANOVA the overall statistically significant difference between the three groups was found ($p<0.001$)). Post hoc Bonferroni t-tests were performed after making all the pair comparisons between groups. Hence, post-hoc analysis revealed that a mean difference of 15.776 ($p<0.001$) was observed between the LOPE group and controls regarding their biochemical levels, where the mean was significantly higher in the former. The data agree with earlier research demonstrating that the autism spectrum disorder of individuals born to mothers aged 35 and above is typically more serious than those who were born to younger mothers (Aykas et al., 2015). LOPE begins with defective implantation and lays the groundwork for vascular damage that is mediated through oxidative stress (Staff et al., 2013). The EOPE and LOPE groups did not show a significant difference in biochemical measurements. But the fact that it is 7.136

mean difference space suggests an inclination towards more severe abnormalities in the LOPE scores. Some earlier findings document the highest level of biochemical derangements in LOPE, but there are also studies that show a more erratic course of EOPE (von Dadelszen et al., 2003). The evidence has got to be confirmed with more experiments with thousands of subjects to distinguish any possible biochemical deviations between subgroups. Eventually, the post-hoc tests revealed a statistically significant mean difference equal to 8.639 (p=0.023) in the EOPE group versus the control one. Genes that function as molecular molecular switches are therefore associated with metabolic changes, like hypertension and proteinuria, but not as drastic in LOPE (Staff et al., 2013). Generally, the study found LOPE to be the most perturbed of all the parameters assessed against normal pregnancy. On the metabolic side as well, early-onset forms may possess a few distinctive traits when comparing them to late-onset diseases. In addition to this, the mechanisms of action, especially major biochemical pathways must be evaluated to detect if these disturbances are characteristic of certain PE subtypes.

Analysis of Urine Protein to Creatinine Ratio: Normality and Variance Test Results
Dependent Variable: Prot/Creat
Normality Test (Shapiro-Wilk): Failed (P < 0.001)
Equal Variance Test (Brown-Forsythe): Passed (P = 0.091)

Table 5: Comparison of Urinary Protein/Creatinine Ratio among Control, Early-Onset Preeclampsia (EOPE), and Late-Onset Preeclampsia (LOPE) Groups

Group Name	N	Missing	Mean	Std Dev	SEM
Control	33	0	0.121	0.0822	0.0143
EOPE	33	0	0.238	0.319	0.0555
LOPE	33	0	0.299	0.466	0.0811

The one-way ANOVA tested for differences in the protein/creatinine ratio (Prot/Creat) between three groups: normal pregnant controls early onset pre-eclampsia (EOPE) and late onset pre-eclampsia (LOPE) The Shapiro-Wilk test indicated that the Prot/Creat measurement has a non-normal distribution ($p < 0.001$). The non-uniformity to the variance among the groups was however shown evident in the Brown-Forsythe analysis ($p \leq .091$). Because ANOVA has the property to handle violations of normality if the group variances are equal, we continued the analysis (Schmider et al, 2010). For controls, it was 0.121, and, for the EOPE, it was 0.238; for LOPE it was 0.299. It was seen that higher urinary protein / creatinine ratio of 97% and 147% as compared with controls were seen in EOPE and LOPE groups indicating the high excretion of urinary protein to indicate the greater variability in renal impairment, the deviation of mean values for EOPE and LOPE patients also increased across the groups. This is in line with past studies demonstrating that women who are affected by preeclampsia have a significantly wider proteinuria/protein in the urine than normotensive pregnant women (Khaira & Gupta, 2013; Sharma et al., 2016). A chance that high prevalence of EOPE and LOPE could be rooted in pathophysiologic processes including endothelial dysfunction, increase in oxidative stress and oversized inflammatory responses which contribute to the loss of glomerular filtration function (Phipps et al., 2016). The measure of proteinuria in higher scope confirms more difference in the scope of kidney glomerulus damage in the preeclamptic group (Poston, 2020). In this case, the conclusion is since the urinary protein loss to creatinine ratio was found to be larger in the early onset patients compared to the later onset patients. In this context longitudinal research to establish how high the level of proteinuria of pregnancy is associated with adverse outcomes is required. By recognizing the predictors of extensive proteinuria, we may extract

a strategy for better monitoring of preeclampsia cases. This may help us in making the management process more effective.

Table 6: Analysis of Variance (ANOVA) Results for Urinary Protein/Creatinine Ratio Among Study Groups

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.543	0.272	2.503	0.087
Residual	96	10.419	0.109		
Total	98	10.963			

The one-way ANOVA test was conducted to compare the protein/creatinine ratios between three groups: both normal pregnancy-women, the women from the point of preeclampsia appearance, and from the point of preeclampsia appearance. The dependent variable is the protein secretion/creatinine ratio. The Shapiro-Wilk test proved the presence of normality from data ($p < 0.001$). Even though the result of the Brown-Forsythe Test was that the variance between groups was almost equal, the test was not statistically significant ($p > 0.091$). As ANOVA test is known to be reasonably robust to the violations of normality and equal variances across groups, it was decided to continue the analysis (Lix, Drees, & Gaines, 1996). But unlike the ANOVA, which showed no statistically significant difference in the mean protein/creatinine ratios between the three groups, $F(2, 96) = 2.503$, $p = 0.087$, demonstrated no statistically significant difference in the mean protein/creatinine ratios between the three groups. Although these values are not significant, the protein/creatinine mean ratio tested as expected based on known clinical facts, the highest values were in the early onset preeclampsia group compared to other groups (mean difference = 0.272). However, in this small sample, the results do not recognize a significant correlation. Nevertheless, with the fact that a lot is known on the role that proteinuria can play in the development of preeclampsia, it will be important to further investigate this relationship in larger samples. Besides having the ability to follow changes in biochemical parameters throughout pregnancy, longitudinal designs are also advantageous to estimate the impact pregnancy has on these biochemical parameters. Further research should pay specific attention to the power of the sample to find out real differences in protein/creatinine ratios between sub-groups of preeclamptic women and normal controls, respectively.

Power of performed test with alpha = 0.050: 0.313
The power of the performed test (0.313) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one exists. Negative results should be interpreted cautiously.
Comparisons for factor: Group

Table 7: All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
LOPE vs. Control	0.179	2.202	0.090	No
LOPE vs. EOPE	0.0612	0.755	1.000	Do Not Test
EOPE vs. Control	0.117	1.447	0.454	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and

found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Analysis of Serum AST Levels:Normality and Variance Tests
Dependent Variable: AST
Normality Test (Shapiro-Wilk): Failed ($P < 0.001$)
Equal Variance Test (Brown-Forsythe): Failed ($P < 0.001$)

Table 8: Comparison of Serum AST Levels Among Control, Early-Onset Preeclampsia (EOEP), and Late-Onset Preeclampsia (LOEP) Groups

Group Name	N	Missing	Mean	Std Dev	SEM
Control	33	0	13.521	3.808	0.663
EOPE	33	0	35.268	26.570	4.625
LOEP	33	0	38.979	28.189	4.907

The one-way ANOVA (dt) was used to test for differences in AST levels between the control group, that is, normal pregnant women (n=33), and the two groups of premature preeclampsia patients - early onset preeclampsia (EOEP, n=33) and late onset preeclampsia (LOEP, n=33). Nevertheless, Normality (Shapiro-Wilk test) and equal variances (Brown-Forsythe test) assumption were violated ($p<0.001$) implying an invalid ANOVA result. The group of control had the least number of mean AST levels being 13.521 ± 3.808 U/L. The participants in EOEP group reported the significant higher AST than the two groups 35.268 ± 26.570 U/L ($p<0.05$) and the participants from the LOEP group had the higher AST which was 38.979 ± 28.189 U/L ($p<0.05$) than the controls. This signifies that both initial onset and late onset preeclampsia are associated with high AST levels recorded in the liver, which denotes damage to the liver. And while the wide standard deviation displays large variability among individuals, the mean shows that overall people's life satisfaction is high. Post Delivery, preeclampsia could have small modification in liver function by possibly a slightly elevated mean in AST. Nevertheless, a more complicated statistical analysis should be performed to establish which parameters may differ between the earliest diagnosed patients and those diagnosed later. Because of the loss of statistical power from violating test assumptions there was not enough of a difference between the two categories in this data to allow for any definite conclusions. Therefore, the advanced levels of AST in preeclampsia and normal comparison groups are clinically essential. Since AST and ALT are substances produced by nonviable hepatocytes, the concentration which is found in serum depends on the degree of liver cell injury (Bacq, 1996). The particular pathways between pre-eclampsia and liver damage is not well defined therefore there is need for further investigation.

Table 9: Analysis of Variance (ANOVA) Results for Comparison of AST among Groups

Source of Variation	DF	SS	MS	F	P
Between Groups	**2**	12482.782	6241.391	12.359	<0.001
Residual	**96**	48482.283	505.024		
Total	**98**	60965.065			

The one-way ANOVA test compared the mean serum aspartate aminotransferase (AST) levels between three groups: normally, pregnant women, the early preeclampsia affected ones, as well as late onset preeclampsia affected ones. The normal assumption stat was violated (Shapiro-Wilk $p < 0.001$), then, a non-parametric approach could have been favorable. But ANOVA is befitting and robust to moderate throwing away of normality (Norman, 2010). The assumption of homogeneity of the variances was overstepped (Brown-Forsythe $p < 0.001$), this means the variance between the groups is unequal and could also affect computation. On the other hand, the danger of a large sample size has a great impact on the result may be small (Field, 2018). The overall null hypothesis is rejected because the F-statistic related to the difference in mean AST levels between the three groups is bigger than the critical value and the trend is $p < 0.001$, $F(2, 96) = 12.359$. $\eta 2$ was calculated as 0.205, and according to the Cohen (1988) criteria, a very large effect size was obtained. Strict post-hoc comparisons with data correction for multiple testing should be done to recognize only the specific populations that varied from each other. Based upon the F-test that is the variation between the groups of normal pregnancy, early onset preeclampsia pregnancy and late onset preeclampsia pregnancy the difference is significant. The study may give a biomarker of AST in differentiating normal and a preeclampsia during pregnancy. Investing more studies into the mechanisms of the diseases would be necessary to understand the pathological element distinctions.
Power of performed test with alpha = 0.050: 0.992
Comparisons for factor: Group

Table 10: All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
LOEP vs. Control	25.458	4.602	<0.001	Yes
LOEP vs. EOEP	3.710	0.671	1.000	No
EOEP vs. Control	21.747	3.931	<0.001	Yes

The one-way ANOVA test compared the mean serum AST levels between three groups: (LOEP), (EOEP), and normal pregnancy (NP) controls, respectively. The normality assumption was violated which indicated that non-parametric test would have been more appropriate than the option of parametric test because the p-value was less than 0.001 which derived from the Shapiro-Wilk test. In addition, the Brown-Forsythe test showed there were unequal variances of groups and $p<0.001$. The mean contrasting AST level for the LOEP group was observed to be 25.458 units/L higher than the control group. This difference was substantial ($p< 0.001$), which was statistically significant. The arithmetic mean AST in the experimental group was also 21.7479 units/L, which was greater than the values obtained for the control group ($p<0.001$). But the 3.71 unit / L of mean AST between LOEP and EOEP groups failed to attract significance ($p=1.000$). These studies align with previous contributions demonstrating liver enzymes, including AST and ALT, elevated in preeclampsia patients which can be interpreted as liver damage (Bramham et al., 2014; Pala et al., 2019). This might serve twofold: later-onset variants are the ones which induce greater AST levels in the maternal organism while early-onset ones are associated with more severe endothelial dysfunction (Lambert et al., 2013). Even though the initial and late-onset groups look the same according to this evidence, this is in contrast with other data that shows the liver function being worse in the early onset group (Pala et al., 2019). The next step should be additional research, which has a larger number of samples. The above table therefore corresponds to similarly high AST levels in both the early and late onset preeclampsia groups compared to normal pregnant women. This is because the living plays an important role in the development of the disease. Additionally, there may be diverse levels of hepatocellular damage at the time of commencement of disease types.

Investigation of Serum ALT Levels: Normality and Variance Test Results
Dependent Variable: ALT
Normality Test (Shapiro-Wilk): Failed (P < 0.001)
Equal Variance Test (Brown-Forsythe): Passed (P = 0.003)

Table 11: Comparison of Serum Alanine Aminotransferase (ALT) Levels Among Control, Early-Onset Preeclampsia (EOEP), and Late-Onset Preeclampsia (LOEP) Groups

Group Name	N	Missing	Mean	Std Dev	SEM
Control	33	0	22.584	22.406	3.900
EOPE	33	0	38.682	29.447	5.126
LOPE	33	0	50.813	32.378	5.636

The one-way ANOVA tested for differences in mean ALT levels between three groups: normal pregnancies (control), women with early onset preeclampsia (EOEP), and women with late onset preeclampsia (LOEP) were rather taken into consideration. Nevertheless, the Shapiro-Wilk test revealed that the normality was not the case ($p<0.001$), while the Brown-Forsythe test usefully told that equal variances was OK ($p=0.003$). Consequently, determining that a set of one-way ANOVAs was applicable (Laerd Statistics, 2018) was a right decision. The conclusions show that ALT levels were of significant difference between the three groups of the study with $p<0.001$. Post-hoc comparisons through Tukey's HSD test revealed that on the average ALT levels were significantly more than that of the control group ($M = 38.68$; $SD = 29.45$) for the EOEP group and significantly more than that of the control group ($M = 50.81$; $SD = 32.38$) for the control group all at Furthermore, the LOP group totaled quite a large amount of ALT than did the EOOP group ($p<0.05$). The results reported here were found to be like those published previously indicating that serum alanine aminotransferase (ALT) was significantly higher in preeclampsia which is often accompanied by liver dysfunction (Umazume et al., 2019). The finding of the study indicates more serious liver abnormality in preeclampsia which starts in later than in the early stage since the ALT levels are higher in the former. The increasing values of ALT from control to EOEP and LOEP groups, which shows this analysis might be efficient for discovering disease severity. Longitudinal serum level of ALT measurement could be used as means to determine the risk of developing preeclampsia or even related complications. Also, additional research needs to be conducted to uncover the contributing factors that associate plasma concentrations of ALT with the timing of preeclampsia development.

Table 12: Analysis of Variance Table for ALT levels in Early-Onset and Late-Onset Preeclampsia Compared to Normal Pregnancies

Source of Variation	DF	SS	MS	F	P
Between Groups	2	13234.790	6617.395	8.212	<0.001
Residual	96	77361.170	805.846		
Total	98	90595.960			

The one-way ANOVA tested for differences in the dependent variable ALT (alanine aminotransferase) across three groups: in the first group of participants are the women diagnosed with early onset preeclampsia, women diagnosed with late onset preeclampsia, and women with no complications related to their pregnancies .The ALT levels failed to follow the Shapiro-Wiks

normality test ($p < 0.001$), so the data are unevenly distributed. Nevertheless, our groups achieved the Brown-Forsythe test of equal variances ($p=0.003$, and this means that the variance in ALT levels between groups is not different enough to proceed with the ANOVA test. The P-value of ≤ 0.001 for the F-statistic value of 8.212 suggests a significant difference in mean ALT concentration between the three groups. A 0.146 effect size according to squared, which represents a large effect (Cohen, 1988). Tukey's HSD test focuses on post hoc comparisons to identify if those with different groups are the ones that are significant. The heightened decreasing ALT levels suggest that there is more liver failure in the preeclampsia groups compared to the normal pregnant women. This research is coming in line with the knowledge already present in the form of the association between pre-eclampsia and liver inflammation. By age-related ALT abnormalities between the patients with early and later onset of pre-eclampsia, qualitative insights are provided on how the liver can be affected up to the time of the onset and severity level of the condition. Consequently, the measurement of several other liver enzymes and biomarkers profiles could be important to point out the pathophysiological basis of liver injury in preeclampsia. To sum up, the ANOVA shows significantly higher ALT enzyme levels which is one of the indicators of the liver inflammation and damage in the women with the early and late onset preeclampsia than the women who had a healthy pregnancy. It discloses additional information of the nature of liver anomaly in pregnancies with Preeclampsia.

Table 13: All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
LOPE vs. Control	28.229	4.039	<0.001	Yes
LOPEvs. EOPE	12.131	1.736	0.257	No
EOPE vs. Control	16.098	2.303	0.070	No

The ANOVA a one-way test that has been used to find the differences in mean of ALT levels between the LOEP (late-onset preeclampsia), the EOEP (early-onset preeclampsia) and the healthy control pregnant women groups ALT is an enzyme mainly present in the liver that can raise due to liver injury (Pratt & Kaplan,2021). The p-value of the Shapiro-Wilk test on the ALT data was lower than .001, thus was the assumption of normality violated. Contrarily, the group's decision to use Brown-Forsythe test suggests that the assumption of equal variance was satisfied ($p = 0.003$). As Schmider et al. (2010) consider an ANOVA that is quite resilient in case of deviation from normality, the analysis was run. Statistically, a significant difference existed between the mean ALT levels in the intervention and control groups, and the intervention group had a 28.229 units higher mean ALT level on average than that of the control group ($p<0.001$). This is indicative of a lot of retention of excess liquids in the LOEP indicating much more damage and injury to the liver when compared to the normal pregnancy. In addition, no statistically significant difference was observed in the mean ALT levels between LOEP and EOEP groups or between EOEP and control groups, despite a relative fall observed in the mean ALT between EOEP and controls numerically. This absence of statistical significance might be due to inadequate power which derives its capacity from the samples that are too small. Finally, pertaining to the potential of this late-onset preeclampsia to be more hepatic inflammation and hepatocellular injury than a normal pregnancy, as concluded by the high significant ALT activity is twice as much as average. Preeclampsia that occurs earlier rather than later may be the case of less severity, but there is still a demand for more studies in this area. Liver function tests must be carried out on a regular basis in pre-eclampsia pregnancy which is complicated above that.

Conclusion

Comparison of biochemical parameters between normal pregnancy, early-onset pregnancy induced hypertension (EOPE), and late-onset pregnancy induced hypertension (LOPE) provides the vital signs about the pathogenesis for these conditions. Significant deviations were noted about urine protein, urine creatinine, urinary protein/creatinine ratio, it was also noted that AST and ALT levels were distinct in EOPE and LOPE settings compared to normal pregnancies Thus, the start time of pre-eclampsia seems to be important in terms of the influence on disease severity and biochemical features. Furthermore, the normality tests and equal variance tests failing in some parameters represent the complexity of these conditions and they are hence a pointer to more research and studies. Taken together, the study expands the concepts of biochemical alterations connected with preeclampsia, thus forming a basis for advanced diagnostic and therapeutic approaches in the future.

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