

Implication of Albumin, Microalbumin and Alpha-Feto Protein Variation in Preeclamptic Pregnant Women in the Third Trimester of Pregnancy

Edebiri O.E.*¹, Akpe C. I.², Onwuka K. C.³, Ehigiamusoe A. O.⁴, Okike P.I.⁵, Ohiwerei W.O.⁶, Nze P.O⁷

¹Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria ²Department of Clinical Pharmacy and Pharmacy Practice, University of Benin, Benin City, Edo state, Nigeria

 ^{3,7}Department of Physiology, Abia State University, Uturu, Abia State, Nigeria
⁴Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
⁵Department of Physiology, Abia State University, Uturu, Abia State, Nigeria
⁶Department of Research and Training, Ohilux Global Research Diagnostic and Training Centre, Ekpoma, Edo State, Nigeria

Author Corresprodence : <u>edebiriduncan@gmail.com</u>*

Abstract. The presence of Albumin, Microalbumin and Alpha-Feto Protein increase activity in third trimester. The aims of this study is to determine the Implication of Albumin, Microalbumin and Alpha-Feto Protein Variation in preeclamptic pregnant women in the third trimester of pregnancy. Forty (40) consenting pregnant women were recruited from St. Philomina Catholic Hospital, Edo State, Nigeria. Blood samples was spun in a bucket centrifuge at 2500 RPM (rounds per minute) for 10 minutes after which plasma was collected and stored frozen in plain sample bottles and was analyzed for liver enzymes (albumin, Microalbumin and Alpha-fetoprotein) levels by fluorescence immunoassay method. Data obtained from this study were analysed using Graph Pad Prism 9. Results generated were expressed as mean \pm SEM and a P-value of ≤ 0.05 were considered statistically significant. The present study showed that there was statistically significant increase in Albumin, Micro albumin were observed in preeclamptic women compared to normotensive pregnant women, indicating various underlying pathophysiological processes such as liver dysfunction. These findings suggest albumin and Micro albumin are implicated as potential biomarkers in identifying and monitoring preeclamptic and normotensive women.

Key Words: Albumin, Alpha-Feto Protein, Microalbumin, Preeclampsia.

1. INTRODUCTION

The implication of Albumin, Microalbumin, and AFP as predictors of preeclampsia offers a promising approach, as they are widely available and cost-effective biomarkers (S. Rafaqat, 2023). This study's focus on these proteins provides a nuanced understanding of their predictive role, enabling healthcare providers to make informed decisions and improve patient care. Furthermore, the study's findings will have implications for the development of personalized medicine approaches to preeclampsia diagnosis and management.

Preeclampsia, it affects approximately 2-8% of pregnancies worldwide, making it a leading cause of maternal and fetal morbidity and mortality (L. Duley, 2009). Early detection and prediction of preeclampsia are crucial to prevent severe complications and ensure timely interventions (A. Asmanidar, 2024).

Proteinuria, or the presence of excess protein in the urine, is a hallmark of preeclampsia (M. F Bartal, 2022). Albumin, Microalbumin, and Alpha-Fetoprotein (AFP) are proteins that

have been implicated in the pathophysiology of preeclampsia. Albumin is a protein produced by the liver that helps maintain fluid balance in the body, while Microalbumin is a smaller protein that can be used to detect early kidney damage (E. K Kumahor, 2024). AFP is a protein produced by the fetus that can be used to detect fetal distress.

The aims of this study is to determine the Implication of Albumin, Microalbumin and Alpha-Feto Protein Variation in preeclamptic pregnant women in the third trimester of pregnancy.

2. RELATED WORKS

Serum albumin, produced by the liver, is the most abundant protein in blood plasma, crucial for maintaining fluid balance, transporting hydrophobic molecules, and regulating osmotic pressure. Abnormal levels, like albumin in urine, can indicate kidney issues. Albumin's flexible structure binds substances like fatty acids and drugs, and its levels are regulated by factors like nutrition and disease. Low albumin levels are linked to liver disease, malnutrition, and nephrotic syndrome. Microalbuminuria, early evidence of kidney dysfunction, is important for diagnosing diabetes-related kidney issues and cardiovascular risks. Treatment focuses on managing blood pressure and diet.

Alpha-fetoprotein (AFP) is a protein produced during fetal development and serves as a marker for liver cancer and fetal abnormalities. Elevated AFP levels are used in diagnosing hepatocellular carcinoma (HCC) and monitoring liver diseases. In pregnancy, abnormal AFP levels can suggest neural tube defects or Down syndrome . AFP is measured using techniques like ELISA and mass spectrometry, and its levels help guide the management of liver and pregnancy-related conditions.

3. MATERIALS AND METHODS

Geographical Description of the Study Area

This research was carried out among Third Trimester Pregnant women in St. Philomina Catholic Hospital, Edo State, Nigeria. Lies longitudinally at 04°E and 43°E and Latitude 05°44°N and 07°34°N. It geopolitical location is the South South and it has a population of 3.5 million people. Oredo land, Benin City, the State capital, is 100 km long. Edo State, South-South, Nigeria. Oredo is a Local Government Area of Edo State, Nigeria. Its headquarters are in the town, Benin city. It has an area of 502 km² and a population of 500,000 at the 2006 census.

Majority of which are civil servants, traders, businessmen/women, transporter, farmers, teachers/lecturers and students by occupation. Oredo, since after its designation as headquarters and as the host of Oba of Benin Palace, the town has grown into an urban center.

Research Design

Fourty (40) consenting pregnant subjects were recruited from St. Philomina Catholic Hospital, Edo State. These subjects consisted of twenty (20) normotensive pregnant women in their third trimester of pregnancy with blood pressure between 120/80mmHg to 130/90 mm/Hg without presence of proteinuria and twenty (20) preeclamptic women in their third trimester of pregnancy classified as having preeclampsia according to their blood pressure measured was above 130/90 mm/Hg with the presence of proteinuria taken two consecutive times at presentation at the antenatal clinic of the hospital

Sample Size

The Population of study was determined using the formula;

 $N = Z^2 pq/d^2$

Where N= the desired sample size (when population is greater than 10,000)

Z= is a constant given as 1.96 (or more simply at 2.0) which corresponds to the 95% confidence level.

P= previous survery prevalence of 2.23%

```
q= 1.0-p
```

d= acceptable error 5%.

Where N= sample size, Z=1.96, p=0.1% (0.01) and d=5% (0.05)

N=39.8 subject.

Therefore, the sample for this study is 40 respondents who are normotensive and preeclamptic pregnant women from Oredo town, Benin City.

Ethical Approval and Informed Consent

Ethical clearance (REC Approval No:RECC/10/2023(07)) was obtained from the Research Ethics Committee of St. Philomina Catholic Hospital, Edo State.

Written informed consent was obtained from subjects prior to commencement of the study.

Blood Sampling

10 milliliters (10 ml) of venous blood was drawn from consenting participants and placed in a lithium heparin sample bottles. Blood samples was spun in a bucket centrifuge at 2500 RPM (rounds per minute) for 10 minutes after which plasma was collected and stored frozen in plain sample bottles and was analyzed for Liver enzymes (**albumin, Microalbumin and Alpha-fetoprotein**)

Experimental Protocols

After the subjects were identified and recruited into the study, they were taken to the lab where their vital signs was taken, after which blood samples were collected by venipuncture and taken to the chemistry laboratory for analysis.

Study Area/Population

The study were conducted for three months at St. Philomina Catholic Hospital, Edo State, Nigeria.

Inclusion Criteria

Normotensive and Preeclamptic pregnant women in the third trimester of pregnancy, within the age range of 25 to 35 years was used for this study. Pregnant women were recruited for this study and women who had given birth before and were pregnant for the second time.

Exclusion Criteria

Normotensive and Preeclamptic pregnant women who were on drugs and with a known history of hyperlipidemia, gestational Diabetes and other comorbidity.

Biochemical Examination

Measurement of Liver Enzymes (albumin, Microalbumin and Alpha-fetoprotein) by fluorescence immunoassay

Principle: The albumin, Microalbumin and Alfa-fetoprotein Rapid Quantitative Test is a fluorescence immunoassay using a competitive method for quantitative analysis of MAU and AFP.

Procedure

Step 1: Preparation: Allow the test cassette, detection buffer and specimen to equilibrate to room temperature prior to testing. Take out the ID chip, make sure that the ID chip is consistent with the batch number of test cassette and insert the ID chip into the chip port of the instrument. **Step 2: Sampling:** Draw 75 μ L of urine with a transfer pipette and add it to the buffer tube.

Step 3: Loading: Pipette 75 μ L of sample mixture and load it into the sample well of the test cassette.

Step 5: Testing: There are two modes for FIA Meters, Standard Test mode and quick test mode. Please refer to the operation manual of FIA meters for details.

- a. For Standard Test mode: Insert the test device onto the test cassette holder of FIA Meter right after adding sample mixture to the sample well. Press "Test" to start testing. The reaction time is 3 minutes.
- b. For Quick Test mode: Set the timer and count down right adding sample mixture into the sample well and leave it at room temperature for 15 minutes. Then insert the test cassette onto the test cassette holder of FIA Meter. Press "Test" to start testing. FIA Meter will start scanning the sample- loaded test cassette immediately.

Step 6: Reading result: Results are displayed on the main screen of meter and can be printed out by press "Print".

Step 7: Withdraw: Discard the used test kit according to local regulations and procedure after released from the meter.

Data Analysis

Data obtained from this study were analysed using Graph Pad Prism 9. Results generated were expressed as mean \pm SEM and a P-value of ≤ 0.05 were considered satistically significant. The significance of difference among the groups were used to assess the repeated-measures analysis of variance (ANOVA). Independent students' t-test were used to compare normotensive and preelclamptic pregnant women group.

4. RESULTS AND DISCUSSION

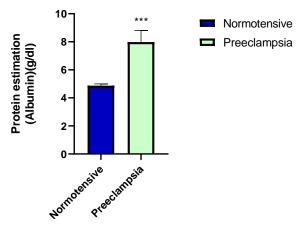


Figure 1. Mean ± SEM of Protein estimation (Albumin) level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference. *** represents p<0.001

Figure 1 shows the Protein estimation (Albumin) in Normotensive and pre-eclamptic women in their third trimester of pregnancy. Protein estimation (Albumin) increased from 4.87 \pm 0.12g/dl in Normotensive women to 7.98 \pm 0.83g/dl in pre-eclamptic women. This increase was found to be statistically significantly (p<0.05; 0.0007).

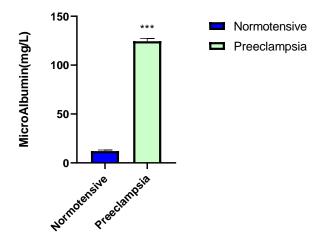


Figure 2. Mean \pm SEM of Micro-Albumin level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference. *** represents p<0.001

Figure 2 shows Micro-albumin levels in Normotensive and pre-eclamptic women in their third trimester of pregnancy. There was a high increase in micro albumin from $12.02 \pm 1.11 \text{ mg/L}$ in normotensive women to $124.4 \pm 2.80 \text{ mg/L}$ in pre-eclamptic women. When both groups where compared, this increase was found to be statistically significant (p<0.05; <0.0001).

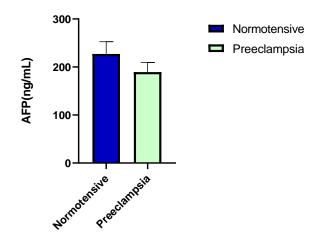


Figure 3. Mean \pm SEM of Alfa- Fetoprotein (AFP) level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference.

Figure 3 shows the levels of Alfa- Fetoprotein (AFP) in Normotensive and preeclamptic women in their third trimester of pregnancy. Alfa- Fetoprotein (AFP) decreased from 227.1 ± 25.38 mg/ml in Normotensive women to 189.5 ± 20.12 mg/ml in pre-eclamptic women. However, when both means were compared, there was no statistically significant difference observed (p> 0.05; 0.2521)

Discussion

Alpha-fetoprotein (AFP), albumin, and microalbumin are collectively referred to as "serum proteins" or "blood proteins". These proteins are found in the blood plasma and play essential roles in various physiological functions, including transporting nutrients, hormones, and waste products, maintaining osmotic pressure, and regulating immune responses. While AFP, albumin, and microalbumin have distinct functions and clinical significance, they are all classified as serum proteins due to their presence in the blood plasma. The contrasting trends observed in alpha-fetoprotein (AFP), albumin, and microalbumin levels between normotensive and pre-eclamptic women provide insights into their potential predictive roles in preeclampsia. Although AFP is a marker of fetal liver function, and its levels are typically elevated in conditions such as neural tube defects and fetal liver dysfunction. The non-significant decrease in AFP levels in pre-eclamptic women suggests that fetal liver function may not be significantly affected in preeclampsia. However, further research is needed to confirm this observation and explore the potential role of AFP as a predictive marker for preeclampsia. Albumin is a marker of liver function and blood volume status. The increase in albumin levels observed in pre-eclamptic women may reflect hemoconcentration due to fluid retention and plasma volume

expansion associated with preeclampsia. Figure 1, the significant increase in albumin levels in pre-eclamptic women compared to normotensive pregnant women suggests a potential predictive role of albumin in preeclampsia. This is in line with previous research done by and he reported elevated albumin levels in women with preeclampsia and are associated with adverse maternal and fetal outcomes. Microalbuminuria is a marker of endothelial dysfunction and liver injury, both of which are characteristic features of preeclampsia. Similarly, Figure 2, there was significant increase in microalbumin levels in pre-eclamptic women compared to normotensive women, suggests a potential predictive role of microalbuminuria in preeclampsia. Elevated microalbumin levels have been consistently reported in women with preeclampsia and are associated with an increased risk of maternal and fetal complications. Overall, the observed changes in albumin and microalbumin levels highlight the importance of assessing liver function and vascular health in the prediction and management of preeclampsia.

5. CONCLUSION

The present study showed that there was statistically significant increase in Albumin, Micro albumin were observed in preeclamptic women compared to normotensive pregnant women, indicating various underlying pathophysiological processes such as liver dysfunction. These findings suggest albumin and Micro albumin are implicated as potential biomarkers in identifying and monitoring preeclampsia. However, levels of alpha-fetoprotein (AFP) did not show significant differences between preeclamptic and normotensive women, suggesting that this biomarker may not be implicated in the pathophysiology of preeclampsia based on the current study's findings.

REFERENCES

- Asmanidar, A., & Emilda, E. (2024). Optimizing maternal healthcare: Holistic strategies for early detection and management of preeclampsia. Science Midwifery, 12(1), 158-167.
- Bartal, M. F., Lindheimer, M. D., & Sibai, B. M. (2022). Proteinuria during pregnancy: Definition, pathophysiology, methodology, and clinical significance. American Journal of Obstetrics and Gynecology, 226(2), S819-S834.
- Berezowsky, A., Pardo, J., Ben-Zion, M., Wiznitzer, A., & Aviram, A. (2019). Second trimester biochemical markers as possible predictors of pathological placentation: A retrospective case-control study. Fetal Diagnosis and Therapy, 46(3), 187-192.
- Chen, J., Wang, J., Li, K., Wang, Y., Gruebele, M., Ferguson, A. L., & Zimmerman, S. C. (2019). Polymeric "clickase" accelerates the copper click reaction of small molecules, proteins, and cells. Journal of the American Chemical Society, 141(24), 9693-9700.

- Chen, Y., Wang, X., Lu, S., Huang, J., Zhang, L., & Hu, W. (2020). The diagnostic accuracy of maternal serum alpha-fetoprotein variants (AFP-L2 and AFP-L3) in predicting fetal open neural tube defects and abdominal wall defects. Clinica Chimica Acta, 507, 125-131.
- Conde-Agudelo, A., Romero, R., & Roberts, J. M. (2015). Tests to predict preeclampsia. In Chesley's hypertensive disorders in pregnancy (pp. 221-251).
- Dangana, A., Okoronkwo, I. M., Onoja, S. O., Okonkwo, I. N., Egenti, N. B., Azolike, S. C., ... & Bakare, M. (2021). Urine-albumin and creatinine ratio among apparently healthy individuals in South Eastern, Nigeria. GSC Biological and Pharmaceutical Sciences, 16(1), 105-114.
- Duley, L. (2009, June). The global impact of pre-eclampsia and eclampsia. In Seminars in perinatology (Vol. 33, No. 3, pp. 130-137). WB Saunders.
- Galle, P. R., Foerster, F., Kudo, M., Chan, S. L., Llovet, J. M., Qin, S., ... & Zhu, A. X. (2019). Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver International, 39(12), 2214-2229.
- Glowska-Ciemny, J., Szymański, M., Pankiewicz, J., Malewski, Z., von Kaisenberg, C., & Kocylowski, R. (2023). Influence of selected factors on serum AFP levels in pregnant women in terms of prenatal screening accuracy—Literature review. Ginekologia Polska, 94(2), 158-166.
- Jelin, A. C., Sagaser, K. G., & Wilkins-Haug, L. (2019). Prenatal genetic testing options. Pediatric Clinics, 66(2), 281-293.
- Khatun, M. R., Khatun, A., & Ali, M. N. (2020). Comparison of liver function tests in normal pregnancy with non-pregnant matched controls. TAJ: Journal of Teachers Association, 33(1), 17-24.
- Kumahor, E. K. (2024). The biochemical basis of renal diseases. In Current trends in the diagnosis and management of metabolic disorders (pp. 185-200). CRC Press.
- Mathieu, M., Névo, N., Jouve, M., Valenzuela, J. I., Maurin, M., Verweij, F. J., ... & Théry, C. (2021). Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9. Nature Communications, 12(1), 4389.
- Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. Biological Psychiatry, 65(9), 732-741.
- Nijst, P., Olinevich, M., Hilkens, P., Martens, P., Dupont, M., Tang, W. W., ... & Mullens, W. (2018). Dermal interstitial alterations in patients with heart failure and reduced ejection fraction: A potential contributor to fluid accumulation? Circulation: Heart Failure, 11(7), e004763.
- Pande, P. D., & Nugraha, J. (2023). The maternal serum alpha-fetoprotein for congenital anomalies screening. WMJ (Warmadewa Medical Journal), 8(1), 9-15.

- Priyadarshini, A. I. (2022). A study on maternal serum glycosylated fibronectin as a predictor of pre-eclampsia in antenatal women between 20-36 weeks of gestational age: A prospective study (Doctoral dissertation, Madurai Medical College, Madurai).
- Provenzano, M., Coppolino, G., Faga, T., Garofalo, C., Serra, R., & Andreucci, M. (2019). Epidemiology of cardiovascular risk in chronic kidney disease patients: The real silent killer. Reviews in Cardiovascular Medicine, 20(4), 209-220.
- Qiu, Y., Myers, D. R., & Lam, W. A. (2019). The biophysics and mechanics of blood from a materials perspective. Nature Reviews Materials, 4(5), 294-311.
- Rafaqat, S., Sattar, A., Khalid, A., & Rafaqat, S. (2023). Role of liver parameters in diabetes mellitus A narrative review. Endocrine Regulations, 57(1), 200-220.
- Zen, M., Padmanabhan, S., Cheung, N. W., Kirby, A., Jesudason, S., Alahakoon, T. I., & Lee, V. W. (2019). Microalbuminuria as an early predictor of preeclampsia in the pregestational diabetic population: A prospective cohort study. Pregnancy Hypertension, 15, 182-188.
- Zheng, J., Zhang, L., Zhou, Y., Xu, L., Zhang, Z., & Luo, Y. (2022). Development and evaluation of a nomogram for adverse outcomes of preeclampsia in Chinese pregnant women. BMC Pregnancy and Childbirth, 22(1), 504.