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Trimester-Specific Patterns of Maternal Serum Alpha-Fetoprotein Among Pregnant Women Attending a Tertiary Hospital in Southeast Nigeria

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Abstract

Alpha-fetoprotein (AFP) is a fetal-derived glycoprotein widely utilized in prenatal screening and increasingly recognized as a biomarker of placental integrity and fetal well-being. Despite its established clinical value, locally derived trimester-specific reference data remain limited in Nigeria. This study evaluated maternal serum AFP concentrations across different trimesters among pregnant women attending antenatal care at a tertiary hospital. A hospital-based cross-sectional study design was employed, involving 50 healthy pregnant women and 40 healthy non-pregnant women of reproductive age. Venous blood samples were collected, and serum AFP concentrations were measured using a standard immunoassay method. Gestational trimester was determined clinically using ultrasound scanning, and pregnant women were categorized into first, second, and third trimesters. AFP values were compared using one-way ANOVA with a significance threshold of $p < 0.05$. Serum AFP levels were markedly higher in pregnant women than in non-pregnant controls across all age categories ($p < 0.05$), while AFP values in controls remained low and relatively uniform. The concentrations of AFP increased gradually across the trimesters (28.90 ± 5.62 ng/mL in the first trimester, to 98.80 ± 7.31 ng/mL in the second trimester, and rose to 112.60 ± 11.31 ng/mL in the third trimester, ($p < 0.05$). The AFP levels in this study rose significantly with gestational age and are substantially elevated in pregnancy compared with non-pregnant women. These findings provide baseline trimester-specific AFP data for pregnant women at various trimesters and support the potential use of AFP as a locally interpretable biochemical marker for risk-stratification and screening during antenatal visits.

Keywords: Alpha-fetoprotein; Maternal serum AFP; Trimester Variation; Antenatal screening; Gestational age.

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INTRODUCTION

Alpha-fetoprotein (AFP) is a major fetal glycoprotein synthesized predominantly by the fetal liver and gastrointestinal tract. It enters the maternal circulation via transplacental transfer and can be quantified in maternal serum during pregnancy (Chen et al., 2025; Głowska-Ciemny et al., 2023). For decades, maternal serum AFP (MSAFP) has constituted a central component of second-trimester prenatal screening programs, particularly for neural tube defects and chromosomal abnormalities, commonly incorporated into triple and quadruple marker screening panels (Chen et al., 2025; Głowska-Ciemny et al., 2023; Liu et al., 2024). With advances in ultrasonography and cell-free fetal DNA testing, the clinical interpretation of AFP has evolved beyond structural anomaly detection to include broader assessments of placental function and fetal health (Dai et al., 2023; Głowska-Ciemny et al., 2024). Emerging evidence demonstrates that abnormal MSAFP levels, in the absence of major fetal malformations, are associated with adverse pregnancy outcomes such as preeclampsia, hypertensive disorders, preterm birth, intrauterine growth restriction, and low birth weight (Chen et al., 2021; Dai et al., 2023; Ozdemir et al., 2022; Sovio et al., 2023). Elevated AFP, particularly when combined with biomarkers such as D-dimer, free β -human chorionic gonadotropin, and pregnancy-associated plasma protein-A, has shown predictive value for ischemic placental disease and hypertensive disorders of pregnancy (Chen et al., 2021; Dai et al., 2023). Conversely, both abnormally low and elevated AFP levels have been linked to fetal growth abnormalities and congenital heart defects (Aghaamoo et al., 2021; Ozdemir et al., 2022; Sovio et al., 2023). A major limitation in AFP interpretation is its dependence on gestational age, maternal characteristics, and clinical context. Most established reference values originate from high-income populations Głowska-Ciemny et al. (2023) and Liu et al. (2024), limiting their generalizability to sub-Saharan African settings. Limited Nigerian data suggest that maternal conditions such as HIV infection may influence AFP levels Udeogu et al. (2021), further emphasizing the need for population-specific baseline data. There remains a paucity of trimester-specific AFP data in southeastern Nigeria. Establishing locally relevant reference patterns is essential for accurate interpretation, early detection of deviations, and integration into structured risk-based antenatal care. Therefore, this study aimed to evaluate maternal serum AFP concentrations across trimesters among pregnant women attending the antenatal clinic at Enugu State University Teaching Hospital (ESUTH). The objective was to generate trimester-specific baseline values that may inform the future development of context-appropriate screening tools in this tertiary hospital setting.

Materials and Methods

Study design and setting

This was a hospital-based cross-sectional study conducted at the antenatal clinic of Enugu State University Teaching

Hospital in Enugu, Southeast Nigeria, between May and September, 2022

Study population

The study included 50 apparently healthy pregnant women attending routine antenatal care and 40 healthy non-pregnant women of reproductive age serving as controls. Participants represented both urban and rural populations. Pregnant women were categorized into trimesters based on ultrasound-confirmed gestational age

Participant selection criteria

Inclusion criteria for the pregnant group were singleton pregnancy, reliable dating (last menstrual period corroborated by ultrasound), and absence of known fetal anomalies. Exclusion criteria for all participants included a history of malignancy, chronic liver disease, multiple gestation, vaginal bleeding, and recent invasive procedures.

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of Enugu State University Teaching Hospital (ESUTH). Written informed consent was obtained from all participants before enrolment, and strict confidentiality of patient's information was maintained throughout the study.

Sample size

A total sample size of 90 (50 pregnant, 40 controls) was obtained using a convenience sampling approach, informed by similar biomarker studies evaluating AFP in pregnancy and in Nigerian populations

Blood sample collection

Five milliliters of venous blood were collected into plain tubes. Samples were allowed to clot and centrifuged at 3000 rpm for 10 minutes. Serum was separated and stored at -20°C until analysis.

AFP assay

Serum AFP concentrations were measured using a commercial sandwich ELISA kit according to manufacturer instructions. Calibration curves were constructed using supplied standards, and quality control sera were included in each analytical run. The assay covered the physiological range of AFP concentrations observed in both non-pregnant and pregnant populations.

Data analysis

Data were analyzed using IBM SPSS version 22. Continuous variables were expressed as mean \pm standard deviation (SD). Independent-samples t-test was used to compare pregnant and control groups. One-way ANOVA with Duncan post-hoc testing was used to evaluate trimester-specific differences. Statistical significance was set at $p < 0.05$.

Results

Participant characteristics

Ninety women participated in the study: 50 pregnant (55.6%) and 40 non-pregnant controls (44.4%). Participants were aged 18–33 years, with no statistically significant

difference in age distribution between groups. Mean serum AFP concentration was significantly higher in pregnant women (80.58 ± 5.31 ng/mL) compared with controls (5.87 ± 0.38 ng/mL; $p < 0.05$) [Table 1](#).

Age-stratified comparison of maternal AFP levels

Across all maternal age categories, pregnant women demonstrated consistently higher AFP levels compared with controls. No statistically significant age-dependent variation was observed within either group [Table 1](#).

Trimester-specific AFP levels

Alpha-fetoprotein concentrations increased gradually with gestational age, according to the mean values of AFP concentrations, which were 28.90 ± 5.62 ng/mL in the first trimester, 98.80 ± 7.31 ng/mL in the second trimester, and 112.60 ± 11.31 ng/mL in the third trimester. All groups showed a significant difference at $p < 0.05$. Alpha-fetoprotein levels in each trimester were significantly ($p < 0.05$) higher than those in the controls (control 9.20 ± 1.11 ng/mL), and levels in the second and third trimesters were significantly ($p < 0.05$) greater than those in the first trimester ($p < 0.05$) [Table 2](#).

Table 1. Comparison of Alpha-fetoprotein levels by age groups

Age group (years)	Pregnant	AFP (ng/mL)	Non-pregnant	AFP (ng/mL)
18–20	4	53.5	8	4.6
21–23	8	67.6	10	3.5
24–26	7	93.1	5	7.2
27–29	11	86.3	7	7.9
30–32	11	95.3	7	6.9
33–35	7	71.3	3	7.7
36–38	2	63.0	–	–

Table 2. Comparison of Mean AFP by Trimester.

Trimester	AFP pregnant women (ng/mL)	AFP non-pregnant (ng/mL)	p value
1st trimester	28.90 ± 5.62	9.20 ± 1.11	< .05
2nd trimester	98.80 ± 7.31	9.20 ± 1.11	< .05
3rd trimester	112.60 ± 11.31	9.20 ± 1.11	< .05

Trimester-based comparison of AFP in cases vs controls

Discussion

This study demonstrates that maternal serum AFP concentrations are strongly influenced by pregnancy status and gestational age among women attending ESUTH. The markedly elevated AFP levels observed in pregnant women relative to non-pregnant controls align with established physiological principles of fetal hepatic synthesis and placental transfer ([Głowska-Ciemny et al., 2023](#)). Minimal variation across maternal age categories suggests that chronological age exerts limited influence compared with gestational age, consistent with findings from international screening studies ([Chen et al., 2025](#); [Dai et al., 2023](#)). This reinforces the importance of gestational age-specific reference ranges in clinical interpretation. The progressive increase in AFP from first to third trimester reflects expected fetoplacental maturation and expanding fetal hepatic production ([Głowska-Ciemny et al., 2023](#)). Contemporary screening strategies often employ multiples of the median (MoM) to identify risk thresholds ([Hu et al., 2014](#)). Elevated second-trimester AFP MoM values have been associated with ischemic placental disease and adverse outcomes ([Hu et al., 2014](#); [Dai et al., 2023](#)). The trimester-specific means generated in this study therefore provide foundational data for deriving locally relevant MoM calculations. By establishing baseline physiological patterns, this study creates an essential framework for future investigations linking AFP deviations to pregnancy outcomes such as

preeclampsia, fetal growth restriction, and preterm birth. Such progression would transition AFP assessment from descriptive biochemistry to predictive risk stratification. The single-center design and modest sample size may limit generalizability. The study did not correlate AFP concentrations with pregnancy outcomes, restricting clinical predictive interpretation. Furthermore, AFP values were expressed as absolute concentrations rather than gestational age-adjusted MoM values, limiting direct comparison with international screening algorithms. Larger multicenter Nigerian studies incorporating outcome data and standardized MoM adjustments are warranted.

Conclusion

Maternal serum alpha-fetoprotein levels are significantly elevated in pregnancy and demonstrate a clear gestational age-dependent increase. Maternal chronological age appears to exert minimal influence relative to gestational progression. The findings provide trimester-specific baseline reference data for women attending ESUTH and represent an important step toward developing population-specific antenatal screening tools. Integration of AFP into structured, risk-stratified antenatal assessment frameworks in Nigeria should be guided by further outcome-linked research.

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Conflict of interest

The authors declare no conflict of interest in the publication of this manuscript

Informed consent

Not applicable

References

- Aghaamoo, S., Lotfi, M., Mirmohammadkhani, M., & Hemmatian, M. N. (2021). Evaluation of second-trimester maternal serum AFP value in the detection of congenital heart defects. *Middle East Journal of Rehabilitation and Health Studies*, 8(4), e111917. <https://doi.org/10.5812/mejrh.111917>
- Chen, Y., Chen, Y., Wang, X., Chu, X., Ning, W., Gu, L., Li, L., Xie, Z., & Wen, C. (2021). Second-trimester maternal serum D-dimer combined with alpha-fetoprotein and free β -subunit of human chorionic gonadotropin predict hypertensive disorders of pregnancy: A systematic review and retrospective case-control study. *Journal of Translational Medicine*, 19(1), 94. <https://doi.org/10.1186/s12967-021-02718-4>
- Chen, Y., Ning, W., Wen, C., Gu, L., & Chu, X. (2025). Predictive value of the maternal alpha-fetoprotein variant, L2, during the first trimester of pregnancy screening for fetal trisomy 21, trisomy 18, and neural tube defects. *BMC Pregnancy and Childbirth*, 25(1), 844. <https://doi.org/10.1186/s12884-025-07893-6>
- Dai, X., Zhang, H., Wu, B., Ning, W., Chen, Y., & Chen, Y. (2023). Correlation between elevated maternal serum alpha-fetoprotein and ischemic placental disease: A retrospective cohort study. *Clinical and Experimental Hypertension*, 45(1), 2175848. <https://doi.org/10.1080/10641963.2023.2175848>
- Głowska-Ciemny, J., Szmyt, K., Kuszerska, A., Rzepka, R., von Kaisenberg, C., & Kocylowski, R. (2024). Fetal and placental causes of elevated serum alpha-fetoprotein levels in pregnant women. *Journal of Clinical Medicine*, 13(2), 466. <https://doi.org/10.3390/jcm13020466>
- Głowska-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. <https://doi.org/10.5603/GP.a2022.0148>
- Hu, Z., Liu, X., Li, L., Jia, C., Li, D., & Liu, R. (2014). Predictive value of abnormal second-trimester maternal serum triple screening markers for adverse pregnancy outcomes. *Zhonghua Fu Chan Ke Za Zhi*, 49(10), 749-753. <https://pubmed.ncbi.nlm.nih.gov/25537246/>
- Liu, D., Kaplan, L. A., & Tolou, M. (2024). The effect of in-vitro fertilization procedures on alpha-fetoprotein levels in second-trimester screening for fetal defects. *Obstetrics, Gynecology and Reproductive Sciences*, 8(3). <https://doi.org/10.31579/2578-8965/212>
- Ozdemir, S., Şahin, O., Acar, Z., Demir, G. Z., Ermin, E., & Aydın, A. (2022). Prediction of pregnancy complications with maternal biochemical markers used in Down syndrome screening. *Cureus*, 14(3), e23115. <https://doi.org/10.7759/cureus.23115>
- Sovio, U., Gaccioli, F., Cook, E., Charnock-Jones, D. S., & Smith, G. C. S. (2023). Association between adverse pregnancy outcome and placental biomarkers in the first trimester: A prospective cohort study. *BJOG: An International Journal of Obstetrics & Gynaecology*, 130(12), 1567-1578. doi: 10.1111/1471-0528.17691.
- Udeogu, C. H., Ugwu, M. C., Okwara, J., Ohale, A. C., Ike, O. C., Amadi, N., Ugwu, O., & Eguogwu, F. C. (2021). Levels of maternal serum alpha fetoprotein and beta human chorionic gonadotropin in HIV seropositive pregnant women attending antenatal care at Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria. *Journal of Advances in Medicine and Medical Research*, 33(12), 32-38. DOI: [10.9734/JAMMR/2021/v33i1230939](https://doi.org/10.9734/JAMMR/2021/v33i1230939)