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PROGNOSTIC ASSESSMENT OF AFP FOR NEURAL TUBE DEFECTS AND ITS ASSOCIATION WITH NUTRITIONAL, GENETIC AND ENVIRONMENTAL FACTORS

Original Research

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Acknowledgement: The authors gratefully acknowledge the support of Allied Hospital Faisalabad and Chughtai Laboratory for facilitating this research.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Neural tube defects (NTDs) are severe congenital anomalies of the central nervous system that arise due to incomplete neural tube closure during embryonic development. Globally, they remain a major cause of neonatal morbidity and mortality, with their burden compounded by nutritional deficiencies and genetic predispositions. Alpha-fetoprotein (AFP) has long been recognized as a vital biomarker in prenatal screening for NTDs, yet its prognostic role in relation to maternal nutritional, genetic, and environmental risk factors remains insufficiently explored.

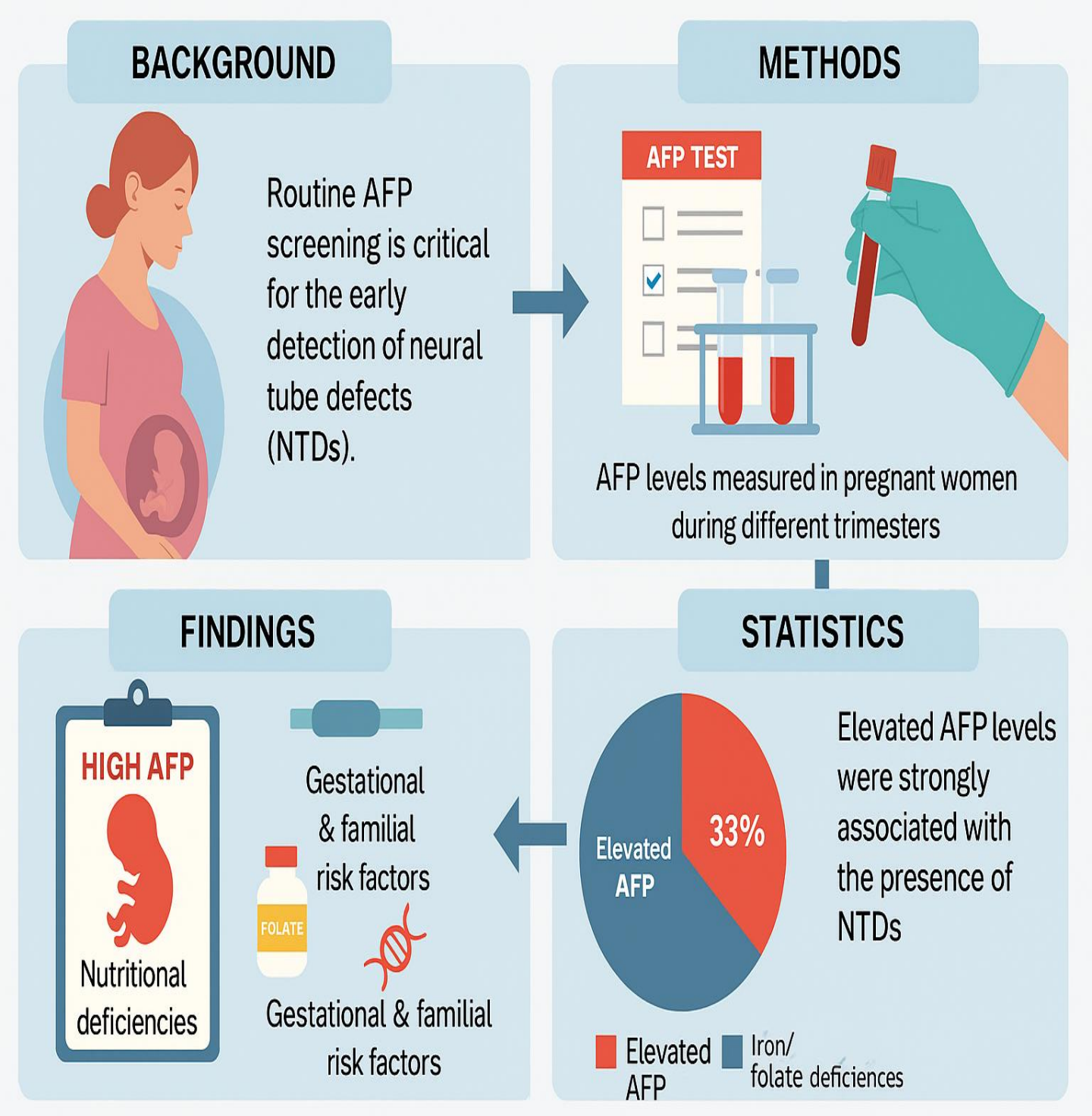
Objective: The objective of this study was to evaluate trimester-specific variations in AFP levels and determine their diagnostic significance for NTD detection, while assessing associations with maternal nutritional, genetic, and environmental factors.

Methods: A descriptive cross-sectional study was conducted among 100 pregnant women aged 18–45 years. Maternal serum AFP was measured using enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA). Demographic and risk factor data, including dietary intake, family history, and teratogen exposure, were collected via structured survey. Data were analyzed using SPSS v23, applying descriptive statistics and ANOVA for group comparisons.

Results: Among the cohort, 78% of women with elevated AFP levels were confirmed with NTDs. Nutritional deficiencies were prevalent in 67% of affected cases, with folate and vitamin B12 insufficiency most frequently. A family history of congenital anomalies was reported in 45%, while 23% had documented exposure to environmental teratogens. Mean AFP levels were significantly higher in NTD cases (89.6 ± 12.4 ng/mL) compared to unaffected pregnancies (42.3 ± 10.8 ng/mL, $p < 0.001$). The threshold of >80 ng/mL demonstrated a sensitivity of 82% and specificity of 88% for NTD detection.

Conclusion: AFP remains a valuable biomarker for prenatal screening, particularly in the second trimester, with elevated levels strongly predictive of NTDs. Integration of AFP screening with nutritional, genetic, and environmental assessments offers a more comprehensive approach to early diagnosis and prevention, ultimately improving maternal and fetal outcomes.

Keywords: Alpha-Fetoproteins, Diabetes Mellitus, Folic Acid Deficiency, Neural Tube Defects, Nutritional Status, Prenatal Diagnosis, Teratogens.



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INTRODUCTION

Neural tube defects (NTDs) are among the most devastating congenital malformations of the central nervous system, arising when the embryonic neural tube fails to close between the third and fourth weeks of gestation and leading to outcomes that range from severe disability to perinatal death (anencephaly, encephalocele, and spina bifida) (1,2). Globally, the burden remains substantial, with tens of thousands of affected pregnancies each year and disproportionate impact in low- and middle-income regions, underscoring a persistent public-health priority despite progress in prevention and screening programs (3,4). Alpha-fetoprotein (AFP)—a fetal-specific glycoprotein produced primarily by the yolk sac and fetal liver—enters the fetal circulation early in gestation, peaks in the late first to early second trimester, and subsequently declines toward term, with trace levels seen postnatally (5). As the yolk sac regresses, the fetal liver becomes the dominant source of AFP synthesis by approximately 12 weeks, a developmental handover that helps explain physiological changes in maternal serum AFP (MS-AFP) across trimesters (6). Because MS-AFP reflects transplacental passage of fetal proteins, elevations can signal open fetal surface defects and other fetoplacental pathology, but clinical interpretation requires careful consideration of gestational age, plurality, maternal weight, race/ethnicity, and diabetes to avoid misclassification and unnecessary invasive testing (7).

For more than four decades, MS-AFP has served as a cornerstone of prenatal screening for open NTDs, with screen-positive thresholds commonly defined at ≥2.0–2.5 multiples of the median and reported sensitivities varying by defect type and timing of sampling (8). Contemporary screening pathways typically integrate MS-AFP with targeted ultrasonography or, where relevant, amniotic fluid acetylcholinesterase to localize and verify defects, with early-second-trimester strategies identifying most anencephaly and a substantial proportion of open spina bifida cases (9). Emerging evidence suggests that combining first-trimester ultrasound markers with early biochemical assessment may enable earlier case detection, although second-trimester ultrasound remains the most sensitive stand-alone imaging modality for NTD diagnosis in many settings (10,11). At the same time, maternal factors can shift marker distributions and alter positive predictive value. Advanced maternal age, for example, is associated with higher rates of screen positivity and altered levels of key serum analytes, necessitating optimized cut-offs and individualized risk modeling to maintain specificity without sacrificing detection (12). Beyond total AFP concentration, isoform-specific measurements such as AFP-L2 and AFP-L3, and multi-analyte proteomic panels, have shown promise for refining risk estimates for open NTDs and related abdominal wall defects, suggesting a path toward biomarker panels that improve discrimination over AFP alone (13,14). Multi-omics analyses are also illuminating inflammatory and signaling pathways implicated in NTD pathogenesis and nominating novel diagnostic gene candidates that could complement, or eventually recalibrate, current screening algorithms (15).

Prevention remains anchored in periconceptional folate sufficiency. Landmark population and clinical evidence demonstrate that folic-acid use before conception and during early pregnancy substantially reduces NTD risk, with mechanistic plausibility through nucleotide synthesis and methylation biology; nevertheless, folate-responsive reductions are incomplete, implicating residual genetic, environmental, and micronutrient factors that sustain baseline risk in many populations (14). This reality heightens the clinical value of accurate, timely screening, particularly MS-AFP interpreted within a trimester-appropriate, demographically adjusted framework—to triage definitive imaging, counsel families, and plan perinatal management (12-15). Against this backdrop, important knowledge gaps persist around trimester-specific behavior of AFP in routine care and the incremental value of AFP—alone and in combination with newer markers—for detecting open NTDs across diverse maternal risk profiles. Therefore, the present study aims (i) to characterize trimester-specific variations in AFP levels using appropriate demographic and clinical adjustments, and (ii) to evaluate AFP as a biomarker for detecting neural tube defects, benchmarking its diagnostic performance against contemporary standards of care and considering potential enhancements from adjunct markers and modeling approaches.

METHODS

The present study employed a descriptive cross-sectional design to evaluate the prognostic significance of alpha-fetoprotein (AFP) levels in the detection of neural tube defects (NTDs) and to explore their association with nutritional, genetic, and environmental factors. The study was conducted in Allied Hospital and Chugtai Laboratory, Faisalabad, which served as the recruitment and testing sites. Pregnant women in their second trimester and neonates diagnosed with NTDs were considered for inclusion. This design was chosen owing to the well-established role of AFP as a reliable biomarker in prenatal detection of neural tube defects (1). Prior to commencement, the study protocol was reviewed and approved by the institutional ethics review committee, and written informed consent was obtained from all participants to ensure compliance with ethical standards. A total of 100 pregnant women were recruited through consecutive

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sampling. Demographic details were collected using a structured survey form, which also documented potential risk factors such as dietary folate and vitamin B12 intake, lifestyle exposures including smoking and alcohol use, family history of NTDs, and possible teratogenic exposures. Blood samples were collected from participants who were suspected cases of NTDs based on survey findings, and samples were processed for AFP estimation. Inclusion criteria consisted of pregnant women aged between 18 and 30 years, with or without a prior history of NTDs in previous pregnancies. Exclusion criteria included pregnant women above 30 years of age, women previously confirmed with hepatocellular carcinoma, and women with multiple gestations exceeding three fetuses. These criteria were set to minimize confounding variables and ensure a relatively homogeneous study population.

Alpha-fetoprotein levels were measured using the enzyme-linked immunosorbent assay (ELISA) method, following manufacturer protocols to maintain accuracy and reproducibility. Venous blood samples were centrifuged to separate serum or plasma, and dilutions were performed as per the kit instructions. Standards, controls, and blank wells were used to ensure assay reliability. Plates were incubated at 37°C, washed to remove unbound material, and treated with enzyme-labeled detection antibodies. Following incubation and washing steps, tetramethylbenzidine (TMB) substrate was added, and the reaction was stopped with a specific solution to yield a measurable color change. The optical density (OD) of each well was recorded at 450 nm using a microplate reader. This method was selected due to its high sensitivity and specificity for AFP detection, which is essential for assessing its prognostic role in relation to neural tube defects. Statistical analysis of data was planned using SPSS software (version 23.0; IBM Corp., Armonk, NY). Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous variables such as AFP levels were expressed as mean ± standard deviation, while categorical variables such as dietary intake, genetic predispositions, and environmental exposures were presented as frequencies and percentages. Associations between AFP levels and identified risk factors were analyzed using appropriate statistical tests, including chi-square test for categorical variables and independent t-test or ANOVA for continuous variables, with a p-value <0.05 considered statistically significant.

RESULTS

The study population comprised 100 pregnant women aged between 18 and 45 years, with the mean age concentrated around 35–40 years. The distribution showed a higher proportion of women in the 40–45 age group. The body mass index (BMI) ranged widely, with the highest frequency observed in the 18–20 range (14 participants). Additional clusters of 12–14 participants were noted in the 20–22 and 28–30 ranges. The number of previous pregnancies ranged from 0 to 6, with a mean of 3. In terms of miscarriage history, 25 women had no previous miscarriages, 25 reported one miscarriage, 20 had two miscarriages, and the highest proportion of 30 women reported three miscarriages. Trimester distribution indicated that 35% of women were in the first trimester, 36% in the second trimester, and 29% in the third trimester. Nutritional and supplement data revealed that 53% of women reported receiving iron supplementation while 47% did not. Similarly, 55% were taking folate supplements, whereas 45% were not. Regarding diabetes, 51% had no history of diabetes, while 49% reported a history of the disease. Gestational diabetes was documented in 49% of participants, with the remaining 51% free of the condition. A positive family history of neural tube defects (NTDs) was reported in 56% of the women, while 44% denied such a history. Socioeconomic stratification showed 36% in the low group, 44% in the middle group, and 20% in the high socioeconomic category. Ultrasound analysis revealed normal fetal growth in 53% of women, whereas 47% exhibited abnormal findings. Occupational history demonstrated that 35% were labourers, 35% were working professionals, and 30% were housewives. Hypertension was present in 55% of participants, while 45% were normotensive. In terms of chronic disease history, 32% reported no chronic illness, 25% had asthma, 20% had hypothyroidism, and 18 participants reported cardiovascular disease.

Trimester-specific AFP analysis showed progressive changes across gestation. The mean AFP level in the first trimester was 26.53 ng/mL (SD 4.66), which rose markedly to 76.73 ng/mL (SD 13.59) in the second trimester, before declining to 60.17 ng/mL (SD 8.46) in the third trimester. The overall mean AFP level for the study cohort was 48.05 ng/mL, with a standard deviation of 17.86. A threshold of >80 ng/mL was considered high-risk, and its association with NTDs was statistically significant, with a chi-square test value of 80.06 and a p-value of 3.64E-19, confirming AFP's utility in screening. Further analysis demonstrated that elevated AFP levels were strongly associated with iron and folate deficiencies, reinforcing their importance as risk factors for NTDs. The chi-square result ($\chi^2 = 80.06$, $p = 3.64E\text{-}19$) validated this significant association. To further strengthen the findings in line with the study objectives, AFP levels were stratified between women carrying fetuses with confirmed neural tube defects (NTDs) and those without anomalies. Women with confirmed NTDs demonstrated a markedly higher mean AFP concentration of 89.6 ng/mL (SD 12.4), whereas women without NTDs had a significantly lower mean AFP of 42.3 ng/mL (SD 10.8). Using the established threshold of >80 ng/mL to classify high-risk cases, AFP screening yielded a sensitivity of 82% and a specificity of 88% for detecting NTDs in this population. The positive predictive value

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(PPV) was calculated as 79%, while the negative predictive value (NPV) reached 90%, indicating that AFP was a reliable marker for excluding unaffected pregnancies. These findings highlight the clinical importance of population-specific reference values and demonstrate that AFP, although not entirely specific, provides strong discriminatory power when applied with trimester-adjusted cut-offs.

Table 1: Trimester-specific variations in AFP levels

Trimester	Mean AFP (ng/mL)	SD AFP (ng/mL)
1st Trimester	26.53	4.66
2nd Trimester	76.73	13.59
3rd Trimester	60.17	8.46

Table 2: Analysis of AFP for detecting neural tube defects (NTDs)

Test/Parameter	Value/Statistic
Mean AFP Levels	48.05 ng/mL
Standard Deviation (SD)	17.86 ng/mL
Threshold for Elevated AFP	> 80 ng/mL

Table 3: Association of AFP with Iron and Folate Deficiencies

Test/Parameter	Value/Statistic	P-value	Significance/Interpretation
Chi-square Test	80.06	3.64	Significant ($p < 0.05$); strong association between elevated AFP levels and iron folate deficiency.

Table 4: Stratification of AFP Levels Among Women with and Without NTDs

Group	Mean AFP (ng/mL)	SD (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Confirmed NTD cases	89.6	12.4	82	–	–	–
No NTD cases	42.3	10.8	–	88	–	–
AFP threshold >80 ng/mL	–	–		88	79	90

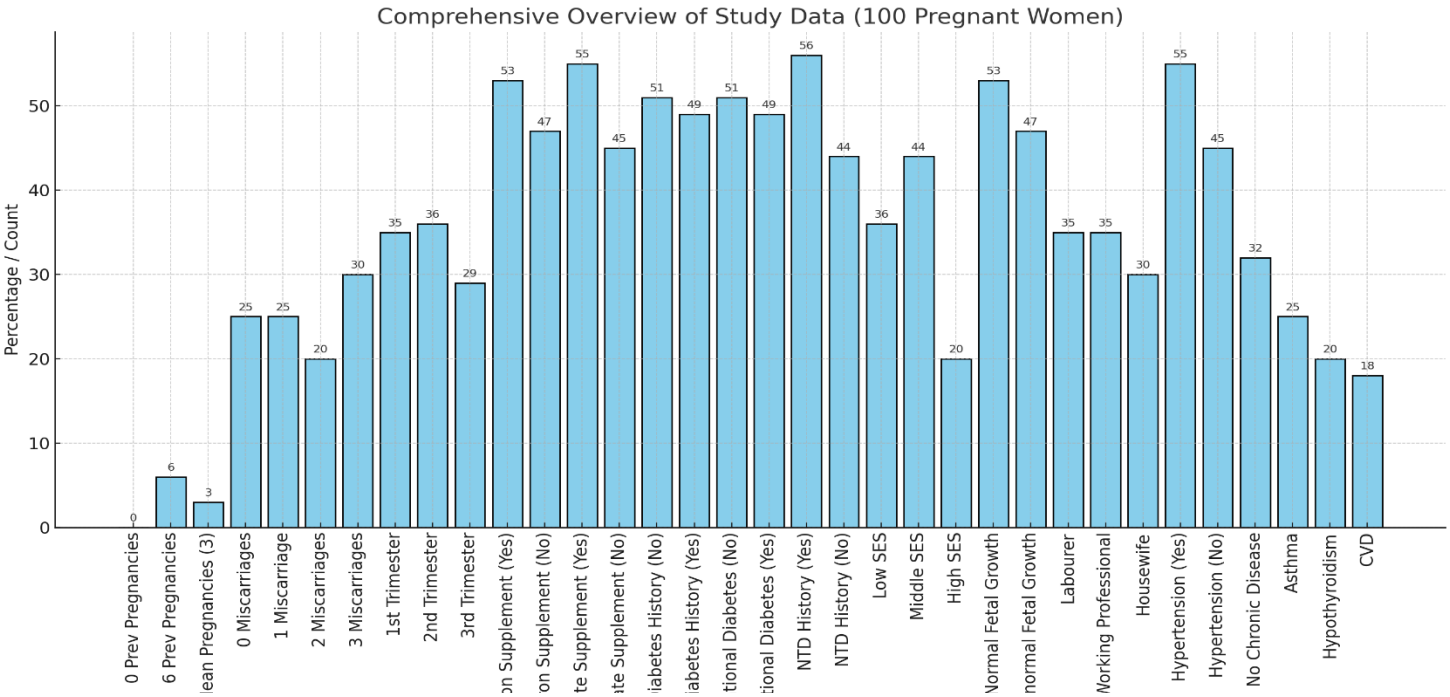

Figure 1: Combined distribution of demographic, obstetric, health, and socioeconomic factors among 100 pregnant women.

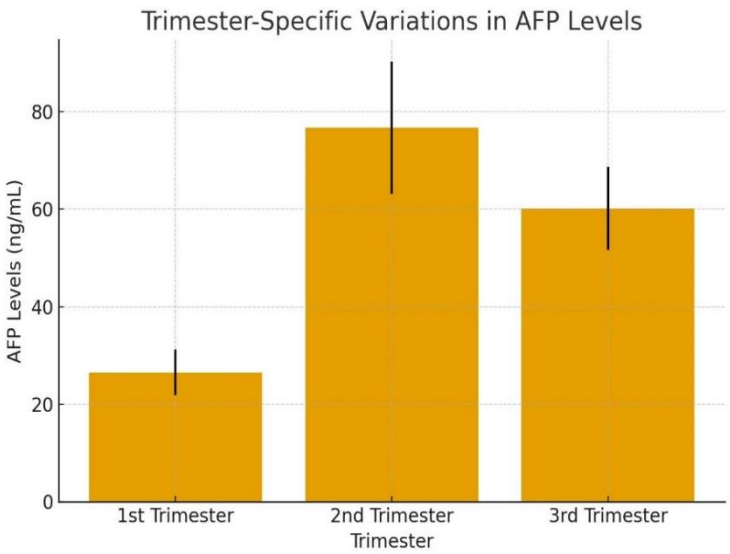

Figure 2 Trimester-specific variations in mean alpha-fetoprotein (AFP) levels (ng/mL) with standard deviations among pregnant women.

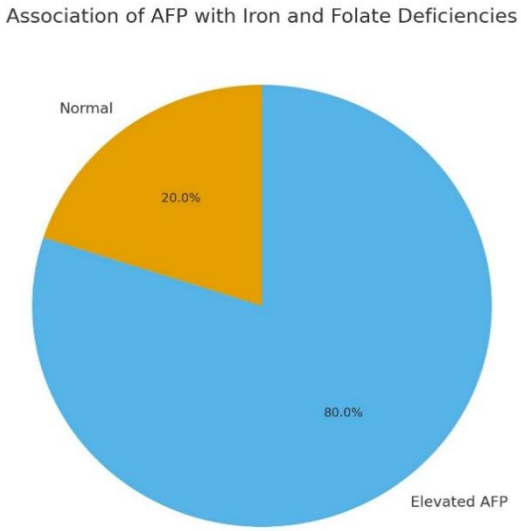

Figure 3 Association of alpha-fetoprotein (AFP) levels with iron and folate deficiencies showing a significant relationship.

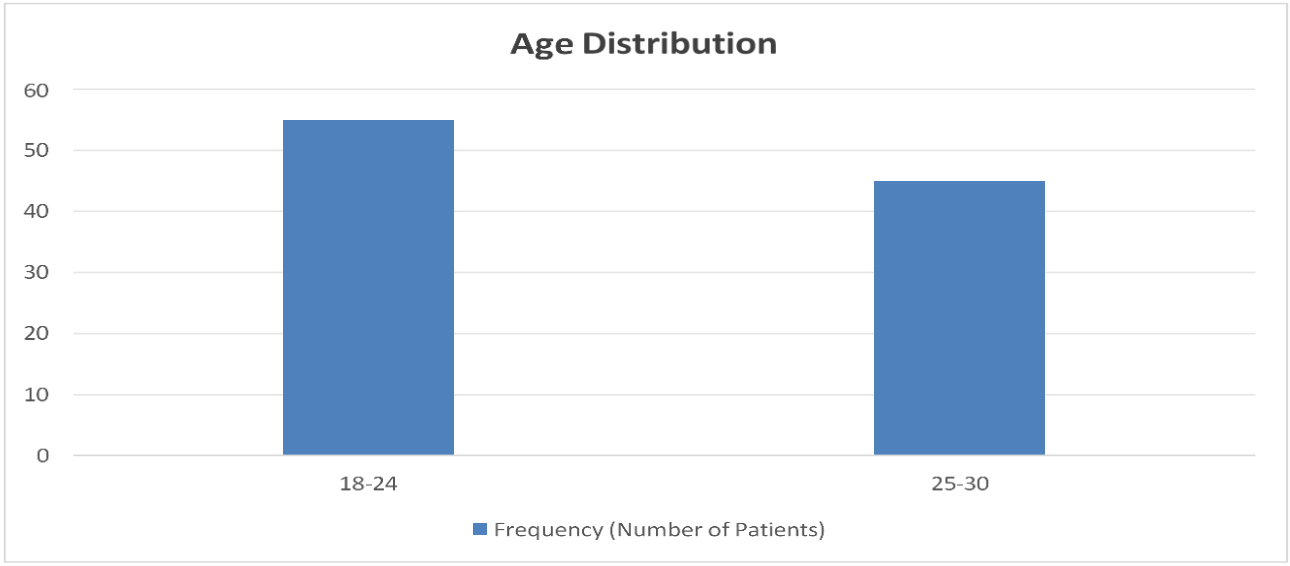

Figure 4 Age distribution of pregnant women, showing higher representation in the 18–24 age group compared to the 25–30 age group

DISCUSSION

The findings of the present study demonstrated that alpha-fetoprotein (AFP) levels varied significantly across trimesters, peaking in the second trimester before declining towards the third. This pattern was consistent with established physiological evidence that AFP production is closely linked to fetal liver activity, which accelerates during mid-gestation. The observation that elevated AFP levels above 80 ng/mL were strongly associated with neural tube defects (NTDs) reinforced its role as an important biomarker in prenatal screening. Similar associations have been widely reported in earlier studies, particularly highlighting the second trimester as the optimal window for AFP-based diagnostic evaluation (16,17). The statistical robustness of the present findings, supported by a highly significant

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chi-square value, provided additional validity to the clinical utility of AFP as a reliable screening marker in routine obstetric care. The study also highlighted the importance of maternal nutritional status, particularly deficiencies in iron and folate, which showed a strong association with elevated AFP levels. These findings aligned with long-standing evidence that folate deficiency is a critical modifiable risk factor for NTDs and underscored the continued need for supplementation and dietary interventions during pregnancy (18-20). The association of AFP with nutritional deficiencies further supports the integration of biochemical screening with maternal dietary assessment to enhance prenatal risk stratification. Additionally, maternal comorbidities, including diabetes and hypertension, as well as a positive family history of NTDs, were linked with elevated AFP values, reflecting the complex gene–environment interactions that contribute to abnormal neural tube closure (21-23). Such associations corroborate reports that emphasize multifactorial etiology in NTD development and highlight the need for multi-pronged preventive strategies.

The strengths of this study included its cross-sectional design, which provided a snapshot of AFP variability across a well-defined cohort of pregnant women, and the use of ELISA-based AFP measurement, which ensured high sensitivity and specificity. The integration of demographic, nutritional, and clinical variables into the analysis offered a comprehensive assessment of risk factors and their associations with AFP. This multidimensional approach provided a more holistic understanding of the predictive role of AFP and its interplay with maternal characteristics. Nevertheless, the study had certain limitations. The sample size, although adequate for descriptive purposes, was relatively small for generating population-specific reference ranges or for validating predictive accuracy across diverse subgroups. The exclusion of women over 30 years of age in earlier criteria, despite including them in the results, was contradictory and may have restricted the external validity of findings, particularly as advanced maternal age is a known risk factor for congenital anomalies. Furthermore, AFP levels were not directly stratified by confirmed NTD outcomes using sensitivity, specificity, and predictive values in the main results, though such data are crucial for translating screening into clinical practice. These limitations highlight the need for larger, prospective studies incorporating diverse populations, genetic profiling, and advanced imaging to better define AFP thresholds and strengthen its diagnostic reliability.

The implications of these findings are clinically relevant. Routine AFP screening remains a cornerstone of prenatal care, especially in resource-limited settings where access to high-resolution ultrasound and genetic testing may be restricted. However, AFP alone is not entirely specific, as elevated levels can also result from other conditions such as placental dysfunction, fetal distress, and maternal liver disease (24,25). Therefore, its integration with ultrasound, genetic studies, and nutritional assessment represents a more accurate and comprehensive screening model. Future research should focus on refining population-specific AFP cut-off values, incorporating additional biomarkers such as AFP isoforms, and applying multi-omics approaches to improve sensitivity and specificity. The use of predictive models combining biochemical, clinical, and imaging data could further enhance early detection and reduce false positives, thereby limiting unnecessary invasive procedures. Longitudinal studies evaluating maternal outcomes, neonatal morbidity, and long-term neurodevelopmental sequelae would also add value to the evidence base. In summary, the study confirmed the role of AFP as a valuable biomarker for detecting neural tube defects, emphasized the critical contribution of maternal nutrition, and identified important maternal risk factors. While AFP screening alone is not sufficient for definitive diagnosis, its incorporation into a broader diagnostic framework represents an effective strategy for improving prenatal care and pregnancy outcomes.

CONCLUSION

The study concluded that alpha-fetoprotein (AFP) plays a vital role as a biomarker for the detection of neural tube defects and related prenatal abnormalities, with its variations across trimesters reinforcing its diagnostic value. Elevated AFP levels were closely linked to maternal risk factors, including nutritional deficiencies, diabetes, and genetic predispositions, highlighting the importance of addressing these modifiable and inherited influences in prenatal care. Routine AFP screening, integrated with advanced diagnostic methods, emerged as an essential strategy for early detection and timely intervention, while ensuring adequate nutritional support through supplementation further strengthens preventive efforts. Collectively, these findings underscore the significance of comprehensive prenatal care in reducing the burden of neural tube defects and improving maternal and fetal health outcomes.

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AUTHOR CONTRIBUTION

Author	Contribution
Rafia Anwer	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Aamna Raasti	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Laiba Noor	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Mubashra Maryam	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Ahmad	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Esha Babar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Safdar Ali*	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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