ORIGINAL ARTICLE

The Relationship between Second Trimester Maternal Serum Alpha Fetoprotein with Adverse Pregnancy Outcome in a Tertiary Care Centre

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ABSTRACT

Background: Maternal serum alpha-fetoprotein (AFP) screening in the second trimester was initially discussed as a method of detecting neural tube defect and trisomy 21. But it has also been noticed that high AFP level is linked to other poor pregnancy outcomes, such as intrauterine growth restriction (IUGR), congenital anomalies, and preeclampsia. **Objectives:** To establish the correlation between the level of AFP in the maternal serum in the second trimester and poor pregnancy outcomes among pregnant women that presented at Usmanu Danfodiyo University Teaching Hospital during antenatal clinic, Sokoto. **Methods:** This was a prospective study in the form of a cohort study, in which selected pregnant patients who booked antenatal care at the teaching hospital were enrolled in the study. The enrolment of participants took place at 16-18 weeks of gestation and they were followed until the parturition. Socio-demographic and clinical data were gathered with the help of a self-structured questionnaire. The measurement of maternal serum AFP was carried out by a completely automated chemiluminescence (Roche) test at 16 and 18 weeks of gestation. When levels of AFP were above 110 ng/ml (>2MOM), an elevated level was determined. The analysis of data was performed with the help of SPSS version 20, and the Chi-square test was employed to test the correlation between AFP levels and pregnancy outcomes, setting the significance level equal to $p < 0.05$. these women were followed till delivery and any adverse maternal and foetal outcomes were documented and correlated with the alpha-fetoprotein levels. **Results:** AFP level was on average 89.01 ng/ml; median of 55 ng/ml. The option of increased AFP was found among 28.6 percent (115/402) of participants. A significant association between elevated AFP and most fetal and maternal outcomes was not identified ($p > 0.05$), whereas the prevalence of IUGR (50%) and congenital anomalies (50%) outcomes among women with elevated AFP levels was higher, so a possible clinical association was considered, and was identified. **Conclusion:** In the present cohort, although elevated maternal serum AFP in second trimester was not significantly related to adverse fetal or maternal outcome, trends were towards elevated risk of IUGR and congenital abnormalities in women with elevated AFP levels, indicating that AFP may still have a role as risk stratification agent. Routine monitoring and closer antenatal surveillance are recommended in cases of unexplained elevated AFP to improve perinatal outcomes.

Keywords: Second Trimester, Maternal, Alpha Fetoprotein, Adverse Outcome, Pregnancy

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Introduction

Alpha-fetoprotein (AFP) in the form of a glycoprotein is the major second-trimester prenatal screening test whose production is primarily facilitated by the fetal liver and yolk sac.[1] Even though initially AFP in maternal serum has been tested in connection with the identification of neural tube malformation and also with non-random trisomies 21, high levels of the AFP in the maternal serum have been later in connection with several other poor pregnancy outcomes, which include intrauterine fetal demise (IUFD), preeclampsia, intra-uterine growth restriction (IUGR), and early birth.[2]

Second-trimester AFP screening is useful in a clinical setting especially in the tertiary care where the purpose of the test is to detect potential bad pregnancy outcomes. Increased levels of AFP (which are sometimes considered to be more than 2 multiples of the median, or MoM) can be a sign of placental deficiency, birth defects, or other minimal disorders of the maternal-fetal physiologic equilibrium.[3] Despite the fact that the exact biomechanisms remain to be investigated, pathways through placental vascular malperfusion, inflammation, and compromise of maternal-fetal exchange have been postulated.[4]

Multiple studies have indicated the risk of poor fetal outcomes, including low birth weight, congenital anomalies, preterm birth, in the case of elevated AFP.[5] Nevertheless, AFP predictive value of such complications is incongruent among different populations and healthcare settings and its non-routine application aside of neural tube defect screening remains controversial. Moreover, although an increase in AFP can be a sign of fetal or placental pathology, an increase in AFP can be a non-specific condition that may be found in otherwise normal pregnancy.[6]

Considering that uncertainty remains and few regional data, particularly in sub-Saharan African populations, additional assessment of AFP as a predictive marker should be performed. This paper thus set out to establish the association between maternal serum alpha-fetoprotein levels second-trimester and adverse pregnancy outcome in women seeking antenatal care in Usmanu Danfodiyo University Teaching Hospital, Sokoto. The result of the study can help increase early detection of complicated deliveries and facilitate appropriate clinical responses to provide better maternal and fetal outcomes under similar scenarios.

Method

Design/Setting of the Study

This research was prospective cohort study carried out at the antenatal unit of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, in the North Western region of Nigeria. The study took Six months on a continuous enrolment of the participants.

Study population and Sampling Method

The population of the study included pregnant women who booked and attend antenatal care in UDUTH. A convenient sampling methodology was used such that an average of 20 women have been recruited in a week out of the number of women in the clinic that was around 40-45 per week, the participants were selected randomly. The final analysis included 402 women and was monitored accordingly so as to see the objective of the study.

Inclusion and Exclusion criteria

Inclusion criteria :

- Gestation period of 16-18weeks as determined by the last menstruation period or early ultrasonography (less than 18 weeks)
- Single pregnancies
- A signed informed consent was obtained by women

Exclusion criteria:

- Twin pregnancies
- Existing chronic ailments (e.g., diabetes, high blood pressure, sickle cell sickness)
- Fetal malformations known as a result of early ultrasonography
- loss to follow up or incomplete data

Data Collection instruments and procedures

A questionnaire was designed and applied as a self-administered questionnaire with a structured format, collecting socio-demographic features, obstetric history, and any other related medical history. Biometrics Bodyweight and height were measured and Body Mass Index (BMI) was obtained.

Blood (5ml) was obtained through sterile tubes between the 16th and 18th week of gestation by venous blood sampling in all individuals. Blood cells were left to clot in the room temperature before centrifuging at a speed of 3,000 rpm within 10 minutes to bring out clear serum. The fractions separated were transferred promptly into labeled cryovials and kept in -20^{0}C until the time of analysis.

The determination of AFP was carried out on fully automated chemiluminescence immunoassay analyzer (Roche Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany). It was performed by well trained laboratory scientists in the UDUTH Chemical Pathology Laboratory under normal biosafety conditions. The internal quality control measures were implemented daily through delivery manufacturer controls to get accuracy and precision of results. External quality control was assured by the involvement in laboratories in proficiency testing. The samples with haemolysis, lipaemia, or turbidity were not subjected to an analysis as it removes the integrity of a specimen.

Key Terms Definition

Fetal outcomes: Encompassed live birth, Intrauterine fetal demise (IUFD), and Intrauterine growth restriction (IUGR)

Maternal outcomes: captured preeclampsia, abruptio placenta, PROM, post delivery bleeding (PPH) and anaemia

Pregnancy outcomes: This is the mode of delivery and the total rate of complication throughout the time of pregnancy or delivery.

AFP levels-elevated: Elevated AFP was determined by cutoff in serum levels above the value of 110 ng/ml which is greater than 2 MoMs as based on the distribution of AFP in the study group. The median value of AFP in the present study was 55 ng/ml; hence, 2x55 ng/ ml = 110 ng/ml was utilized to establish the cut-off level of AFP as elevated. This approach complies with the available literature, as there are values >2.0 MoM defined as abnormal and clinically meaningful in determining pregnancies with a risk of adverse outcomes[7].

Factors Affecting AFP and How They Were Controlled

The values of the AFP in maternal serum may be affected by various physiological and pathological conditions, such as multiple gestation, inaccuracy in gestational dating, the weight of mothers, diabetes in mothers, fetal malformations, and abnormalities in the placenta.[8] In order to reduce confounding:

Women who carried singleton were selected only.

The gestational age was properly calculated by first trimester ultrasound (<18 weeks) or dating last menstrual period (LMP).

Women with known chronic diseases (diabetes e.g., hypertension, sickle cell disease) or identified congenital fetus defects via ultrasound were not included.

The BMI of the mothers was noted and high outliers were checked upon the analysis of the data.

Samples exhibiting gross hemolysis, lipaemia or turbidity were not analyzed for AFP because of the doubts about the integrity of the samples.

Determination of the sample size

Using the Cochran formula, the study population was computed at 95 percent confidence level, 5 percent margin of error and 50 percent prevalence and means that a sample size of 384 is needed. To offset the anticipated 10 per cent attrition rate, however, 402 patients were recruited to take part in the study. Four hundred and two participants were successfully recruited and it was followed to delivery.

2.7 Follow-up and outcome measurement

The participants were followed up till delivery and the results evaluated on the clinical records. The fetal outcomes were devised in terms of IUFD, IUGR, and birth asphyxia through use of standard obstetric and pediatric terms. Birth asphyxia was described as Apgar score <7 after 5 minutes of delivery. Troublesome issues like preeclampsia, anaemia and PPH in the mothers were identified with meet and greet criteria.

Analysis of data

The data were evaluated on SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous measures (e.g. the AFP levels) were expressed as the mean, and those categorical (e.g. fetal outcomes) were expressed as frequencies and percentages. Associations between AFP levels and pregnancy outcomes were evaluated using Chi-square or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant.

Ethical considerations

The Ethical Review Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto gave ethical approval. Every participant was enrolled after written informed consent. The study did not compromise data confidentiality and anonymity.

Results

Socio-Demographic Data

The number of pregnant women that took part in the study was 402. The percentage of age distribution indicated that most were under the age of 20-29 years (29.72%) followed by 37.3 per cent under the age of 30-39 years with only 1.5per cent in the age bracket of 40-49 years. The age had a mean of 30.4 ± 4.5 years (range 18-45 years).

Regarding ethnic distribution, majority of the respondents were of Hausa/Fulani background (66.7%), the Igbo/Yoruba ethnic group made 12.2% and 10.2% respectively. The rest of 10.9 percent were other ethnics. Concerning the educational level, 59.2 percent of the respondents had tertiary education, 24.4 percent had secondary education, 9.7 percent had primary education and only 6.7 percent were without any educational level.

The same social history was associated with low reported prevalence of substance use: 0.7 percent of

patients responded to cigarette smoking and 3. 0 percent responded to alcohol intake. The rest 96.3 percent did not claim to use any substances. Nonetheless, excessive screening on the presence of other forms of psychoactive substances like marijuana, shisha or non-prescribed drugs was not done in the questionnaire.

As far as the medical history is concerned, 91.0 percent of the respondents did not have any chronic medical conditions. Out of 9 percent of comorbidities, the most prevalent ones were hypertension (3.7 percent) and diabetes mellitus (2.0 percent). These underlying medical conditions were mentioned even though they are not the major outcomes of this study, but have a potential implication of pregnancy outcomes. Table I shows a full disintegration of the socio-demographic features.

Table I. Socio-demograhic characteristics of the participants

Socio-demographic characteristics	Frequency	Percent (%)
Age in years		
< 20 years	26	6.5
20 – 29	220	54.7
30 – 39	150	37.3
40 – 49	6	1.5
Total	**402**	**100**
Tribe		
Hausa/Fulani	268	66.7
Igbo	49	12.2
Yoruba	41	10.2
Others	44	10.9
Total	**402**	**100**
Educational status		
No formal education	27	6.7
Primary	39	9.7
Secondary	98	24.4
Tertiary	239	59.2
Total	**402**	**100**
Social history		
Smoking	3	0.7
Alcohol	12	3.0
None	387	96.3
Total	**402**	**100.0**
Past medical history		
Diabetes	8	2.0
Hypertension	15	3.7
Sickle cell disease	7	1.7
Asthma	6	1.5
None	366	91.0
Total	**402**	**100**

The average maternal serum value of alpha-fetoprotein (AFP) consist of 89.01 ng/ml (SD: 51.3), meanwhile, the median was 55 ng/ml. Taking two times multiples of the median (MoM, or 110 ng/ml) as a cutoff, 28.6 percent (n=115) of the participants were ruled as possessing high AFP levels, whereas 71.4 percent (n=287) were given normal AFP.

Correlation of AFP Levels with the Fetal Results

Fetal outcomes were considered as any condition which has direct fetal or neonatal consequence (premature birth, prematurity (delivery <37 weeks); birth asphyxia (Apgar score <7 at 5 minutes); intrauterine fetal demise (IUFD), intrauterine growth restriction (IUGR: birth weight <10th percentile of gestational age), or congenital anomaly (structural defect diagnosed at birth). A statistically significant association between having an elevated AFP and individual fetal complications was not found among women with an elevated AFP.

About three-quarters of normal participants (70.8 %) indicated no complication of the fetus in comparison to a quarter of participants with abnormal AFP (29.2 %) and this provided no significant correlation between fetal outcome and AFP level (P=0.34).

Correlation of AFP Levels to Maternal Data

Maternal outcomes were diseases directly involving the pregnant woman i.e. preeclampsia, postpartum hemorrhage (PPH), anaemia (PCV <30%), chorioamnionitis and abruptio placentae.

Abruptio placentae was the only condition that had a potential link (50.0 percent of incidences were in the high AFP group) although this was not statistically significant (p=0.06).

Association Between AFP Levels and Composite Pregnancy Outcomes

Composite pregnancy outcomes factored in both fetal and maternal issues such as mode of delivery, IUFD, IUGR, congenital anomaly, preeclampsia, PROM, placenta previa, and perinatal morbidity (this is any adverse neonatal condition that has to be admitted in the NICU). The result was not statistically significant, with no significant association with overall pregnancy outcome between high AFP and lower AFP (p=0.17, 2 x test). Cases of IUGR and congenital anomalies were slightly higher in elevated AFP (50.0 percent each, but cases were not statistically significant).

Table 2. Relationship Between AFP Levels and Pregnancy Outcomes

Outcome	Normal AFP (n, %)	Elevated AFP (n, %)	p-value	X^2/Fisher's
Fetal Outcomes				
Prematurity	8 (80.0%)	2 (20.0%)	0.33	2.55*
Birth Asphyxia	4 (57.1%)	3 (42.9%)	0.42	1.34
IUFD	3 (75.0%)	1 (25.0%)	0.67	3.02
Congenital anomaly	2 (50.0%)	2 (50.0%)	1.00	1.00
IUGR	10 (66.7%)	5 (33.3%)	0.51	1.10*
No complication	194 (70.8%)	80 (29.2%)	0.34	93.20*
Maternal Outcomes				
Preeclampsia	27 (71.1%)	11 (28.9%)	0.45	6.38*
Anaemia	3 (42.9%)	4 (57.1%)	0.27	4.76
Abruptio placenta	1 (50.0%)	1 (50.0%)	0.06	2.47
PPH	2 (100.0%)	0 (0.0%)	0.49	1.02
Chorioamnionitis	1 (100.0%)	0 (0.0%)	1.00	1.00
Preterm labor	2 (100.0%)	0 (0.0%)	0.40	1.14
No complication	185 (70.3%)	78 (29.7%)	0.39	92.55*
Composite Outcomes				
Any adverse outcome	155 (67.7%)	74 (32.3%)	0.17	89.80*

Key: IUGR: Intrauterine growth restriction, IUFD: Intrauterine Fetal Demise. PPH: postpartum haemorrhage
*Chi-square (X^2), Unmarked=Fishers exact test

Discussion

This research intended to establish the relationship between second trimester- serum alpha- fetoprotein (Alpha-fetoprotein AFP) levels and adverse outcome of pregnancies among women who received antenatal clinical services in a UDUTH. The AFP result showed a prevalence of raised values to be 28.6%, i.e. above 110 ng/ml (>2 multiples of the median in this population). Although this proportion is rather high, no statistically significant relations between increased AFP and multiple single adverse fetal or maternal outcomes have been identified.

We found different results than the studies carried out before, such as the Głowska at al that revealed a close relationship between high AFP and early-onset gestational hypertension and preeclampsia.[9] The mismatch may be related to gestational age at AFP samples, population-based variables; or due to statistical power of the involved study. Though our study has shown a moderate increase in the prevalence of preeclampsia and anemia in patients with high levels of AFP, such results failed to obtain a statistical significance ($p > 0.05$) possibly because of insufficient quantities of the experienced events in the respective subgroups.

Though a high level of AFP were found in 50 percent of women, who had developed abruptio placentae, this association was not statistically significant. Nevertheless, the described tendency is consistent with the well-recognized pathophysiological processes between placental malfunction and placental leakage of maternal serum AFP. Abruption might indicate insufficiency or destruction of the placenta, which may elevate transplacental distribution, consequence, and less fetal AFP might suffer the situation of shipping to the maternal system.[10]

Admittedly, more women with a high level of AFP had babies with intrauterine growth restriction (IUGR) and structural and functional impairments, which confirms earlier studies which identify the connection between the abnormalities and AFP and fetal depredation.[11]

In this cohort, the results were not statistically significant and this was probably because of the low counts, but the same direction of association is evident which highlights the likelihood of AFP as a surveillance marker. High AFP has been hypothetically attributed to the placental insufficiency, which can adversely affect fetal development and fetal defects, especially a defect in the abdominal wall or neural tube, both of which contribute to increased leakage of AFP into the fetus. Our research failed to show any substantial relationship between AFP and preterm birth, IUFD, or birth asphyxia. However, this is contrary to a few reports in literature which may be attributed to different definition of the outcome, different cohort size and whether the elevated AFP was isolated or within a panel of biomarkers.[12] It is agreeable to note that in most studies, the predictive value of AFP is enhanced when used alongside other analytes namely hCG, inhibin A, and uterine artery Doppler indices.

Concerning delivery mode, a correlation between AFP levels to incidence of caesarean delivery was not indicated. Obstetric conditions like cephalopelvic disproportion or fetal distress seem to be the major determinants of the mode of delivery in the current study instead of biochemical risk stratification.

On the whole, the outcomes in the majority of this cohort were not dramatic but the fact that there was a tendency to adverse outcomes in women with high AFP shows its usefulness in risk stratification. Nevertheless, its standalone predictive value does not seem to be large, which is also indicated by previous meta-analyses.[13]

Conclusion

This study was made with an aim of examining an association between second trimester maternal levels of alpha-fetoprotein (AFP) and adverse pregnancy outcome among patients attending antenatal clinic in Usmanu Danfodiyo University Teaching Hospital, Sokoto. The average AFP value was 89.01 ng/ml and highly AFP (>110 ng/ml) in 28.6 percent of the participants. According to the outcomes of the analyzed information, no statistically significant correlation with an increased AFP level was identified with most of the negative fetus/mothers outcomes. Nevertheless, there was a possible correlation between the abnormal level of AFP and some diseases, including the intrauterine growth restriction (IUGR), and congenital abnormalities.

Most of the pregnancies had positive outcomes and the proportion of spontaneous vaginal delivery and minimal obstetric complications was also noted. The particular outcome of the delivery, fetal death and preeclampsia were shown to be not significantly related to the maternal levels of AFP in this group. This implies that although increased AFP can be a factor of clinical concern, it does not always have predictive value of poor maternal and fetal outcomes on its own.

Strengths

The prospective form of the study allowed real-time collection of information and follow-up.

The use of AFP was a fully standardized assay using a validated and fully automated platform, chemiluminescense.

Both the maternal and fetal outcome measures can be included, which makes the analysis more complete.

Limitations

Only one of the tertiary hospitals was used to provide a sample that might be raised as a limit in generalizing populations.

The possible confounding variables of unreported substance use, nutritional condition or infections were not fully measured.

The sample size might have reduced statistical power in the detection of rare outcome associations.

Recommendations

An abnormal screening of maternal serum AFP must be corroborated with other clinical, biochemical and imaging results.

In subsequent researches, it is necessary to utilize cohorts of larger size and greater multicenters and further designate other confounding variables to improve certainty of AFP predictive character on various populations.

It is necessary that the clinicians do not use only the increase of AFP levels to diagnose pregnancy outcome, but combine it into a more comprehensive risk assessment model.

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