American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Evaluation of Some Biochemical Birth Defects Biomarkers (Triple Tests) Neglected Routinely in Ante-Natal Clinic Screening in Older Pregnant Women in Benin City, Nigeria

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Received:24 November 2022; | Revised:27 November 2022; | Accepted:13 December 2022

Abstract

Prenatal triple marker serum screening has become a standard tool used in obstetrical care to identify pregnancies that may have an increased risk for certain birth defects like neural tube defect, trisomy 21 and trisomy 18. According to the American College of Obstetricians and Gynecologists, it has become standard in prenatal care to offer screening tests for neural tube defects and genetic abnormalities. This study therefore aims to evaluate some biochemical birth defects biomarkers (Triple Tests) neglected routinely in Ante-Natal Clinic Screening in older pregnant women from 35 years and above in Benin City (serum levels of alpha feto-protein, estriol and beta human chorionic gonadotropin).

The blood samples were collected from one hundred (100) subjects recruited for the study. Serum was processed from clotted blood samples and analyzed using Enzyme Linked Immunosorbent Assay (ELISA) method. Ultrasound scan was also adopted to confirm the laboratory results. All the results were analyzed statistically using student T-test and Chi square. A prevalence of $P \leqslant 0.05\%$ was considered as statistically significant.

From the study, the results obtained showed significant difference in the values of alpha-feto protein, 141.02 ± 80.19ng/ml and 163.11 ± 112.69ng/ml, at $P < 0.026$ for test and control subjects respectively. The estriol, also, exhibited a significant difference in the result of test subjects (2108 ± 194.82) and control (1957.14 ± 422) at $P< 0.023$. The ultrasound scan used to confirm the laboratory results showed some abnormalities like neural tube defects, hydrocephalus, anencephaly and polyhydramnios. The study concluded that abnormal pregnancy outcome is strongly associated with increase in maternal age. Also, it

showed that triple screen is to be performed during pregnancy preferably in the second trimester to classify a patient as either high or low risk for chromosomal abnormalities and neural tube defects. Thus, there is need to include these biomarkers in routine antenatal screening, particularly as women advances in age.

Keywords: birth defect, trisomy, hcg, alpha-feto-protein

1. Introduction

Prenatal screening has become an important part of the care of pregnant women in our society. It allows parents to make informed decisions regarding the care of their baby [1] . It is a non-invasive method. It is an important prenatal screening as this helps to reduce the proportion of women who have to undergo invasive diagnostic testing or give birth to babies with some congenital abnormalities.

According to the American College of Obstetricians and Gynecologists, it has become standard in prenatal care to offer screening tests for neural tube defects and genetic abnormalities. The current maternal serum analytes in use in most areas are alpha feto protein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol. Measurement of AFP alone can detect the vast majority of neural tube defects and a small portion of trisomy 21-affected pregnancies in patients of all ages. Adding hCG and unconjugated estriol to this screen increases the rate of detection of trisomies 21 and 18 [2] . The most commonly used test for genetic diagnosis is amniocentesis, but the rate of spontaneous fetal loss related to amniocentesis averages about one in every 200 procedures. Because of this risk, serum analyte testing has become an important, noninvasive first step in detecting patients at risk for congenital abnormalities. Current maternal serum analyte screening helps identify women at risk for neural tube defects(NTDs), trisomy 21 and trisomy 18 [3].

Neural tube defects (NTD) are birth defects of the brain, spine or spinal cord. They usually happen in the first month of pregnancy. These defects include anencephaly, spina bifida and encephalocele. Worldwide, the prevalence of NTD is approximately 1-5/1000 livebirths and the risk of recurrence is 2-3%. An incidence of 0.6 to 1.3 has been reported in USA, whereas in Nigeria, rates of 2.75 to 7/1000 births have been reported. In anencephaly, most of the brain and skull do not develop. Babies with anencephaly are usually either stillborn or die shortly after birth [4].

Down's syndrome also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21 [5]. It is associated with mental retardation, malformation of the heart, gastrointestinal tract, eyes and ears, and early Alzheimer's disease. The parents of the affected individual are usually genetically normal. The second trimester risk is 1 in 270 in women 35 to 40 years of age and 1 in 100 in women older than 40 years [3]. The probability increases from less than 0.1% in 20 year old mothers to 3% in those of age 45. It has long been accepted that women who are 35 years or older at the time of delivery should be offered prenatal diagnosis with amniocentesis or chorionic villus sampling. Although the risk for trisomy 21 increases with maternal age, an estimated 75% of affected fetuses are born to mothers younger than 35 years. Because of this risk, it is important to provide pregnant women who are younger than 35 years with noninvasive screening for this trisomy [3].

Trisomy 18 also known as Edward's syndrome (after the doctor who first described it) is a chromosomal abnormality in which the fetus has three copies of chromosome 18. Among liveborn children, trisomy 18 is the second most common autosomal trisomy after trisomy 21. It occurs in every 6,000 births and is associated with low birth weight, mental retardation and cranial, cardiac and renal malformations. Most infants affected with this trisomy die within the first year of life [6] .

Prenatal triple test is a panel of blood tests that serves as an initial broad medical screening tool, usually done in the second trimester to detect neural tube defect, trisomy 18 and 21. The test include: alpha feto protein, estriol and maternal β -human chorionic gonadotropin (β -hCG). These markers have been shown to be of value in evaluating pregnancies at risk for various anomalies and aneuploidies [7].

Alpha-feto protein (AFP) is a glycoprotein molecule, which has similarity to albumin. AFP was first recognized as a fetal-specific globulin in 1956. It is initially synthesized in the yolk sac and liver of the fetus. A small amount also is produced by the gastrointestinal tract. By the end of the first trimester, nearly all of the AFP is produced by the fetal liver. AFP was one of the first serum tumour markers to serve in the dual capacities of tumour marker and fetal defect marker. Maternal serum alpha-feto protein levels during the first and or second trimester of pregnancy are altered in pregnancies with aneuploidy, neural tube defects and adverse pregnancy outcome [8].

Estriol, the principal circulatory estrogen hormone in the blood during pregnancy, is synthesized by the intact feto-placental unit. Estriol is an estrogen produced by both the fetus and the placenta. Decreased unconjugated estriol has been shown to be a marker for trisomy 21 and trisomy 18. Low levels of estriol also have been associated with overestimation of gestation.

β -human chorionic gonadotropin (β -hCG) is a hormone produced within the placenta. It consists of 2 non-covalently bound subunits. Elevations of hCG are the most specific markers for fetal trisomy 21. Pregnancies affected by fetal trisomy 18 also have reduced levels of uE3 and hCG [9].

2. Justification Of The Study

Pregnant women especially those that are 35 years and older, with family history of birth defects or viral infection during pregnancy, are mostly predisposed to having babies with neural tube defect and other chromosomal abnormalities. These of course cannot be detected by the routine antenatal screening tests done in the hospitals or laboratories; this explains why in advanced countries like UK, the triple test is included as part of the screening tests for pregnant women in order to detect these abnormalities early and good decisions taken. The result of the triple marker screen test show the likelihood of an infant having a genetic disorder, and thus help prepare parents for birth.

The use of these biomarkers is not yet a common practice in Nigeria; hence the need to embark on this study, as there is paucity of information in this area obstetrical practice in Nigeria.

2.1 Aims and objectives

The aim of the study was to evaluate the serum levels of AFP, estriol and β -hCG in older pregnant women, and compare with non-pregnant women,as part of some biochemical birth defects biomarkers (Triple Tests) neglected routinely in Ante-Natal Clinic Screening in older pregnant women from 35 years and above in Benin City

2.2 Materials and methods

The study population consisted of 100 pregnant women, consisting of 50 older women (35 years and above) as test, and 50 younger pregnant women (34 years and below) as control. Informed consent was obtained from each participant after proper notification and information on the nature of the research, benefits as well as confidentiality by using a questionnaire.

2.3 Study design

This is a randomized cross-sectional case control study. A well-structured questionnaire was the instrument of data collection. Informed consent was obtained from each participant after proper notification and information on the nature of the research, benefits as well as confidentiality by using a questionnaire. The questionnaire has two main sections i.e the demographic variables such as age, sex, weight, tribe and basic medical history such as history of birth defects, gestation week of the participants.

Inclusion criteria: Pregnant women from 35 years and above, those with family history of genetic problem, and pregnant women in their second trimester and early third trimester.

Exclusion criteria: Non-pregnant women and pregnant women in their early first trimester and late third trimester

Ethical approval: Ethical approval was sought and obtained from the Research/Ethics Committee of Edo State Ministry of Health, Benin City with reference number- HM.1208/259.

2.4 Sample size and sampling technique

The minimum sample size was obtained using the formula described by [10].

The study population consisted of 100 pregnant women, consisting of 50 older women (35 years and above) as test, and 50 younger pregnant women (34 years and below) as control. About five (5) milliliters of venous blood was drawn from the antecubital vein of each subject using a sterile needle and syringe into a plain container. The blood sample was allowed to clot for fifteen (15) minutes, after which it was centrifuged at 5,000 rpm for five minutes to separate serum from clot. The serum was dispensed into a clean and dry plain tube and freezed immediately at -50C until they are set for analysis.

Estimation of Serum Alpha Feto Protein [11]

The AFP ELISA test kit is based on the principle of a solid phase enzyme-linked immunosorbent assay.

Normal range: Men and non-pregnant women: 0-10ng/ml

15-20 gestation weeks: 10-150ng/ml [11]

Estimation of Serum Estriol [12] .

This is done using enzyme linked immunosorbent assay (ELISA). The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

Normal range: 1st Trimester: 188-2497pg/ml

2nd Trimester: 1278-7192pg/ml

3rd Trimester: 6137-3460pg/ml [12].

Determination of Serum β -hCG using Immunoenzymometric Assay Type 3 [13]

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen.

Normal range: 1st Trimester: 10-1,000mIU/ml

2nd Trimester: 10,000-30,000mIU/ml

3rd Trimester: 5,000-15,000mIU/ml [13].

2.5 Statistical Analysis

The results were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (Chicago IL). Values obtained in this study were presented as mean ± standard error of mean (SEM). Student T-test and Chi square were used. A prevalence of 0.05% was considered as statistically significant.

3. Results

A total of 100 pregnant women were used for the study. 50 were older pregnant women from 35 years and above (test); while the other 50 were younger pregnant women from 34 years and below (control).

Table 1 shows the serum levels of AFP, Estriol and β -hCG, in older pregnant women and younger pregnant women. It was deduced that there was a significant difference between the serum levels of AFP and estriol in older pregnant women compared to younger pregnant women respectively. There was no significant difference in the serum levels of β -hCG of the test subjects and the control subjects.

Table 1: Age, AFP, β-hCG, and estriol levels in test and control subjects

Parameters	Test group (n=50)	Control group (n=50)	t-test	p-value
Age	27.20±4.65	38.48±3.12	-14.253	0.0001
AFP (ng/ml)	141.02±80.19	163.11±112.69	-1.130	0.026*
β-hCG (mIU/l)	653.42±108.38	621.78±138.94	1.270	0.207
Estriol (pg/ml)	2108.86±194.82	1957.14±422.38	2.306	0.023*

*= significant

Table presented in mean ±SEM, p< 0.05 is significant.

p= 2tail probability at α= 0.05.

All values expressed in mean ± SEM (standard error of mean).

Table 2, shows levels of AFP, β -hCG and Estriol in 2nd and 3rd trimester in Test subjects

It was deduced that the serum levels of AFP (113.89 ± 48.71) and estriol (2083.86 ± 186.33) were significantly lower at the 2nd trimester in the test subjects compared to the 3rd trimester ($p < 0.05$). There was no significant difference in the serum levels of β -hCG between the 2nd trimester and 3rd trimester respectively.

Table 2: Levels of AFP, β-hCG and Estriol in 2nd and 3rd trimester in Test subjects

Parameters	2nd trimester (n=35)	3rd trimester (n=15)	t-test	p-value
AFP (ng/ml)	113.89±48.71	204.31±103.03	-3.247	0.004
β –hCG (mIU/l)	657.95±120.26	642.87±76.27	0.447	0.657
Estriol (pg/ml)	2083.86±186.33	2167.20±208.12	-3.400	0.05

Table 3 shows the levels of AFP, β -hCG and Estriol in 2nd and 3rd trimester in control subjects. The serum AFP (110.11 ± 92.38) and estriol (1750.80 ± 429.66) levels were significantly lower at the 2nd trimester in the control subjects than at the 3rd trimester respectively ($p<0.05$). There was no significant difference in the serum levels of β -hCG between the 2nd trimester and 3rd trimester.

Table 3: Levels of AFP, β -hCG and Estriol in 2nd and 3rd trimester in control subjects

Parameters	2nd trimester (n=25)	3rd trimester (n=25)	t-test	p-value
AFP (ng/ml)	110.11±92.38	220.53±105.63	-3.921	0.0001
β -hCG (mIU/l)	599.38±145.58	646.06±130.03	-1.192	0.239
Estriol (pg/ml)	1750.80±429.66	2180.67±281.34	-4.147	0.0001

Table 4, shows the Correlation of age and gestational age with AFP, β -hCG and estriol in test subjects. There was a positive and significant correlation between age and serum AFP (r= 0.279, p= 0.049). There was no significant correlation between the ages of the subjects and serum β -hCG (r= 0.068, p= 0.640).

There was no significant correlation between the age and serum estriol levels of the subjects (r= 0.112, p=0.440). There was a positive and significant correlation between gestational age of the subjects and serum AFP (r= 0.631, p= 0.0001). There was also a positive and significant correlation between the gestational age of the subjects and serum estriol (r= 0.329, p= 0.002). There was a negative correlation between the gestational age and serum levels of β -hCG of the subjects (r= -0.126, p= 0.383).

Table 4: Correlation of age and gestational age with AFP, β-hCG and estriol in test subjects

Parameters	R	p-value
Age vs AFP	0.279	0.049
Age vs β-hCG	0.068	0.640
Age vs Estriol	0.112	0.440
Gestational age vs AFP	0.631	0.0001
Gestational age vs β-hCG	-0.126	0.383
Gestational age vs Estriol	0.329	0.020

Table 5 shows Comparison of ultrasound findings in test and control subjects. It was deduced that there was no significant difference between the findings of ultrasound scan in test and that of control.

Table 5: Comparison of ultrasound findings in test and control subjects with that of serum levels

		Control Group			Chi-square	p-value
		Polyhydramnios N(%)	Normal N(%)	Total		
Test Group	Polyhydramnios N(%)	0(0.0)	5(100.0)	5(100)	0.231	0.630
	Normal N(%)	2(4.4)	43(95.6)	45(90.0)		
	Total	2(4.4)	48(96.0)			

4. Discussion

In this present study, some preliminary and baseline data such as age, tribe, occupation, medical history, among others were collected to see their possible influences on the outcome of the study.

As observed in this study, the rate of having a child with anomalies was statistically significant among women at age 35 years and above, when compared to the younger pregnant women ($p< 0.05$). The three markers of the triple screening test yield results that fall within the normal range in the control group. In contrast, varying results were obtained in the study group.

This is in agreement with the study done by [13] in 1909 who was the first to observe the association between increased maternal age and Down syndrome after examining 350 cases.

According to [15], the prevalence of Down syndrome has been found to increase with maternal age.

This may be as a result of the increasing strong desire to complete formal education before conception and the rising incidence of infertility [16].

In this study, there was a significant difference between the serum levels of AFP in the test subjects compared to the control subjects.

This is in agreement with [17] which stated that there was increased serum AFP in neural tube defect. This is because AFP is thought to leak directly into the amniotic fluid, thereby causing unexpectedly high concentrations of AFP; while

low values of serum AFP may mean that the baby has Down syndrome. The cause of this decrease may be due to the production of an altered AFP molecule with modified turnover or transport properties, or a reduction in the level of AFP synthesis [18]. Thus, too much or too little AFP in a mother's blood may be a sign of a birth defect or other conditions. The serum AFP results lower than normal AFP levels, may mean that the baby has a genetic disorder such as Down syndrome; while those that showed higher than normal AFP levels may mean that the baby has a neural tube defect (an abnormal fetal brain or spinal cord that is caused by folic acid deficiency during pregnancy) such as spina bifida, a condition in which the bones of the spine do not close around the spinal cord, or anencephaly, a condition in which the brain does not develop properly [2]. Women carrying twins or other multiples may also have elevated levels of AFP due to the presence of multiple fetus producing AFP.

According to [19], 2008, significant amount of patients with elevated maternal AFP do not develop birth defects, but there may be an increased risk of obstetric complications like premature rupture of membrane, placenta accreta, increta and packet, also, certain uncommon birth defects of the abdominal wall, as well as certain kidney and bowel defects. In rare cases, maternal serum AFP level is elevated because the fetus is dying or is dead. In addition, maternal weight can have a significant effect on the screening process [20]. AFP, being an overspill protein, having no real function in maternal blood, has no regulated levels. Thus, if the volume of distribution is greater, the concentration of AFP will be smaller [21]. A similar effect but with a lesser degree of correlation is found for hCG [22], but there is no correlation for unconjugated estriol [23].

According to [3], 1995, although the risk for trisomy 21 increases with maternal age, an estimated 75% of affected fetuses are born to mothers younger than 35 years.

Little or no study from Nigeria has confirmed this trend, hence the need for this research.

It was also discovered that there was a significant difference between the serum levels of estriol in the test subjects compared to the control subjects.

Unconjugated estriol levels are decreased in trisomy 21 and trisomy 18. The addition of unconjugated estriol to hCG and AFP screening increases the detection of trisomy 21 in women younger than 35 years [24]. Also, maternal serum uE3 decreases during the second trimester of pregnancy in case of fetal growth restriction [25]. However, low levels of estriol also have been associated with overestimation of gestation, Smith-Lemli-Opitz syndrome (defect in cholesterol biosynthesis) and fetal loss [26].

Furthermore, there was a negative correlation between the maternal age and serum levels of β -hCG of the subjects. This was in agreement with the study by[27], which stated that there was a significant negative association of maternal age with log-transformed hCG concentrations. Thus, high maternal age is associated with low serum β -hCG concentrations. Since hCG is synthesized in trophoblast cells only, the lower hCG concentrations in women of advanced age may reflect functional impairment or delayed proliferation of trophoblast cells in early pregnancy in these women. In addition, a rapid increase in maternal hCG concentrations is seen during first trimester in a normal pregnancy, followed by a decrease in concentrations in the second trimester

It has been suggested that substantially higher hCG concentrations are needed in older women for the pregnancy to succeed [28]. It is known that hCG has angiogenic properties in itself [29]. It also has a role in modulating the effect of adipokines and other angiogenic growth factors [30]. This hypothesis is supported by the larger placentas seen in older women [27], and placental growth is largely dependent on angiogenesis. Thus, hCG may be reduced in older mothers, possibly indicating reduced placental capacity for hormone production and synthesis [27].

In addition, low hCG can be caused by underlying problems such as gestational age miscalculation, which usually occurs if one has a history of irregular periods. An ultrasound and further hCG tests can be used to calculate the gestational age correctly. This is usually the first step when low hCG levels are detected. Another thing that can cause low serum hCG levels in older pregnant women is blighted ovum. This is when a fertilized egg attaches to the wall of the womb, but

does not continue to develop. Thus, when the gestational sac develops, hCG hormone can be released, but the level does not rise since the egg does not develop. Other things that may cause low hCG are ectopic pregnancy, preeclampsia, high maternal body mass index and miscarriage [31].

Also, it was deduced that the serum levels of AFP were significantly lower at the 2nd trimester in the test subjects compared to the 3rd trimester. Also, since there was a significant difference in the serum AFP and estriol, the associated possible condition is Down syndrome. According to [32] 2016, low maternal serum AFP may be suggestive of risk for Down syndrome. This is in conjunction with studies done by [33,34] which stated that the levels of AFP and estriol are abnormally low, while β -hCG is high in trisomy 21 (Down syndrome) and trisomy 18. This is also in conjunction with the research done by [35], which reported that maternal serum AFP in the second trimester from pregnancies affected with fetal trisomy 21 was lower compared to normal pregnancies.

Contrary to these finding, is the study done by [36], which stated that serum AFP increases with increasing gestational age and decreases with greater maternal weight.

In this study, the serum levels of estriol were significantly lower at the 2nd trimester in the test subjects compared to the control subjects. This was in agreement with the research done by [37], which stated that decreased second trimester serum, estriol level has been shown to be a marker for Down syndrome and trisomy 18 syndromes. Also, according to [38], a very low unconjugated estriol level in the second trimester is associated with increased risk for early death and placental sulfatase deficiency.

Furthermore, it was deduced from this study that the abnormalities is highest at the 2nd trimester in both AFP and β -hCG, for the control samples, while there was no significant difference in estriol with respect to gestational age. Thus these parameters increase with gestational age. This implies that serum AFP and β -hCG is best measured within the 2nd trimester.

However, according to [39], a significant amount of patients with elevated maternal AFP do not develop birth defects, but there may be an increased risk of obstetrics complications like premature rupture of membrane, placenta accrete, increta and packet.

It was also deduced in this study that there is a significant difference in the AFP of older pregnant women unlike in serum β -hCG and Estriol. This probably means that older pregnant women are more likely to have babies with birth defects like neural tube defect. This was also confirmed in the ultrasound scan carried out on these subjects.

This finding is in agreement with the research done by [40] which stated that serum AFP is significantly increased in pregnant women with babies with neural tube defect.

This is contrary to findings of [41], which stated that AFP, estriol and β -hCG are only weakly correlated with one another, and their values are all independent of maternal age. Also, according to the research made by [3], the decrease in maternal serum AFP was independent of maternal age. Thus, making prenatal screening for fetal Down's syndrome possible in women less than 35 years old.

Also, there was a positive and significant correlation between gestational age of the subjects and serum AFP and estriol respectively. The low levels of AFP and unconjugated estriol, and high β -hCG in Down syndrome can be explained as reflecting inappropriate immaturity or dysmaturity of the fetus [42]. The studies by [43], showed a correlation between low estriol and trisomy 21, with a high level of correlation between serum AFP and estriol. Estriol is produced by the placenta from maternal substrates (cholesterol and pregnenolone). Estriol diffuses from the placenta into the maternal blood where it can be measured as unconjugated uE3. Second trimester maternal serum uE3 in Down's syndrome pregnancies are approximately 75% of the values expected in normal pregnancies [44]. Maternal serum levels of unconjugated estriol are lower in Down's syndrome pregnancies than in unaffected pregnancies in the second trimester. In the second trimester, estriol is synthesized in the placenta and secreted into the maternal circulation. 16alpha-hydroxyepiandrosterone sulphate (16alpha-OH-DHEAS) is formed in the fetal liver by hydroxylation of dehydroepiandrosterone sulphate (DHEAS) and transported to the placenta where it undergoes desulphation by steroid sulphatase (STS) and aromatization to estriol. Thus, a diminished supply of the fetal precursor DHEAS may be the

cause of the decreased placental production of unconjugated estriol in Down's syndrome pregnancies in the second trimester [45].

According to [34], Down syndrome (trisomy 21) is accompanied by decreased serum AFP and Estriol, with increased β -hCG. The increased serum β -hCG could be due to transcriptional hyper-activation of the CGB (hCG beta) gene, or an increased half-life of glycosylated hCG hormone, or both. Another possibility is that serum hCG levels remain high due to reduced availability of the hormone's cognate receptor, LHCGR, leading to lack of hormone utilization [46]. In addition, a rapid increase in maternal hCG concentrations is seen during first trimester in a normal pregnancy, followed by a decrease in concentrations in the second trimester

Furthermore, there was a positive correlation between the gestational ages of the subjects and serum β -hCG. hCG levels usually consistently rise until around week 10-12 of pregnancy, when the levels plateau or even decrease. This is the reason why pregnancy symptoms are mostly greater in the first trimester and ease off after that time.

Lastly, it can be deduced from table 5 that there was a significant correlation between the findings of ultrasound scan in test and that of control. Thus, recent advances in real-time sonography and the use of maternal serum pregnancy testing now permit reliable identification of some congenital abnormaities. Also, there was no significant difference between the findings of ultrasound scan and that of laboratory. This inferentially implies that triple test for pregnant women can be used to confirm cases of neural tube defect and other chromosomal abnormalities after ultrasound scan. Using these two will help avoid some potential damaging procedures for the unaffected fetuses [47].

5. Conclusion

The triple test remains relevant because it is the foundation upon which current antenatal screening tests for Down syndrome and some other fetal abnormalities are rooted. It can be deduced from this research that serum AFP has the highest predictive value. However, the combination of the three biomarkers gives a better diagnostic performance.

Patients with abnormal findings of triple test are under the risk of obstetric complications. Because of this risk, it is important to provide not only pregnant women who are 35 years and above but also pregnant women who are younger than 35 years with noninvasive screening for this trisomy. The use of only ultrasound is less accurate for determination of chromosomal abnormalities such as Down syndrome, than it is for neural tube defect, hence the need for both triple screening tests and ultrasound scan.

Recommendation

Invasive testing remains the gold standard for prenatal diagnosis of chromosomal syndromes. Thus, triple screening should be requested for those pregnant women with abnormal triple test results. There is need for triple test to be included in routine antenatal screening, particularly for older pregnant women. Also, improvement in maternal nutrition and early antenatal folate intake is recommended.

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