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POLISH GYNECOLOGY
GINEKOLOGIA POLSKA
ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGICZNEGO
THE OFFICIAL JOURNAL OF THE POLISH GYNECOLOGICAL SOCIETY

ISSN: 0017-0011

e-ISSN: 2543-6767

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DOI: 10.5603/GP.a2022.0148

Article type: Review paper

Submitted: 2022-08-22

Accepted: 2022-11-21

Published online: 2022-12-29

Articles in "Ginekologia Polska" are listed in PubMed.

[REVIEW PAPER / OBSTETRICS]

Influence of selected factors on serum AFP levels in pregnant women in terms of prenatal screening accuracy — literature review

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ABSTRACT

Alpha-fetoprotein (AFP) is one of the biochemical components of the triple (T-3) and quadruple (T-4) test used so far in prenatal screening mainly for trisomy 21 (T21) and neural tube defects (NTDs). Based on many years of experience and data collected during these studies, a variety of factors have been identified that can affect a pregnant woman's serum AFP level, and thus the risk assessment of trisomy 21 (T21) and neural tube defects. These include both unaccounted for purely medical data (e.g., from baseline information about the patient, assisted reproduction methods used, comorbidities and emerging pregnancy pathologies) and errors made during statistical analysis. Since the triple or quadruple test is usually performed between 15 and 20 weeks of pregnancy, most scientific studies are based solely on results from this period of pregnancy — limited data are available for the first and third trimesters of pregnancy. In the era of new improved screening tests, AFP has the potential to become an independent marker for pregnancy well-being evaluation.

Key words: prenatal screening; false positive/negative; triple/quadruple test; alpha-fetoprotein; AFP MoM; trisomy 21; neural tube defects

INTRODUCTION

In use since the late 1980s and early 1990s, the triple test [alpha-fetoprotein (AFP), human beta chorionic gonadotropin beta-hCG, estriol E3] and then the quadruple test (AFP, beta-hCG, E3, inhibin A) was a milestone in the biochemical diagnosis of aneuploidy. However, despite the undeniable advantages demonstrated by years of use, it is burdened with certain drawbacks. In the era of the spread of fetal deoxyribonucleic acid (DNA), the sensitivity of the triple test at about 67–73% and quadruple test at 75–85% (depending on the cutoff point for false positive rate, FPR) is no longer particularly awe-inspiring. Another problem is the 4–8% false-positive rate for trisomy 21 (as high as 21–25% after an age of 35), and 5–14% for the quadruple test, causing concern in patients whose first trimester composite test result [according to Fetal Medicine Foundation (FMF)] and second trimester fetal anatomy evaluation were normal [1–3]. In addition, like any laboratory test, this test is susceptible to the influence of individual factors specific to the patient, her comorbidities, diagnostic and statistical errors. This review paper aims to systematize and characterize the factors that directly influence AFP (and AFP multiple of the median — MoM) levels, and ultimately the incidence of false-positive and negative prenatal screening tests based on its evaluation.

The available data suggest the possibility of using AFP as a separate marker of pregnancy well-being indicating pregnancy abnormalities other than those already present after considering all known distractors of its serum levels in pregnant women [4].

BASELINE INTERVIEW

Race

Black and yellow race patients have higher AFP levels than white Caucasians. The difference in AFP levels between black and Caucasian races decreases with increasing gestational age [5–7]. In the first trimester of pregnancy, the difference in AFP levels between black and Caucasian is about 23%, and in the second trimester it is 10–20% [8]. The exact

mechanism of these disparities besides the effect of body mass index (BMI) for the yellow race has not yet been determined. The reasons for this are not reported in the literature. Some possible mechanisms, in our opinion, are presented below.

BMI

The higher the pre-pregnancy BMI, the lower the maternal serum AFP concentrations. This is due to the dilution effect in the larger plasma volume [6]. If one were to take this fact into account for Caucasians and correct the AFP results based on the BMI of pregnant women, it would turn out to be at a similar level to Caucasians [5].

Parity

In multiparous women, AFP concentrations are lower than in primiparous women of the same gestational age. The explanation for this phenomenon is believed to be the relationship between synthesis of AFP and estriol. In animal experiments, estriol has been shown to be responsible for AFP synthesis. With successive pregnancies, estriol levels decrease and AFP levels decrease in parallel [9]. AFP levels are related to the length of the interval between successive pregnancies (it is lower the shorter the interval) and the weight of the newborn from the previous birth [10].

Gestational age

AFP levels increase as pregnancy progresses by 37% per week [8]. In the context of underestimation of AFP results expressed in MoM, attention should be paid to redating the gestational age in case of discrepancies between last menstrual period (LMP) and first trimester ultrasound. This would improve the accuracy of calculating AFP MoM values [11]. The cases of amenorrhea, where the crown rump length (CRL) measurement will be underestimated on average by a value equivalent to two days, will be problematic [12].

Twin pregnancies

In twin pregnancies, AFP levels are doubled as are inhibin-A levels, which is explained by the existence of a dual source in the form of two fetuses, while beta-hCG and

estriol are not doubled. The results of the research showed that chorionicity has no influence on the AFP level in the maternal serum. Neither does maternal age nor maternal weight. There is a scarcity of randomized controlled trials about marker levels in twin pregnancies in situations with one affected fetus. Besides, even assuming that the fetuses are of equal weight, it is not possible to fully investigate the exact contribution of each fetus to the total measured AFP level. The presence of a healthy fetus can disturb the detection of the sick fetus because of averaged results of AFP level. The main problem is the lack of specially dedicated software to calculate the risk for multifetal pregnancies so currently calculators for single pregnancies are used. To calculate the risk of T21 in twin pregnancies in the second trimester one half of a specific marker is used. As a result, false positive and negative results are numerous and the sensitivity of this test for multifetal pregnancies is lower. To avoid this, the separate calculation risk model for twin pregnancy based on the data from local populations should be created but since the NIPT (non-invasive prenatal testing) becomes a more accurate form of the aneuploidy detection the twin calculator based on biochemical markers is not first line goal [13–17].

Cigarettes and alcohol

Cigarettes

In this regard, the results of scientific studies do not agree. Higher levels of MoM AFP by 11% in the 1st trimester and by 2–6% in the 2nd trimester compared to the population of non-smoking women were observed [8, 18–21]. In contrast, a study by Bredaki et al. [10] found an effect of cigarette smoking on AFP levels only in the 1st trimester, no longer in the 2nd and 3rd. Higher AFP levels in the umbilical cord blood of newborns of mothers who smoke cigarettes were also reported, depending on the amount of cigarettes smoked. High levels of AFP in the umbilical cord were inversely correlated with the weight and length of the newborn [22]. Smoking impairs blood flow in the maternal-fetal unit by affecting placental morphology: blocking cytotrophoblast differentiation, decreasing placental villi volume and their invasiveness.

Smoking-induced tissue hypoxia and the presence of methemoglobin and toxins impairs vascularization of placental villi. It probably also affects, in fetal hepatocytes, the expression of genes responsible for post-translational modifications of proteins responsible for detoxification and secretion, among others [22].

Alcohol

There is little data on the effect of alcoholism on AFP levels. High levels of AFP are found in the serum of chronic and heavy drinking mothers, this happens in the mechanism of fetal liver damage [23].

Fetal weight from a previous birth

No correlation was found between neonatal weight and maternal serum AFP levels in physiological pregnancy. In newborns with low birth weight, elevated AFP levels in pregnant women's serum are related to associated pregnancy pathology. High AFP levels are the result of damage to the placenta and an increase in its permeability such as in preeclampsia and are not a factor per se due to fetal weight [7].

Gender of fetus

Female fetuses have slightly lower AFP values (about 5%) than male fetuses, regardless of racial origin, but in turn are found to have higher levels of beta-hCG [23]. It was suspected that this might result in more frequent false-positive triple test results in mothers of girls and false-negative results in mothers of boys. It turned out that in contrast to pregnancies with genetically normal fetuses, no statistically significant relationship was found between fetal sex and maternal serum AFP and beta-hCG levels in pregnancies with T21 [24]. There are also studies in which the relationship between AFP and gender was not confirmed [7]. Lower AFP values in female fetuses have been associated with lower fetal liver weight, which was not confirmed in studies on terminated fetuses. Another explanation for the higher AFP production in male fetuses is that AFP plays a protective role against brain exposure to circulating maternal estrogen (it is a carrier protein for it), preventing its feminization [9].

INTERNAL DISEASES

Diabetes mellitus (DM) types 1 and 2

Both groups have lower serum AFP MoM values than women without diabetes. Abnormalities in the development and function of fetal hepatocytes of mothers with insulin-dependent diabetes and immaturity of placental villi in insulin-dependent and independent diabetes have been cited as potential reasons [25–27]. In the 1990s, a 20% correction of AFP MoM levels for diabetes alone was applied. Nowadays, it is believed that only the correction

related to the inclusion of BMI is sufficient. Without weight-related correction of AFP MoM, patients with DM 2 have lower values than those with DM 1 because they often have higher body weight. In contrast, after correction using maternal weight, pregnant women with both types of DM have similar levels, but still lower than healthy patients [25]. There is a hypothesis that this approximately 20% lower AFP MoM level is transformed alpha-fetoprotein (tAFP) arising from chronic fetal stress.

The relationship between AFP levels and glycated hemoglobin (HbA1c) levels was also studied. Early reports suggested that patients with poorly controlled diabetes (high HbA1c) had low AFP levels, but this was later not confirmed. On the other hand, most of the studies on the relationship between AFP and diabetes date from the 1980s and 1990s, and given the now much better glycemic control, use of insulins and surveillance of pregnant women with diabetes, there is a need for more recent data. Adequate assessment of AFP levels in these patients is important because the incidence of neural tube defects in children of patients with insulin-dependent diabetes is 3–4 times higher [6].

Chronic hypertension

There was no effect on AFP levels, even in renal transplant patients or those with end-stage renal failure, where kidney function is significantly altered [28].

Systemic lupus erythematosus (SLE)

Pregnant patients with lupus have higher AFP levels. This may be related to the use of glucocorticosteroids (see impact of medicines and supplements) [29]. The second theory is that the underlying cause of high AFP is increased permeability of placental vessels associated with their inflammation in SLE [30].

Thrombophilia

The effect of thrombophilia on AFP levels is still unclear. Congenital thrombophilia either showed no difference in AFP levels compared to controls [31, 32] or showed reduced levels [33] or elevated levels [31]. Some authors contribute that some patients with high AFP levels are carriers of the C677T methylenetetrahydrofolate reductase (MTHFR) mutation [34]. In antiphospholipid syndrome (acquired thrombophilia), elevated AFP levels are noted [35].

The immunosuppressive effect of high AFP concentrations helps reduce the level of antibodies to b2-glycoprotein in antiphospholipid syndrome [32, 31]. It is likely that in addition to increased synthesis as a target for the immunosuppressive effect, elevated maternal serum AFP levels result from increased leakage through a functionally or structurally abnormal placenta. Microscopically, the placentas of patients with thrombophilia may show acquired intervillous thrombosis, chronic chorioamnionitis and placental vascular infarction [36].

There has also been no evidence of any effect of heparins used to treat congenital and acquired thrombophilia on AFP levels, although data on the timing of treatment with these drugs is also often lacking [33].

Obstructive sleep apnea (OSA)

Obstructive sleep apnea (OSA) mainly affects obese pregnant women with an initial physiologically increased respiratory effort. Episodes of apnea cause stimulation of the sympathetic nervous system with concomitant hypoperfusion at the tissue level up to ischemia, and consequently tissue hypoxia and oxidative stress. Clinically, OSA is associated with obstetric complications like hypertension and FGR, among others. High levels of markers of chronic hypoxia: erythropoietin, IL-6 and nuclear forms of red blood cells have been found in the cord blood of newborns of mothers suffering from OSA. When studying the relationship between AFP levels and OSA, it was expected to be high due to ischemic placental damage. Ultimately, however, after using weight correction in calculating the AFP MoM (OSA patients generally have higher BMIs), no statistically significant difference was shown [37].

PATHOLOGICAL CONDITIONS OF PREGNANCY

Gestational diabetes mellitus (GDM)

GDM affects 1–3% of pregnant women. These patients have lower AFP values, and weight correction for AFP MoM is required. There are reports that very low AFP levels are associated with high birth weight of newborns [38].

Hypertension in pregnancy (PIH) and preeclampsia (PE)

Elevated AFP is not found in pregnancy-induced hypertension, but in preeclampsia it is. The greater the severity of preeclampsia, the higher the AFP concentrations in the pregnant woman. This is a consequence of progressively more severe damage to the placental fetal-maternal barrier and increased permeability to AFP. Abnormal placentation and the subsequent maternal inflammatory response (overproduction of anti-angiogenic factors) are the basis for this pathology [30, 38, 39].

Intra- hepatic cholestasis of pregnancy (ICP)

The effect of maternal cholestasis on the liver and biliary functions of the newborn has been demonstrated in an animal model. In the fetus, utilization of bile acids is impossible due to the immaturity of the liver to secrete them and the small intestine to absorb them, and accumulation in the fetus would be toxic. Typically, bile acids from the fetus are transported across the placenta to the mother, where they are excreted into bile in the case of a properly functioning maternal liver [40]. Pregnancy cholestasis impairs maternal and indirectly fetal excretion of bile acids, leads to their accumulation in the fetal liver and impairs its metabolism. However, human studies have not confirmed the effect of this disease on maternal serum AFP levels [41, 42].

Hyperemesis gravidarum

They occur in the first and early second trimester of pregnancy and affect about 3% of pregnant women. No effect on maternal serum AFP levels has been demonstrated [43].

Maternal anemia

Placental volume gain in the second trimester is inversely proportional to maternal hemoglobin levels. In cases of high maternal hemoglobin and accompanying reduced placental volume and dimensions, fetal-maternal AFP transport decreases. It was therefore expected that in cases of maternal anemia, increased placental volume would result in increased transport of AFP to the mother and elevated AFP concentrations in her blood. However, contrary to assumptions, maternal anemia was found to result in low AFP levels. This is attempted to be explained by a dilution of AFP concentrations due to an increase in

plasma volume, but on the other hand, high AFP levels should be found in patients with polycythemia and hemoconcentration, and this is not the case [44].

Serological conflict

In the 1970s, the use of AFP was proposed to monitor the course of serological conflict, as its levels were increased in maternal serum and amniotic fluid during this condition. However, due to the development of ultrasonography and Doppler techniques (middle cerebral artery peak systolic velocity — MCA-PSV evaluation), the use of AFP was discontinued.

Two theories attempt to explain the increased serum AFP levels in Rh-negative pregnant women. The first (more likely) speaks of increased AFP production as a side effect of increased hematopoiesis involving a functionally immature fetal liver. The other says there is increased transfer of AFP through a large and swollen placenta, but this does not explain the high levels in amniotic fluid. It seems that increased AFP production precedes the onset of fetal anemia, as an expression of effective adaptation to hemolysis. The more advanced the hemolytic anemia and increasing fetal edema, the paradoxically lower the level of AFP in the mother's blood, due to the breakdown of hepatic hematopoiesis [45].

ASSISTED REPRODUCTION METHODS

In vitro fertilization (IVF)

It has been studied that the FPR in the triple test for assisted reproduction methods is 19%, and the highest at 30.8% in the group using frozen embryos [46]. Pregnancies obtained from IVF have about 10% higher AFP levels in the first trimester of pregnancy compared to those conceived naturally [8]. It seems a fair observation that the number of embryos transferred into the uterus may affect the results of biochemical tests [47]. On the other hand, in the second trimester, pregnancies from IVF have AFP levels about 5% lower than those conceived naturally — hence the higher risk of false positive results in the triple test. Pregnancies from IVF-ICSI (in vitro fertilization by intracytoplasmic sperm injection) have lower AFP MoM than those from conventional IVF (by 16%) and natural conception (by 19%) [48].

Frozen embryos

Patients in IVF pregnancies from frozen embryos have higher serum AFP levels compared to natural conceptions. This is especially true for frozen embryos obtained by conventional IVF (by 20%); frozen embryos obtained by ICSI have standard levels as from natural conceptions. This is influenced by the freezing and thawing process [46, 48, 49].

Oocyte donation

In these pregnancies, AFP levels are higher compared to pregnancies obtained from own oocytes (IVF and natural conception) regardless of the age of the oocyte donor or recipient. This is likely due to impaired implantation, immunoregulation and subsequent vascular disorders in the maternal-fetal unit of oocyte donor pregnancies, resulting in pregnancy pathologies such as pre-eclampsia (PE) or fetal growth restriction (FGR) [47, 50].

Vanishing twin syndrome

When one of the twins dies and is absorbed in the first trimester of pregnancy, AFP levels are elevated by about 10%. The death of one of the twins before the date of prenatal testing can affect the results of biochemical tests of the remaining single fetus. Data obtained over pregnancies after selective termination of one of the fetuses in a twin pregnancy show that AFP levels are elevated for about eight weeks after the procedure. In assessing the genetic risk in this case, two facts would have to be considered. The first is that AFP levels undergo a slow gradual decrease from the time of termination until AFP is measured, and the second is that the half-life of AFP in maternal serum is 59–133 h [51].

DISORDERS OF THE CHORION, PLACENTA AND UTERUS

Chorionic villus sampling (CVS)

Patients after chorionic villus biopsy, as well as any other genetic invasive procedure, show an increase in serum AFP levels, which occurs immediately after the procedure. Maximum values are noted about 1h after the procedure, after which the level stabilizes. The increase in AFP levels is due to damage to the maternal-fetal barrier. Based on the rise in AFP after the procedure, it is possible to estimate the amount of fetal blood that has entered the

mother's bloodstream. This method is comparable to the Kleihauer-Betke test (98% vs 100%). There is a correlation between the weight of the collected villi and the AFP increase. It has not been reported that high AFP values after chorionic villi biopsy persist until 16–18 week of pregnancy, i.e., the standard period for performing a triple or quadruple test [52, 53].

Subchorionic hematoma

In about 20% of women, bleeding in the first trimester of pregnancy is associated with a subchorionic hematoma. The presence of hematoma is associated with an increased risk of miscarriage, preterm labor and FGR. Elevated AFP levels in these patients are due to extravasation of blood into the space between the chorionic villi and the uterine wall, which allows a certain amount of AFP to enter the mother's serum [54].

Placental mesenchymal dysplasia (PMD)

It is a rare vascular pathology of the placenta resulting in placentomegaly with multiple cystic areas preceded by hypoechoic areas. The etiology is unknown. Histopathologically, aneurysmal dilatation of chorionic vasculature with villous proliferation is described, without trophoblastic proliferation. It is often mistaken on ultrasound and histopathological examination for a hydatidiform mole (due to numerous small cystic lesions), in contrast to which there is no risk of transformation into gestational trophoblastic disease and fetal development is usually normal. A high AFP level is accompanied by a normal beta-HCG level for a given gestational age and a normal fetal karyotype (helps distinguish it from hydatidiform mole). High AFP levels are due to the large surface area of the placenta made up of thin-walled vessels, which increases fetal-maternal transfer of AFP. More than 90% of pregnancies with PMD are characterized by complications including pregnancy-induced hypertension (PIH), preeclampsia (PE), hemolysis, elevated liver enzymes, low platelets (HELLP), fetal growth restriction (FGR), fetal anemia, thrombocytopenia, intrauterine fetal demise (IUFD) and preterm birth (PTB) [55].

Placenta accreta spectrum (PAS)

Under normal conditions, the basal decidua is a barrier to the ingrowth of placental villi into the myometrium. If there is even the slightest damage to the basal decidua (e.g., by

cesarean section, curettage, endometrial ablations), the decidualization process is disrupted and invasion into the myometrium occurs. It has been studied that the endometrium within the lower uterine segment is less well nourished. Hence, in the case of implantation in this area of the uterus, further damaged by surgery, there is a greater risk of placenta ingrowth. In addition, abnormal placentation is accompanied by pathologically enhanced angiogenesis and hyperperfusion, which increases the absorption of AFP and beta-hCG into the maternal circulation [56].

Circumvallate placenta

Is a pathology of the shape of the placenta. It arises when the chorionic plate in the placenta is smaller than the basal plate, resulting in the formation of a characteristic ring at the periphery on the fetal side. The ring that forms the bulwark is made up of two layers of amniotic fluid and chorionic villi with a layer of degenerated decidua between them and the presence of hematomas and areas of infarction. One theory of this abnormality origin is that the blastocyst implants too deeply into the uterine muscle. It can result in recurrent bleeding from the first trimester of pregnancy, thrombocytopenia, premature rupture of the amniotic membranes, premature separation of the placenta, preterm labor, or fetal growth restriction. The elevated AFP levels found in this case are due to hypoperfusion of the placental plate and the presence of infarcted areas [57].

Uterine myomas

They account for 95% of benign uterine tumors. They occur in 30% of Caucasian women over the age of 35, and an increasing number of pregnant women are now being reported in this age group. Co-occurrence of uterine myomas with pregnancy occurs in about 1–4%. No effect has been shown on AFP levels in pregnancy [58].

INFECTIONS

Human immunodeficiency virus (HIV)

About 90% of HIV-infected women are of reproductive age. Previous scientific studies have shown that there is an effect of HIV infection and the drugs used in the infection on the results of biochemical screening tests, although there are no studies that provide answers as to

how HIV affects trophoblast cells [9]. High AFP levels correlate with high viral load and low CD4+ levels, which in this situation increases the risk of a false-negative result in the triple test. HIV+ pregnant women who are untreated have lower AFP levels than cART-treated and healthy women [59]. This is explained by the impaired effect of the virus on placental transport of AFP. Treatment with the combination antiretroviral therapy (cART) regimen increases AFP levels, but they remain within normal limits [60]. These are important data because of the risk of viral transmission during amniocentesis [9].

Intrauterine herpes simplex virus type 2 (HSV-2) infection

It is accompanied by changes in ultrasound images: hyperechoic intestines, hyperechoic foci in the myocardium, differentiated echogenicity of the fetal liver. The source of high AFP can be found in the damage to the fetal liver by the herpes virus in an already advanced infection. High levels of AFP in maternal serum and amniotic fluid in the second trimester are accompanied by high levels of acetylcholesterase in the amniotic fluid (which is typical of open neural tube defects) but in this disease is associated with fetal central nervous system (CNS) damage and necrosis [61].

Parvovirus B19 (B19V)

Parvovirus B19 infects rapidly dividing cells, and this is what attempts to explain its preference for the fetus. Typically, B19V attacks precursor cells of the erythroid lineage in the bone marrow and liver of the fetus leading to complete blockade of erythrocyte production and anemia. The target receptor for B19V is erythrocyte P antigen, which is also found on megakaryocytes, endothelial cells, in the fetal liver and heart. High AFP levels occur based on fetal liver damage and increased transudate through the placenta, which is swollen due to anemia [30].

MEDICINES AND SUPPLEMENTS

The following drugs were not found to affect AFP levels: heparins, antibiotics, painkillers, antidepressants, antiemetics, anti-asthmatics, hypotensives [62, 63].

Antiepileptic drugs such as lamotrigine, levetiracetam, carbamazepine, oxcarbamazepine, valproic acid and clonazepam affect the increase in AFP and E3 levels in the second trimester of pregnancy [64].

Immunosuppressants, particularly corticosteroids, cause elevated AFP levels based on effects on metabolic pathways. Corticosteroids are utilized in the liver involving cytochrome P450, and induction of this group of enzymes has a parallel effect on increasing AFP synthesis. In addition, corticosteroids affect placental perfusion, thereby increasing fetal-maternal transfer of AFP [62].

Protease inhibitors used to treat HIV infection cause AFP levels to decrease. This is important because of the risk of exposing HIV+ pregnant patients to invasive testing for in-depth diagnosis of trisomy 21 in the case of an abnormal triple test [62].

Drugs for ovulation induction: higher AFP levels are found with clomiphene citrate (Clostilbegyt) and lower with gonadotropins, e.g., Pergonal, Menopur, Mensinorm [8].

Folic acid: when food fortification with folic acid began in the US in 1998, it helped reduce the number of patients with AFP levels > 3 MoM by more than 42% [65].

STATISTICAL ERRORS IN RISK ESTIMATION

The basis for estimating the risk of T21 in biochemical tests is the calculation of multiples of the median (MoM) for each component of the test. This allowed us to obtain values comparable for laboratories using the same immunoassay. The calculated MoM value is expected to be corrected for gestational age, patient weight, race, smoking, comorbidities, and periodically monitored to see if it is near 1. Errors in the calculation of the MoM can occur in a number of ways including incorrect calculation of medians (medians must be representative of the population), changes in reagent lot quality, immunoenzymatic assay performance, and human error. Accurate determination of gestational age and reliable regression coefficients for the relationship between biochemical test results and gestational age are also important. Problems with immunoenzymatic assay and incorrectly determined regression equations can result in deviations in the determination of MoM values. It has been found that a 10% error in calculating MoM values for a single marker (which is reportedly quite common) can result in a 1–2% increase in FPR in a triple test. If it involves all three markers, then the FPR can even double [66].

CONCLUSIONS

1) The AFP level, like other biochemical markers, should not be analyzed only as the current serum concentration without considering the influence of demographic factors, ongoing diseases, medications used, type of pregnancy, pathological conditions and abnormalities of pregnancy and other disturbances that imply an appropriate correction of the concentration expressed by MoM to increase the accuracy of prenatal testing.
2) The impact of statistical errors in estimating the risk of T21 and neural tube defects is little appreciated. Failure to account for the above factors increases false-positive high-risk results and following unnecessary invasive testing for T21.
3) AFP, due to a number of correlations with disease states in pregnancy, shows potential as a more universal marker of fetal and maternal well-being.
4) Patients should be informed of the sensitivity and limitations of each screening test to avoid unnecessary stressful situations.
5) There is a lack of research on the effects of various factors on AFP levels in the first and third trimesters of pregnancy.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Pregnancy periods during which the effects of selected factors on AFP levels were studied

Factor	I trimester	II trimester	III trimester
Mother race	√	√	√
Mother BMI	√	√	√
Parity	√	√	√
Pregnancy age	√	√	√
Cigarettes	√	√	√
Alcohol		√	
Fetus gender		√	
DM 1 and DM 2	√	√	
SLE		√	
Thrombophilia		√	
Hypertension		√	
OSA		√	
GDM		√	
PIH/PE	√	√	√
ICP		√	
Hyperemesis gravidarum		√	
Hemoglobin level		√	
Serological conflict		√	
IVF	√	√	
IVF-ICSI		√	
IVF — frozen embryos		√	
IVF — oocytes donation		√	
Vanishing twin	√	√	
CVS	√		
Subchorionic hematoma	√		
PMD		√	
PAS		√	
Circumvallate placenta		√	
Uterine myomas		√	
HIV+		√	
HSV2+ (fetal infection)		√	

Parvovirus B19		√	
Antiepileptic immunosuppressants, used to treat HIV+, inducing ovulation		√	
Folic acid deficiency		√	

BMI — body mass index; CVS — chorionic villus sampling; DM — diabetes mellitus; GDM — gestational diabetes mellitus; HIV — human immunodeficiency viruses; HSV2 — herpes simplex virus type 2; ICP — intrahepatic cholestasis of pregnancy; IVF — *in vitro* fertilization; PAS — placenta accreta spectrum; PIH/PE — pregnancy-induced hypertension/preeclampsia; PMD — placental mesenchymal dysplasia; OSA — obstructive sleep apnea; SLE — systemic lupus erythematosus

Table 2. Factors affecting maternal serum AFP levels (MS-AFP)

↑ higher AFP values	**↔ no effect on AFP level**	**↓lower AFP values**
Male fetuses	Hyperemesis gravidarum	Female fetuses
Vanishing twin	Uterine myomas	Multiparity#
Clack and yellow race #	OSA	Obesity#
Alcohol	ICP	DM 1 and 2#
Cigarettes#	Weight of a newborn from a previous birth	GDM
SLE	Hypertension	Maternal anemia
PE	End-stage renal failure	Maternal polycythemia
Serological conflict	PIH	Folic acid supplementation
Parvovirus B19		HIV
HSV-2		Protease inhibitors
CVS		IVF#
Subchorionic hematoma		IVF-ICSI
PMD		
PAS		
Circumvallate placenta		
Antiphospholipid		

syndrome		
IVF — frozen embryos		
IVF — oocytes donation #		
Immunosuppressants		
Antiepileptics		

— factors currently included in the risk calculation of trisomy 21 and neural tube defects in the triple test; AFP — alpha-fetoprotein; CVS — chorionic villus sampling; DM — diabetes mellitus; HIV — human immunodeficiency viruses; HSV-2 — herpes simplex virus type 2; ICP — intrahepatic cholestasis of pregnancy; IVF — *in vitro* fertilization; IVF-ICSI — *in vitro* fertilization by intracytoplasmic sperm injection; GDM — gestational diabetes mellitus; PAS — placenta accreta spectrum; PE — preeclampsia; PIH — pregnancy-induced hypertension; PMD — placental mesenchymal dysplasia; OSA — obstructive sleep apnea