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The effect of a vanishing twin on first- and second-trimester maternal serum markers and ultrasound screening for aneuploidy

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A vanishing twin (VT) is the early demise of a twin fetus. It is estimated to occur in 20-30% of pregnancies associated with assisted reproductive technology. VT becomes increasingly prominent when assisted fertilization is used, because one or more embryos are transferred to the uterus. Maternal serum screening tests during pregnancy can screen for trisomy chromosomes 21, 18, and 13 and are divided into first- and second-trimester tests. In singleton pregnancies, the first trimester screening test is performed at 11-13 weeks and 6 days of gestation. It consists of two serum markers, pregnancy-associated plasma protein A and β-human chorionic gonadotropin (β-hCG), and measures nuchal translucency thickness. The second-trimester screening test was performed at 15-20 weeks and 6 days of gestation. It consists of four serum markers: alpha-fetoprotein, β-hCG, unconjugated estriol, and inhibin A. More effective screening for trisomy 21 in singleton pregnancies is achieved by analyzing cell-free DNA in the maternal blood. A VT includes a demise of the fetus. Although it affects maternal serum markers, it has not been corrected. Five studies examined the effect of VT on maternal serum markers, but the results were controversial. This study aimed to review the patterns of changes in maternal serum markers in VTs, interpret prenatal tests for pregnant women with VTs in clinical practice, and consider what information should be provided.

Keywords: Prenatal diagnosis; Down syndrome; Aneuploidy; Pregnancy-associated plasma protein-A; Chorionic gonadotropin

Introduction

A vanishing twin (VT) is the early demise of a twin fetus. It is estimated to occur in 20-30% of pregnancies associated with assisted reproductive technology (ART). Owing to the increasing number of assisted reproductive treatments, the incidence of twin pregnancies and VT syndrome has increased because one or more embryos are transferred to the uterus [1,2]. The diagnosis of VT is more frequent with the increased use of ultrasonography in early pregnancy [3].

Remarkable progress has been made in screening for fetal chromosomal abnormalities over the past few decades. Various screening tests are already widely used in clinical practice, and each screening test has advantages and limitations; counseling on this must be provided before screening for fetal chromosomal abnormalities [4].

Maternal serum marker screening for trisomy was per-

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formed in the first and second trimesters of pregnancy. First trimester serum tests (pregnancy-associated plasma protein A [PAPP-A] and the free β subunit of human chorionic gonadotropin [β-hCG]) and ultrasonography for nuchal translucency (NT) measurements were performed at 10 weeks and 3 days through 13 weeks and 6 days of gestation. At 15-18 weeks of gestation, a second-trimester quadruple screening test (alpha-fetoprotein, total human chorionic gonadotropin, unconjugated estriol, and inhibin A) was performed to screen for aneuploidy [5]. Screening for fetal chromosomal abnormalities that is currently used in clinical practice by combining the serum markers described above includes the first trimester test, quad screening, integrated test, sequential stepwise screening, and contingent screening. In addition to serum markers, cell-free DNA (cfDNA) screening, which involves the analysis of fetal DNA fragments in the maternal blood, is a single time-point screening approach that has recently been used [4].

However, screening for fetal chromosomal abnormalities in twin pregnancies is not optimal [6]. Furthermore, if one of the twins died, the results may have been inaccurate [7].

Therefore, it is necessary to educate pregnant women about the differences in maternal serum markers between VT and singletons. This study aimed to review the patterns of changes in maternal serum markers in VT, interpret prenatal tests for pregnant women with VT in clinical practice, and consider what information should be provided.

First trimester screening for aneuploidy in VT

Pregnancy-associated plasma protein-A

PAPP-A, synthesized in the placental syncytiotrophoblast, is a metalloprotease that circulates in the maternal blood. PAPP-A is used for trisomy screening because its maternal serum concentration decreases during the first trimester in fetal trisomy 21 [8,9].

Chasen et al. [10] reported that PAPP-A concentration in pregnant women diagnosed with VT was higher than that in normal singleton women. In another retrospective analysis, Spencer et al. [11] reported that PAPP-A levels were not altered in VT with an empty gestational sac. However, in VT with dead embryos, PAPP-A was increased, and the increase in PAPP-A was related to the time interval between fetal demise and blood sampling. Huang et al. [7] reported that the multiple of the median (MoM) values of PAPP-A increased in pregnant women diagnosed with VT within 28 days before blood sampling, but not in those diagnosed more than 28 days before blood sampling. Chaveeva et al. [12] reported that PAPP-A MoM was higher in VT with empty gestational sacs and dead embryos. In the latter group, the effect of VT on PAPP-A levels was inversely related to the interval between the estimated gestational age at embryonic demise and blood sampling.

However, another prospective cohort study by Gjerris et al. [3] reported no significant differences in the MoM values of PAPP-A between women with VT and singleton pregnancies, regardless of whether VT occurred at <9 or at 9-13 weeks of gestation.

β-human chorionic gonadotropin hormone

Human chorionic gonadotropin (hCG) is a glycoprotein hormone found in the blood and urine only during pregnancy, and is produced by the syncytiotrophoblast cells of the placenta. Marternal serum β-hCG rises rapidly during the 8-10 weeks of gestational age. Then, it decreases, reaches a plateau at 18-20 weeks of gestation and remains low until term. Maternal serum-hCG levels are elevated in pregnant women with trisomy 21 fetuses compared to those with normal fetuses. Therefore, it is used as a screening test during the first trimester of pregnancy [13].

Chasen et al. [10] reported that VT within 28 days prior to blood sampling was associated with an increase in free β-hCG levels. Another prospective cohort study reported no significant differences in MoM values of β-hCG between VT and singleton pregnant women regardless of whether the embryo death occurred less than 9 weeks or 9-13 weeks of gestation [11]. Other retrospective analyses have reported no significant differences in the MoM of free-hCG. Huang et al. [7] reported that the MoM values of first- and second-trimester β-HCG did not change. Chaveeva et al. [12] reported that free-hCG MoM in VT was not significantly different from that in normal singleton pregnancies.

Nuchal translucency

NT is the subcutaneous space overlying the cervical spine of the fetus. NT thickness was measured by ultrasound at 11-13 weeks and 6 days of gestation, and fetuses with trisomy 21 tended to have increased NT. The first-trimester screening

test consisted of maternal serum markers and NT thickness [14,15].

Gjerris et al. [3] assessed the impact of VT on first-trimester maternal serum markers and NT in pregnancies conceived after *in vitro* fertilization and intracytoplasmic sperm injection. They reported that first trimester maternal serum markers were not significantly increased compared to singleton ART pregnancies. The NT measurements were similar.

Second trimester screening for aneuploidy in VT

α-fetoprotein (AFP)

AFP is a glycoprotein synthesized in the yolk sac at an early gestational age and in the liver of the fetus at a late gestational age [16]. Some AFP pass through the placenta and maternal blood. In 1984, Merkatz et al. [17] reported that maternal serum AFP levels in the second trimester of pregnancies affected by fetal trisomy 21 were lower than those in normal pregnancies. It is also used in aneuploid screening tests during the second trimester of pregnancy [17,18].

Huang et al. [7] conducted a retrospective case-control study on the concentration of second trimester maternal serum markers in pregnancies with VT. The results showed that the second trimester AFP level was elevated in VT pregnancies.

Inhibin-A

Inhibin levels were compared in Down syndrome and unaffected pregnancy samples using radioimmunoassay. Inhibin A levels have been reported to be higher than total inhibin levels in Down syndrome pregnancies [19].

In a retrospective case-control study, inhibin A concentration was elevated in pregnancies [7]. However, Chen et al. [19] found that maternal serum inhibin A levels in twin pregnancies and multiple pregnancies were reduced to those in twins in the second trimester. Maternal serum inhibin levels in multifetal reduction-to-twin pregnancies were lower than those in twin pregnancies during the second trimester [19].

Unconjugated estriol

Estriol is produced from maternal cholesterol and pregnenolone in the placenta. Estriol diffuses from the placenta into the maternal blood, where it is measured as unconjugated estriol (uE3). Maternal serum uE3 concentrations are lower in pregnancies with Down syndrome than in unaffected pregnancies during the second trimester [20]. There were two studies on uE3 in VT. In a retrospective case-control study by Huang et al. [7], the concentration of uE3 did not change during the second trimester. In addition, the study reported by Lee et al. [21] reported the same result that the uE3 concentration did not change in VT [21].

β-human chorionic gonadotropin hormone in the second trimester

Bogart et al. [22] reported elevated second trimester hCG levels and Down syndrome pregnancies. β-HCG is a glycoprotein composed of two subunits, α and α. It is produced by the trophoblast of the blastocyst and, later in pregnancy, by the chorion and placenta. Serum hCG levels increase exponentially between 3 and 10 weeks of gestation. Levels peaked during the first trimester and decreased during the second and third trimesters. The same retrospective case-control study showed that the concentrations of total hCG in VT remained unchanged [7].

Non-invasive prenatal testing in VT

The detection of VT is clinically important because it can affect the outcomes of the non-invasive prenatal testing (NIPT). The presence of DNA and serum markers released from the residual trophoblast and embryonic tissues of the VT significantly influences the risk assessment of aneuploidy in other fetuses. Aneuploidy is common in VT, and a single-nucleotide polymorphism (SNP)-based NIPT can be used to confirm the presence of VT in undetected dizygotic twins [23].

The results of NIPT in VT were not as accurate as those of singletons. Recently, a large cohort study on the NIPT in vanishing twins was published [24]. According to this study, the positive predictive value was 50% in the vanishing twin for trisomy 12, and there were no false negative results. Additionally, one study showed that cfDNA from the VT can be present in the maternal blood and can lead to false-positive or false-negative results for fetal aneuploidy up to 15 weeks after ultrasound identification [25]. cfDNA testing is currently not recommended in the presence of VT in the gestational sac because the persistent detection of cfDNA derived from VT is imprecise [26].

Conclusion

The currently used tests for screening fetal chromosomes have been studied for singletons. cfDNA showed the highest detection rate (99%) of trisomy 21 [27]. In the integrated test, if NT was included, the detection rate of trisomy 21 was relatively high at 96% [5]. In contrast, if only the first trimester screening is performed, the detection rate is as low as 82-87%, and if only the quad screening is performed in the second trimester, the detection rate is as low as 81% [4]. Sequential stepwise is known to have a detection rate of approximately 95% as a test in which the first trimester test is performed first and then whether the next test is performed, and the type is determined according to the test result [4].

The significance of the serum markers included in the screening test was as follows: all three trisomies were associated with increased NT thickness and decreased levels of PAPP-A; however, in trisomy 21, serum free-hCG was increased, whereas in trisomies 18 and 13, serum free-hCG was decreased. In the second trimester, AFP and uE3 levels decrease, but-hCG and inhibin A levels increase in Down syndrome [13].

AFP, uE3, and free-hCG levels were reduced in trisomy 18 pregnancies; however, there was no statistically significant difference in inhibin A levels. Conversely, in trisomy 13 pregnancies, inhibin-A levels increased; however, AFP, uE3, and free-hCG levels did not significantly change [13]. In twin pregnancies, trisomy screening is performed by adding maternal serum markers to maternal factors and fetal NT thickness. However, chorionic adjustments were required [28].

VT syndrome occurs before the first trimester during screening. Therefore, VT might affect the expression of maternal serum markers [7]. If obstetricians interpret the screening test results as single fetuses without knowing the existence of VT, they may mistaken a normal fetus for aneuploidy. In addition, even if VT is known, the accuracy of the test results decreases because it is not corrected in the aneuploidy screening test. Furthermore, cfDNA tests cannot be performed in VT pregnancies because of the overflow of cfDNA from necrotic trophoblasts into the maternal plasma, which can persist for at least 15 weeks after fetal death [29].

If VT persists, it may affect the serum test results of the first or second trimester tests; this study compared and reviewed several studies to determine the effect of each. Several reports have shown serum markers in VT, and the PAPP-A

Table 1. Comparison with results of previous studies about vanishing twin (VT) and maternal serum makers

	Total number	Type of studies	Serum markers				
			PAPP-A	AFP	Inhibin A	β-hCG	uE3
Chasen et al. [10] (2006)	VT 41; control 4,536	Retrospective cohort	Increased			Increased	
Chen et al. [19] (2007)	VT 35; singleton 20; twin 37	Case control study			Not changed		
Gjerris et al. [3] (2009)	VT 56; control 897	Retrospective cohort study	Not changed			Not changed	
Spencer et al. [11] (2010)	VT 193; control 1,361	1:5 matching case-control study (matched for the same screening period)	Increased			Not changed	
Huang et al. [7] (2015)	VT 174; control 858	1:5 matching case-control study (matched for ethnicity, maternal age, gestational age, and blood sampling date)	Increased	Increased	Increased	Not changed	Not changed
Lee et al. [21] (2018)	VT 65; control 5,315	Prospective cohort study	Not changed	Increased	Increased	Not changed	Not changed
Chaveeva et al. [12] (2020)	VT 528; control 5,280	Retrospective cohort study	Increased			Not changed	

PAPP-A, pregnancy-associated plasma protein-A; AFP, α-fetoprotein; β-hCG, β-human chorionic gonadotropin; uE3, unconjugated estriol.

level was found to increase in four studies but not in two other ones. Two studies compared this, and both showed an increase. Inhibin A showed an increase in two of three studies, but there was no difference in another study. There was no change in the β-hCG levels in five studies, except for an increase in the results in only one study. Both studies investigating uE3 leves reported no change (Table 1).

If an increase or decrease in these serum markers is observed, VT may have an effect when screening for chromosomal abnormalities, which needs to be considered, and caution is required when interpreting the results. According to the recent American College of Obstetricians and Gynecologists' Committee (ACOG) practice bulletin, if VT or an anomaly is confirmed during multiple gestations, this information should be consulted with the patient because there is a significant risk of inaccurate test results using maternal serum marker screening tests for trisomy or cfDNA. Therefore, appropriate diagnostic tests should be performed [4].

In 2021, an expert review paper was published in ACOG on screening tests for prenatal chromosomal abnormalities in twins [26]. Some studies on VT have shown that hCG, with or without PAPP-A and NT, may be considered for aneuploidy screening [11,12]. cfDNA from the VT may be present in the maternal circulation and may lead to false-positive or false-negative results for up to 15 weeks after ultrasound identification [26]. One study suggested delaying cfDNA testing until 14 weeks gestation. VT improves accuracy owing to the fading of contaminated DNA [30]. However, cfDNA testing is not currently recommended in the presence of VT because the persistence of cfDNA with the disappearance of VT is not known. Additional studies are needed to validate cfDNA in VT. Thus, an SNP-based cfDNA approach may be useful for screening for aneuploidy in VT. However, this test is not currently commercially available [26].

Serum markers in pregnant women with VT are controversial because the results differ slightly between studies. There is no method to accurately predict fetal genetic abnormalities in pregnant women with VT, and further research is needed. In pregnant women with VT, it is necessary to keep in mind that the test results for existing serum markers or cfDNA may change, and appropriate counseling is necessary.

In addition, structural abnormality on ultrasound, such as NT, is not affect survived VT. In Korea, several reports about aneuploidy screening with structural abnormalities [31,32]. In the future, further studies about major and soft markers, such as structural markers on second trimester ultrasound with serum markers in VT, will be more helpful in screening aneuploidy in VT.

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The study is not applicable to Institutional Review Board.

Patient consent

No informed consent was obtained from the patients because the study was retrospective.

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