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Original Article

Can Serum Biomarker Values from Second-Trimester Aneuploidy Screening Predict the development of Retinopathy of Prematurity in Premature Infants?

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Abstract

Objectives: This study aimed to evaluate serum biomarker values measured during second-trimester aneuploidy screening in terms of their predictive ability for the development of retinopathy of prematurity (ROP) in premature infants.

Methods: This retrospective cohort study evaluated the data of 1985 idiopathic premature infants who underwent ROP screening from 2016 to 2022. The infants were divided into two groups according to the presence of ROP, and those with ROP were further evaluated in two subgroups based on the presence of proliferation. Comparisons were made concerning the serum multiple of the median values of unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and alpha-fetoprotein (AFP) among aneuploidy screening biomarkers.

Results: While 1628 premature infants were in the non-ROP group, 357 were in the ROP group. Of the infants with ROP, 72 were in the proliferative ROP group and 285 in the non-proliferative ROP group. There was no significant difference in the multiple of the median values of the evaluated serum biomarkers (uE3, hCG, and AFP) between the ROP and non-ROP groups or between the proliferative ROP, non-proliferative ROP, and non-ROP groups.

Conclusion: The multiple of the median values of second-trimester aneuploidy screening serum biomarkers were not able to predict the development of ROP in premature infants. This result may have been caused by the fact that the blood tests were taken only once and in the same weeks.

Keywords: Alpha-fetoprotein, human chorionic gonadotropin, premature retinopathy, unconjugated estriol.

Introduction

Retinopathy of prematurity (ROP) is a vasoproliferative disorder that causes serious morbidity in premature newborns. Despite all the developments, ROP continues to be a very serious health problem. Small for gestational age (SGA), defined as a birth weight below the 10th percentile on the growth curve, is among the most important causes of perinatal morbidity and mortality. Although many maternal and fetal causes are considered risk factors for ROP, low birth weight and premature birth are the two most important risk

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factors. Although some studies conducted in recent years have shown that second-trimester fetal aneuploidy screening tests, including the measurement of maternal serum alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE3), can also provide insight into preterm birth, there are some conflicting reports in the literature (1-4). Fetal aneuploidy screening and the measurement of AFP, uE3, and hCG are routinely undertaken in pregnant women in most countries to detect the risk of chromosomal anomalies and neural tube defects in newborns (5). AFP production begins in the yolk sac during fetal life. Later, with the disappearance of this structure, it is synthesized in the fetal liver. Fetal AFP serum values and maternal serum AFP values tend to increase and decrease at different times. Fetal serum AFP values usually peak at the 9th week and then gradually decrease until birth. Maternal serum AFP values begin to increase in the 10th week, when fetal serum AFP values begin to decrease, and continue to increase steadily until the 25th week of pregnancy. After the 30th week, maternal AFP begins to decrease steadily, returning to normal values shortly after birth. The hCG test is a test used to detect pregnancy and gestational age and identify ectopic and molar pregnancies, certain malignancies, and chromosomal anomalies during pregnancy. hCG is synthesized by the syncytiotrophoblast cells of the placenta during pregnancy. hCG is at its maximum level in the ninth to 10th week of pregnancy.

The level of uE3 is measured in the blood of pregnant women during the second trimester to screen for trisomy. The majority of estriol production is carried out by the placenta, and the level of free estriol in the blood increases until the last period of pregnancy.

Although the relationship between the risk factors of ROP and systemic disorders that may occur during pregnancy and fetal aneuploidy screening biomarkers has been directly investigated, the direct relationship of these markers with ROP has not yet been evaluated. In this study, we aimed to examine the relationship between second-trimester aneuploidy biomarkers (hCG, uE3, and AFP) and ROP.

Methods

A study plan was created by retrospectively examining the records of 1985 idiopathic premature newborns (<34 weeks) and their mothers who were examined in our ROP clinic between January 1, 2016, and March 15, 2022. Pregnant women whose laboratory results could not be obtained properly, multiple pregnancies, pregnancies with fetuses with chromosomal and structural anomalies, those whose data were obtained from different laboratories, those with a history of systemic diseases during pregnancy (gestational diabetes, preeclampsia, eclampsia, etc.), those exposed to radiation during pregnancy, those who used drugs or alcohol, those with a history of drug use, those exposed to teratogenic effects, smokers, and those of non-Caucasian ethnicities were excluded from the study. The multiple of median values of uE3, AFP, and free hCG, which are second-trimester fetal aneuploidy screening biomarkers, were obtained for all mothers included in the study. Mothers who gave birth to infants with ROP were evaluated as the ROP group, and those who gave birth to healthy infants were evaluated as the non-ROP group. Hormone levels were measured with the electrochemiluminescence method using the Roche Cobas e 601 (Switzerland) device with a Roche commercial kit (Switzerland).

All infants were examined using indirect ophthalmoscopy and RetCam, and the images obtained with RetCam were recorded. ROP staging was performed according to the International Committee for the Classification of ROP. The infant's gestational age, birth weight, and accompanying systemic diseases were recorded. Among the infants with ROP, the presence of proliferation and any treatment applied for ROP were also noted. Using these data, the relationship between the presence of ROP and second-trimester fetal aneuploidy screening biomarkers was compared. The parents of all infants signed an informed consent form. Ethics committee approval with decision number 14/7 was received from the local ethics committee on July 28, 2022.

Statistical Analysis

IBM SPSS Statistics 25.0 (New York, USA) was used for statistical analyses. As descriptive statistics, frequency, percentage, mean±standard deviation, median, and minimum-maximum values were used. The Shapiro–Wilk and Kolmogorov–Smirnov tests were conducted to evaluate whether the data conformed to a normal distribution ($P>0.05$). The Mann–Whitney U test was used in the comparison of non-normally distributed variables between two groups, while the Kruskal–Wallis test was conducted in the comparison among three groups (post hoc test: Dunnett). $P<0.05$ was considered statistically significant.

Results

Among the 1985 infants included in the study, ROP was detected in 357, while it was not detected in 1628. Seventy-two of the infants with ROP had proliferative ROP, and 285 had non-proliferative ROP. In the ROP group, there were 169 girls and 188 boys. The mean gestational age at birth was 30.00±3.06 weeks in the ROP group and 32.50±2.13 weeks in the non-ROP group. The mean birth weight values of the infants with and without ROP were determined to be 1.474.49±484.26 g and 1.882.86±448.07 g, respectively. In the ROP group, 36 infants were born through normal delivery and 321 by cesarean section. In the ROP group, there were 110 normal births and 1518 cesarean births. The num-

ber of cases intubated after birth was 214 in the ROP group and 632 in the non-ROP group, and the number of infants with sepsis was 178 and 632, respectively (Table 1).

The mean age of the mothers at the time of birth was 30.61±5.42 years for the ROP group and 30.32±5.77 years for the non-ROP group. Assisted reproductive techniques were used by 53 mothers in the ROP group and 90 mothers in the non-ROP group. Although the mean maternal AFP value of the ROP group was higher than that of the non-ROP group, no statistically significant difference was detected (1.417±0.759 vs. 1.388±0.746, p=0.593). The mean maternal uE3 value of the ROP group was lower than that of the non-ROP group, but there was no significant difference (1.008±0.327 vs. 1.116±0.575, p=0.585). Similarly, the total hCG value was found to be higher in the ROP group compared to the non-ROP group without any significant difference (1.438±1.085 vs. 1.353±0.872, p=0.826) (Table 2).

The infants with ROP were further divided into proliferative and non-proliferative subgroups, and no statistically significant difference was detected in the comparison of their maternal AFP, uE3, and hCG values. There was also no significant difference between the maternal AFP, uE3, and hCG values of the proliferative ROP and non-ROP groups (Table 3).

Discussion

Given that ROP is an important cause of blindness in premature newborns in many countries, the main motivation for this study was to investigate whether fetal aneuploidy screening tests could also provide an idea about the possibility of ROP development.

Panova et al. (6) demonstrated the presence of AFP in

Table 1. Demographic characteristics and systemic clinical findings of premature infants according to the presence of retinopathy of prematurity

	ROP (357 premature infants)	Non-ROP (1.628 premature infants)
Gender		
Female	169 (47.50)	821 (50.49)
Male	188 (52.50)	807 (49.51)
Gestational age at birth (week)	30.00±3.06 (24–36)	32.50±2.13 (26–40)
Birth weight (g)	1.474.49±484.26 (700–2.400)	1.882.86±448.07 (1.040–3.500)
Mode of delivery		
Vaginal/natural	36 (10.00)	110 (6.80)
Cesarean section	321 (90.00)	1.518 (92.23)
Assisted vaginal delivery (breech)	0	16 (0.98)
Intubation	214 (60.00)	632 (38.83)
Sepsis	178 (49.85)	632 (38.83)
Necrotizing enterocolitis	44 (12.32)	31 (1.90)
Intracranial hemorrhage	25 (7.00)	0

Data are presented as mean±standard deviation (range) or n (%). ROP: Retinopathy of prematurity.

Table 2. Comparison of the multiple of the median values of aneuploidy screening serum biomarkers according to the presence of retinopathy of prematurity in premature infants

	ROP group (357 premature infants)			Non-ROP group (1,628 premature infants)			
	Mean±SD	Min	Max	Mean±SD	Min	Max	p*
AFP MoM	1.41±0.75	0.43	4.03	1.38±0.74	0.60	5.62	0.59
uE3 MoM	1.00±0.32	0.49	1.92	1.11±0.57	0.21	3.78	0.58
Total hCG MoM	1.43±1.08	0.37	6.37	1.35±0.87	0.01	6.21	0.82

*Mann-Whitney U test. MoM: Multiple of the median; SD: Standard deviation; ROP: Retinopathy of prematurity; AFP: Alpha-fetoprotein; uE3: Unconjugated estriol; hCG: Human chorionic gonadotropin.

Table 3. Comparison of the multiple of the median values of aneuploidy screening serum biomarkers between the proliferative ROP, non-proliferative ROP, and non-ROP groups

	P-ROP group (72 premature infants)			ROP group (357 premature infants)			Non-ROP group (1.628 premature infants)			p			
	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	P*	P1§	P2§	P3§
AFP MoM	1.35±0.94	0.50	4.03	1.44±0.68	0.43	3.44	1.38±0.74	0.60	5.62	0.33	0.98	0.99	0.97
uE3 MoM	0.97±0.35	0.64	1.92	1.02±0.32	0.49	1.67	1.11±0.57	0.21	3.78	0.67	0.95	0.52	0.62
Total hCG MoM	1.23±0.70	0.41	2.68	1.50±1.19	0.37	6.37	1.35±0.87	0.01	6.21	0.51	0.78	0.94	0.89

Bold values represent statistical significance ($p<0.05$). *Kruskal-Wallis test; §Dunnett test. P: Comparison between the three groups; P1: Comparison between the P-ROP and NP-ROP groups; P2: Comparison between the P-ROP and non-ROP groups; P3: Comparison between the NP-ROP and non-ROP groups; MoM: Multiple of the median; ROP: Retinopathy of prematurity; P-ROP: Proliferative ROP; NP-ROP: Non-proliferative ROP; AFP: Alpha-fetoprotein; uE3: Unconjugated estriol; hCG: Human chorionic gonadotropin.

the fetal vitreous at various stages of the prenatal period and reported that this marker might be important in the differentiation of photoreceptors and retinal neurons. The authors also reported that while the fetal serum and vitreous concentrations of AFP increased together until the 20th week of pregnancy, they both started to decrease after the 24th week, which they attributed to the decrease in AFP concentration in serum and the formation of the blood-retina barrier. It has been reported that AFP is one of the positive acute-phase reactants in the fetus (7). In cases where the fetus is under stress, the synthesis of AFP may increase in the fetal liver, but it may also increase in maternal serum, although its passage to the vitreous is limited due to the blood-retina barrier. In cases of premature birth, retinal development may be negatively affected by the decrease in the synthesis of AFP and its concentration in the vitreous.

Waller et al. (8) also found high serum AFP values to be associated with preeclampsia and preterm birth. In another study conducted by Boonpiam et al., (9) AFP was found to be significantly associated with SGA. However, neither of these studies considered conditions such as preeclampsia, eclampsia, autoimmune diseases, chronic hypertension, and alcohol and cigarette use, which can negatively affect the maternal serum AFP test and also lead to premature birth.

hCG is both a first-trimester and second-trimester fetal aneuploidy screening biomarker, and conflicting results have been reported regarding this measurement. Although Sirikunalai et al. (2) found that low and high hCG values measured in the second trimester were associated with preterm birth, Boonpiam et al. (9) reported that high values were associated with preterm birth and SGA. Another study showed hCG release from Müller cells and retinal pigment epithelium (RPE) in the human retina and hCG receptors in cone photoreceptor cells and suggested that hCG functioned as a neuroactive molecule (10). In our study, no significant relationship was found between maternal serum hCG levels and the probability of giving birth to an infant with ROP. Controlled vascular endothelial growth factor (VEGF) release, which occurs under hypoxic conditions, is important in retinal vascularization in intrauterine life (11). In the case of premature birth, VEGF release is the first to decrease under hyperoxic conditions and vasoconstriction, and obliteration occurs in the vascular structures that have already developed. As a result, VEGF, which is secreted excessively and uncontrollably from the immature avascular retinal tissue that remains ischemic, thus causing pathological vascularization, has an important place in the pathogenesis of ROP (12). Based on all these, increasing VEGF levels in the vitreous trigger the development of ROP (13). After understanding the importance of increased vitreous VEGF concentration in the pathophysiology of ROP, intravitreal anti VEGF treatments have become almost the main treatment in ROP cases requiring treatment, and laser photocoagulation treatment of the avascular retina is mostly used as a complementary treatment to anti VEGF treatment (14,15).

Movsas et al. (16) reported 15% less VEGF and decreased retinal vascular density in experimentally genetically modified mice lacking the chorionic gonadotropin (CG) receptor and raised under normoxic conditions compared to normal mice and stated that CG and luteinizing hormone contributed to retinal vascularization. In another study by Movsas et al. (17) the blood hCG levels measured at the 1st and 4th weeks in the early newborn and non-proliferative ROP groups were found to be significantly lower compared to the non-ROP group. However, in that study, unlike our investigation, the hCG levels were measured in the serum of early newborns. In a study conducted by Dukic-Stefanovic et al. (10) it was reported that the sources of hCG were Müller cells and RPE, and hCG might affect photoreceptors. The authors concluded that it did not affect the vascular

structures. Losordo et al. (18) suggested that hCG could affect the VEGF level. Based on these findings, we consider that the direct relationship between hCG and ROP remains uncertain, and further studies including more participants are needed.

Estrogen receptors have been detected in vascular endothelial and smooth muscle cells. Various studies have shown that estrogen affects angiogenesis through vascular endothelial cells (17). In a study conducted by Grigsby et al. (19) exogenous estrogen administration resulted in an increase in the number of endothelial cells in a culture of capillary endothelial cells from the retina of rhesus monkeys. When the placenta, which is responsible for producing estrogen during pregnancy, separates prematurely during birth, it can lead to the interruption of retinal blood vessel formation. This interruption can cause the release of VEGF from the immature retina, which may result in the growth of new blood vessels in the retina, a condition known as retinal neovascularization. In our study, although the free estriol levels of the mothers who gave birth to infants with ROP were lower than those of the mothers in the non-ROP group, the difference was not statistically significant.

A review of the literature reveals that many studies have investigated the relationship between fetal aneuploidy biomarkers and ROP risk factors, namely, low birth weight and gestational age at preterm birth. However, while some of these studies have reported the presence of a significant relationship, contradictory results are also present. In most of these studies, pregnant women with diseases that could affect fetal aneuploidy test results were included in the sample, which may have affected the results. Therefore, our exclusion of pregnant women with such diseases and inclusion of only idiopathic preterm births in our sample allowed for the investigation of the relationship between fetal aneuploidy biomarkers and ROP in a more isolated manner. The internationally accepted reference values of fetal aneuploidy tests are established based on the Caucasian race. Thus, we consider that all pregnant women participating in our study being of Caucasian origin was an advantage of our study.

One of the limitations of our study is that only one measurement was made during the 16th–20th weeks of gestation. Large-scale studies, including measurements at different gestational weeks, are needed. At the same time, investigating the possibility of ROP disease with second-trimester aneuploidy tests from maternal serum is another limitation of this study. In more cases, applying these tests to blood samples taken by cordocentesis may enable us to obtain more accurate results. In addition, the small number of pregnant women participating in our study may have had an effect on our non-significant results.

Conclusion

We found no statistically significant relationship between the second-trimester fetal aneuploidy biomarkers (AFP, hCG, and uE3) and the incidence of ROP, although these biomarkers were reported to have a significant relationship with ROP risk factors in most previous publications. This discrepancy may be due to the inclusion of pregnant women with various disorders in previous studies. Our study is noteworthy as it is the first to examine the relationship between ROP and plasma AFP, hCG, and uE3 levels in pregnant women. In light of our results, we think that measuring fetal aneuploidy parameters not only in the second trimester but also at different gestational weeks and including a larger number of sample groups, especially measuring samples taken by cordocentesis, may reveal the relationship between ROP and these markers more clearly.

Disclosures

Ethics Committee Approval: Ethics committee approval with decision number 14/7 was received from the local ethics committee on July 28, 2022.

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