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THE IMPACT OF INHERITED THROMBOPHILIA ON FIRST TRIMESTER COMBINED ANEUPLOIDY SCREENING PARAMETERS

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Abstract. Objective. *To investigate the impact of thrombophilia on pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) and the nuchal translucency (NT) during the first trimester of the pregnancy.* ***Material and Methods.*** *A case-control research study was conducted at a prenatal outpatient unit of a tertiary referral hospital in Burgas, Bulgaria, between January 1st, 2021 and March 31st, 2023. A total of 309 pregnant women patients with congenital thrombophilia took part in the experimental research of the study, while 150 healthy pregnant women patients without evidence of thrombophilia were the control sample.* ***Results.*** *A statistically significant difference in the pregnancy-associated plasma protein A (PAPP-A), $t(369) = 1.028$, $p < 0.05$ between the two groups, with the experimental group reporting lower multiples of median (MoM) values as compared to the control group. The results showed statistically significant differences in the median values of PAPP-A and NT between the different types of inherited thrombophilia but no statistically significant difference in the median values of HCG. The results showed no statistically significant difference in age, gravidity, or parity between the experimental and control groups.* ***Conclusion.*** *The first trimester combined aneuploidy screening parameters are important in prenatal detection of the pregnancy status for identification of any variations in terms of chromosomal and fetal structural anomalies. Inherited thrombophilia adversely impacts the aneuploidy screening parameters during the first trimester of pregnancy.*

Key words: *pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (HCG), nuchal translucency (NT), trisomy, preeclampsia.*

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INTRODUCTION

An understanding of the risk factors associated with recurrent pregnancy loss (RPL) provides the perfect benchmark for the formulation of interventions to address the related complications at specific stages of the pregnancy. In human reproduction, recurrent pregnancy loss is the occurrence of two or more consecutive pregnancy losses within the first 20 weeks of gestation [1, 2]. The occurrence of RPL is a culmination of the complex interaction and interplay between a wide variety of genetic, immunologic and environmental factors [2-4]. In the first trimester, the first 13 weeks since conception, both

the mother and fetus undergo delicate biological and physiological changes that can easily be disrupted by related genetic, immunological and environmental risk factors leading to pregnancy loss [5, 6]. Specifically, thrombophilia, an inheritable genetic risk factor, has been identified as a possible cause of biological and physiological events that can cause pregnancy loss during the first trimester [6-8]. Notably, such events are related to the potential impairment of blood flow by directly affecting the vascularized systems, leading to pregnancy loss [9]. Further, it is important to note that thrombotic events are not only limited to the vascularized systems but have also been associated with the immune system, which has led to immunologic changes that can cause pregnancy loss [10-12]. As a causative risk factor, inherited thrombophilia can impact the different biological and physiological parameters of the pregnancy during the first trimester, which highlights the importance of understanding the impact of its related events on the screening parameters.

The combined aneuploidy screening test is used for early detection of any existing risks that can affect the normal gestational process. Appropriate first-trimester screening can be achieved through tests that combine maternal age, serum biochemical testing and sonographic markers to determine whether the mother and fetus are at risk of adverse genetic and immunologic factors [13]. The primary biomarkers that can be tested during the first trimester include the pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), and the nuchal translucency (NT), and the collection of the blood for testing should be done between 11^{+0} and 13^{+6} weeks of gestation [13, 14]. The nuchal translucency (NT) is the sonolucent space behind the fetal neck, which is present in all fetuses and a higher measurement is strongly associated with aneuploidy forms such as trisomies as well as other fetal structural anomalies such as congenital heart defects, diaphragmatic hernias, skeletal dysplasias, and a variety of genetic syndromes [15, 16]. While thrombophilic events significantly impact these biochemical markers during the first trimester of pregnancy, few research studies have investigated the impact of the condition on combined aneuploidy screening parameters, leaving a considerable research gap on the topic. To this end, the present research study aims to fill the existing gap by investigating the impact of thrombophilia on pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG), and the nuchal translucency (NT), and whether they contribute to recurrent pregnancy loss.

MATERIAL AND METHODS

The research adopted a case-control methodological approach to determine the impact of inherited thrombophilia on first-trimester combined aneuploidy screening parameters, including the pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), and the nuchal translucency (NT). The research was conducted at a prenatal outpatient unit of a tertiary referral hospital in Burgas, Bulgaria, between January 1st, 2021 and December 31st, 2023. A total of 309 pregnant women patients with inherited thrombophilia took part in the experimental research of the study, while 150 healthy pregnant women patients without evidence of thrombophilia were the control sample. The study participants included women who applied for first-trimester aneuploidy screening during the first trimester period and had been diagnosed as having inherited thrombophilia. The exclusion criteria included women with systemic diseases, chromosomal or structural fetal anomalies, and those who discontinued gestational follow-up at the facility during the study. All ethical approvals were performed before the start of the study. Demographic factors and anamnestic data were used to characterize the experimental group. In terms of genetic factors, the study participants were classified on the basis of genetic factors associated with thrombophilia including Factor V Leiden, mutation G20210A in the prothrombin gene, genetic variant C677T in the Methylenetetrahydrofolate reductase gene, Genetic variant in the gene of Plasminogen activator inhibitor 1 (PAI-1) (carriage of genotype 4G/4G) and angiotensin-converting enzyme ACE D/D genotype. Other anamnestic factors for participant characterization included gravidity, parity and blood parameters.

Upon application to the study facility unit, the participants underwent ultrasonographic evaluation, after which the crown-rump length (CRL) and the nuchal translucency (NT) were measured after the confirmation of a viable foetus. The maternal serum pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) were measured using a solid-phase enzyme-labeled chemiluminescent immunometric assay method. The expression of the maternal serum markers was done on the basis of gestational age-specific multiples of median (MoM) values. The statistical calculation for prenatal risk was done using the PRISCA 4.0 software, and any participant with a risk of 1/200 for chromosomal anomaly was informed and advised accordingly. The patients who accepted invasive diagnosis were offered the choice of chorionic villus sampling (CVS) or amniocentesis (A/S). All the parameter measure-

ments were recorded in Microsoft Excel and the analysis was done using IBM Statistical Package for Social Sciences (SPSS) 25.0 software. The continuous variables, including age, weight, gestational period, gravidity, parity and abortion, were analyzed using descriptive statistics, including mean and standard deviation, while the categorical variables, including smoking status and thrombophilia type, were expressed using frequencies or percentages. An independent t-test was performed to compare the continuous variables between the groups, and correlation analysis was used to determine the strength and direction of the relationship between inherited thrombophilia and the combined aneuploidy screening parameters. The level of statistical significance was set at a 95% confidence interval, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Based on the inclusion and exclusion criteria, a total of 309 participants were eligible for analysis and were included in the experimental group. On the other hand, a total of 150 healthy pregnant women patients without evidence of thrombophilia were included in the control group. In terms of the type of the thromboliphic mutation, 33.7 percent of the experimental group had MTHFR C677T while 28.5 percent had Plasminogen activator inhibitor 1(PAI – I) 4G/4. Only 12.29 percent of the participants had ACE D/D genotype, with 12.29 percent having Factor V Leiden and 12 percent having Mutation G20210A in the Prothrombin gene. The frequency statistics for the type of thromboliphic mutation are shown in Table 1 below.

Table 1. Frequency for Participants in terms of types of thromboliphic gene mutation

Thrombophilic gene mutation	N = 309	(%)
Factor V Leiden	38	12.29
Mutation G20210A in Prothrombin gene	39	12.62
MTHFR C677T	104	33.65
Plasminogen activator inhibitor 1(PAI – I) 4G/4G	88	28.47
ACE D/D genotype	38	12.29

In terms of demographic characteristics, the mean age for the experimental group was 31.75 years (S.D = 5.89), while the mean age for the control group was 30.75 years (S.D = 5.53). The mean BMI for the experimental group on the first visit was 25.19 (S.D = 3.38), while the mean BMI for the control group was 24.99 (S.D = 2.49). The t-test results showed no statistically significant difference in the age, gravidity and parity between the experimental and control groups ($p > 0.05$). Also, there was no statistically significant difference between the BMI levels of the experimental group and the control group visit ($F(2,307) = 1.28163$, $p = 0.0576$.

Table 2. Demographic characteristics of participants

Factors	n = 309; SD; %	n = 150; SD; %
Age (years)	31.75 ± 5.89	30.75 ± 5.53
BMI (kg/m^2) on 1st visit	25,19 ± 3.38	24,99 ± 2.49
Smokers	122 (39.50%)	36 (24.00%)
Non – smokers	187(60.50%)	114(76.00%)
Married	168 (54.40%)	64 (42.70%)
Non married	141 (45.60%)	86 (57.30%)

In relation to the first trimester combined aneuploidy screening parameters, the results show a statistically significant difference in the pregnancy-associated plasma protein A (PAPP-A), $t(369) = 1.028$, $p < 0.05$ between the two groups, with the experimental group reporting lower multiples of median (MoM) values as compared to the control group. Similarly, there was a statistically significant difference in the hCG median MoM values between the experimental and the control groups, $t(369) = 2.918$, $p < 0.05$, with the control group reporting higher values as compared to the experimental group. However, the results indicate no statistically significant difference in the nuchal translucency (NT) MoM median values between the two groups, $p < 0.05$. The Pearson correlation analysis indicates a statistically significant moderate positive correlation between the gestational age of the participants and the pregnancy-associated plasma protein A (PAPP-A) for the experimental group. The results further show a statistically significant weak negative correlation between the gestational age of the participants and the hCG and the NT MoM values for both the experimental and control groups. The correlation results between inherited thrombophilia and the aneuploidy screening parameters are shown in Table 3 below.

Table 3. Correlation between gestational age-specific multiples of median and aneuploidy parameters

	Gestational Age (Correlation Coefficient)	p-value
PAPP-A	0.201	< 0.001
hCG	-0.105	< 0.001
NT	-0.119	< 0.001

An analysis of variance (ANOVA) was used to compare the levels of the aneuploidy parameters between the different types of inherited thrombophilia. The

results showed statistically significant differences in the median values of PAPP-A and NT between the different types of inherited thrombophilia but no statistically significant difference in the median values of HCG. For PAPP-A, the participants with MTHFR C677T reported the lowest mean value of 0.5 (S.D = 2.156), while those with Factor V Leiden reported the highest mean value of 0.725 (S.D = 1.0825). In terms of the nuchal translucency (NT) measurements, the participants with Mutation G20210A in the Prothrombin gene reported the highest mean value of 3.6mm (S.D = 1.078), while those with Factor V Leiden reported the lowest mean value (M = 3.2 mm, S.D = 0.925). The median values of the PAPP-A and NT for the different types of inherited thrombophilia are shown in Table 4 below.

DISCUSSION

The first trimester combined aneuploidy screening parameters, which are important in prenatal detection of the pregnancy status, for the identification of any variations in terms of chromosomal and fetal structural anomalies. The primary aneuploidy screening parameters include the pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), and the nuchal translucency (NT), and any abnormal values or changes might be an indication of gestational complications such as congenital heart defects, diaphragmatic hernias, skeletal dysplasias, and a variety of genetic syndromes. The pregnancy-associated plasma protein A (PAPP-A) is produced by the trophoblastic cells, and its quantity during the pregnancy increases with the gestational age [12, 14]. The PAPP-A levels are used for the detection of aneuploid fetuses with low values of below 0.5 MoM being directly associated with adverse pregnancy outcomes, including pregnancy loss, stillbirths and preeclampsia [13, 14, 15]. On the other hand, the free or the total human chorionic gonadotropin (hCG) is produced by the placental syncytiotrophoblastic cells and can be used as biochemical markers of trisomic risk [16]. A high value of the hCG during the aneuploidy test is an indication of a high risk of trisomy 21, and a lower value is an indication of a high risk for trisomy 18 and 13. As an aneuploidic parameter, the nuchal translucency (NT) is used as an indicator for fetal structural anomalies, with higher measurements being directly associated with fetal lymphatic or venous congestion, anemia, cardiac dysfunction, and congenital infections. The present study investigated the effect of different types of inherited thrombophilia on the PAPP-A, HCG and NT values during the first trimester of the pregnancy.

According to the research findings, the experimental group with inherited thrombophilia reported lower multiples of the median (MoM) of PAPP-A compared to the control group of healthy pregnant women patients without evidence of thrombophilia. In gestational research, the PAPP-A can be found in the mother's blood at 12 weeks of the pregnancy since it is produced by the placenta, and low values have often been associated with prenatal complications such as preeclampsia, fetal growth restriction, preterm birth, and even pregnancy loss [19]. Existing research has shown that women with PAPP-A levels ≤ 0.4 MoM in the first trimester have higher chances of a combined composite pregnancy outcome and is directly associated with chronic uteroplacental hypo-perfusion, which can lead to preeclampsia and intrauterine growth restriction (IUGR) [20-22]. Lower levels of PAPP-A in the experimental group are an indication that inherited thrombophilia has a negative impact on the PAPP-A levels and is, therefore, associated with adverse pregnancy outcomes such as preeclampsia and stillbirths. Further, the findings show a statistically significant difference in the HCG median MoM values between the experimental and the control groups, with the experimental group reporting higher values as compared to the control group. Higher levels of HCG during pregnancy have been associated with different types of trisomies, including 21, 18 and 13, all of which are chromosomal anomalies, an indication of an adverse effect of thrombophilia on pregnancy outcomes. However, the results indicate no statistically significant difference in the nuchal translucency (NT), which shows that thrombophilia

Table 4. Comparison of mean values of aneuploidy parameters based on type of thrombophilia

	Factor V Leiden (Mean)	Mutation G20210A in Prothrombin gene (Mean)	MTHFR C677T (Mean)	Plasminogen activator inhibitor 1(PAI – I) 4G/4G (Mean)	ACE D/D genotype (Mean).	P-value
PAPP-A	0,62	0,52	0,78	0,625	0,48	< 0.001
hCG	32 420	35 250	33 280	36 295	34 050	> 0.05
NT	1,2	1,3	1,5	1,4	1,2	< 0.001

does not directly affect the NT measurements during pregnancy.

The research findings show that different types of inherited thrombophilia have different effects on the levels of the combined first-trimester aneuploidy screening parameters. According to the findings, the participants with MTHFR C677T reported the lowest mean value of PAPP-A levels, while those with Factor V Leiden reported the highest value of PAPP-A. Based on the results, it is hypothesized that the MTHFR C677T variant of inherited thrombophilia has a significant impact on the gestational processes associated with the production of PAPP-A and is therefore associated with adverse pregnancy outcomes such as early fetal loss or premature births. However, it is important to note that the other types of inherited thrombophilia have also been associated with PAPP-A pregnancy-related complications but not as significantly as the MTHFR C677T variants [24, 25]. Further, the results show that the participants with Mutation G20210A in the prothrombin gene reported the highest mean value of nuchal translucency while those with Factor V Leiden reported the lowest mean value, which indicates significant differences between the impacts of the different types of inherited thrombophilia on NT. However, the results showed no statistically significant difference in the HCG values between the different types of thrombophilia. The results showed no statistically significant difference in age, gravidity, or parity between the experimental and control groups. Also, there was no statistically significant difference between the BMI levels of the experimental group and the control group visit, which indicates that inherited thrombophilia does not affect the age, gravidity, parity and BMI of the patients.

CONCLUSION

In the first trimester of pregnancy, both the mother and fetus undergo delicate biological and physiological changes that can easily be disrupted by related genetic, immunological and environmental risk factors leading to pregnancy loss. The combined aneuploidy screening test is used for early detection of any existing risks that can affect the normal gestational process and can be achieved through tests that combine the maternal age, serum biochemical testing and sonographic markers to determine whether the mother and fetus are at risk of adverse genetic and immunologic factors. The study investigated the impact of inherited thrombophilia on aneuploidy screening parameters, including the pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), and the nuchal translucency (NT). The results showed that inherited thrombophilia reported lower multiples of the median (MoM) of PAPP-A, which is associated with chronic uteroplacental hypo-perfusion that can lead to preeclampsia and intrauterine growth restriction. The results further show that the MTHFR C677T variant of inherited thrombophilia has a significant impact on the values of PAPP-A as compared to other types of inherited thrombophilia. Based on the findings, it can be concluded that inherited thrombophilia adversely affects the aneuploidy screening parameters during the first trimester of pregnancy.

Conflict of interests: *The authors declare that they have no conflict of interest.*

Authorships: *Zlatko Kirovakov contributed to the literature review and wrote the article. Nadezhda Hinkova and Emiliana Konova provided critical revision and final approval of the finalized manuscript. All authors have read and approved the final manuscript.*

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