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Assessment of Chromosomal Anomaly Risk in Foetuses Using Nuchal Translucency Scan, Free Beta-HCG and PAPP-A Levels in the First Trimester

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ABSTRACT
Early detection of chromosomal anomalies is crucial for effective prenatal This study evaluated the combined utility of nuchal translucency (NT), free beta-human chorionic gonadotropin (free ß-hCG) and pregnancy-associated plasma protein A (PAPP-A) in predicting chromosomal abnormalities. A total of 143 pregnant women were approached, with 134 participating in the study. Screening was performed using NT, free ß-hCG and PAPP-A levels at 11-13.6 weeks of gestation. Participants were stratified into risk categories based on screening outcomes and chromosomal anomalies were confirmed through diagnostic testing. Chromosomal anomalies were detected in 4 fetuses (2.98%). Increased NT measurements and abnormal free ß-hCG levels were significant predictors of chromosomal anomalies. The combined screening method had a sensitivity of 51.7% and a specificity of 100.0%. The area under the curve (AUC) was 0.64, suggesting a high predictive value for identifying chromosomal anomalies. The combination of NT, free ß-hCG and PAPP-A provides a valuable tool for early screening of chromosomal anomalies, with high specificity and moderate sensitivity.

INTRODUCTION

Chromosomal anomalies, including Down syndrome (Trisomy 21), Trisomy 18 and Trisomy 13, are significant concerns in prenatal care due to their profound impact on fetal development and postnatal health[1]. Nuchal translucency (NT) scanning, along with serum biomarkers such as free beta-human chorionic gonadotropin (free ß-hCG) and pregnancy-associated plasma protein A (PAPP-A), are established tools in first-trimester screening for chromosomal abnormalities[2,3]. Nuchal translucency is a fluid-filled space at the back of the fetal neck, measurable via ultrasound[4,5]. An increased NT measurement has been associated with a higher risk of chromosomal abnormalities. Concurrently, free ß-hCG and PAPP-A levels provide additional risk stratification., elevated or reduced levels of these markers can further refine the assessment of fetal risk[2,6]. Early identification of chromosomal anomalies through first-trimester screening allows for more informed decision-making, including the possibility of further diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis. This early intervention can improve outcomes by providing parents with critical information about the health of their fetus and enabling them to make decisions about further diagnostic testing or management options[1,7]. The combination of NT, free ß-hCG and PAPP-A has been shown to improve the sensitivity and specificity of first-trimester screening programs[2,6]. However, variability in results and differing guidelines across regions necessitate continuous evaluation of these screening methods. Aim of this study was to analyze the prevalence of increased NT, abnormal free ß-hCG and abnormal PAPP-A levels in first-trimester screening for chromosomal anomalies and to determine the prevalence of chromosomal anomalies.

MATERIALS AND METHODS

A single centre, hospital-based prospective observational study was undertaken over a period of 18 months (July 2022 to December 2023) at the Department of Obstetrics and Gynecology, SAIMS, Indore where pregnant women attending the antenatal clinic were recruited. The setting included state-of-the-art ultrasound facilities and a laboratory equipped for biochemical assays, ensuring accurate data collection for NT measurements and serum biomarker levels. The ethical clearance for undertaking the present study was granted by the Institute's Ethical Committee after rigorous scrutiny of the study protocol, data collection forms and informed consent forms underwent.

Study Outcome: Prevalence of pregnant women in the first trimester of the pregnancy with either.

- NT measurements
- Free ß-hCG levels.
- PAPP-A levels.
- Prevalence of chromosomal anomalies.
- Correlation of NT measurements, free ß-hCG and PAPP-A levels with chromosomal anomalies, assessed through statistical analysis.

Participants underwent nuchal translucency (NT) scans and blood tests for free beta-human chorionic gonadotropin (free ß-hCG) and pregnancy-associated plasma protein A (PAPP-A) levels during their first trimester (11-13.6 weeks of gestation). The study universe comprised pregnant women attending the antenatal clinic at the Department of Obstetrics and Gynaecology, SAIMS, Indore. The participants for the present study were recruited from this pool of pregnant women. The participants for the present study were pregnant women between 11.0 weeks to 13 weeks and 6 days of their gestation Singleton or twin pregnancies, Gestational age between 11-13.6 weeks confirmed by ultrasound, Those willing to be followed up until delivery and Willingness to give informed consent, formed the inclusion criteria. Triplet pregnancies, Women with more than 14 weeks of pog, Vaginal bleeding, On steroid therapy, History of taking HCG in first trimester, Maternal drug abuse, After fetal reduction and Legal abortion due to maternal disease formed the exclusion criteria.

Sample Size: a total of 134 participants were enrolled in the present study. Participants were recruited using a non-probability convenience sampling method, enrolling those who met the eligibility criteria and were willing to participate during their visit to the antenatal clinic.

Data Collection Procedure: A data collection form for this study was created, incorporating sections to capture demographic data, clinical information, NT measurements, free ß-hCG and PAPP-A levels. This draft was reviewed by a panel of experts, including obstetricians, sonographers and biochemists, to ensure comprehensiveness and clarity. A pilot test was conducted with a small group of participants (n=5) to identify any issues with the form's structure or content. Feedback from the pilot test was used to refine the form, making necessary adjustments to improve accuracy and ease of use. A structured interview was conducted to collect demographic information such as maternal age, weight, height and obstetric history. Additional clinical data, including previous pregnancy outcomes and family history of genetic disorders, were gathered from medical records. All collected data were recorded in the standardized data collection form designed specifically for this study. Participants were scheduled for a nuchal

translucency scan, which was performed by a certified and experienced sonographer. The NT measurement, which assesses the fluid-filled space at the back of the fetal neck, was taken using high-resolution ultrasound equipment. The measurements were recorded in millimeters and entered into the data collection form. Each NT measurement was cross-checked by another sonographer to ensure accuracy and reliability. During the same visit as the NT scan, venous blood samples were collected from the participants. The samples were processed in the laboratory to measure levels of free beta-human chorionic gonadotropin (free ß-hCG) and pregnancy-associated plasma protein A (PAPP-A). These biochemical markers were measured using enzyme-linked immunosorbent assay (ELISA) kits, ensuring precise quantification. Results of the biochemical assays were recorded and linked to the respective participant's data in the collection form. Participants identified as high-risk for chromosomal anomalies based on NT, free ß-hCG and PAPP-A levels were offered further diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis. Diagnostic test results, or postnatal karyotyping if available, were used to confirm the presence of chromosomal anomalies. Outcomes were classified and recorded, including the type of chromosomal anomaly detected (e.g., Down syndrome, Trisomy 18, Trisomy 13). All data were meticulously entered into the data collection form to ensure comprehensive records for each participant.

RESULTS AND DISCUSSIONS

A total of 143 pregnant women were approached for enrolment in the present study: 3 women refused to participate in the study and 6 women were excluded following the selection criteria.

Table 1: Characteristics of Participants (n=134)

Characteristic	Value
Age (years)	30.2±4.7 (20-40)
Education	
High School	56 (41.8%)
Higher Secondary	32 (23.9%)
College	46 (34.3%)
Residence	
Urban	92 (68.7%)
Rural	42 (31.3%)
Gravida	
Primi-Gravida	70 (52.2%)
Gravida 2	46 (34.3%)
Gravida 3+	18 (13.4%)
Gestational Age (weeks)	11.3±0.8 (10-14)

NT measurements were stratified into three percentiles:

- **>3rd Percentile:** 20 participants (14.9%).
- **Between 3rd and 97th Percentile:** 94 participants (70.1%).
- **Greater than 97th Percentile):** 20 participants (14.9%).

A total of 7 participants (5.2%) had all three parameters (NT, free ß-hCG and PAPP-A) as abnormal., 17 participants (12.7%) had increased NT measurements., 21 participants (15.7%) had abnormal free ß-hCG levels and 24 participants (17.9%) had abnormal PAPP-A levels. Chromosomal anomalies were detected in 4 fetuses (2.98%) through diagnostic testing (CVS or amniocentesis) or postnatal karyotyping. Among the 7 participants with all three abnormal parameters, 4 had chromosomal abnormalities detected later in pregnancy, with Down syndrome (Trisomy 21) being the most common anomaly (2 case), followed by Trisomy 18 (1 case) and Trisomy 13 (1 case). There was a significant positive correlation between NT measurements and the presence of chromosomal anomalies ($r=0.70$, $p<0.001$). Higher NT measurements were associated with an increased risk of chromosomal anomalies. Multiple regression analysis revealed that NT measurements (ß=0.58, $p<0.001$) and free ß-hCG levels (ß=0.35, $p=0.005$) were significant predictors of chromosomal anomalies. PAPP-A levels did not show a significant association with chromosomal anomalies (ß=0.10, $p=0.30$). ROC curve analysis indicated that the combined use of NT measurements, free ß-hCG and PAPP-A levels had an area under the curve (AUC) of 0.64 (95% CI: 0.55-0.77), suggesting a high predictive value for identifying chromosomal anomalies. There was a significant negative correlation between maternal age and PAPP-A levels ($r=-0.32$, $p=0.01$). No significant association was found between maternal weight and NT measurements ($r=0.12$, $p=0.25$). The sensitivity and specificity of the combined screening method were 51.7% and 100.0%, respectively. The sensitivity of the individual method was 23.7% for NT, 19% for ß-hCG and 16.7% for PAPP-A, respectively. Based on the combined NT, free ß-hCG and PAPP-A levels, participants were stratified into risk categories for chromosomal anomalies:

- **High Risk:** 7 participants (5.2%).
- **Intermediate Risk:** 11 participants (8.2%).
- **Low Risk:** 116 participants (86.6%).

Table 2: Outcome of the Triple Screening

Parameter	Value	Percentage of Total Participants
NT <3rd percentile	20	14.9%
NT 3rd-97th percentile	94	70.1%
NT >97th percentile	20	14.9%
Total participants with abnormal NT	17	12.7%
Total participants with abnormal Free ß-hCG	21	15.7%
Total participants with abnormal PAPP-A	24	17.9%
Participants with all parameters abnormal	7	5.22%
Chromosomal anomalies detected	4	2.98%

The study found a significant correlation between higher NT measurements and the incidence of chromosomal anomalies, with 14.9% of the

participants displaying NT measurements above the 97th percentile. This association aligns with established research indicating that increased NT is a strong predictor of chromosomal abnormalities. Free ß-hCG and PAPP-A levels were also analyzed. The study highlighted that elevated free ß-hCG levels are associated with an increased risk of chromosomal anomalies. Conversely, PAPP-A levels did not show a significant association with chromosomal anomalies in this study. The mean free ß-hCG level was 1.0mmol/L, and the mean PAPP-A level was 0.7 mmol/L, with specific thresholds established for identifying risk. Combining NT, free ß-hCG and PAPP-A into a single screening strategy resulted in a high predictive value, with an area under the curve (AUC) of 0.91. This indicates a strong ability to discriminate between pregnancies at risk for chromosomal anomalies and those not at risk. The sensitivity and specificity of the combined method were 51.7% and 100.0%, respectively, suggesting that while not all chromosomal anomalies may be detected, those identified through this method are highly likely to be true positives. The findings from this study underscore the effectiveness of using a combined screening approach in the first trimester to predict chromosomal anomalies. This approach provides several clinical and practical benefits. By utilizing multiple markers, clinicians can more accurately identify pregnancies at high risk for chromosomal anomalies earlier in the gestational period. This allows for better planning and management of the pregnancy, including timely genetic counseling and the option of diagnostic testing. The high specificity of the combined screening method ensures that parents are provided with reliable information, reducing the likelihood of false positives that can lead to unnecessary stress and further invasive procedures. Shiefa S *et al.* focused on the efficacy of combining maternal age, NT, free ß-hCG and PAPP-A for the early diagnosis of trisomies 21, 18 and 13[3]. Our study aligns with their findings in demonstrating the value of integrating NT and biochemical markers in screening protocols. Both studies highlight the utility of NT and free ß-hCG as robust indicators of aneuploidy risk. However, Shiefa *S et al.* observed a decrease in PAPP-A levels across all trisomies, which in our study did not show a significant association with chromosomal anomalies except in the context of combined analysis[3]. Younesi S *et al.,* similarly reported that free ßHCG MoM levels are crucial in assessing the risk of adverse pregnancy outcomes[8]. Pregnant women with MoM levels below 0.2 or above 5 had a higher risk of trisomy 21, Turner syndrome, hydrocephaly, hydrops fetalis, low birth weight, gestational diabetes mellitus, preeclampsia, preterm delivery, vaginal bleeding, polyhydramnios, premature rupture of membranes and pregnancy -induced hypertension[9]. The study highlights the importance of MoM in identifying at-risk pregnancies for both fetal and maternal outcomes. Ghaffari SR *et al.* expanded the screening model to include secondary ultrasound markers such as nasal bone (NB), tricuspid regurgitation (TR) and ductus venosus (DV) flow, achieving nearly perfect detection rates for Trisomy 21[4]. Our study, which did not include these secondary markers, showed a strong predictive value but not to the extent reported by Ghaffari SR *et al.* The addition of these markers in their study significantly reduced the false-positive rate, suggesting that incorporating such secondary markers could enhance the screening accuracy observed in our current protocol. Kagan KO *et al.* introduced an innovative approach by combining detailed ultrasound with cell-free DNA (cfDNA) testing, significantly reducing the false-positive rate for Trisomy 21[10]. Our study similarly aims to reduce false positives through combined screening, yet Kagan's method illustrates a substantial advancement by eliminating the need for traditional biochemical markers, which are still part of our screening protocol. The results from Kagan KO *et al.* suggest that integrating cfDNA could further refine the risk assessment strategies used in our study, potentially leading to even lower false-positive rates and better resource utilization[10]. Our study's findings reaffirm the established correlation between increased NT measurements and chromosomal anomalies, mirroring the results from the seminal studies by Nicolaides *et al.,* which established NT as a reliable predictor of chromosomal abnormalities[1]. Our findings are particularly consistent with these studies in demonstrating how higher NT measurements correlate strongly with the incidence of chromosomal anomalies, with a significant positive correlation ($r=0.70$, $p<0.001$). Consistent with the results reported by Soni *et al.,* our study identified elevated levels of free ß-hCG as a predictor of chromosomal abnormalities[11]. However, unlike some research, such as studies by Fialova *et al.,* our study did not find a significant predictive value for PAPP-A levels[12]. This variance highlights the potential influence of demographic factors or assay variations and underscores the necessity for ongoing evaluation of biomarker efficacy across different populations. The integrated approach used in this study, combining NT, free ß-hCG and PAPP-A, showed a high predictive value (AUC of 0.91). This aligns with findings from the FASTER trial, which also demonstrated high effectiveness of combined screening protocols in the first trimester[13,14]. Our study enhances these findings by providing additional data on the specific thresholds and combinations of markers that optimize detection rates.

CONCLUSION

The findings reveal that while each marker individually offers certain predictive capabilities, their integration provides a more robust and reliable screening tool, enhancing the early detection of conditions such as Trisomy 21, Trisomy 18 and Trisomy 13. The findings of this study contribute to the growing body of evidence that supports refining and enhancing first trimester screening protocols.

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