

FIRST-TRIMESTER PAPP-A, FREE β -HCG, AND NUCHAL TRANSLUCENCY FOR PREDICTING FETAL GROWTH RESTRICTION

PAPP-A, SLOBODNI β -HCG I NUHALNA TRANSLUCENCIJA U PRVOM TRIMESTRU ZA PREDVIĐANJE RESTRIKCIJE FETALNOG RASTA

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Summary

Background: Early identification of pregnancies at risk for fetal growth restriction (FGR) is essential for appropriate prenatal monitoring and timely intervention. First-trimester screening markers, including nuchal translucency (NT), pregnancy-associated plasma protein A (PAPP-A), and free β -human chorionic gonadotropin (free β -hCG), are routinely used in clinical practice and may provide early insight into placental function.

Methods: This cohort study analysed first-trimester biochemical and ultrasound markers in pregnancies that later developed FGR and compared them with those of uncomplicated control pregnancies. NT was measured between 11 and 13+6 gestational weeks, while PAPP-A and free β -hCG concentrations were expressed as multiples of the median (MoM). Correlation analyses and logistic regression models were applied to evaluate associations and predictive value.

Results: NT values were significantly higher in pregnancies complicated by FGR. Mean PAPP-A and free β -hCG levels did not differ significantly between the FGR and control groups. Correlations among first-trimester markers were more consistent in the control group, whereas disrupted relationships were observed in FGR pregnancies. Although mean PAPP-A values were similar, very low PAPP-A levels (<5th percentile) were associated with an increased risk of FGR. The combined presence of elevated NT and low PAPP-A improved the identification of high-risk pregnancies.

Kratak sadržaj

Uvod: Rana identifikacija trudnoća sa povećanim rizikom za restrikciju fetalnog rasta (FGR) od ključnog je značaja za unapređenje prenatalnog praćenja i perinatalnih ishoda. Prvotrimestrijski skrining rutinski uključuje merenje nuhalne translucencije (NT), kao i određivanje serumskih koncentracija pregnancy-associated plasma protein A (PAPP-A) i slobodnog β -humanog horionskog gonadotropina (β -hCG), koji mogu odražavati ranu placentalnu funkciju.

Metode: U ovoj kohortnoj studiji analizirani su biohemijski i ultrazvučni markeri iz prvog trimestra u trudnoćama koje su kasnije bile komplikovane FGR-om i upoređeni sa nekomplikovanim kontrolama. NT je merena između 11. i 13+6. gestacione nedelje, dok su vrednosti PAPP-A i slobodnog β -hCG izražene kao multiplikati medijane (MoM). Korišćene su korelacione analize i logistička regresija radi procene povezanosti i prediktivne vrednosti.

Rezultati: Vrednosti NT bile su značajno više u grupi sa FGR-om. Prosečne vrednosti PAPP-A i slobodnog β -hCG nisu se statistički značajno razlikovale između grupa. Korelacije između markera bile su konzistentnije u kontrolnoj grupi, dok su u FGR trudnoćama uočeni poremećeni odnosi. Iako prosečne vrednosti PAPP-A nisu pokazale razliku, veoma niske vrednosti PAPP-A (<5. percentila) bile su povezane sa povećanim rizikom od FGR-a. Kombinacija povišenog NT i niskog PAPP-A poboljšala je identifikaciju trudnoća sa visokim rizikom.

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Conclusion: NT may serve as a potential early indicator of FGR risk, particularly when interpreted within specific clinical sub-stratifications rather than as a standalone screening tool. Extremely low PAPP-A values may further contribute to early risk stratification. The combined interpretation of biochemical and sonographic markers in the first trimester may support earlier identification of pregnancies requiring closer surveillance.

Keywords: fetal growth restriction, nuchal translucency, PAPP-A, free β -hCG, first-trimester screening

Introduction

Fetal growth restriction (FGR), historically referred to as intrauterine growth restriction (IUGR), remains one of the leading causes of perinatal morbidity and mortality worldwide and is most commonly associated with impaired placentation and chronic uteroplacental insufficiency (1). Because clinical manifestations often appear only in the later stages of pregnancy, early identification of pregnancies at increased risk remains a major challenge in obstetric care. Earlier risk recognition would allow closer surveillance, timely clinical interventions, and improved perinatal planning. While historical literature and traditional registries frequently utilise the term Intrauterine Growth Restriction (IUGR), contemporary clinical guidelines increasingly favour 'Fetal Growth Restriction (FGR)'. Throughout this manuscript, both terms are considered interchangeable and refer to pathological fetal growth restriction, in which birthweight falls below established population-specific percentiles.

First-trimester combined screening was initially developed for the detection of fetal aneuploidies and routinely includes assessment of maternal serum pregnancy-associated plasma protein A (PAPP-A), free β -human chorionic gonadotropin (free β -hCG), and fetal nuchal translucency (NT). Growing evidence suggests that these markers reflect early placental and fetal development and may therefore be associated with subsequent adverse pregnancy outcomes, including FGR (2, 3).

Among biochemical markers, low maternal PAPP-A has been most consistently linked to placental dysfunction and later fetal growth restriction. Large cohort studies and meta-analyses have shown that PAPP-A values in the lowest percentiles are associated with increased risks of FGR, preterm birth, and other placental-mediated complications (4–6). However, the predictive performance of PAPP-A alone is limited, suggesting that its clinical relevance lies primarily at extreme values and in combination with other parameters (7–9).

The association between free β -hCG and fetal growth has been less consistent. While some studies

Zaključak: NT može poslužiti kao potencijalni rani indikator rizika za FGR, posebno kada se tumači unutar specifičnih kliničkih podgrupa, a ne kao samostalan skrining alat. Izrazito niske vrednosti PAPP-A mogu dodatno doprineti ranoj stratifikaciji rizika. Kombinovano tumačenje biohemijskih i ultrazvučnih markera u prvom trimestru može omogućiti raniju identifikaciju trudnoća koje zahtevaju pojačano praćenje.

Ključne reči: restrikcija fetalnog rasta, nuchalna translucencija, PAPP-A, slobodni β -hCG, prvotrimestrijski skrining

report associations between abnormal free β -hCG levels and adverse outcomes, others demonstrate minimal independent predictive value once maternal and clinical confounders are considered (10–12). These inconsistencies indicate that free β -hCG should be evaluated as part of a combined screening approach rather than as a standalone marker.

NT measurement, although primarily used in aneuploidy screening, has also been explored as an indirect marker of early fetal and placental development. Increased NT thickness has been associated with adverse pregnancy outcomes, including FGR, even in chromosomally normal fetuses (13). Despite some variability related to gestational age and measurement technique, NT remains a readily available and clinically relevant parameter in early pregnancy assessment.

Recent research has therefore focused on integrated screening models that combine biochemical markers, sonographic measurements, and maternal characteristics to improve early risk stratification for FGR (10, 11). Population-specific analyses are particularly important, as baseline risks and biomarker distributions may vary between settings. The present study aimed to evaluate the association between first-trimester PAPP-A, free β -hCG, and NT and the subsequent development of FGR in a Montenegrin cohort, and to explore whether combinations of these markers improve early risk assessment.

Materials and Methods

Ethics statement

The study was approved by the Health Centre Filipovic, Montenegro (approval number 403/25) and conducted in accordance with the principles of the Declaration of Helsinki.

Participants and procedures

This prospective observational cohort study included 120 pregnant women attending routine first-trimester prenatal visits at the Centre for

Reproductive Health (Health Centre Podgorica) and the Filipovic Polyclinic. All participants had a negative first-trimester combined screening result for trisomies 21, 18, and 13, performed between 10 and 13 gestational weeks. To ensure a homogeneous study population and reduce potential confounding, strict exclusion criteria were applied. Multiple pregnancies, pregnancies conceived by assisted reproductive technologies (IVF), fetuses with confirmed chromosomal abnormalities, and those with major structural anomalies detected on early ultrasound were excluded. Furthermore, patients with pre-existing maternal conditions known to severely impact fetal growth – such as chronic hypertension, pre-gestational diabetes mellitus, active autoimmune diseases, and known thrombophilia – were excluded at recruitment. Information on maternal weight, BMI, and smoking status was inconsistently recorded in the screening registry, a limitation acknowledged.

Serum concentrations of PAPP-A and free β -hCG were measured using the AutoDELFIA immunofluorometric assay. NT was measured during a standard obstetric ultrasound examination. All biochemical results were expressed as multiples of the median (MoM) adjusted solely for gestational age using native software baselines, without further adjustments for maternal weight or smoking status.

Demographic and clinical data, including maternal age, parity, previous miscarriages, and pregnancy outcomes, were obtained from medical records. Fetal growth restriction (FGR/IUGR) was clinically defined as a confirmed birthweight below the 10th percentile for gestational age at delivery, as established by population-specific local growth charts. Gestational age at delivery was recorded for all cases.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk test and visual inspection. Due to the non-normal distribution discovered across biochemical and sonographic variables, non-parametric methods were consistently deployed. Descriptive statistics were calculated for all variables. Group comparisons were performed using the non-parametric Mann-Whitney U test for continuous variables and Pearson's Chi-square test for categorical demographic variables. Associations between variables were evaluated using Spearman's correlation coefficient.

Principal component analysis (PCA) was applied to explore multivariate relationships among PAPP-A, free β -hCG, and NT. Logistic regression analysis was used to assess predictors of IUGR. Receiver operating characteristic (ROC) curve analysis was performed for selected markers, with the area under the curve (AUC) used to evaluate predictive performance. Optimal operating cut-off values for discriminatory performance were determined using the Youden index. Statistical significance was defined as $p < 0.05$.

Results

Maternal characteristics

Baseline maternal demographic and obstetric characteristics are presented in *Table 1*. A total of 120 pregnant women were included in the study, comprising 60 women in the control group and 60 women in the FGR group. As shown in *Table 1*, the median maternal age was statistically significantly higher in the control group compared with the FGR group (40.00 (IQR 2.00) vs. 36.00 (IQR 2.00) years, $p < 0.001$). The distribution of parity ($p = 0.835$) and the prevalence of previous abortions ($p = 0.281$) were comparable between the two groups.

Table 1 Demographic and obstetric characteristics of the study population.

Variable	Control Group (n=60)	FGR Group (n=60)	p-value
Age (years), Median (IQR)	40.00 (2.00)	36.00 (2.00)	< 0.001
Age range (years)	30–42	29–40	-
Nulliparous, n (%)	24 (40.00%)	22 (36.67%)	0.851
Parity 1, n (%)	21 (35.00%)	19 (31.67%)	0.835
Parity 2, n (%)	14 (23.33%)	17 (28.33%)	-
Parity >2, n (%)	1 (1.67%)	2 (3.33%)	-
Previous abortion, n (%)	11 (18.33%)	17 (28.33%)	0.281

****Note:** Data are presented as median (interquartile range, IQR) for continuous variables and as count (percentage, %) for categorical variables. Statistical comparisons between groups were performed using the non-parametric Mann-Whitney U test for continuous data and Pearson's Chi-square test for categorical data. Significance level was set at $p < 0.05$.

Table II Comparison of biochemical and ultrasonographic parameters between study groups.

Parameter	Control Group (n=60)	FGR Group (n=60)	p-value
NT level (mm)	1.09 (0.88–1.30)	1.22 (0.96–1.48)	0.022
PAPP-A level (mIU/L)	3.10 (1.53–4.65)	2.56 (0.92–4.20)	0.156
Free β -hCG level (ng/mL)	72.79 (44.60–101.00)	71.06 (13.00–129.10)	0.867
NT MoM	0.99 (0.73–1.25)	1.05 (0.78–1.32)	0.322
PAPP-A MoM	1.07 (0.60–1.54)	0.81 (0.40–1.23)	0.207
Free β -hCG MoM	1.14 (0.63–1.65)	1.03 (0.53–1.53)	0.674

Note: All data are presented as Median (Interquartile Range, IQR) because the variables did not follow a normal distribution. Group comparisons were performed using the non-parametric Mann-Whitney U test. NT: Nuchal Translucency; PAPP-A: Pregnancy-Associated Plasma Protein A; Free β -hCG: Free beta human chorionic gonadotropin; MoM: Multiples of the Median. $p < 0.05$ indicates statistical significance.

Table III Spearman's rank correlation coefficients (rho) for first-trimester markers instudy (FGR) and control groups.

Variable	Group	NT	PAPP-A	F β -hCG	NT MoM	PAPP-A MoM	F β -hCG MoM
NT	FGR (\n=60\))	-	0.247 (p=0.027)	-0.054 (p=0.638)	0.613 (p<0.001)	-0.082 (p=0.468)	0.062 (p=0.586)
	Control (\n=60\))	-	0.230 (p<0.001)	-0.214 (p<0.001)	0.763 (p<0.001)	-0.019 (p=0.730)	-0.084 (p=0.121)
PAPP-A	FGR (\n=60\))	0.247 (p=0.027)	-	-0.062 (p=0.587)	-0.160 (p=0.157)	0.672 (p<0.001)	0.157 (p=0.164)
	Control (\n=60\))	0.230 (p<0.001)	-	-0.151 (p<0.01)	-0.070 (p=0.197)	0.618 (p<0.001)	0.045 (p=0.408)
F β -hCG	FGR (\n=60\))	-0.054 (p=0.638)	-0.062 (p=0.587)	-	0.081 (p=0.477)	0.093 (p=0.416)	0.867 (p<0.001)
	Control (\n=60\))	-0.214 (p<0.001)	-0.151 (p<0.01)	-	-0.061 (p=0.261)	0.008 (p=0.888)	0.858 (p<0.001)
NT MoM	FGR (\n=60\))	0.613 (p<0.001)	-0.160 (p=0.157)	0.081 (p=0.477)	-	-0.033 (p=0.773)	-0.073 (p=0.523)
	Control (\n=60\))	0.763 (p<0.001)	-0.070 (p=0.197)	-0.061 (p=0.261)	-	0.023 (p=0.668)	-0.151 (p<0.01)
PAPP-A MoM	FGR (\n=60\))	-0.082 (p=0.468)	0.672 (p<0.001)	0.093 (p=0.416)	-0.033 (p=0.773)	-	0.131 (p=0.247)
	Control (\n=60\))	-0.019 (p=0.730)	0.618 (p<0.001)	0.008 (p=0.888)	0.023 (p=0.668)	-	0.047 (p=0.389)
F β -hCG MoM	FGR (\n=60\))	0.062 (p=0.586)	0.157 (p=0.164)	0.867 (p<0.001)	-0.073 (p=0.523)	0.131 (p=0.247)	-
	Control (\n=60\))	-0.084 (p=0.121)	0.045 (p=0.408)	0.858 (p<0.001)	-0.151 (p<0.01)	0.047 (p=0.389)	-

Note: Data represent Spearman's rank correlation coefficients (rho) with their exact p-values in parentheses, displayed separately for the fetal growth restriction group (FGR, \n=60\)) and the uncomplicated control group (Control, \n=60\)). Statistical significance is defined as (p<0.05).

Abbreviations: NT: Nuchal Translucency; PAPP-A: Pregnancy-Associated Plasma Protein A; F β -hCG: Free beta human chorionic gonadotropin; MoM: Multiples of the Median.

First-trimester biomarkers

Comparisons of first-trimester biomarkers between the control and IUGR groups are shown in Table II. NT values were significantly higher in the IUGR group than in the control group [1.22 (0.96–1.48) vs 1.09 (0.88–1.30), $p=0.022$]. No statistically significant differences were observed between the groups for PAPP-A, free β -hCG, or their corresponding MoM values ($p>0.05$ for all comparisons).

Correlation analysis

Spearman's correlation analysis within the FGR group is presented in Table III. A weak but statistically significant positive correlation was observed between NT and PAPP-A ($\rho=0.247$, $p=0.027$), along with a strong positive correlation between NT and NT MoM ($\rho=0.613$, $p<0.001$). A very strong positive correlation was found between free β -hCG and free β -hCG MoM ($\rho=0.867$, $p<0.001$). No other statistically significant correlations were identified.

In the control group (Table III), NT showed a significant positive correlation with PAPP-A ($\rho=0.230$, $p<0.001$) and NT MoM ($\rho=0.763$, $p<0.001$), as well as a weak negative correlation with free β -hCG ($\rho=-0.214$, $p<0.001$). Free β -hCG demonstrated a strong positive correlation with its MoM value ($\rho=0.858$, $p<0.001$) (Table III).

Principal component analysis

Principal component analysis (PCA) identified three components explaining 100% of the total variance. The first principal component (PC1) accounted for 43.5% of the variance and was primarily loaded by NT and PAPP-A. The second component (PC2) explained 31.7% of the variance. It was predominantly associated with free β -hCG MoM, while the third component (PC3) accounted for 24.8% of the variance (Table – PCA factor loadings). The relationships between PC1 and PC2 and between PC1 and PC3 are illustrated in Figures 1 and 2, respectively.

Logistic regression analysis for FGR prediction

The results of the multiple logistic regression analysis are summarised in Table IV. NT emerged as a statistically significant predictor of the outcome ($\beta=1.344$, $OR=3.833$, $p=0.008$), whereas PAPP-A showed a negative association ($\beta=-0.156$, $OR=0.856$, $p=0.047$). Free β -hCG was not significantly associated with the outcome.

Further logistic regression analysis stratified by MoM categories is presented in Table V. NT

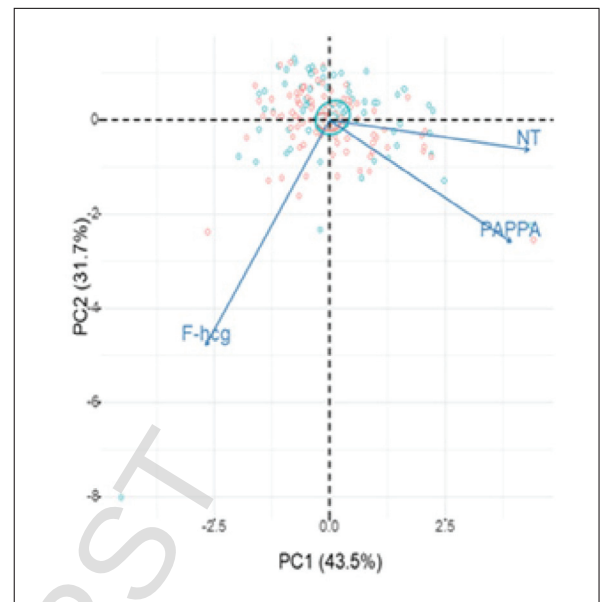


Figure 1 Relationship between PC1 and PC2.

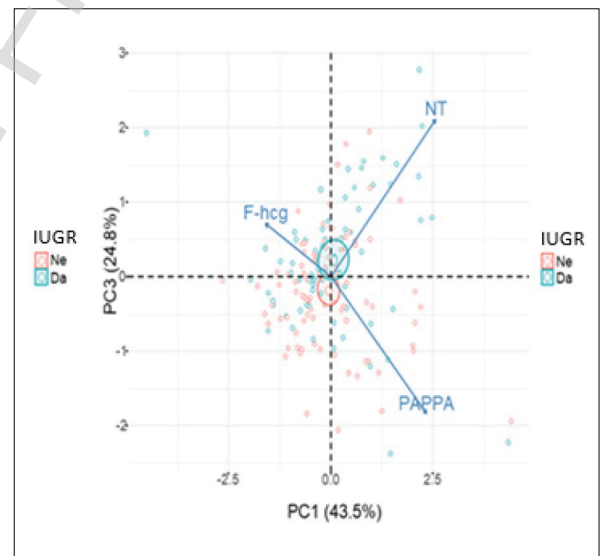


Figure 2 Relationship between PC1 and PC3.

PCA Factor Loadings and Explained Variance			
Variable	PC1 (loading)	PC2 (loading)	PC3 (loading)
NT	0.672	0.152	
0.735			
PAPP-A	0.589	0.472	
0.635			
F β -hCG MoM	0.274	0.874	
0.096			
Component	Eigenvalue	% Variance	
Explained			
PC1	1.353	43.5%	
PC2	0.958	31.7%	
PC3	0.747	24.8%	

Table IV Multiple logistic regression analysis for predicting FGR.

Variable	B (SE)	Wald statistic	OR (95% CI)	p-value
NT	1.344 (0.510)	6.936	3.833 (1.411–10.417)	0.008
PAPP-A	-0.156 (0.078)	3.951	0.856 (0.735–0.997)	0.047
Free β -hCG	0.000 (0.003)	0.011	1.000 (0.994–1.006)	0.916

Note: Data represent unstandardised regression coefficients (B) with their standard errors (SE) in parentheses, Wald statistics, odds ratios (OR), and 95% confidence intervals (CI).

Abbreviations: NT: Nuchal Translucency; PAPP-A: Pregnancy-Associated Plasma Protein A; Free β -hCG: Free beta human chorionic gonadotropin; FGR: Fetal Growth Restriction. Significance level was set at $(p < 0.05)$.

Table V Logistic regression analysis according to MoM stratification.

MoM Category Group	Independent Variable	Beta	OR (95% CI)	p-value
NT MoM category				
Subgroup <1.2	NT level	0.670	1.954 (0.543–7.029)	0.305
	PAPP-A level	-0.107	0.898 (0.755–1.068)	0.225
	F β -hCG level	0.001	1.001 (0.996–1.006)	0.762
Subgroup 1.2–2	NT level	2.440	11.477 (1.343–98.101)	0.026
	PAPP-A level	-0.087	0.917 (0.699–1.203)	0.532
	F β -hCG level	-0.013	0.987 (0.970–1.005)	0.163
PAPP-A MoM category				
Subgroup <1.2	NT level	1.200	3.321 (1.073–10.288)	0.037
	PAPP-A level	-0.271	0.763 (0.564–1.030)	0.077
	F β -hCG level	0.001	1.001 (0.996–1.007)	0.655
Subgroup 1.2–2	NT level	1.343	3.832 (0.277–52.985)	0.316
	PAPP-A level	-0.101	0.904 (0.578–1.413)	0.659
	F β -hCG level	-0.001	0.999 (0.985–1.014)	0.921
Subgroup >2	NT level	0.129	1.137 (0.111–11.658)	0.914
	PAPP-A level	0.058	1.060 (0.839–1.340)	0.626
	F β -hCG level	-0.021	0.979 (0.954–1.005)	0.107
F β -hCG MoM category				
Subgroup <1.2	NT level	0.944	2.570 (0.456–14.498)	0.285
	PAPP-A level	-0.205	0.815 (0.427–1.556)	0.534
	F β -hCG level	-0.016	0.984 (0.965–1.004)	0.122
Subgroup 1.2–2	NT level	1.286	3.617 (0.687–19.068)	0.130
	PAPP-A level	-0.044	0.957 (0.745–1.230)	0.733
	F β -hCG level	-0.018	0.983 (0.954–1.012)	0.239
Subgroup >2	NT level	1.251	3.495 (0.275–44.434)	0.335
	PAPP-A level	-0.319	0.727 (0.416–1.267)	0.261
	F β -hCG level	0.005	1.005 (0.994–1.015)	0.373

Note: Data represent standardised logistic regression coefficients (Beta), odds ratios (OR), and their corresponding 95% confidence intervals (CI) calculated across prospective maternal serum marker sub-stratifications.

Abbreviations: NT: Nuchal Translucency; PAPP-A: Pregnancy-Associated Plasma Protein A; F β -hCG: Free beta human chorionic gonadotropin; MoM: Multiples of the Median. Statistical significance is defined as $(p < 0.05)$.

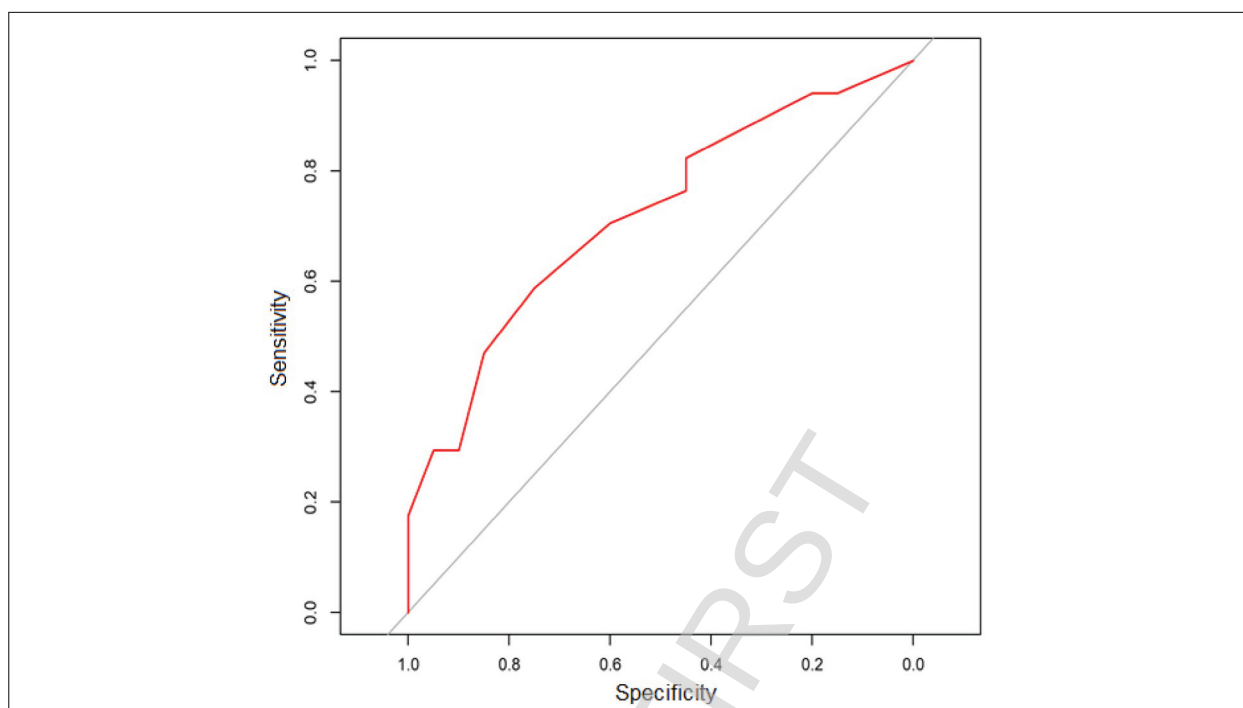


Figure 3 Receiver operating characteristic (ROC) curve demonstrating the predictive performance of nuchal translucency (NT) restricted within the NT MoM 1.2–2 subgroup category for fetal growth restriction (FGR).

The area under the curve (AUC) is 0.719 (95% Confidence Interval: 0.589–0.849, $p=0.003$). Based on the Youden index, the optimal cut-off value corresponds to a sensitivity of 82.2% and a specificity of 55.0%.

demonstrated a statistically significant association in the NT MoM 1.2–2 category ($OR=11.477$, $p=0.026$). Additionally, NT was a significant predictor in the PAPP-A MoM <1.2 category ($OR=3.321$, $p=0.037$). No statistically significant associations were observed in the remaining MoM categories.

Predictive potential of parameters for FGR (ROC curves)

The ROC curve illustrates the predictive potential of nuchal translucency (NT) as an individual predictor for FGR (Figure 3). In the unselected study cohort analysis, the AUC of NT as an isolated marker was 0.581 (95% CI: 0.438–0.724, $p=0.285$), indicating limited standalone discriminatory capacity, close to random classification. This finding must be interpreted alongside the multivariable logistic regression model (Table IV), which demonstrated a statistically significant association ($OR=3.833$, $p=0.008$). This indicates that within our framework, a 1 mm increase in absolute NT thickness increases the odds of developing FGR by approximately 3.8 times.

In the subsequent subgroup analysis restricted to the NT MoM 1.2–2 category, the ROC curve demonstrated enhanced performance. The area under the curve (AUC) was 0.719 (95% CI:

0.589–0.849, $p=0.003$), indicating moderate predictive accuracy. Based on the Youden index, the optimal operating cut-off within this high-risk sub-stratification yielded a clinical sensitivity of 82.2% and a specificity of 55.0%. However, this subgroup interpretation requires extreme caution due to the sample size contraction, which naturally increases the risk of mathematical overfitting. Within this specific boundary, for every 0.1 mm increase in NT, the odds of subsequent FGR increased 1.276 times.

Discussion

In this study, we examined first-trimester biochemical and sonographic markers in pregnancies that later developed intrauterine growth restriction and compared them with those of uncomplicated controls. The principal finding was that nuchal translucency measured between 11 and 13+6 gestational weeks was significantly increased in the FGR group. In contrast, mean values of PAPP-A and free β -hCG did not differ significantly between groups, highlighting the complexity of early placental markers and their interpretation.

An unexpected baseline finding was that the median maternal age was significantly higher in the control group than in the FGR group.

While advanced maternal age is an established independent risk factor for adverse pregnancy outcomes, this distribution reflects the specific design of our screening registry, in which older mothers who achieved uncomplicated outcomes exhibited exceptionally high screening compliance and rigorous early monitoring, thereby upwardly shifting the control baseline.

Although average PAPP-A levels were similar between groups, a greater proportion of FGR pregnancies exhibited very low PAPP-A values. This observation aligns well with previous studies demonstrating that the clinical significance of PAPP-A is most pronounced at the lower extreme of its distribution rather than across the entire range (6, 14). Low PAPP-A is believed to reflect impaired trophoblastic invasion and early placental insufficiency. These mechanisms may ultimately lead to suboptimal nutrient and oxygen transfer to the foetus, resulting in subsequent growth restriction (15, 16). Our findings therefore support the concept that PAPP-A should be interpreted as a categorical or threshold-based risk marker rather than a linear predictor.

The absence of a significant association between free β -hCG and FGR in our cohort is consistent with the heterogeneous evidence reported in the literature. While some studies have suggested that abnormal free β -hCG concentrations may be associated with adverse pregnancy outcomes, others have failed to demonstrate independent predictive value once maternal characteristics and other biomarkers are taken into account (2, 5, 17). These discrepancies may be related to differences in study design, population characteristics, and gestational age at sampling. In this context, our results suggest that free β -hCG alone has limited utility for early prediction of FGR but may still contribute to multivariable risk models.

An important observation of the present study was the disruption of inter-marker correlations in pregnancies complicated by FGR. In the control group, biochemical and sonographic markers demonstrated stronger and more consistent relationships. In contrast, these associations were attenuated or absent in the FGR group. This finding supports the hypothesis that placental dysfunction is already present in early gestation in pregnancies destined to develop growth restriction and may manifest as altered regulatory interactions among first-trimester markers (17–19).

Nuchal translucency emerged as the most consistent and robust predictor of FGR in our analysis. Logistic regression demonstrated a significant association between increased NT and subsequent growth restriction, even within MoM ranges generally considered normal. This finding

is in line with previous reports suggesting that subtle increases in NT thickness may reflect early disturbances in fetal cardiovascular adaptation or extracellular fluid balance related to placental insufficiency (10, 19). The improved predictive performance observed within specific NT MoM categories further underscores the potential clinical relevance of NT beyond its traditional role in aneuploidy screening.

The combined interpretation of NT and PAPP-A appears particularly informative. Pregnancies characterised by both elevated NT and low PAPP-A may represent a subgroup with early and more pronounced placental dysfunction, placing them at increased risk for FGR and potentially other placental-mediated complications. This synergistic effect has been suggested in previous studies. It supports the use of combined biochemical and sonographic assessment in early pregnancy (20–22).

Despite these associations, the overall predictive performance of individual first-trimester markers in our study remained moderate. This limitation is consistent with large cohort studies and systematic reviews indicating that no single marker provides sufficient sensitivity or specificity for universal screening (23–25). Therefore, earlier statements implying strong standalone predictive value for isolated markers like NT across unselected cohorts must be heavily moderated. Consequently, current evidence favours integrated screening strategies that incorporate maternal characteristics, biochemical markers, sonographic parameters, and, where available, uterine artery Doppler indices or additional placental biomarkers such as placental growth factor.

From a clinical perspective, identifying pregnancies at increased risk of FGR in the first trimester may enable tailored antenatal surveillance, including closer fetal growth monitoring and timely referral to specialised care. Population-specific evaluation of screening markers, as performed in this Montenegrin cohort, is essential to ensure appropriate calibration of MoM values and clinically meaningful risk thresholds.

In summary, our findings contribute to the growing body of evidence supporting the role of first-trimester markers as early indicators of placental dysfunction and subsequent fetal growth restriction. While their standalone predictive value is limited, the combined assessment of NT and extreme PAPP-A values may help identify a subset of pregnancies that would benefit from enhanced follow-up and individualised prenatal management.

Limitations

The main limitations of this study include the relatively small sample size and single time-point measurement. Furthermore, crucial maternal clinical confounding variables – specifically maternal pre-pregnancy BMI, precise gestational weight gain, and smoking status – were not uniformly catalogued within the biochemical screening software database at the time of sample compilation. Consequently, we were unable to adjust MoM calculations or construct multivariable prediction models incorporating these established risk factors. Future larger, multicentre prospective studies with comprehensive clinical registries and serial biomarker measurements are required to validate these population-specific trends fully.

Conclusion

This study supports the value of nuchal translucency as an early indicator of FGR risk in the Montenegrin population. While mean PAPP-A and free β -hCG levels did not differ significantly between groups, extremely low PAPP-A values were associated with increased FGR risk. Considering extreme biomarker values and combining biochemical and sonographic markers may enhance early identification of high-risk pregnancies.

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Authors' contribution

All authors contributed to the study conception and design. SV and AS performed material preparation and data collection. SP, SS and SV performed the analysis. SV and SS wrote the first draft of the manuscript. Statistical analysis was done by SS. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement

A dataset is provided after the data were anonymised and de-identified to protect participants' privacy.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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