

**METHODOLOGICAL ARTICLE**

**COMPARISON OF ELISA AND CHEMILUMINESCENCE IMMUNOASSAY METHODS FOR QUANTIFICATION OF HUMAN PLACENTAL GROWTH FACTOR IN SERUM**

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**Abstract:** Placental growth factor (PIGF) is crucial during placental development in early pregnancy. Several studies in pregnancies with complications such as preeclampsia or small for gestational age neonates find that PIGF levels are significantly lower in the first trimester, which implies that the concentration of PIGF could be used as an early screening biomarker for these conditions. This study aimed to compare the performance of chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA) for the quantification of human PIGF in serum. This is a comparative study on 88 pregnant women in the first trimester subjected to measurement of PIGF in serum using two commercially available kits: Human PIGF Quantikine HS ELISA (R&D Systems) and PIGF CLIA (Snibe). The overall coefficient of correlation between the tests was 0.93. When the cut-off value of 40 pg/mL was applied, it dropped significantly to 0.50 towards the lower values, while remaining an excellent 0.91 in the group with higher concentrations of PIGF. While R&D Systems's ELISA seems to have better sensitivity, it is not very convenient to use for a small number of samples. Snibe's CLIA automated method is user-friendly, fast and powerful. Both tests show excellent performance when indicating risk-free pregnancies.

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**INTRODUCTION**

Placental growth factor (PIGF) is a member of the vascular endothelial growth factor (VEGF) family, with an important role in angiogenesis.<sup>1</sup> The main source of PIGF is the placental trophoblast, and it has been shown that abundant expression of PIGF in the trophoblast is crucial for the trophoblastic invasion of maternal spiral arteries during placental development in early pregnancy.<sup>2</sup> The concentrations of PIGF physiologically increase throughout pregnancy, with a peak in the third trimester (approximately week 30), after which they decrease as a sign of placental maturation. Several studies have found that in pregnancies with complications such as preeclampsia or small for gestational age neonates, the PIGF levels are significantly lower in the first trimester, which implies that the concentration of PIGF measured in first trimester (11<sup>+1</sup> – 14<sup>+1</sup> gestational week) could be used as screening biomarker for these conditions.<sup>3-5</sup> The possibility of effective prediction of preeclampsia would have a substantial impact on the improvement of outcome by establishing intensive antenatal monitoring for the pregnant women recognized as high risk patients and by undertaking direct measures (low dose Aspirin) for the prevention of the disease.<sup>6</sup> In order to develop a comprehensive algorithm which will be able to use the PIGF concentration for the discrimination between affected and unaffected pregnancies and as a predictor of pregnancy complications, reference ranges based on large scale data should be established by every laboratory.<sup>7</sup> Furthermore, the relative contribution of other associated conditions and/or habits, such as maternal age and smoking status, method of conception, parity,

should be taken into account and converted to multiples of medians (MoMs).<sup>8-9</sup> These goals are beyond the scope of our study, and they are currently being analyzed and are in process of preparation (personal communication).

Many different methods for the determination of the PIGF concentration in human plasma/serum have been already proposed and are available on the market.<sup>10-13</sup>

The aim of our study was to evaluate two commercially available immunoassay methods, enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA), and to calculate the correlation between the results.

## MATERIAL AND METHODS

This study is part of a larger research project, entitled "Screening for preeclampsia in the first trimester: Serum levels of PIGF as opposed to mother characteristics", whose aim is to establish the national reference range of PIGF levels in pregnant women between 11<sup>+1</sup> - 14<sup>+1</sup> of gestational age. This project lasted from July 2018 to December 2019, and it included more than 800 pregnant women. The material in this study comprised pregnant women who met the following criteria: singleton pregnancy, gestational age between 11<sup>+1</sup> - 14<sup>+1</sup> weeks, crown rump length (CRL) 45-84 mm and maternal age of at least 18 years. The exclusion criteria were as follows: fetal demise, congenital fetal anomalies, serious mental illness of the woman, communication difficulties. At the starting point of the study, all women were questioned about their medical history and signed an informed consent at the Special Hospital for Obstetrics and Gynecology "Mother Teresa" in Skopje, Republic of North Macedonia. Data were collected following the NICE (National Institute for Health and Care Excellence) protocol for screening of preeclampsia, and venous blood was drawn. Six milliliters of venous blood were drawn in plain vacutainer (no anticoagulant). After 30 min. at room temperature, the tubes were centrifuged for 10 minutes at 1500 rpm, the serum was transferred into 2 fresh tubes and stored at -20°C until analysis of the PIGF concentration, but for no more than 3 months. Measuring of the concentration was performed at the Institute for Immunobiology and Human Genetics at the Medical Faculty in Skopje. The specimens were transported to the Institute frozen in accordance with the cold chain rule. For quantitative analysis of PIGF, two commercially available kits were used: Human PIGF Quantikine HS ELISA kit from R&D Systems, (Minneapolis, Minnesota, United States) and PIGF CLIA kit from Snibe (Shenzhen, China).

For comparison, sera from 88 pregnant women, most of them (68) selected on the basis of the data collected from the NICE protocol for screening of preeclampsia, and 20 randomly selected, were subjected to measurement using both methods, according to the manufacturer's instructions, briefly explained below.

### **Human PIGF Quantikine HS ELISA (R&D)®**

This manually performed immunoassay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human PIGF has been pre-coated onto a microplate. In the first step, 100 µL of RD1-22 diluent (a buffered protein base) is added to all wells. In the second step, 100 µL of standards and samples are then pipetted into the wells and left for incubation 1 hour at room temperature. Following a wash step, 200 µl of PIGF HS conjugate (horseradish peroxidase labeled) is added to all wells, and the plate is incubated 1 hour at room temperature. Another step of washing follows and then 50 µl of substrate solution is added to the wells, and the plate is left for an incubation of 1 hour at room temperature protected from light. In the next step, 50 µl of amplifier solution is added to the wells, and the plate is incubated for 30 minutes at room temperature. The color development is stopped with 50 µl Stop solution to each well. Absorbance was measured at 490 nm using Wallac 1420 Victor 2 ELISA plate reader from Perkin Elmer (Waltham, Massachusetts, United States). The concentrations of the measured samples were calculated using a standard curve that was obtained using dilution series (3.13, 6.25, 12.5, 25, 50, 100 and 200 pg/mL) from a standard sample with defined concentration of 2000 pg/mL.

### **PIGF CLIA (Snibe)®**

This automated immunoassay is performed on MAGLUMI 1000 fully automated chemiluminescence immunoassay analyzer from Snibe (Shenzhen, China). In the first step, 50 µl of sample (or calibrator/ control), ABEI labeled with anti-PIGF polyclonal antibody and magnetic microbeads coated with anti PIGF polyclonal antibody are mixed thoroughly and incubated at 37°C, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted, and a washing cycle is performed. Subsequently, Starter 1+2 are added to initiate a flash chemiluminescence reaction. The light signal is measured by a photomultiplier within 3 seconds as relative light units (RLUs) which are proportional to the concentration of PIGF present in the sample (or calibrator/ control). The serum PIGF levels are determined using previously generated 2-point calibration curve and a master curve (10 calibrations) provided by the manufacturer of the kit.

The main characteristics of both assays used are summarized in Table 1.

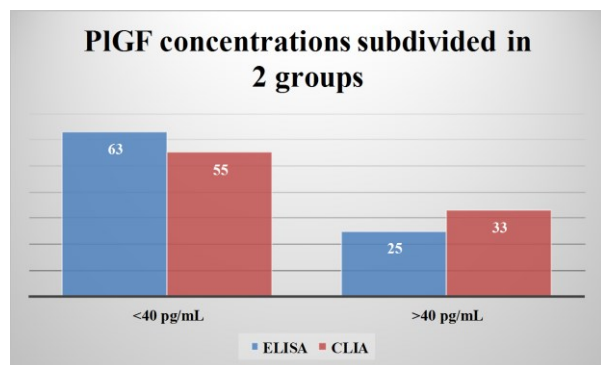
## RESULTS AND DISCUSSION

The mean PIGF concentration for the 88 analyzed samples using ELISA was 43.67 pg/mL, and using CLIA it was 46.45 pg/mL. When compared, the overall

**Table 1. Assay characteristics**

Assay characteristics	ELISA (R&D)	CLIA (SNIBE)
Assay platform	Manual	Automated
Minimum sample required	100 µL	50 µL
Time to results	~4 h	~1.5 h
Assay measuring range	3.1-200 pg/mL	2.0-10000 pg/mL
Technical expertise required	High	Low
Number of controls provided by the producer	None	2

coefficient of correlation between the tests was 0.93, indicating very strong concordance. Keeping in mind the relevance of the PIGF concentration for the prediction of the pregnancy outcome, we further subdivided the results in two groups according to the first obtained results using the ELISA kit. Several studies analyzing the potential use of PIGF concentration as an early screening biochemical marker used in the first trimester of the pregnancy for predicting later preeclampsia or small for gestational age fetus have used cut-off value of 40 pg/mL. Accordingly, we have subdivided the results into two subgroups, the low concentration group (<40 pg/mL) – indicative of pregnancy complications, and the high concentration group (>40 pg/mL) – not likely to be associated with preeclampsia or small for gestation age fetus (Figure 1).



**Figure 1. Distribution of PIGF concentrations in two groups, low and high**

In the first group of samples with low concentrations of PIGF (<40 pg/mL), a total of 63 samples were detected in the ELISA measurement, and 55 in the CLIA measurement, while the coefficient of correlation for this group of results was 0.50. The coefficient of correlation between the groups with high concentrations was an excellent 0.91, calculated on 25 results obtained using ELISA and 33 samples analyzed with the CLIA method.

Despite the excellent correlation between overall results, it is worth noting that this correlation significantly dropped towards the lower values of PIGF (Table 2).

Based on simple observation, one could notice that the results for PIGF concentration obtained with the two methods are perfectly concordant towards higher concentrations and would most efficiently rule-out the pregnancies in which no complications are to be expected. On the other hand, the two methods perform more differently when measuring lower concentrations of PIGF in serum, and, while still keeping the coefficient of correlation of 0.5 within the limits of moderate correlation, it is significantly lower than when analyzed on entire cohort and again on samples with concentration above 40 pg/mL.

**Table 2. Median value and coefficient of correlation according to two subgroups of results, low and high**

Group of results	Assay*	N	M	CC
Low (<40 pg/mL)	R&D	63	26.2	0.50
	Snibe	55	29.51	
High (>40 pg/mL)	R&D	25	57.72	0.91
	Snibe	33	59.07	
Overall	R&D	88	32.22	0.93
	Snibe		33.13	

Legend: \* - assay manufacturer; N - number of results; M - median; CC - coefficient of correlation

However, without any intention to neglect authors advocating taking into account all the important associated characteristics and their conversion to Multiples of Medians, by simply counting the analyzed pregnancies, we might conclude that by simply applying the cutoff value of 40 pg/mL on our cohort of 88 analyzed samples, we would be able to recognize 15 out of 19 pregnancies actually associated with high risk. Within these 15 positively selected patients, likely to develop pregnancy complications, 3 are detected only when using ELISA, while one patient stratified in the non-risk group according to the ELISA result was correctly detected with the CLIA method (Figure 2).

Our study has its limitations. Some of them are the size of the cohort, the heterogeneity of the patients and different ages within the studied group. Including as many samples as possible for parallel analysis with both methods will produce much more objective insights. We are certain that the ongoing inclusion and conversion of all contributing factors into MoMs will greatly improve the delineation capacity and will add power to discriminate between both immunoassays. However, this is influenced by limited funds. In the meanwhile, we were able to conclude that both tests have their strengths and weaknesses. For example, when taking into consideration of the outcome of the pregnancy, ELISA seems to have better sensitivity

### Non-risk pregnancies according to PIGF concentration using R&D

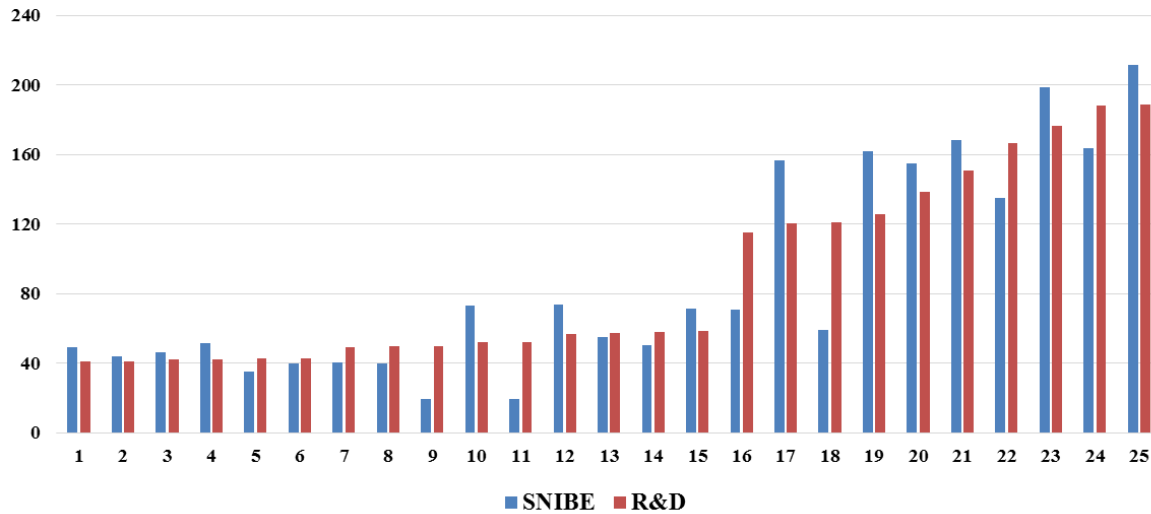


Figure 2. High concentration of PIGF (>40 pg/mL) as measured by R&D

since it detects truly high-risk pregnancies more efficiently. On the other hand, it is not very convenient for a small number of samples. Inclusion of calibrators and negative controls in every single run greatly increases the price per test. Higher level of lab experience is critical for yielding quality results. The CLIA automated method is very user-friendly; almost no experience on the part of the lab technician is needed. The technique is fast and powerful, and results are obtained within 90 minutes. Its sensitivity was a bit lower than the ELISA method, having measured as normal a few samples from women as finished their pregnancies with preeclampsia. But again, a better evaluation on a larger cohort is needed for a definitive conclusion. Both tests show excellent performance when indicating risk-free pregnancies, while more attention and possibly repetitive measurements could help in identifying pregnancies at risk solely based on concentration of PIGF in the first trimester of pregnancy.

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