Online publication ahead of print, AJMHS Vol 57, 2021

Association of adverse pregnancy outcome with the values of serum biomarkers of Quadruple test

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Abstract

Background: Prenatal test includes prenatal screening and diagnosis that aims to find different changes in fetus and mother during the pregnancy. Prenatal screening is focused in finding any possible pathology in the wide population using some noninvasive methods.

The changes of utero-placental blood flow lead to utero-placental insufficiency, which will be manifested by pregnancy induced hypertension, preeclampsia, intrauterine growth retardation or/and small for gestation age fetus, preterm birth, etc.

Aim: of this research is to perform a prenatal screening of serum biomarkers from Quadruple test in the second trimester of pregnancy, in order to predict early diagnosis of eventual adverse pregnancy outcome.

Material and method: This prospective study is realized in the Special Hospital for Gynecology and Obstetrics "Mother Theresa", Skopje, during the period November 2019 to June 2021. It includes 673 pregnant women, between 18-23.6 gestational weeks, followed up and monitored till delivery. We followed up the values of serum biomarkers from Quadruple test, fetal biometry, quantity of amniotic fluid and gestational week and bodily measures of the fetus in delivery.

Statistical processing: It was conducted a statistical analysis of maternal characteristics in research group and the group with no adverse pregnancy, determining the variables which significantly associate with adverse pregnancy outcome.

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Results: Within 673 respondents, 523 (77.7%) had favorable pregnancy outcome, while 150 (22.3%) of pregnant women had adverse pregnancy outcome (they made up the research group). From the group with no adverse pregnancy, 48 (32%) had preeclampsia, 32 (21.3%) had pregnancy induced hypertension, 20 (13.3%) had small fetus according to the gestational age, and 50 (33.3%) had intrauterine growth retardation.

Conclusion: Inhibin A as a single marker for adverse pregnancy outcome was the best predictor for differentiation of pregnant women with adverse and favorable pregnancy outcome.

Keywords: Quadruple test, pregnancy outcome, Inhibin A

INTRODUCTION

Prenatal screening is focused in finding any possible pathology in wide population using some noninvasive methods. Placenta and feto-placental circulation has a crucial role in perinatal growth of the fetus and the pregnancy outcome. (1) During pregnancy, inhibin A is mainly derived from the placenta and regulates the implantation and differentiation of embryos. (2) The fetal compromise and the utero-placental blood flow changes can result to utero-placental insufficiency. Placenta, produces several specific proteins that flow in the maternal serum, in different quantity and they have a very important role in normal development of the fetus. (3) According to the literature data, there is a correlation between the maternal serum protein values and the pregnancy outcome, thus the prediction of potential placental insufficiency is very important. A shallow implantation leads to pregnancy induced hypertension, preeclampsia, intrauterine growth retardation, preterm birth, etc. The risk of pregnancy induced hypertension is due to placental ischemia, restrictive changes in intrauterine fetal growth and is an indication for preterm delivery. (4)

Usually, preeclampsia is found after the 20^{th} gestational week, in previously normotensive women, but it can be found also during delivery and after it (ACOG, 2020). The certain etiology isn't known, but it is met usually in women who have previous preeclampsia, hypertensive disease, gestational and familiar hypertension, in oldest pregnant women (up to 40 years), in black women, obese pregnant women, multiple pregnancies, etc. Pathophysiology is due to immunological and genetic factors, placental ischemia, oxidative stress and other factors that result in spiral arteries abnormality, dysfunction or inadequate trophoblastic invasion and shallow implantation. Final result is placental hypo perfusion and ischemia, producing different substances that enter the maternal blood circulation. Except high blood pressure, as a first sign of preeclampsia, appearance of proteins in urine, more than 0.3 g./24h and joint and face swelling are the other accompanying signs. If not diagnosed on time, it can lead to eclampsia, a state of high risk for both mother and baby, and may have fatal end. (5) A study by Park Hea Ree, (2021) concluded that Inhibin A and other second-trimester serum markers may be useful for early detection of preeclampsia. (6)

Intrauterine growth retardation is a complex complication or fetal growth restriction under the 10^{th} percentile, accompanied with other pathological restrictions and perinatal risk, due to shallow placentation. In case of IUGR (Intrauterine Growth Retardation), an induction of preterm delivery is indicated very often. (7)

The cause of preterm delivery is utero-placental ischemia, preeclampsia, preexisting hypertension of the pregnant women, gestational diabetes, obesity or malnutrition, cervical insufficiency, infections, etc. (8)

The quadruple marker test or the second trimester screen is a prenatal test that measures levels of four substances in pregnant women's blood:

- Alpha-fetoprotein (AFP), a protein produced by the fetus,
- Human chorionic gonadotropin (HCG), a hormone produced by the placenta,
- Estriol, a hormone produced by the placenta and the fetus' liver,
- Inhibin A, a hormone produced by the placenta.

The same biomarkers are used as indicators and predictors that refer to risk, not just for the numeric chromosomic aberration but also to correlation with other pathophysiological fetal changes, pregnancy outcome, preeclampsia, pregnancy induced hypertension, preterm birth, intrauterine growth retardation, etc. Down syndrome biochemical markers levels are altered in those patients who subsequently developed preeclampsia and may be a useful screening test for preeclampsia. Inhibin-A is the most predictive marker and correlates with the severity of subsequent preeclampsia and inversely with the week of occurrence of preeclampsia. (9)

The aim of this study was to define the role of biomarkers of Quadruple test in prediction of adverse pregnancy outcome.

MATERIALS AND METHODS

This prospective study was realized in a Special Hospital for Gynecology and Obstetrics "Mother Theresa", Skopje, from November 2019 to June 2021. It includes 673 second trimester pregnant women, followed till delivery. Firstly, were taken the bodily measures and blood pressure, after that was taken 2 ml venous blood for Quadruple test and ultrasound for fetal biometry and amount of amniotic fluid. All participants were monitored till end of pregnancy by regular measuring blood pressure and biochemical analysis. Inclusion criteria were: singleton pregnancy, age at least 18 years old of pregnant women, gestational age 18-23.6 week, previously excluded fetal anomalies by ultrasound. Exclusion criteria were: twin or multiple pregnancy, fetus mortus in utero, findings of fetal anomalies, pre-existed hypertension, other diseases in pregnant woman (diabetes, autoimmune diseases, etc), pregnant women that used Aspirin.

Statistical processing was done by conducting a statistical analysis of maternal characteristics in all patients included in the study and determining the variables which significantly associate with adverse pregnancy outcome.

RESULTS

From 673 respondents, 523 (77.7%) had favorable pregnancy outcome, while 150 (22.3%) of pregnant women had adverse pregnancy outcome (they made up the research group). From the group with no adverse pregnancy, 48 (32%) had preeclampsia, 32 (21.3%) had pregnancy induced hypertension, 20 (13.3%) had fetus small for gestational age, and 50 (33.3%) had intrauterine growth retardation. (Table 1)

Table 1. Mother's characteristics in the two groups.

Mother's characteristics	Statistical parameters	Adverse pregnancy outcome	No adverse pregnancy outcome	p-level
Age Years	mean ±SD	27.3 ± 3.8	27.8 ± 4.5	t=1.22
	min – max	22 – 37	18 – 48	p=0.22 ns
BMI kg/m^2	mean ±SD	28.10 ± 2.8	27.02 ± 3.8	t=3.22
	min – max	23 – 34.5	18 – 45.1	**p=0.0014 sig
TA systolic (mmHg)	mean ±SD	132.50 ± 10.8	124.72 ± 9.4	t=8.61
	min – max	105 – 155	102 – 145	***p=0.000000 sig
TA diastolic (mmHg)	mean ±SD	85.67 ± 7.5	78.77 ± 6.2	t=11.47
	min – max	70 – 105	64 – 95	***p=0.000000 sig
Gestational week /in second trimester	mean ±SD	21.1 ± 1.0	20.9 ± 1.3	t=2.05
	min – max	18.6 – 23	17.1 – 23.6	*p=0.041 sig
Gestational week /delivery	mean ±SD	37.5 ± 1.1	39.7 ± 1.05	t=21.9
				***p=0.000000 sig

t(Student t-test) *p<0.05; **p<0.01; ***p<0.0001

Table 1 presents pregnant women with adverse and no adverse pregnancy outcome were mean age was 27.3 ± 3.8 and 27.8 ± 4.5 years, in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome, respectively, without statistical significance (p=0.22)

The body mass index has a significant higher value in the group with adverse pregnancy outcome than in the group with no adverse pregnancy outcome (difference value was 28.10 ± 2.8 vs 27.02 ± 3.8, p=0.0014).

The systolic and diastolic pressure were significantly higher in the group with adverse pregnancy outcome comparing to the group with no adverse pregnancy outcome (p<0.0001); 132.50 ± 10.8 and 124.72 ± 9.4 mean values for systolic pressure, respectively in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome; 85.67 ± 7.5 and 78.77 ± 6.2 mean values of diastolic pressure, respectively in research and control group.

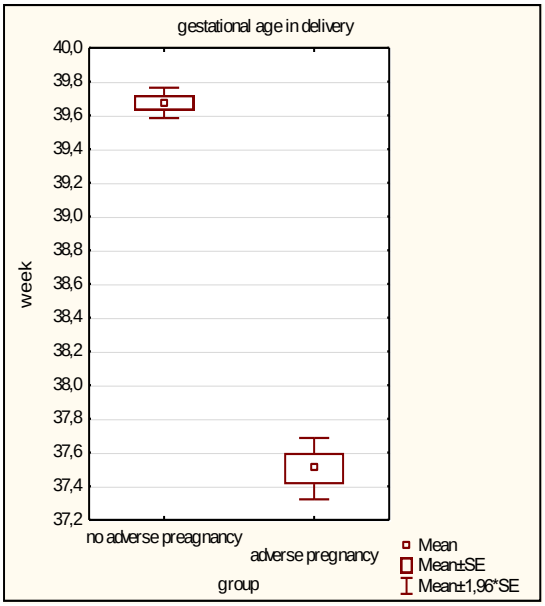

Figure 1. Graphic presentation of gestational week in the group with no adverse pregnancy outcome and the group with adverse pregnancy outcome in delivery

Figure 1 presents the gestational week at delivery for both groups included in the study. The gestational week was significantly different in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome (21.1 ± 1.0 vs 20.9 ± 1.3, p=0.041, and 37.5 ± 1.1 vs 39.7 ± 1.05, p<0.0001, consequently).

adverse and no adverse pregnancy outcome doesn't show any statistical difference in relation to parity (p=0.62). It was a domination of pregnant women with one delivery in both groups - 56% and 55.4%, respectively in groups with adverse and no adverse outcome.

Pregnant women from the group with adverse and

Table 3. Fetal body measures in delivery in both groups

Obsterical caracteristics	Statistical parameter	**Adverse pregnancy outcome**	**No adverse pregnancy outcome**	**p-level**
Body weight (gr)	mean ±SD	2596.5 ± 364.4	3441.5 ± 344.6	t=26.13
	min - max	1460 – 3450	2340 – 4520	***p=0.000000 sig
Body length (cm)	mean ±SD	47.0 ± 1.5	50.7 ± 1.7	t=24.01
	min - max	45 – 52	46 – 55	***p=0.00000 sig

Table 2. Parity in pregnant women in both groups

Parity	**Groups**	
	Adverse pregnancy outcome n(%)	**No adverse pregnancy outcome** n(%)
1	84 (56)	289 (55.36)
2	43 (28.67)	160 (30.65)
3	14 (9.33)	55 (10.54)
4	7 (4.67)	15 (2.87)
5	2 (1.33)	2 (0.38)
6	0	1 (0.19)
p-level	X^2=3.52 p=0.62 ns	

X2(Pearson Chi-square)

Table 2. presents the newborn's body measures in both groups included in the study. Newborns, that have adverse pregnancy outcome, has significantly lower bodily measures comparing to those from mother with no adverse pregnancy outcome (2596.5 ± 364.4 vs 3441.5 ± 344.6 gr/cm, and, 47.0 ± 1.5 vs 50.7 ± 1.7 cm; p<0.0001, respectivly).

Table 3 presents pregnant women in both groups included in the study. Pregnant women with

no adverse pregnancy outcome has significantly different values for serum biomarker Inhibin A, (p<0.0001). Median serum concentration of this biomarker were significantly higher in the group with adverse pregnancy outcome in relation to the group with no adverse pregnancy outcome – 489 vs 203.7 pg/mL, and 1.12 vs 0.45 MoM, (table 4).

Table 4. The value of Inhibin A in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome

Variable	calculated parameter	Groups		p-level
		Adverse pregnancy outcome	No adverse pregnancy outcome	
Inhibin A (pg/mL)	mean ±SD	520.22 ± 269.3	241.96 ± 138.4	Z=12.07
	median (IQR)	489 (267 – 659)	203.7 (156 – 294)	***p=0.00000 sig
Inhibin A (MoM)	mean ±SD	1.19 ± 0.6	0.54 ± 0.3	Z=12.56
	median (IQR)	1.12 (0.62 – 1.46)	0.45 (0.34 – 0.64)	***p=0.000000 sig

Z(Mann-Whitney U Test) ***p<0.0001

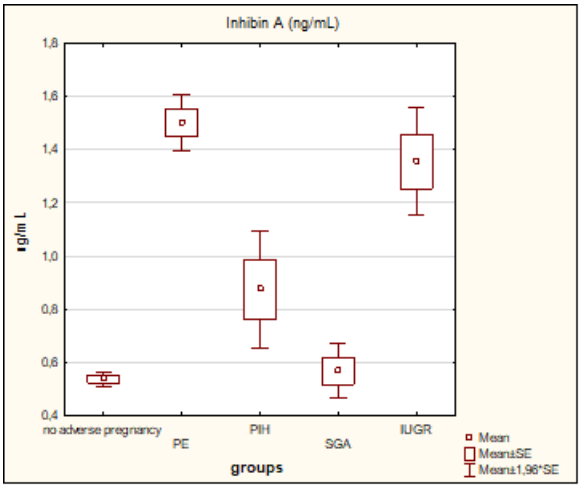

Figure 2. Inhibin A in all groups

Figure 2. presents the values of Inhibin A in pregnant women with adverse pregnancy outcome and the group with no adverse pregnancy outcome. Serum Inhibin A has the highest medial values in group with preeclampsia (1.37 MoM), followed by the group with IUGR (1.16 MoM), PIH– (pregnancy induced hypertention) (0.81 MoM), SGA (small for gestational age)(0.55MoM) and the lower values were measured in the group with no adverse pregnancy outcome (0.45 MoM). There were confirmed statistically significant higher values of Inhibin A in group with preeclampsia comparing to the group with no adverse pregnancy outcome, PIH versus group with no

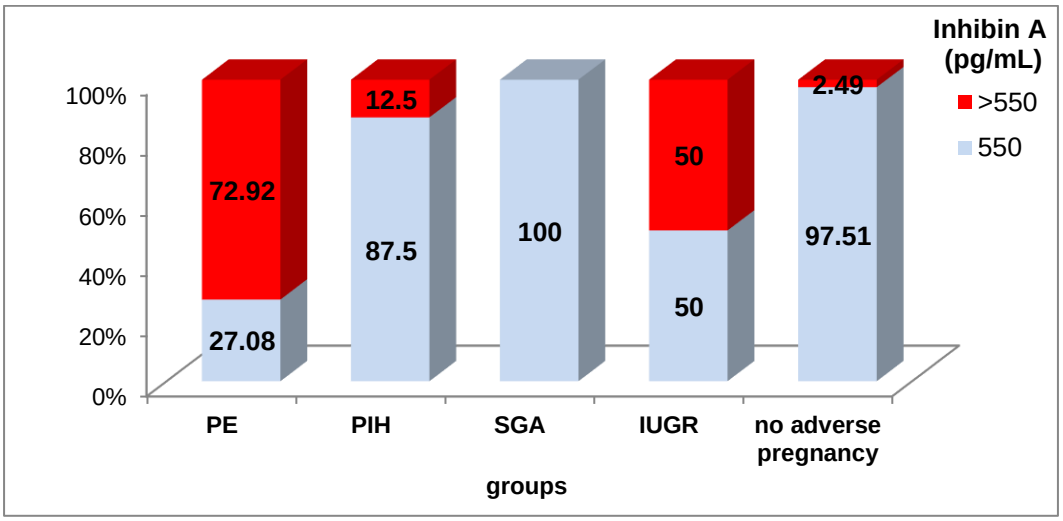

Figure 3. Inhibin A in all research groups in relation to the group with no adverse pregnancy outcome

adverse pregnancy outcome and IUGR versus group with no adverse pregnancy outcome.

Figure 3 represent the values of Inhibin A in the groups with adverse pregnancy outcome in relation to the group with no adverse pregnancy outcome.

Pregnant women with PE, significantly less frequently than women from the group with no adverse pregnancy has decreased values of uEstriol (68.75% vs 82.41%), while pregnant women with SGA more frequently than pregnant women from the group with no adverse pregnancy outcome has decreased values of uEstriol. (fig. 4).

Medial values of serum HCG were 50 780, 20 844, 33 172, 50 780 and 24 113 IU/mL, respectively in groups with PE, PIH, SGA, IUGR and the group with no adverse pregnancy outcome. In relation to the group with no adverse pregnancy outcome, the values were significantly

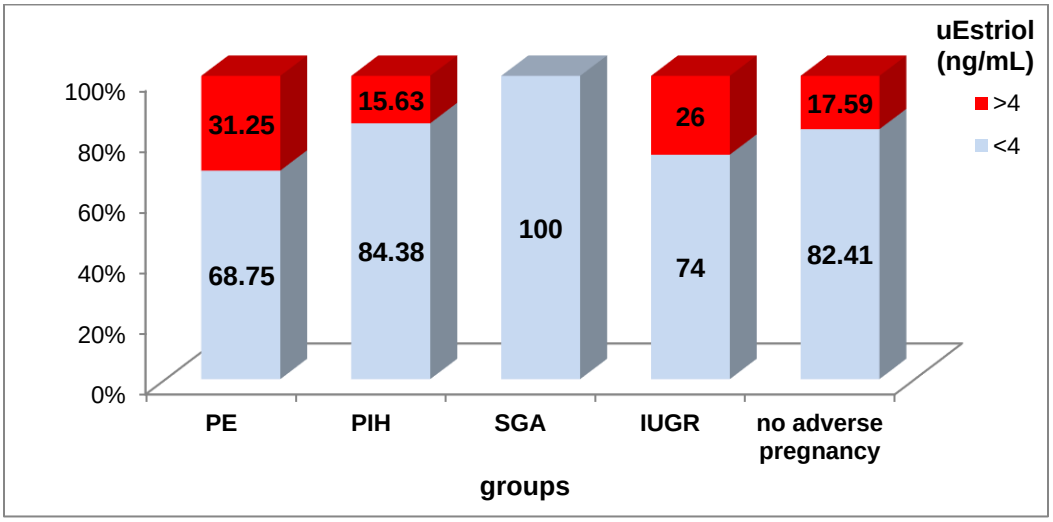

Figure 4. Presentation of uEstriol in all groups

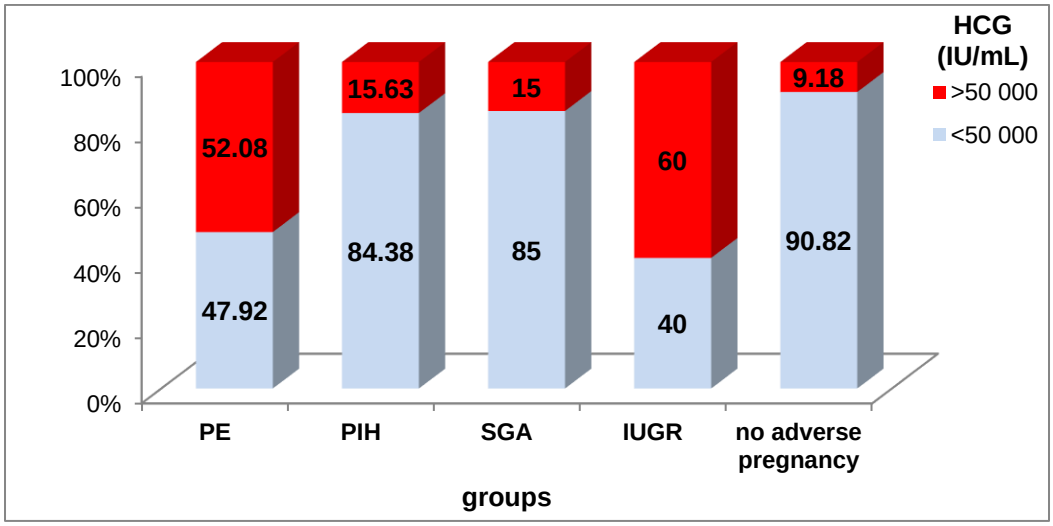

Figure 5. Presentation of HCG in all groups

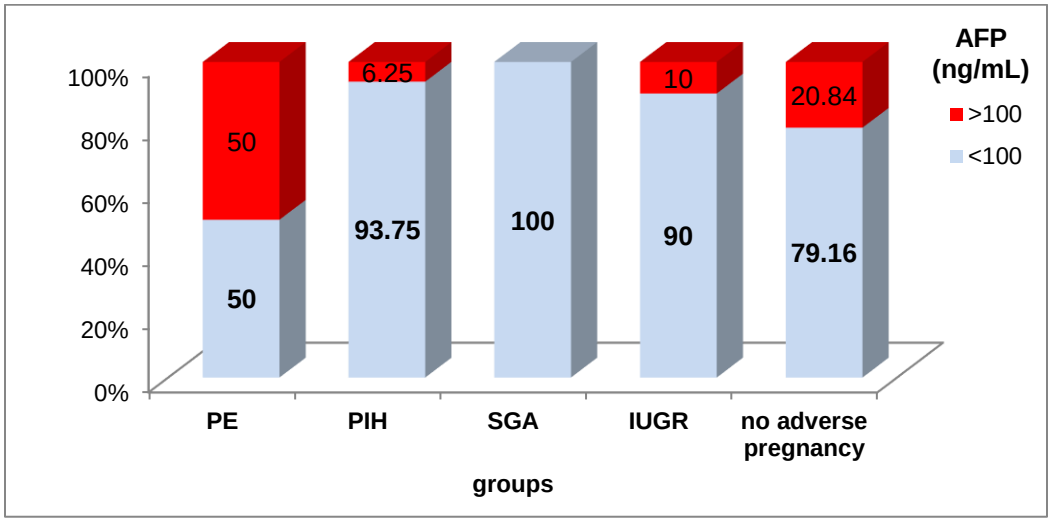

Figure 6. Presentation of AFP in all groups

higher in pregnant women with PE (p<0.0001), in pregnant women with SGA (p=0.0019) and in pregnant women with IUGR (p<0.0001), (fig. 5).

Alpha-fetoprotein (AFP) has significantly higher mean serum concentration in group with PE comparing to the group with no adverse pregnancy outcome (94.32± 47.5 vs 78.09±38.1,p=0.0059), non significantly higher in group with IUGR comparing to the group with no adverse pregnancy outcome (81.68±23.1 vs 78.09±38.1,p=0.51), non significantly lower in group with PIH comparing to the group with no adverse pregnancy outcome (74.83± 41.2 vs 78.09±38.1,p=0.64) and non significantly lower in group with SGA comparing to the group with no adverse pregnancy outcome (75.04± 16.7 vs 78.09±38.1,p=0.51) (fig. 6).

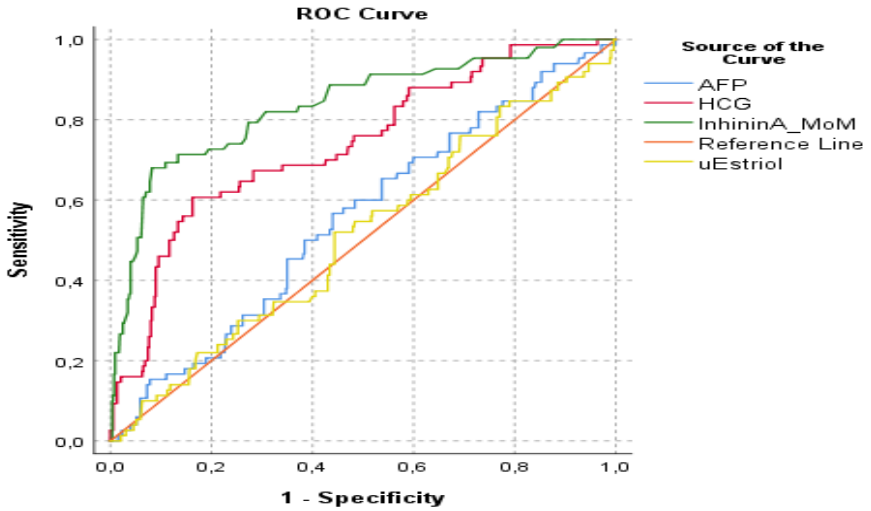

Figure 7. Performances of serum biomarkers of Quadruple test

Figure 7. presents the results for performances of serum biomarkers of Quadruple test. According to the size of area under the ROC curve, Inhibin A as a single biomarker has the best differential ability in diagnosing pregnant women with adverse pregnancy, AUC=0.701.

The combination of Inhibin A, uEstriol, HCG and AFP has the biggest area under the ROC curve (AUC=0.792) that is, this combined model represents the test with the best differentiation ability for pregnant women with adverse and favorable pregnancy outcome

DISCUSSION AND CONCLUSIONS

This study analyzed biomarkers from Quadruple test; Inhibin A, HCG, AFP and uEstriol and fetal biometry in second trimester of pregnancy and their relation to pregnancy outcome. According to Chowdhary et al. (2017), the use of Inhibin A as a predictor for IUGR has a great importance. Increased value of maternal serum Inhibin A in second trimester of pregnancy has an important correlation with abnormal placentation. (10) In our study the level of Inhibin A in serum of pregnant woman has the highest values in group with preeclampsia, followed by the group with IUGR, PIH and SGA. More frequently the increased serum Inhibin A values in pregnant women with adverse outcome versus those with no adverse pregnancy outcome, statistically was confirmed as significant, $p<0.0001$, while the performances of the test showed a good differentiation ability.

It is confirmed by Mazhari (2018) and Sanayukta (2019), that the serum level of HCG in second trimester of pregnancy is a good predictor for PIH. (11), (12) In our study, median values of HCG in serum were significantly higher in the group with preeclampsia, followed by the group with SGA, IUGR versus control group. More than half of respondents with preeclampsia and IUGR (52.1% and 60% respectively), has increased level of serum HCG, while in other groups, that was much lower, 15.6% in PIH, 15% SGA and 9.2% in the control group.

According to Hu et al. (2020), increased level of AFP in maternal serum is associated with a big risk for adverse pregnancy outcome. (13) This was confirmed partially in our study, because just women with preeclampsia has significantly higher values of AFP, comparing to pregnant women with PIH, IUGR and control group. Also, our study didn't find any statistically significant difference between median values of uEstriol, as a single biomarker in all four research groups and control group. The area under ROC curve showed that uEstriol has a weak discriminatory ability, thus this biomarker, independently does not allow a prediction of pregnancy outcome.

The results of Quadruple test represented significantly higher values of Inhibin A, in the group with adverse pregnancy outcome (median 1.12 vs 0.45 MoM), and significantly higher values of HCG in the same group (median 45684.5 vs 24113), while uEstriol and AFP values were non significantly higher in the group with adverse pregnancy outcome.

Inhibin A has increased serum level in the group with preeclampsia (659.2 pg/mL), followed by the group with IUGR (524.65pg/mL), PIH (376.2 pg/mL) and SGA (237.pg/mL), while the lowest serum level of Inhibin A has CG (203.7pg/mL). It was found a statistically significance between groups with PE versus CG, PIH versus CG and IUGR versus CG.

uEstriol has no statistically significant difference in median values of uEstriol in four research groups comparing to the control group 3.64, 2.92, 2,71, 3.04 vs 2.85, respectively for PE, PIH, SGA, IUGR and CG).

The results for validity of using serum biomarker in our study, for diagnosing adverse pregnancy outcome, showed that Inhibin A as a single marker has the best diagnostic performances (AUC=0.701, sensitivity 65%, specificity 76%). These biomarkers may be predictive of adverse pregnancy outcome, first of all in prediction of preeclampsia, but the prediction value is low.

Combination of Inhibin A, u Estriol, HCG and AFP has the biggest area under the ROC curve (AUC=0.792), so this combination of test represents the test with the best ability of differentiation between pregnant women with adverse and no adverse pregnancy outcome.

Acknowledgments: None declared.

Conflict of interest: No conflict of interest.

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