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Association Between Serum Markers Used in the Routine Prenatal Screening with Pregnancy Outcomes: A Cohort Study

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Abstract

Background Early detection of adverse pregnancy outcomes is an important topic in prenatal care. This study aimed to evaluate the association between maternal serum markers and pregnancy outcomes.

Materials and Methods This hospital-based cohort study was performed according to the national Down syndrome screening protocol on 2923 eligible pregnant women. Pregnancies were classified into three groups of based on each biomarker. The participants were followed up until delivery, and the pregnancy outcomes were identified by hospital discharge records.

Results High levels of free BHCG were significantly associated with an increased risk of preterm birth ($B = -0.31$, $SE = 0.158$, $OR = 0.730$; $P = 0.046$). Based on multivariate analysis the high levels of MSAFP had a direct relationship with premature birth ($B = -0.84$, $SE = 0.361$, $OR = 0.431$, $P = 0.020$), gestational hypertension ($B = -0.59$, $SE = 0.354$, $OR = 0.549$. $P = 0.091$), IUGR ($B = -1.46$, $SE = 0.433$, $OR = 0.231$, $P = 0.001$), and fetal death ($B = -1.50$, $SE = 0.533$, $OR = 0.223$, $P = 0.005$). Furthermore, an increase in the levels of Inhibin-A could more likely lead to gestational hypertension ($B = -0.63$, $SE = 0.235$, $OR = 0.533$).

Discussion According to the result, maternal biomarkers, especially MSAFP, can be beneficial in identifying high-risk cases, in addition to examining the possibility of Down syndrome, facilitating achievement of the desired pregnancy outcomes.

Keywords Prenatal screening · Pregnancy outcomes · Serum marker

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Introduction

Prenatal screening using biochemical markers, including six biomarkers of alpha-fetoprotein, human chorionic gonadotropin, pregnancy-associated protein, unconjugated sterol, and Inhibin-A, was first introduced to detect aneuploidy and neurological defects [1–3]. However, over the last 3 decades, the progress made by the use of these biomarkers led to the utilization of numerous ultrasound and biochemical markers in the first and second trimesters. Such markers are not only used to identify Down syndrome, trisomy 13, trisomy 18, and nervous system abnormalities, but also, effective in identifying pregnancies that are at risk of adverse pregnancy outcomes [4–6].

The existence of therapeutic potential for the treatment and prevention of pregnancy complications has made it important to evaluate the benefits of serum markers in the early detection of adverse outcomes [7]. The use of these biomarkers gets prominence due to the fact that they are measured by noninvasive methods, which lack any special concern [7, 8]. Various studies have been conducted to

evaluate the benefits of using such biomarkers for early identification, detection, and prediction of adverse pregnancy conditions, such as gestational hypertension, preterm birth, intrauterine growth restriction (IUGR), and intrauterine fetal death (IUFD) [9, 10].

Based on some reports, abnormal maternal serum biomarkers performed for prenatal screening of fetal abnormalities were associated with adverse pregnancy outcomes [9, 11]. Researchers have examined maternal serum markers separately in the first and second trimesters or in combination with biomarkers to determine their relationship with various complications [7]. In this regard, some researchers have reported the relationship between abnormal amount of each biomarker and some adverse effects and consequences [12, 13]. Nonetheless, numerous inconsistencies are observed in the results of studies [6, 13] and, according to some researchers, screening methods using biomarkers are especially useful in diagnosing various pregnancy complications in severe cases that are less common [7].

There has been a high effort for early detection of pregnancy complications, and maternal serum biomarkers have been used to predict some adverse pregnancy outcomes [10]. A vast body of literature regarding abnormal maternal serum biomarkers has been published in an effort to anticipate the adverse pregnancy outcomes. However, information about the usefulness of serum markers for the prediction of adverse outcomes is scant [14, 15]. The discrepancies in the results of various studies suggest the necessity of performing further studies to obtain the biomarkers appropriate to identify high-risk pregnancies. This study aimed to investigate the relationship between first- and second-trimester biomarkers with adverse pregnancy complications.

Methods

This cohort study was carried out in Al-Zahra Hospital, Tabriz, Iran, from spring 2018 to mid-2019. This hospital is a gynecological-midwifery referral center and provides preventive, diagnostic, treatment, and rehabilitation services for women and neonates in the northwestern region of Iran. The population of this study consisted of all pregnant females referred to the hospital clinic for prenatal care over a period of one year. The population was screened for Down syndrome according to the National Down Syndrome Screening Protocol. This protocol is a contingent sequence that first performs first-trimester tests, including double marker (free β-human chorionic gonadotropin [BHCG] and pregnancy-associated plasma protein A [PAPP]) and ultrasound nuchal translucency examination at the gestational age of 11–13 weeks. According to the results of the first stage of screening, women are classified into three groups of low, moderate, and high risk. The second-trimester screening is

merely carried out for women in the moderate-risk group using quad markers (i.e., Inhibin-A, total BHCG, unconjugated estriol [uE3], and alpha-fetoprotein [AFP]) during the 15–20 weeks of pregnancy.

According to this protocol, low-risk women continue with routine care, while those in the high-risk group undergo diagnostic tests, such as amniocentesis, based on the results of the first-trimester screening. The eligible cases included in the study were Iranian, pregnant of singleton birth, had a gestational age of 11 weeks based on ultrasound, had a medical record in the hospital clinic, did not use in vitro fertilization for recent pregnancy, were not smoking, and lacked pre-pregnancy diseases, including overt diabetes, heart disease, chronic hypertension. On the other hand, the exclusion criteria were unwillingness to continue participation, identification of structural and chromosomal abnormalities, inability to follow up, lack of access to pregnancy data and consequences, and induced and spontaneous abortion before the 21st week of pregnancy. The steps of the study are depicted in Fig. 1.

Based on the results of each biomarker, the pregnant women were classified into three groups, which included low levels (< 0.5 MOM), normal levels (0.5–2 MOM), and high levels (> 2 MOM). Primary outcomes were the rates of preterm delivery, gestational hypertension, IUGR, and IUFD. After obtaining permission from the hospital authorities, all eligible cases were enrolled into the study using the availability sampling method. Afterwords, informed consent was taken from participants and their demographic information, including maternal age, parity, maternal weight, and gestational age, was collected.

The subjects at gestational age of 11–13 weeks were then referred to the hospital laboratory for double marker screening. Based on the results of the first-trimester screening, the females in the moderate-risk group, at gestational age of 15–20 weeks, were referred to a reference laboratory to undergo a quad marker screen. The levels of biomarkers measured were corrected according to maternal weight, smoking, and diabetes and MOM was reported by laboratory devices. The participants, based on the results of each biomarker, were divided into three groups of low-level biomarkers (< 0.5 MOM), natural-level biomarkers (0.5–2 MOM), and high-level biomarkers (> 2 MOM). The cases with low and high levels were considered abnormal.

After the completion of all stages of screening, all pregnant women were followed up until delivery and information about pregnancy outcomes was collected. The information about pregnancy and neonatal outcomes was gathered by the research team by reviewing medical records or contacting mothers by telephone. The considered outcomes in the abnormal groups were compared separately for each biomarker with the corresponding biomarker in the natural group.
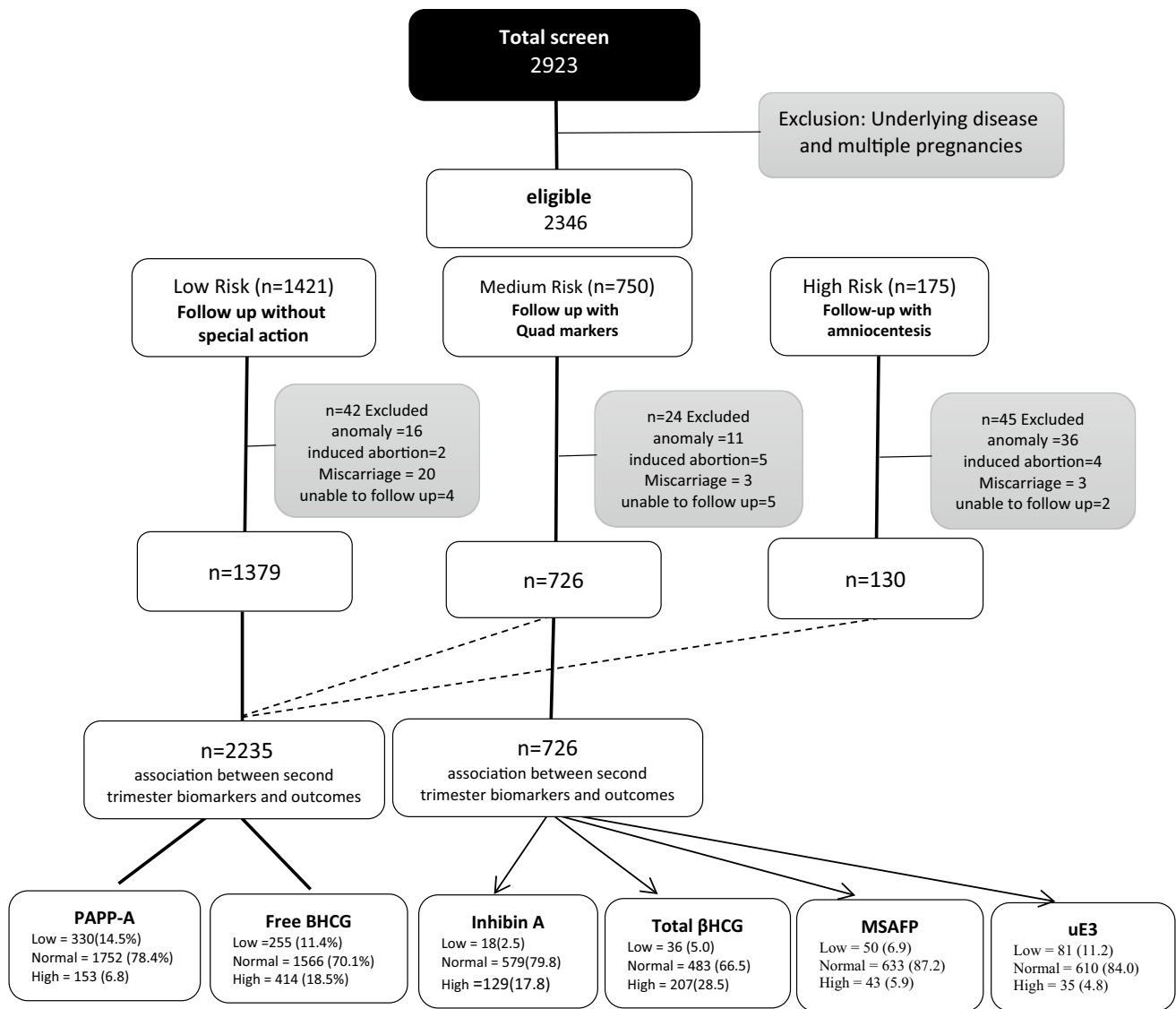
Figure 1 Flowchart of the study

Definitions related to pregnancy outcomes were provided based on (1) premature birth: birth before 37 full weeks of pregnancy, including spontaneous and induced cases (induction of labor due to such cases as severe preeclampsia), (2) IUGR: ultrasound-estimated fetal weight less than the 10th percentile of gestational age, (3) gestational hypertension: systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg with or without proteinuria of 1 + or more in a random urine sample or 24-h urine protein of more than 300 mg after the 20th week of pregnancy, and (4) ultrasound report of IUGR after the 21st week of pregnancy.

Statistical Analysis

Continuous variables were expressed as mean (SD) according to distribution state. Categorical variables were expressed as numbers and percentage. The baseline characteristics between the normal and abnormal groups (low and high) were compared by one-way ANOVA for continuous variables and Chi-square test for categorical data. Results were analyzed for statistical significance with Scheffé test for multiple comparisons. The percentages of preterm birth, preeclampsia, IUGR, and IUFD were compared between the group of abnormal (high and low) Inhibin-A concentrations and the group of normal concentrations, using Chi-square. Binary logistic regression analysis was performed to adjust the confounders of the main outcomes. P-value < 0.05 was

considered statistically significant. The statistical analysis was done with SPSS (IBM Corp. Released 2012; IBM SPSS Statistics for Windows, version 21.0. Armonk, NY).

Result

During the study period, 2923 pregnant women were referred to the clinic for receiving prenatal care and Down syndrome screening, among which 577 cases were excluded due to medical conditions or multiple pregnancy. The remaining 2346 subjects were divided into three groups of low risk, moderate risk, and high risk after the completion of the first-trimester screening. Finally, the subjects with abortion and inability to follow the pregnancy consequences were excluded from the study, and the results of screening in the first and second trimesters of pregnancy were evaluated among 2235 and 726 pregnant women, respectively.

According to the demographic and obstetric characteristics of 2235 pregnant women participating in the first phase of screening, the mean (SD) scores of age and weight were estimated at 30.16 (7.22) and 67.68 (12.68), respectively. Moreover, the majority of cases (42.8%) in this group were 30–40 years old. It was also revealed that 1329 (59.5%) cases were multiparous. The mean (SD) gestational age at the first-stage screening and delivery was 12.27 (16.78) and 37.13 (2.97), respectively. Considering the pregnancy outcomes, 336 (15%), 51 (2.3%), 117 (5.2%), and 283 (12.7%) participants had gestational hypertension, IUGR, IUFD, and preterm birth, respectively. The mean (SD) birth weight of neonates was 2.97 (0.78) kg, and most of them (53%) were males.

Moreover, the results of examining 726 participants in the second-trimester screening showed that the mean (SD) scores of age and weight of pregnant women were, respectively, 31.98 (6.89) and 69.43 (12.35). Based on the results, the mean (SD) age at the second-stage screening, delivery and neonatal weight was calculated at 16.78 (1.31), 36.82 (3.76) weeks, and 2.93 (0.84) kg, respectively. In terms of pregnancy outcomes, 129 (17.8%), 21 (2.9%), 57 (7.9%), and 108 (14.9%) subjects had gestational hypertension, IUGR, IUFD, and preterm birth, respectively.

Comparison of Demographic and Obstetric Characteristics at Different Levels of First-Trimester Biomarkers

The subject groups based on different levels of PAPP-A biomarkers, were placed in the low, normal, and high levels with 330 (14.5%), 1752 (78.4%), and 153 (6.8%) pregnant women, respectively. The comparison of different levels of PAPP-A with demographic and obstetric characteristics showed that all variables showed a significant difference in

the groups, except for gestational age at the time of screening and birth weight ($P = 0.37$ and $P = 0.87$, respectively). In addition, it was revealed that 255 (11.4%), 1,566 (70.1%), and 414 (18.5%) pregnant women had low, normal, and high levels of free BHCG, respectively. The comparison of these three groups in terms of baseline characteristics indicated that there was a significant difference regarding maternal age, parity, gestational age, and birth weight ($P = 0.31$, $P = 0.81$, and $P = 0.23$, respectively) (Table 1).

Comparison of Demographic and Obstetric Characteristics at Different Levels of Second-Trimester Biomarkers

The examination of maternal serum alpha-fetoprotein (MSAFP) biomarker showed that 50 (6.9%), 633 (87.2%), and 43 (5.9%) pregnant women had low, normal, and high levels, respectively. Furthermore, different levels of MSAFP showed no significant difference with baseline variables including the number of pregnancies ($P = 0.91$), gestational age at screening time ($P = 0.144$), and type of delivery ($P = 0.53$). Based on the total-BHCG biomarker examination, 483 and 242 of the cases had normal and abnormal levels, respectively. Accordingly, the results showed that different levels of biomarkers had no significant difference with maternal weight ($P = 0.910$), neonatal weight ($P = 0.757$), and the number of pregnancies ($P = 0.766$). Additionally, the comparison of different levels of uE3 biomarker in the second-trimester screening showed no significant difference between variables, except for maternal weight and number of pregnancies. Finally, the analysis of different groups of the fourth biomarker, Inhibin-A, was indicative of a significant difference between different levels of this biomarker with gestational age at the time of screening and infant weight ($P = 0.001$). All results obtained from the comparisons of different levels of biomarkers with baseline characteristics are reported in Table 1.

Comparison of Adverse Pregnancy Outcomes Between the Groups of Low, Normal, and High Levels of First-Trimester Biomarkers

The investigation of the relationship between PAPP-A biomarkers and the studied outcomes revealed that the rates of gestational hypertension were significantly higher in the group of low levels than those in the normal group (19.1% vs. 14.4%; $P = 0.029$). Nonetheless, the other adverse pregnancy outcomes, including preterm birth, IUGR, and IUFD, showed no significant difference between the groups with normal and abnormal level (Table 2). The results of univariate regression showed that low-level PAPP-A accounted as a risk factor for a 29% gestational hypertension individually ($B = -0.34$, SE $= 0.156$, OR $= 0.712$). Likewise, after

Table 1 Demographic characteristics—obstetrics of pregnant women at different levels of biomarkers

Variable	PAPP-A (n = 2235)			Free BHCG (n = 2235)			MSAFP (n = 726)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Age (year) Mean (SD)	31.23 (6.89)	30.16 (7.26)	27.85 (6.93)	30.93 (6.27)	29.84 (7.52)	30.89 (6.50)	32.81 (5.89)	32.10 (6.92)	29.38 (7.07)
P-Value	<0.001 High < normal < low			<0.001 Normal < high			0.03 Normal > High		
Body Weight (kg) Mean (SD)	70.35 (13.40)	67.60 (12.39)	63.06 (12.96)	68.35 (11.50)	67.41 (12.94)	68.30 (12.32)	68.50 (11.11)	68.95 (12.41)	76.97 (10.96)
P-Value	<0.001 High < normal < low			0.31			<0.001 Normal < High		
Parity									
Nulliparity n (%)	111 (33.6)	714 (40.8)	81 (52.9)	87 (34.1)	669 (42.7)	150 (36.2)	15 (35.7)	243 (38.0)	18 (40.0)
Multiparity n (%)	219 (66.4)	1038 (59.2)	72 (47.1)	168 (65.9)	897 (57.3)	264 (63.8)	27 (64.3)	396 (62.0)	27 (60.0)
P-Value	<0.001			0.005			0.919		
Gestational age at first screening Mean (SD)	12.12 (0.65)	12.29 (0.63)	12.32 (0.62)	12.23 (0.67)	12.29 (0.62)	12.21 (0.72)	12.02 (0.73)	12.20 (0.67)	12.04 (0.63)
P-Value	<0.001 Low < Normal < high			0.18			0.162		
Gestational age at second screening Mean (SD)	–	–	–	–	–	–	16.29 (0.92)	16.66 (1.21)	16.72 (1.34)
P-Value	–			–			0.144		
Gestational age at delivery Mean (SD)	36.87 (3.87)	37.18 (3.61)	37.06 (3.70)	36.68 (3.87)	37.11 (3.66)	37.46 (3.45)	37.80 (2.36)	37.01 (3.58)	33.32 (5.29)
P-Value	0.37			0.02 Low < high			<0.001 Normal > High		
Gestational weight (Kg) Mean (SD)	2.99 (0.82)	2.98 (0.78)	2.94 (0.73)	2.91 (0.81)	2.96 (0.79)	3.07 (0.69)	3.12 (0.70)	2.96 (0.79)	2.17 (1.20)
P-Value	0.87			0.01 Normal < high			<0.001 Normal > High		
Route of delivery									
Normal delivery n (%)	126 (38.2)	639 (36.5)	78 (51.0)	99 (38.8)	603 (38.5)	141 (34.1)	12 (28.6)	219 (34.3)	18 (40.0)
Cesarean delivery n (%)	204 (61.8)	1113 (63.5)	75 (49.0)	156 (61.2)	963 (61.5)	273 (65.9)	30 (71.4)	420 (65.7)	27 (60.0)
P-Value	0.002			0.23			0.53		

Variable	Total BHCG (n = 726)			uE3 (n = 726)			Inhibin-A (n = 726)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Age (year) Mean (SD)	29.72 (5.40)	32.48 (6.90)	31.21 (6.97)	31.94 (7.68)	31.97 (6.75)	32.25 (7.64)	31.23 (6.16)	31.92 (7.02)	32.44 (6.89)
P-Value	0.011 Normal > Low			0.973			0.652		
Body Weight (kg) Mean (SD)	69.33 (14.14)	69.57 (12.55)	69.12 (11.59)	66.23 (11.97)	69.48 (12.23)	76.17 (12.64)	67.83 (13.42)	69.77 (12.62)	68.07 (11.02)
P-Value	0.91			<0.001 Normal < High			0.338		

Table 1 (continued)

Variable	Total BHCG (n=726)			uE3 (n=726)			Inhibin-A (n=726)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Parity									
Nulliparity n (%)	15 (41.7)	186 (38.5)	75 (36.2)	40 (49.4)	228 (37.4)	8 (22.9)	6 (33.3)	230 (39.7)	40 (31.0)
Multiparity n (%)	21 (58.3)	297 (61.5)	132 (62.8)	41 (50.6)	382 (62.6)	27 (77.1)	12 (66.7)	349 (60.3)	89 (69.0)
P-Value	0.766			0.019			0.168		
Gestational age at first screening Mean (SD)	–	–	–	–	–	–	–	–	–
P-Value	–			–			–		
Gestational age at second screening Mean (SD)	17.32 (1.29)	16.68 (1.24)	16.42 (1.05)	16.48 (1.01)	16.64 (1.21)	16.98 (1.55)	16.73 (0.51)	16.73 (1.27)	16.31 (0.87)
P-Value	<0.001 High<normal<Low			0.149			0.001 Normal>High		
Gestational age at delivery Mean (SD)	37.15 (3.19)	36.87 (3.64)	36.65 (4.13)	36.83 (3.25)	36.91 (3.79)	35.37 (4.11)	38.26 (1.86)	36.89 (3.62)	36.31 (4.47)
P-Value	0.681			0.064			0.072		
Gestational weight (Kg) Mean (SD)	2.96 (0.65)	2.95 (0.87)	2.87 (0.79)	2.77 (0.66)	2.95 (0.85)	2.85 (0.98)	3.60 (0.69)	2.93 (0.85)	2.81 (0.76)
P-Value	0.757			0.093			0.001 Normal<Low		
Route of delivery									
Normal delivery n (%)	12 (33.3)	150 (31.1)	87 (42.0)	26 (32.1)	215 (35.2)	8 (22.9)	6 (33.3)	204 (35.2)	39 (30.2)
Cesarean delivery n (%)	24 (66.7)	333 (68.9)	120 (58.0)	55 (67.9)	396 (64.4)	27 (77.1)	12 (66.7)	375 (64.8)	90 (69.8)
P-Value	0.021			0.294			0.555		

adjusting for the potential confounding factors by logistic regression analysis, high blood pressure was not significantly associated with low PAPP-A ($B=-0.24$, SE$=0.168$, OR$=0.780$; $P=0.140$). In addition, the results of investigating free-BHCG biomarker showed that the rate of preterm birth was significantly higher in the group with high-levels of free-BHCG than those in the normal group (15.2% vs. 11.6%; $P=0.048$). Nevertheless, there was no significant difference in other outcomes in different free-BHCG groups (Table 2). Univariate regression analysis showed that at high levels of free BHCG the probability of preterm birth increased by 27% ($B=-0.31$, SE$=0.158$, OR$=0.733$). Furthermore, the analysis of the adjusted risk ratio indicated that the occurrence of preterm birth was still statistically significant ($B=-0.31$, SE$=0.158$, OR$=0.730$; $P=0.046$), as shown in Table 3.

Comparison of Adverse Pregnancy Outcomes Between Groups of Low, Normal, and High Levels of Second-Trimester Biomarkers

The examination of second-trimester biomarkers and their association with pregnancy outcomes showed that the rate of adverse pregnancy outcomes including preterm labor, gestational hypertension, IUGR, and IUFD was higher in the high MSAFP levels as compared with the normal group (30.2% vs. 13.7%, $P=0003$; 33.3% vs. 17.4%, $P=0.008$; 20.9% vs. 6.6%, $P=0.001$; and 14% vs. 2.4%, $P<0.001$).

Table 2 Comparison of adverse pregnancy outcomes between the group of abnormal level and normal group of all biomarkers

Variable	PAPP-A (n = 2235)			Free BHCG (n = 2235)			MSAFP (n = 726)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Gestational hypertension n (%)	63 (19.1)	252 (14.4)	21 (13.7)	36 (14.1)	243 (15.5)	57 (13.8)	4 (8.0)	110 (17.4)	15 (33.3)
Relative risk (95% CI)	1.40 (1.03–1.91)		0.94 (0.58–1.52)	0.89 (0.61–1.30)		0.86 (0.62–1.18)	0.41 (0.14–1.17)		2.37 (1.23–4.56)
P-Value	0.029		0.824	0.565		0.377	0.087		0.008
Preterm birth n (%)	44 (13.3)	213 (12.2)	26 (17.7)	38 (14.9)	182 (11.6)	63 (15.2)	8 (16.0)	87 (13.7)	13 (30.2)
Relative risk (95% CI)	1.11 (0.78–1.57)		1.47 (0.94–2.31)	1.32 (0.91–1.94)		1.36 (1.00–1.86)	1.19 (0.54–2.63)		2.72 (1.36–5.41)
P-Value	0.551		0.083	0.136		0.048	0.657		0.003
Intra uterine death n (%)	12 (3.6)	39 (2.2)	0 (0)	6 (2.4)	33 (2.1)	12 (2.9)	0 (0)	15 (2.4)	6 (14.0)
Relative risk (95% CI)	1.65 (0.85–3.20)		0.91 (0.90–0.93)	1.11 (0.46–2.69)		1.38 (0.71–2.70)	0.92 (0.90–0.94)		6.68 (2.45–18.21)
P-Value	0.128		0.069*	0.802		0.33	0.271		< 0.001
IUGR n (%)	306 (92.7)	84 (4.8)	144 (94.1)	12 (4.7)	90 (5.7)	15 (3.6)	6 (12.0)	42 (6.6)	9 (20.9)
Relative risk (95% CI)	1.56 (0.97–2.49)		1.24 (0.61–2.52)	0.81 (0.43–1.50)		0.61 (0.35–1.07)	1.91 (0.77–4.76)		3.72 (1.67–8.27)
P-Value	0.063		0.549	0.503		0.086	0.153		0.001

Variable	Total HCG (n = 726)			uE3 (n = 726)			Inhibin-A (n = 726)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Gestational hypertension n (%)	3 (8.3)	96 (19.9)	30 (14.5)	10 (13.2)	113 (18.5)	6 (15.4)	3 (16.7)	93 (16.1)	33 (25.6)
Relative risk (95% CI)	0.36 (0.11–1.22)		0.68 (0.43–1.06)	0.66 (0.33–1.33)		0.80 (0.32–1.95)	1.04 (0.30–3.68)		1.79 (1.14–2.82)
P-Value	0.089		0.094	0.252		0.626*	0.945*		0.011
Preterm birth n (%)	9 (25)	75 (15.5)	24 (11.6)	11 (13.6)	89 (14.6)	8 (22.9)	3 (16.7)	93 (16.1)	12 (9.3)
Relative risk (95% CI)	1.81 (1.05–0.97)		0.71 (0.43–1.16)	0.92 (0.46–1.80)		1.73 (0.76 t0 3.94)	1.04 (0.29–3.68)		0.53 (0.28–1.01)
P-Value	0.137		0.177	0.808		0.183	0.945*		0.055
Intra uterine death n (%)	0 (0)	12 (2.5)	9 (4.3)	0 (0)	21 (3.4)	0 (0)	0 (0)	15 (2.6)	6 (4.9)
Relative risk (95% CI)	0.92 (0.90–0.95)		1.784 (0.74–4.30)	0.87 (0.85–0.90)		0.94 (0.92–0.96)	0.969 (0.95–0.98)		1.949 (0.74–5.127)
P-Value	0.039		0.192	0.158*		0.621*	0.492*		0.169

Table 2 (continued)

Variable	Total HCG (n=726)	uE3 (n=726)	Inhibin-A (n=726)
IUGR n (%)	39 (8.1)	44 (7.2)	12 (9.8)
	18 (8.7)	11 (13.6)	3 (16.7)
	0 (0)	2 (5.7)	3 (16.7)
Relative risk (95% CI)	0.925 (0.90–0.94)	2.02 (0.99–4.09)	1.39 (0.71—2.74)
	1.08 (0.60–1.94)	0.78 (0.18–3.35)	2.58 (0.72–9.28)
P-Value	0.766	0.047	0.328
	0.786	0.738*	0.132*

*Fisher exact test

Nevertheless, univariate regression analysis indicated that elevated MSAFP levels increased the probability of gestational hypertension by 59% ($B = -0.93$, $SE = 0.337$, $OR = 0.393$), but after adjusting for other variables. An increase in the level of MSAFP increased the probability of gestational hypertension by 45% ($B = -0.59$, $SE = 0.354$, $OR = 0.549$). At high MSAFP levels, the incidence of preterm delivery increases by 64% ($B = -1.00$, $SE = 0.352$, $OR = 0.368$) and after adjusting for other baseline variables ($B = -0.84$, $SE = 0.361$, $OR = 0.431$), it increases by 57%. The results of univariate regression regarding the relationship between MSAFP and IUGR showed that in cases of high MSAFP levels, the occurrence of IUGR was 74% ($B = -1.31$, $SE = 0.407$, $OR = 0.268$,), increasing to 77% in the adjusted risk ratio ($B = -1.46$, $SE = 0.433$, $OR = 0.231$). Moreover, at high MSAFP levels, the incidence of IUFD increased by 85% ($B = -1.89$, $SE = 0.512$, $OR = 0.150$; $P < 0.001$); after adjusting for other variables, this probability increased to 78% ($B = -1.50$, $SE = 0.533$, $OR = 0.223$) (Table 3).

No significant relationship was found between different levels of total-BHCG and adverse pregnancy outcomes (Table 2). The examination of the relationship between uE3 biomarker levels and outcomes showed a significant difference between normal and abnormal uE3 levels with IUGR. In this respect, cases with low uE3 levels were more likely to experience IUGR than those with normal levels (13.6% vs. 7.2%; $P = 0.047$) (Table 2). Univariate regression analysis revealed that a decrease in uE3 led to IUGR in 64% ($B = -0.70$, $SE = 0.360$, $OR = 0.495$; $P = 0.050$). It was reported that in the adjusted risk ratio, the low-level uE3 was not significant for the incidence of IUGR ($B = -0.67$, $SE = 0.364$, $OR = 0.509$) (Table 3).

Finally, the results of the Inhibin-A biomarker showed only a significant relationship of high and normal levels of this biomarker with gestational hypertension. Pregnancy induced hypertension was higher among subjects with high levels of Inhibin-A (25.6% vs. 16.1%; $P = 0.011$). However, no statistically significant difference was observed between this biomarker and other outcomes (Table 2). Univariate regression analysis showed that an increase in the Inhibin-A levels caused a 45% increase in gestational hypertension ($B = -0.58$, $SE = 0.231$, $OR = 0.557$), which increased to 47% after adjusting other variables ($B = -0.63$, $SE = 0.235$, $OR = 0.533$) (Table 3).

Discussion

An important finding was that, after adjusting other variables, for first-trimester serum biomarkers, elevated free-BHCG levels significantly increased the risk of preterm birth. Among second-trimester serum biomarkers, abnormal

Table 3 Crude and adjusted odd ratios for the risk of preterm birth and preeclampsia derived from logistic regression analysis

Outcomes Potential risk factors	Preterm birth (<37 week)				Gestational hypertension				Intra uterine death				IUGR			
	Crude P-Value	Crude OR (95%CI)	Adjusted P-Value	Adjusted OR (95%CI)	Crude P-Value	Crude OR (95%CI)	Adjusted P-Value	Adjusted OR (95%CI)	Crude P-Value	Crude OR (95%CI)	Adjusted P-Value	Adjusted OR (95%CI)	Crude P-Value	Crude OR (95%CI)	Adjusted P-Value	Adjusted OR (95%CI)
PAPP-A																
Normal	–	Ref	–	Ref	–	Ref	–	Ref	–	–	–	–	–	–	–	–
Low	0.049	0.73 (0.53–0.99)	0.046	0.73 (0.53–0.99)	0.029	0.71 (0.52–0.96)	0.14	0.78 (0.56–1.08)	–	–	–	–	–	–	–	–
Free βHCG																
Normal	–	Ref	–	Ref	–	–	–	–	–	–	–	–	–	–	–	–
High	0.004	0.36 (0.18–0.73)	0.02	0.43 (0.21–0.87)	–	–	–	–	–	–	–	–	–	–	–	–
MSAFP																
Normal	–	–	–	–	–	Ref	–	Ref	–	Ref	–	Ref	–	Ref	–	Ref
High	–	–	–	–	0.005	0.39 (0.20–0.76)	0.091	0.54 (0.27 to1.10)	<0.001	0.15 (0.05–0.40)	0.005	0.22 (0.07–0.63)	0.001	0.26 (0.21–0.59)	0.001	0.23 (0.09–0.54)
uE3																
Normal	–	–	–	–	–	–	–	–	–	–	–	–	–	Ref	–	Ref
Low	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.49 (0.24–1.00)	0.064	0.5 (0.24–1.03)
Inhibin-A																
Normal	–	–	–	–	–	Ref	–	Ref	–	–	–	–	–	–	–	–
High	–	–	–	–	0.011	0.55 (0.35 to0.87)	0.007	0.53 (0.33–0.84)	–	–	–	–	–	–	–	–

OR = Odd Ratio; CI = Confidence Interval; Ref = Reference group; Model 1: Unadjusted; Model2: Adjusted for women's age, women's weight, parity and, Gestational age

levels of MSAFP and Inhibin-A were associated with adverse outcomes. In this regard, elevated MSAFP levels were directly associated with preterm birth, gestational hypertension, IUGR, and IUFD. Additionally, an increase in the levels of Inhibin-A biomarkers increased the probability of developing gestational hypertension among pregnant women.

First-Trimester Serum Biomarkers

First, the cases with low levels of PAPP-A were more likely to develop gestational hypertension; however, no significant relationship was observed with other outcomes and relationship between low PAPP-A levels and gestational hypertension was not significant after considering other potential variables. The results of studies conducted in the recent years in the different countries indicate the same relationship as the current study [16–19]. D'Antonio et al. [20] reported that maternal serum PAPP-A performs poorly as a screening test for PT, SGA, and preeclampsia. Nonetheless, other studies support the association between low PAPP-A levels and risk for poor pregnancy outcome [21, 22]. The results of a study carried out by Dugoff et al. [6] showed that a 10%-MOM reduction in PAPP-A levels significantly increased the risk of adverse pregnancy outcomes. In addition, Yaron et al. [19] reported that in cases of PAPP-A level less than 0.5 MOM, the relative risk of developing IUGR was 3.3 times, which was significant. Furthermore, Morris et al. [4] conducted a meta-analysis on 175,000 pregnancies and proved that lower level of PAPP-A has a relationship with preeclampsia (1.94 odds ratio) [19]. Pakniat et al. [17] also showed that the mean score of PAPP-A had a significant negative relationship with preterm birth ($P < 0.001$). Secondly, analyzing the relationship between first trimester serum free BHCG with pregnancy complications showed that with increasing level of serum free BHCG the probability of premature birth increases; it showed no relationship with other outcomes. However, the results of various studies in this field are different [2, 6]. In a study carried out by Haung et al. [2], it was revealed that pregnancies with free-BHCG level above the 95th percentile were reported to have an odds ratio of 4.2 for preeclampsia and small for gestational age, compared to the control group; nevertheless, the cases in which the free-BHCG level was less than the 5th percentile, the odds ratio of preterm birth was estimated at 2.1. In the mentioned study, the 5th and 95th percentiles corresponded to 0.32 and 2.98 MOM, respectively. These values were different from the values considered in the present study for the low and high free-BHCG groups, which could be the reason for this discrepancy. However, in our study, on comparing the mean gestational age at birth in groups there was a significant difference between the groups. In other study, in which free BHCG was less than the 5th percentile for normal values,

the odds of birth weight less than 10th percentile was 1.55; however, there was no significant relationship between low free-BHCG level and birth weight less than 5th percentile [5]. In the above study, the 5th percentile corresponded to 0.42 MOM, which was lower than the values considered in the present study. The divergent points of maternal serum BHCG and PAPP-A with wide range of cutoff values may be responsible for these results. Also, the difference in sample size could be a contributing factor. In addition, the inconsistency of these results could be related to strict adjustment for confounders and using different types of studies to investigate the relationship between serum marker levels and pregnancy outcomes.

Second-Trimester Serum Biomarkers

First of all, according to the findings of the present study, MSAFP was the only biomarker to be associated with adverse pregnancy outcomes and predicting all four pregnancy complications. This finding was consistent with several studies that have reported MSAFP levels of ≥ 2MOM in the second trimester are associated with an increased risk of adverse pregnancy outcomes such as preeclampsia, IUGR, preterm delivery, placenal abruption, and fetal loss [23–25]. The results obtained from the scans of 225,000 pregnant women showed that 20 to 38% of women with unexplained MSAFP elevation had poor pregnancy outcomes. Similarly, our study results are consistent with the previous findings. Nevertheless, some studies did not find any significant increase in MSAFP levels in women with preeclampsia [8, 16, 17]. Basbug et al. [26] reported that rates of preterm delivery, preterm premature rupture of membranes (PPROM), oligohydramnios, and intrauterine growth restriction (IUGR) were increased in the elevated MSAFP group, but the level of MSAFP did not have relationship with preeclampsia, but this can be due to small sample size of their study.

According to another finding of our study, there was no significant relationship between different levels of total-BHCG biomarker with adverse pregnancy outcomes. This result was not similar to Onderoglu and Kabucu [24]; they showed that with a total BHCG of > 2, the odds of preterm birth, gestational hypertension, preeclampsia, and IUGR were, 5.66, 1.5, 93.5, and 5.34 respectively. They also indicated that an increase in the total-BHCG levels resulted in a 411 g reduction in birth weight, as compared to the control group. In their study, the researchers compared the complications of pregnancy in two groups with high and low HCG levels. Nonetheless, in the present study, pregnancy complications were compared in three groups of abnormal (high and low) and normal total-BHCG levels. Therefore, the use of different levels accounted for the discrepancies in the results. Yaron et al. [27] showed a significant relationship

of total BHCG of > 2.5 with gestational hypertension, preterm birth, and IUFD; nevertheless, it had no relationship with IUGR. The results of other pieces of research were confirmatory of the relationship between high levels of total BHCG and pregnancy complications [28, 29], which were not similar with our study. The inconsistency of these results could be related to consideration of wide range of cutoff values and different classification of groups.

In addition, the subjects with low uE3 levels were more likely to develop IUGR. However, considering the effect of other variables, this probability was not significant. In our study, there was no association between uE3 levels adverse pregnancy/neonatal outcomes unlike earlier reports [6–8, 30]. Furthermore, the results of a study conducted by Odibo et al. [28] revealed that uE3 of < 0.9 had a significant relationship with IUGR, and the odds ratio of IUGR was 2.7 in such conditions. However they did not examine the effect of other variables. On the other hand, Yaron et al. [27] reported a significant relationship of uE3 of < 0.5 with pregnancy-induced hypertension, IUGR, and IUFD. Moreover, they found that uE3 of < 0.5 had no significant relationship with preterm birth. In the mentioned study, pregnancy outcomes were compared in the group with uE3 of < 0.5 and group with uE3 of > 0.5, which seems to account for the discrepancy between the results of the present study. Duric et al. [31] found positive relationship between low unconjugated estriol levels (MOM ≤ 0.74) with intrauterine growth restriction and threatened preterm delivery, but their participants included women with 15–22 weeks of gestation. So, this can be a reason for the difference in results.

Finally, the cases with high levels of Inhibin-A had a higher incidence of gestational hypertension. However, no significant relationship was observed between this biomarker and adverse outcomes. This finding is consistent with the results of other studies [24, 28]. Yazdani et al. [25] reported that preeclampsia was associated with high levels of Inhibin-A. They indicated that high levels of Inhibin-A were associated with IUGR and preterm birth, the difference between their study and the present study is in the selection of normal and abnormal values. Doguoff et al. [6] reported that Inhibin-A of > 2 had a significant relationship with preterm birth, IUGR, preeclampsia, and IUFD. These researchers have compared Inhibin-A of > 2 with those of < 2 in terms of pregnancy complications. However, in the present study, Inhibin-A levels were compared at three levels of low, normal, and high in terms of pregnancy complications, which accounts for the differences in the results of these two studies.

In general, it can be said that the discrepancies between the findings of our study and those of other studies can be attributed to the selection of different cut-off level of biomarkers for normal and abnormal cases and the use of different definitions of pregnancy complications. In addition,

another reason may be due to the fact that these studies were not performed within the same duration or at the exact time. The sample size can also play an important role in achieving the relationship between variables, especially in identifying cases with lower prevalence.

Since this is a hospital-based study, it is not possible to generalize the results to all socioeconomic classes and other parts of the country. One of the limitations of this research was related to its small sample size to investigate the relationship between some less common outcomes. Another limitation of the study was that it did not analyze the biomarkers in combination with each other. However, one of the strengths of the present study was to consider and eliminate the effect of possible confounding variables, such as fetal and chromosomal abnormalities and gynecological medical diseases. Moreover, all experiments were performed at the same center to reduce bias.

Conclusion

According to the results of the study, MSAFP biomarker levels had relationship with the most important complications of pregnancy and could predict adverse outcomes. Nevertheless, other biomarkers had the ability to predict some side effects. The use of maternal serum markers during pregnancy is simple, fast, and relatively inexpensive. Therefore, it seems that these markers can be beneficial in identifying high-risk cases, in addition to examining the possibility of Down syndrome, facilitating achieving the desired pregnancy outcomes.

Declarations

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

Ethics Approval and Consent to Participate This study was approved by the Ethics Committee of the Islamic Azad University Tabriz branch, Iran (code number: IR.IAU.TABRIZ.REC1397.001).

Informed Consent Informed consent was obtained from all participants for being included in the study.

Human and Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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