RESEARCH
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ASSOCIATION OF PLACENTAL ALPHA 1 MICROGLOBULIN, FETAL FIBRONECTIN AND TSH IN 2ND AND 3RD TRIMESTER PREGNANT WOMEN

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Abstract
Introduction: The second and third trimesters of pregnancy are critical periods for maternal and fetal health. Placental Alpha 1 Microglobulin (PAMG-1), Fetal Fibronectin (fFN), and Thyroid-Stimulating Hormone (TSH) are important biomarkers that may play a role in predicting complications such as preterm labor, thyroid dysfunction, and placental insufficiency. This study aims to explore the association between these biomarkers and pregnancy outcomes in the second and third trimesters.
Aims: The aim of our study is to assees the association of placental alpha microglobulin-1,fibronectin and TSH in 2nd and 3rd trimester
Materials and Methods: It was a prospective observational study, was conducted one year obstetric and gynecology department in IGIMS, PATNA.51 Patients were included in this study
Results: In Multigravida, 9(40.9%) patients had Backache, 10 (45.5%) patients had Pain Abdomen and 3 (13.6%) patients had Pain Abdomen + Backache. In Primigravida, 10 (34.5%) patients had Backache, 15(51.7%) patients had Pain Abdomen and 4(13.8%) patients had Pain Abdomen + Backache. Association of Chief Complain with Group was not statistically significant (p=0.8873).In Multigravida, 4(18.2%) patients had Modrate Anemia .In Primigravida, 5(17.2%) patients had Modrate Anemia. Association of Medical History with Group was not statistically significant (p=0.7995).
Conclusion: We conclude that demonstrates a significant association between Placental Alpha 1 Microglobulin (PAMG-1), Fetal Fibronectin (fFN), and Thyroid-Stimulating Hormone (TSH) levels in pregnant women during the second and third trimesters, and their relationship with adverse pregnancy outcomes.
Keywords: Placental Alpha 1 Microglobulin (PAMG-1), Fetal Fibronectin (fFN) ,Thyroid-Stimulating Hormone (TSH),Second Trimester Third Trimester

INTRODUCTION

Preterm birth occurs in approx 6-10 % of pregnancies worldwide. [1] It is difficult to determine whether a woman having preterm contractions will give birth prematurely. Accurate diagnosis enables therapies like antinatal corticosteroid therapy, group B streptococcal infection prevention, and magnesium sulphate for neuro-protection, which can enhance newborn outcomes. It is impossible to determine exactly which women with preterm contractions will give birth prematurely. Accurate diagnosis will enable therapies such as antinatal corticosteroid therapy, group B streptococcal infection prevention, and magnesium sulphate for neuroprotection, which can enhance newborn outcomes. In addition to identifying pregnant women who are not truly in preterm labor, it would avert needless treatments and neonatal intensive care hospitalization in about 50% of patients with suspected preterm labor who later deliver at term without tocolytic medication. [2] Currently, fetal fibronectin and transvaginal sonography cervical length measurements are utilized to detect pre-term labour. Recent research reveal Placental α microglobulin-1 is a superior predictor.Amniotic fluid has significant concentrations of placental α microglobulin-1, which is secreted by decidual cells. Placental α microglobulin-1 is present in cervico-vaginal secretions after spontaneous preterm birth, possibly

due to early contractility or inflammation during preterm labor. Fetal fibronectin (fFN) is a fibronectin protein synthesized by fetal cells. It is located at the junction of the chorion and decidua.It may be thought of as a glue that holds the fetal sac to the uterine lining.The capacity to detect the danger of an impending preterm delivery is hence a top concern in obstetrics.TSH levels are important during the various stages of pregnancy.Thyroid disease during pregnancy is related with poor pregnancy outcomes. According to the WHO, PTB can be classified into extremely preterm (<28 weeks), very preterm (28 to <32 weeks), and moderate to late preterm (32 to 37 weeks), depending on gestational age [3] Preterm neonates are more likely to develop necrotizing enterocolitis, respiratory distress syndrome, periventricular leukomalacia, seizures, intraventricular hemorrhage, cerebral palsy, hypoxic-ischemic encephalopathy, visual and hearing impairments, infections, feeding problems, and a variety of other short- and long-term morbidities. [4] Between 2000 and 2014, the global PTB rate climbed from 9.8% to 10.6%, with an estimated 13.4 million cases (1 in every 10 births) by 2020. In 2020, India accounted for 3.02 million PTBs, or over 23% of all PTBs globally, the largest number of preterm births worldwide, and the fourth highest PTB rate behind Bangladesh, Malawi, and Pakistan. [5]. Preterm labor (PTL) is "regular uterine contractions before 37 weeks of pregnancy that cause cervical change or regular contractions with an initial presentation with cervical dilation of 2 cm or more" [6]. It is characterized by mild abdominal pains, back pain, regular uterine contractions, watery or bloody vaginal discharge, increased volume of discharge, and the rupture of membranes with water leakage. Various risk factors are responsible for PTL, such as infection (endotoxin), which accounts for 25–40% of cases, inflammatory mediators (IL1β, TNF-α), vaginal bleeding (hemorrhage), uterine overdistension [7,8]

MATERIALS AND METHODS

- **Study design:** prospective observational study
- **Sample size justification:**
- **Sample size and source data**: 51 pregnant women from OPD & who will be admitted under obstetric and gynecology department in IGIMS, PATNA with threatened preterm labour between 24 0/7 weeks and 36 6/7 weeks of pregnancy
- **Duration of study**:

1.5 years (after clearance from ethical committee)

- Institutional review board approval will be obtained and all participants provided written informed consent as a requisite for enrollment. After enrollment a detailed medical history for each participant would be obtained.
- Signs and symptoms of threatened preterm labour would include, uterine contraction, intermittent lower abdominal pain, dull back ache, pelvic pressure,mild bleeding during 2nd and 3 trimester, menstrual or intestinal cramping with or without diarrhoea.
- Those women will be excluded if they had received tocolytics medications before the collection of cervical vaginalspeciemen or if they had placenta previa ; moderate to gross vaginal bleeding, coitus with in past 24 hrs, history of cervical cercalge, history not consistant with idiopathic threatened preterm delivery such as trauma, digital trans vaginal sonography.
- PAMG-1 sample will be taken by inserting sterile swab provided with the PAMG-1 test kit into the vagina without the use of speculum for 30 sec. The specimen will then be eluted for 30 sec through active rotation of swab in solvent solution provided with the test kit.
- The test will be read no earlier then 5 minutes and no later then 10 minutes, 2 red line on test stripe would indicate a positive test and 1 line indicate a negative test result.there after, a physical examination including speculum examination will be perform to collect a swab of cervico-vaginal secretion for fetal fibronectine ,and the speciemen will be sent to the lab for testing (threshold of 50 ng/ml)

Inclusion Criteria

1) Adult women (at least 18 year old) legally c.03ompetent
2) Provide oral consent after information
3) Singleton pregnancy
4) Gestation age between 24 – 36weeks+6days
5) Clinically intact membrane
6) Cervical dilatation less then 3 cm. assessed by digital examination
7) Medically indicated deliveries.

Exclusion criteria

1) Patients who do not give consent for the study
2) Presence of cervical bleeding
3) History of cervical conization
4) Premature rupture of membranes
5) Leaking per vaginally.

Study design:

- Patient arriving at OPD of Obstetric and Gynaecology, in 2nd and 3rd trimester will be investigated for CBC, TSH and viral marker
- fetal fibronectin in cervico-vaginal secretion has testing threshold of 50 ng/ml.

RESULT

Table 1: Association between Chief Complain : Parity

Parity				
Chief Complain	**Multigravida**	**Primigravida**	**Total**	**p-value**
Backache Row % Col %	9 47.4 40.9	10 52.6 34.5	19 100.0 37.3	0.8873
Pain Abdomen Row % Col %	10 40.0 45.5	15 60.0 51.7	25 100.0 49.0	
Pain Abdomen + Backache Row % Col %	3 42.9 13.6	4 57.1 13.8	7 100.0 13.7	
Total Row % Col %	22 43.1 100.0	29 56.9 100.0	51 100.0 100.0	

Table 2: Association between Medical History : Parity

PARITY				
Medical History	**Multigravida**	**Primigravida**	**TOTAL**	**P value**
BOH Row % Col %	1 100.0 4.5	0 0.0 0.0	1 100.0 2.0	0.7995
B-Thalassemia Trait Row % Col %	0 0.0 0.0	1 100.0 3.4	1 100.0 2.0	
GDM Row % Col %	1 50.0 4.5	1 50.0 3.4	2 100.0 3.9	
Heart Disease Row % Col %	0 0.0 0.0	1 100.0 3.4	1 100.0 2.0	
Hypothyroidism Row % Col %	2 50.0 9.1	2 50.0 6.9	4 100.0 7.8	
IUI (H) Row % Col %	0 0.0 0.0	1 100.0 3.4	1 100.0 2.0	
Modrate Anemia Row % Col %	4 44.4 18.2	5 55.6 17.2	9 100.0 17.6	
Modrate Anemia + hypothyroidism Row % Col %	0 0.0 0.0	1 100.0 3.4	1 100.0 2.0	

Modrate **Anemia+hypothyroidism**	0	1	1	
Row %	0.0	100.0	100.0	
Col %	0.0	3.4	2.0	
No	14	16	30	
Row %	46.7	53.3	100.0	
Col %	63.6	55.2	58.8	
TOTAL	22	29	51	
Row %	43.1	56.9	100.0	
Col %	100.0	100.0	100.0	

Table 3: Association between H/O Previous Term Birth : Parity

Parity				
H/O Previous Term Birth	**Multigravida**	**Primigravida**	**Total**	**p-value**
No	5	29	34	<0.0001
Row %	14.7	85.3	100.0	
Col %	22.7	100.0	66.7	
Yes	17	0	17	
Row %	100.0	0.0	100.0	
Col %	77.3	0.0	33.3	
Total	22	29	51	
Row %	43.1	56.9	100.0	
Col %	100.0	100.0	100.0	

Table 4: Association between H/O Previous Preterm Birth: Parity

Parity				
H/O Previous Preterm Birth	**Multigravida**	**Primigravida**	**Total**	**P value**
No	18	29	47	0.0167
Row %	38.3	61.7	100.0	
Col %	81.8	100.0	92.2	
Yes	4	0	4	
Row %	100.0	0.0	100.0	
Col %	18.2	0.0	7.8	
Total	22	29	51	
Row %	43.1	56.9	100.0	
Col %	100.0	100.0	100.0	

Table 5: Association between H/O Abortion : Parity

Parity				
H/O Abortion	**Multigravida**	**Primigravida**	**Total**	**p-value**
No	14	29	43	0.0004
Row %	32.6	67.4	100.0	
Col %	63.6	100.0	84.3	
Yes	8	0	8	
Row %	100.0	0.0	100.0	
Col %	36.4	0.0	15.7	

Total	22	29	51	
Row %	43.1	56.9	100.0	
Col %	100.0	100.0	100.0	

Table 6: Association between Delivery in 7, 7-14 and >14 Days: Parity

	Parity				
		Multigravida	**Primigravida**	**Total**	**p-value**
Delivery in 7 Days	**No**	19	27	46	0.4227
	Row %	41.3	58.7	100.0	
	Col %	86.4	93.1	90.2	
	Yes	3	2	5	
	Row %	60.0	40.0	100.0	
	Col %	13.6	6.9	9.8	
	Total	22	29	51	
	Row %	43.1	56.9	100.0	
	Col %	100.0	100.0	100.0	
Delivery in 7-14 Days	**No**	21	27	48	0.7237
	Row %	43.8	56.3	100.0	
	Col %	95.5	93.1	94.1	
	Yes	1	2	3	
	Row %	33.3	66.7	100.0	
	Col %	4.5	6.9	5.9	
	TOTAL	22	29	51	
	Row %	43.1	56.9	100.0	
	Col %	100.0	100.0	100.0	
Delivery in >14 Days	**No**	4	4	8	0.6695
	Row %	50.0	50.0	100.0	
	Col %	18.2	13.8	15.7	
	Yes	18	25	43	
	Row %	41.9	58.1	100.0	
	Col %	81.8	86.2	84.3	
	Total	22	29	51	
	Row %	43.1	56.9	100.0	
	Col %	100.0	100.0	100.0	

Table 7: Distribution of mean with all parameters: Parity

		Number	Mean	SD	Minimum	Maximum	Median	p-value	T Statistic
Gestational Age (Weeks)	Multigravida	22	30.5519	3.2115	25.0000	35.5714	30.3571	0.5080	0.6669
	Primigravida	29	31.2020	3.6141	24.4286	36.2857	30.1429		
Hemoglobin (g/dL)	Multigravida	22	11.0227	1.2675	8.4000	13.1000	11.3000	0.4022	0.8450
	Primigravida	29	10.7379	10.7379	7.9000	12.6000	11.1000		
TLC ($x10^3/mm^3$)	Multigravida	22	9.1950	2.6751	4.3000	15.2500	9.3650	0.2753	1.1032
	Primigravida	29	10.0741	2.9214	4.8000	18.5000	10.4000		
Platelet Count ($x10^3/mm^3$)	Multigravida	22	176.4091	48.7107	102.0000	288.0000	183.0000	0.8945	0.1333
	Primigravida	29	178.6207	65.1353	47.0000	300.0000	169.0000		
TSH (µIU/mL)	Multigravida	22	1.8727	1.3660	0.4000	7.1000	1.6300	0.9009	0.1251
	Primigravida	29	1.8321	.9547	0.5300	4.4000	1.7000		

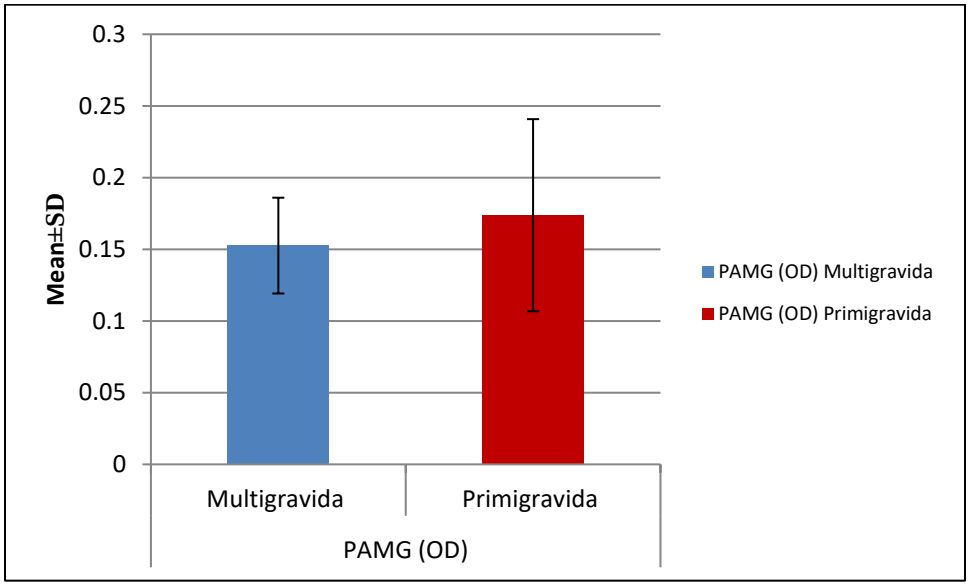

Figure: 1 Distribution of mean PAMG (OD): Parity

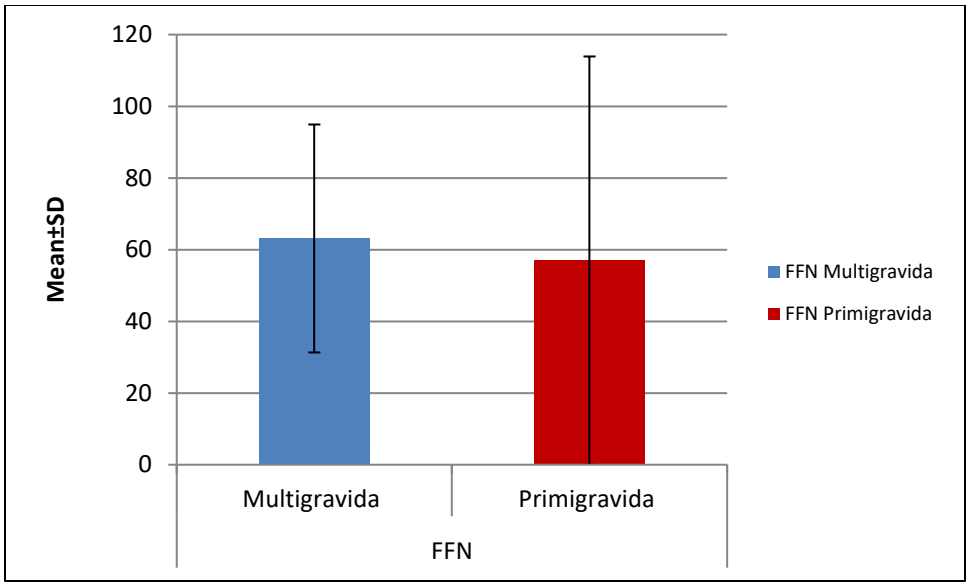

Figure: 2 Distribution of mean FFN: Parity

In Multigravida, 9(40.9%) patients had Backache, 10 (45.5%) patients had Pain Abdomen and 3 (13.6%) patients had Pain Abdomen + Backache. In Primigravida, 10 (34.5%) patients had Backache, 15(51.7%) patients had Pain Abdomen and 4(13.8%) patients had Pain Abdomen + Backache.

Association of Chief Complain with Group was not statistically significant (p=0.8873).In Multigravida, 4(18.2%) patients had Modrate Anemia .In Primigravida, 5(17.2%) patients had Modrate Anemia. Association of Medical History with Group was not statistically significant (p=0.7995).In Multigravida, 17 (77.3%) patients had H/O Previous Term Birth. Association of H/O Previous Term Birth with Parity was statistically significant (p<0.0001). In Multigravida, 4(18.2%) patients had H/O Previous Preterm Birth. Association of H/O Previous Preterm Birth with Parity was statistically significant (p=0.0167).In Multigravida, 8(36.4%) patients had H/O Abortion. Association of H/O Abortion with Parity was statistically significant (p=0.0004).In Multigravida, 3(13.6%) patients had Delivery in 7 Days. In Primigravida, 2(6.9%) patients had Delivery in 7 Days. Association of Delivery in 7 Days with Parity was not statistically significant (p=0.4227).In Multigravida, 1(4.5%) patients had Delivery in 7-14 Days. In Primigravida, 2(6.9%) patients had Delivery in 7-14 Days. Association of Delivery in 7-14 Days with Parity was not statistically significant (p=0.7237).In Multigravida, 18(81.8%) patients had Delivery in >14 Days. In Primigravida, 25(86.2%) patients had Delivery in >14 Days. Association of Delivery in >14 Days with Parity was not statistically significant (p=0.6695).In Multigravida, the mean Age (years) (Mean± SD) of patients was 28.1364 ± 5.5144.In Multigravida, the mean Gestational Age (Weeks) (Mean± SD) of patients was 30.5519 ± 3.2115.In Primigravida, the mean Gestational Age (Weeks) (Mean± SD) of patients was 31.2020 ± 3.6141.Distribution of mean Gestational Age (Weeks) with Parity was statistically significant (p=0.5080).In Multigravida, the mean Hemoglobin (g/dL) (Mean± SD) of patients was 11.0227 ± 1.2675.In Primigravida, the mean Hemoglobin (g/dL) (Mean± SD) of patients was 10.7379 ± 10.7379.Distribution of mean Hemoglobin (g/dL) with Parity was statistically significant (p=0.4022).In Multigravida, the mean TLC ($x10^3/mm^3$) (Mean± SD) of patients was 9.1950 ± 2.6751.In Primigravida, the mean TLC ($x10^3/mm^3$) (Mean± SD) of patients was 10.0741 ± 2.9214.Distribution of mean TLC ($x10^3/mm^3$) with Parity was statistically significant (p=0.2753).In Multigravida, the mean Platelet Count ($x10^3/mm^3$)(Mean± SD) of patients was 176.4091 ± 48.7107.In Primigravida, the mean Platelet Count ($x10^3/mm^3$)(Mean± SD) of patients was 178.6207 ±65.1353.Distribution of mean Platelet Count ($x10^3/mm^3$) with Parity was statistically significant (p=0.8945).In Multigravida, the mean TSH (µIU/mL) (Mean± SD) of patients was 1.8727 ± 1.3660.In Primigravida, the mean TSH (µIU/mL) (Mean± SD) of patients was 1.8321 ± .9547.Distribution of mean TSH (µIU/mL) with Parity was statistically significant (p=0.9009).

DISCUSSION

It was found that, more number of patients had Pain Abdomen [15(51.7%)] in Primigravida Group compared to Multigravida Group [10 (45.5%)] but this was not statistically significant (p=0.8873).

It was found that, higher number of patients had Moderate Anemia [5(17.2%)] in Primigravida Group compared to Multigravida Group [4(18.2%)] but this was not statistically significant (p=0.7995).

Savaliya K et al 9(2021) showed that Of the patients studied, 18.57% had PPH, 15.71% had pre-eclampsia, 8.57% had IUD, and 37.14% newborns were LBW.Multiparity itself is a major risk factor of anemia. Anemia presenting in the third trimester of pregnancy is a proxy indicator of care received by gravid women in the early antenatal period. In combination, a multigravida in the third trimester with less time to restock iron and vitamin stores may result in considerable maternal as well as perinatal mortality and morbidity.

It was found that, out of 51 patients [17 (77.3%)] patients had H/O Previous Term Birth but this was statistically significant (p<0.0001).

Savaliya K et al 9(2021) showed that Pre-eclampsia, placenta praevia, postpartum haemorrhage (PPH), congestive cardiac failure (CHF), neonatal intensive care unit (NICU) admission, preterm birth (PTB), low birth weight (LBW), intrauterine death (IUD), low Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score, and birth asphyxia records were investigated. Of the patients studied, 18.57% had PPH, 15.71% had pre-eclampsia, 8.57% had IUD, and 37.14% newborns were LBW.

It was found that, out of 51patients [4(18.2%)] had H/O Previous Preterm Birth was statistically significant (p=0.0167).

It was found that, out of 51 patients only [8(36.4%)] patients had H/O Abortion in was statistically significant (p=0.0004).

It was found that, higher number of patients had Delivery in 7 Days [3(13.6%)] in Multigravida Group compared to Primigravida Group [2(6.9%)] but this was not statistically significant (p=0.4227).

Wing et al 10 (2017) The coprimary endpoints were positive predictive value (PPV) superiority and negative predictive value (NPV) noninferiority of placental a microglobulin-1 compared with fetal fibronectin for the prediction of spontaneous preterm birth within 7 days and within 14 days. Of 796 women included in the study cohort, 711 (89.3%) had both placental a microglobulin-1 and fetal fibronectin results and valid delivery outcomes available for analysis.

It was found that, majority number of patients had Delivery in 7-14 Days [2(6.9%)] in Primigravida Group compared to Multigravida Group [1(4.5%)`] but this was not statistically significant (p=0.7237).

It was found that, more number of patients had Delivery in >14 Days [25(86.2%)] in Primigravida Group compared to Multigravida Group [18(81.8%)] but this was not statistically significant (p=0.6695).

In our study, Age (years) was higher in Multigravida Group [28.1364 ± 5.5144] compared to Primigravida Group [25.6207 ± 4.1612] but this was statistically significant (p=0.0691).

We found that, Gestational Age (Weeks) was higher in Primigravida Group [31.2020 ± 3.6141] compared to Multigravida Group [30.5519 ± 3.2115] but this was statistically significant (p=0.5080).

We examined that, Hemoglobin (g/dL) was higher in Multigravida Group [11.0227 ± 1.2675] compared to Primigravida Group [10.7379 ± 10.7379] but this was statistically significant (p=0.4022).

In our study, TLC ($x10^3/mm^3$) was higher in Primigravida Group [10.0741 ± 2.9214] compared to Multigravida Group [9.1950 ± 2.6751] but this was statistically significant (p=0.2753). was statistically significant (p=0.2753).

We found that, Platelet Count ($x10^3/mm^3$) was higher in Primigravida Group [178.6207 ±65.1353] compared to Multigravida Group [176.4091±48.7107] but this was statistically significant (p=0.8945).

In our study, TSH (µIU/mL) was higher in Multigravida Group [1.8727 ± 1.3660] compared to Primigravida Group [1.8321 ± .9547] but this was statistically significant (p=0.9009).

We found that, PAMG (OD) was higher in Primigravida Group [.1738±.0670] compared to Multigravida Group [.1526 ± .0334] but this was statistically significant (p=0.1810).

Sosa CG et al 11(**2014)** The PAMG-1 test had a sensitivity of 100.0% [confidence interval (CI) 0.87–1.0], specificity of 99.1% [(CI) 0.95–0.99], positive predictive value of 96.3% [(CI) 0.82–0.99], negative predictive value of 100.0% [(CI) 0.97–1.0], and ± likelihood ratios of 74.6 [(CI) 20.31–274.51] and 0.0 [(CI) 0.00–0.98].

CONCLUSION

We conclude that demonstrates a significant association between Placental Alpha 1 Microglobulin (PAMG-1), Fetal Fibronectin (fFN), and Thyroid-Stimulating Hormone (TSH) levels in pregnant women during the second and third trimesters, and their relationship with adverse pregnancy outcomes. Elevated PAMG-1 and fFN levels were linked to an increased risk of preterm labor, while abnormal TSH levels were associated with thyroid dysfunction, which can affect both maternal and fetal health. These findings highlight the potential of using these

biomarkers in routine prenatal screening to identify high-risk pregnancies and provide timely interventions to improve pregnancy outcomes.

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