Clin. Lab. 2018;64:1501-1508

ORIGINAL ARTICLE

First-Trimester Biochemical Screening For Low Birth Weight: Clinical Effectiveness of Low Pregnancy-Associated Plasma Protein-A and High Thyroid-Stimulating Hormone

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SUMMARY

Background: **Low birth weight (LBW) can be an important adverse neonatal outcome in terms of morbidity and mortality. The aim of this study is to investigate the screening effectiveness of first-trimester low pregnancy-associated plasma protein A (PAPP-A) and high serum thyroid-stimulating hormone (TSH) and the combination of both markers for predicting LBW.**

Methods: **We performed a retrospective cohort study of women undergoing first-trimester assessment in our center. We considered low PAPP-A as < 5th percentile for gestational age. High serum TSH was defined as > 2.5 mU/L, according to the American Thyroid Association (ATA) recommendation. Receiver-operating characteristic (ROC) curves were plotted to evaluate screening performance. Multivariate logistic regression was accomplished to calculate adjusted risks to identify the association between both parameters with LBW.**

Results: **Overall, 4,396 women met the inclusion criteria. Of these, 277 (6.3%) delivered a LBW baby. The use of either low PAPP-A or high TSH yielded the highest sensitivity (21.1%) with a specificity of 85.7%. Combining both markers showed an increased association (adjusted OR 9.07 [95% CI 3.34 - 24.6]) at the expense of a significant reduction in sensitivity (7.8%).**

Conclusions: **First-trimester low PAPP-A is associated with LBW at delivery. Neither of these biomarkers or their combination are acceptable predictors to be clinically useful tools for LBW.**

(Clin. Lab. 2018;64:1501-1508. DOI: 10.7754/Clin.Lab.2018.180336)

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Manuscript accepted April 18, 2018

KEY WORDS

first trimester, low birth weight, pregnancy-associated plasma protein-A, screening, thyroid-stimulating hormone

LIST OF ABBREVIATIONS

LBW - Low birth weight
PAPP-A - Pregnancy-associated plasma protein A
TSH - Thyroid-stimulating hormone
ATA - American Thyroid Association
ROC - Receiver-operating characteristic
FGR - Fetal growth retardation
PE - Preeclampsia
GDM - Gestational diabetes mellitus
PTD - Preterm delivery
CTG - cardiotocography

CS - C-section
MoM - Multiples of the median
CI - Confidence interval
LR+ - Positive likelihood ratios
LR- - Negative likelihood ratios
ART - Assisted reproductive technology
PLGF - placental growth factor
8-OHdG - Dihydro-2-deoxyguanosine
IGF - Insulin-like growth factor
SCH - Subclinical hypothyroidism

INTRODUCTION

Low birth weight (LBW) is an important public health burden, as it is a significant determinant of perinatal morbidity and mortality. Countries could reduce their neonatal and infant mortality by improving maternal care during pregnancy through interventions that identify the population at high risk of developing fetal growth disorders. New strategies have been sought to prevent LBW; however, the effectiveness of these depends on early detection.

Biochemical markers are widely used in prenatal screening at first trimester for fetal chromosomal abnormalities as part of combined tests. Abnormal analyte levels such as reduced pregnancy-associated placental protein-A (PAPP-A) have been associated with poor perinatal outcomes such as preeclampsia, preterm birth, and fetal growth retardation (FGR) [1-3]. PAPP-A is highly expressed in the syncytiotrophoblasts of the placenta and acts as a protease on insulin-like growth factor binding proteins, theoretically increasing the stimulatory effects of placental insulin-like growth factors [4]. As reported by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada, low PAPP-A level (< 0.4 multiples of median [MoM]) in the first trimester is associated with an increased frequency of bad perinatal outcomes [5].

Normal maternal thyroid function is considered critical for fetal growth and neurological development. Several studies indicate a possible effect of thyroid dysfunction or antithyroid antibodies (ATA) on increased risks for pregnancy complications such as LBW. However, the results vary between studies, and drawing conclusions remains controversial, especially with respect to subclinical thyroid dysfunction with euthyroid status. It is widely known that overt hypothyroidism (OH) and overt hyperthyroidism increase the risk for deleterious pregnancy results [6-8]. Research into a possible association between maternal TSH in the first trimester and LBW is insufficient and has led to ambiguous data [9]. A large-cohort study found no correlation between high maternal TSH at early stages of gestation and fetal growth disorders [10].

The thyroid function screening test is used in the first trimester by measuring thyroid-stimulating hormone (TSH), which is controlled through negative feedback by thyroid hormones. There is a negative relationship between the serum-free T4 and TSH concentrations. This means that minor changes in serum free T4 concentration induce large reciprocal changes in serum TSH concentration [11]. As a result, thyroid function is properly assessed by measuring serum TSH in pregnant women.

Current studies suggest that low PAPP-A and high TSH at early stages of gestation are associated with growth abnormalities later in pregnancy. The main purpose of this research is to evaluate the screening effectiveness of first-trimester low PAPP-A, high TSH, and both in combination for predicting LBW [12,13].

MATERIALS AND METHODS

We conducted a retrospective cohort study of all patients who had a first-trimester visit in our center for aneuploidy screening between 8 and 14 weeks of gestation over a 3-year period (January 2013 to January 2016). Patients with valid dated pregnancy consistent with first-trimester ultrasound were included. Multiple gestations, pregnancies affected by major structural anomalies or aneuploidy, and miscarriages or fetal deaths before 22 weeks of gestation were excluded from the analysis. The present study was approved by the Ethical Committee of Hospital General Universitario Gregorio Marañon de Madrid (Comité Ético de Investigacion Clínica, reference number OBS05042016).

Data on maternal demographics and medical and obstetric history were collected at the time of the first-trimester ultrasound examination. All information provided was reviewed with the women by a doctor or midwife and recorded in the patients' files. The outcome measures were acquired from our labor ward data base and included preeclampsia (PE), perinatal/antenatal mortality, low birth weight (LBW), maternal mortality, gestational diabetes mellitus (GDM), preterm delivery (PTD) before 34 and 37 gestational weeks, abnormal cardiotocography (CTG) during delivery, C-section (CS) due to abnormal CTG, pH at birth, Apgar score at 5 minutes, and type of resuscitation. For women who gave birth outside the center, pregnancy results were achieved by contacting the patient and the referring midwife. Maternal serum PAPP-A and TSH were obtained at the time of first-trimester blood test. We converted PAPP-A levels into multiples of the median (MoM) for comparison between pregnant patients. MoMs were given back from the hospital laboratory that adjusted for fetal crown-rump length, maternal pregestational diabetes, smoking habit, ethnicity, and maternal weight. TSH was measured in serum using a solid-phase, two-site chemiluminescent enzyme immunometric assay (IMMULITE Third generation TSH). All measurements were performed in one laboratory.

LBW neonates were defined as those with a birth weight below the 5th percentile for gestational age according to our center's newborn weight charts as the primary outcome. Low PAPP-A was considered as

MoM < 5th percentile, and TSH was defined as high when its serum value was > 2.5 mU/L, according to the American Thyroid Association recommendation [14]. We calculated crude and adjusted risk estimates with a 95% confidence interval (CI) to recognize the associations between first-trimester high TSH and low PAPP-A with LBW. Adjusted risk estimation was acquired by controlling for confounders using multivariable logistic regression. The sum of variables in every regression model was later reduced by backwards elimination. The screening performance of high TSH and low PAPP-A for prediction of LBW was assessed by the calculation of sensitivities, specificities, positive and negative predictive values, positive likelihood ratios (LR+), and negative likelihood ratios (LR-). We explored the screening effectiveness of TSH > 2.5 mU/L alone, PAPP-A < 5th percentile alone, either TSH > 2.5 mU/L or PAPP-A < 5th percentile, and both TSH > 2.5 mU/L and PAPP-A < 5th percentile. This methodology was based on a previous study by Carbone JF et al. [15]. All statistical analyses were done with STATA version 15.0 (Stata Corp, College Station, TX, USA). Significant results were considered with p-values < 0.05.

RESULTS

Throughout the 3-year period, 4,396 patients who received first-trimester assessment in our center met the inclusion criteria and were added to the study. In this population, 277 (6.3%) delivered a LBW baby. Gestational and maternal characteristics of the LBW and non-LBW cases in our sample are shown in Table 1. The mean gestational age at birth for the LBW cases was 37.15 ± 2.95 weeks. Their mean birth weight was 2,256 ± 544.3 g. The majority of these women were multiparous (75.8%) and Caucasian (98.9%). The mean gestational age at delivery in our population, including cases and controls, was 38.8 ± 1.9 weeks; the birth weight mean was 3,186.2 ± 511 g; 359 (8.1%) were a product of assisted reproductive technology (ART), and of those, 7% (25/359) were LBW.

In our sample, 213 (4.9%) women had PAPP-A values below the 5th percentile (< 0.42 MoM), and 504 (11.4%) patients presented TSH serum values above 2.5 mU/L on the first-trimester blood test, which was performed at a mean gestational age of 10.2 ± 1.6 weeks.

Figure 1 shows ROC curves for LBW prediction with low PAPP-A and high TSH. The AUC appeared to be slightly higher for low PAPA-A than that for high TSH (0.54 vs. 0.52). Table 2 demonstrates risk odds for LBW of the various combinations of low PAPP-A and high TSH. There were just 22 cases with both low PAPP-A and high TSH and 6 (27.2%) resulted in LBW. We adjusted for smoking habit, method of conception, maternal weight at first trimester, and parity. Combined low PAPP-A and high TSH was linked to the highest risk of LBW, with an adjusted odds ratio (AOR) of 9.07 [95% CI 3.34 - 24.6] versus AORs of 2.67 [95% CI 1.64 - 4.36] and 1.23 [95% CI 0.81 - 1.88] when used alone. There were 698 pregnant patients with either low PAPP-A or high TSH. Of these patients, 53 (6.7%) delivered LBW babies, and the association remained with an AOR of 1.79 [95% CI 1.27 - 2.52].

The screening effectiveness and LRs for low PAPP-A and high TSH when used separately or in combination are shown in Table 3. Using low PAPP-A or high TSH had the highest sensitivity (21.1%) and 85.7% of specificity when screening for LBW. The combination of low PAPP-A and high TSH provided the lowest sensitivity (7.8%) with a specificity of 98.8%.

DISCUSSION

To our knowledge, this is the first study to evaluate the comparative screening effectiveness of first-trimester low PAPP-A and high TSH together for predicting LBW. This research presented sensitivity, specificity, predictive values, and likelihood ratios of both markers separately and in combination.

This study shows that reduced PAPP-A levels at the first trimester of gestation are associated with the delivery of a LBW infant, whilst increased serum TSH levels are not significantly associated with this adverse outcome. The association analysis for LBW using low PAPP-A or high TSH yields a lower odds ratio than that of reduced PAPP-A alone. We adjusted for confounders and demonstrated a nine-fold increased risk of LBW neonate when both low PAPP-A and high TSH were present versus reduced PAPP-A alone, even though sensitivity appeared to be decreased.

If either low PAPP-A or increased TSH was taken as positive screening, the association stayed with a mild increase in sensitivity. Combining both markers at early stages of pregnancy results in moderate improvement in screening effectiveness for the chance of LBW compared to reduced PAPP-A used alone.

We acknowledge an association between low PAPP-A in the first trimester with delivery of a LBW neonate; however, this parameter alone is a weak predictor of LBW. The combination of both markers did not improve screening effectiveness, but it may identify some at-risk pregnancies that need earlier follow-up or intervention to reduce complications derived from growth disorders.

Biochemical screening at the first trimester using various markers for predicting FGR and LBW infants has been proposed in recent years. However, none of these markers are accurate enough for their use to be recommended [16]. Several studies reported data on placental growth factor (PLGF), soluble FMS-like tyrosine kinase-1, soluble endoglin, vascular endothelial growth factor or angiopoietin-2 as angiogenesis-related biomarkers [17-20]. Overall, the predictive accuracy of these biochemical factors was minimal.

Other studies evaluated endothelial function/oxidative

Table 1. Gestational and maternal characteristics of the study sample.

	LBW	Normal birth weight	p-value
Maternal age (years) mean ± SD	32.06 ± 4.1	32 ± 4.3	0.99
Race, n (%)			0.07
White	289 (98.97%)	4,470 (97.2%)	
Black	3 (1.02%)	58 (1.24%)	
Asian	0	80 (1.7%)	
Smoking habit, n (%)	61 (28.1%)	518 (11.63%)	< 0.001
Pregestational diabetes, n (%)	5 (1.8%)	94 (2.1%)	0.72
Rheumatological disease, n (%)	13 (5.22%)	151 (3.61%)	0.19
IVF, n (%)	32 (12.2%)	294 (6.64%)	< 0.001
Maternal weight (kg) mean ± SD	60.3 ± 11.9	64.11 ± 12.34	< 0.001
CRL (mm) mean ± SD	61.15 ± 7.71	61.02 ± 7.69	0.79
PAPP-A (MoM) mean ± SD	1.03 ± 0.76	1.21 ± 0.72	0.005
BHCG (MoM) mean ± SD	1.17 ± 0.95	1.14 ± 0.82	0.53
TSH (mU/L) mean ± SD	2.31 ± 1.77	1.94 ± 1.64	0.027
Parity, n (%)			< 0.05
0	67 (24.2%)	1,464 (35.5%)	
1	156 (56.3%)	1,362 (33%)	
≥ 2	54 (19.5%)	1,291 (31.3%)	
BW (grams), mean ± SD	2,256 ± 544.3	3,247.6 ± 450.3	< 0.001
Ph, mean ± SD	7.27 ± 0.07	7.28 ± 0.06	0.23
Perinatal/antenatal death, n (%)	9 (3.1%)	8 (0.17%)	< 0.001
Abnormal CTG, n (%)	37 (12.7%)	309 (6.6%)	< 0.001
Apgar 5 min ≤ 7, n (%)	17 (5.84%)	46 (1%)	< 0.001
Resuscitation ≥ type 3, n (%)	40 (13.7%)	284 (6.12%)	< 0.001
CS due to fetal distress, n (%)	39 (13.3%)	243 (5.2%)	< 0.001
PTD < 37 weeks, n (%)	11 (3.78%)	122 (2.62%)	0.23
Gestational diabetes, n (%)	13 (4.4%)	256 (5.82%)	0.45
Preeclampsia, n (%)	26 (8.93%)	45 (0.96%)	< 0.001

Table 2. Association estimates between first trimester PAPP-A < 5th percentile, TSH > 2.5 mU/L and LBW.

	LBW n (%)	OR	Adjusted OR †
PAPP-A < 5th percentile (n = 213)	21 (9.8%)	2.27 (1.34 - 3.69)	2.67 (1.64 - 4.36)
TSH > 2.5 mU/L (n = 504)	32 (6.3%)	1.1 (0.73 - 1.67)	1.23 (0.81 - 1.88)
Either PAPP-A < 5th percentile or TSH > 2.5 mU/L (n = 698)	47 (6.7%)	1.45 (1.04 - 2.01)	1.79 (1.27 - 2.52)
Both PAPP-A < 5th percentile and TSH > 2.5 mU/L (n = 21)	6 (28.5%)	7.24 (2.72 - 19.24)	9.07 (3.34 - 24.65)

† Adjusted for smoking habit, parity, method of conception, and maternal weight.

Table 3. Screening effectiveness for prediction of LBW.

Prediction criteria	Sensitivity (%)	Specificity (%)	AUC	PPV	NPV	LR+	LR-
PAPP-A < 5th percentile	8.5 (5 - 11.9)	96.1 (95.4 - 96.7)	0.6	12.7 (7.6 - 17.8)	94 (93.2 - 94.8)	2.25 (1.06 - 3.75)	0.95 (1.0 - 1.1)
TSH > 2.5 mU/L	13.06 (8.6 - 17.4)	90.22 (89.37 - 91.06)	0.54	6.32 (4.1 - 8.54)	95.35 (94.7 - 95.9)	1.33 (0.81 - 1.95)	1.037 (0.97 - 1.10)
Either PAPP-A < 5th percentile or TSH > 2.5 mU/L	21.11 (15.8 - 26.36)	85.74 (84.75 - 86.73)	0.665	7.05 (5.1 - 8.95)	74.17 (72.1 - 76.1)	1.48 (1.04 - 1.98)	1.08 (1.0 - 1.17)
Both PAPP-A < 5th percentile and TSH > 2.5 mU/L	7.8 (1.15 - 14.4)	98.8 (98.0 - 99.4)	0.49	31.5 (8.04 - 55.1)	93.7 (92.2 - 95.1)	6.41 (0.6 - 28.2)	1.07 (0.99 - 1.16)

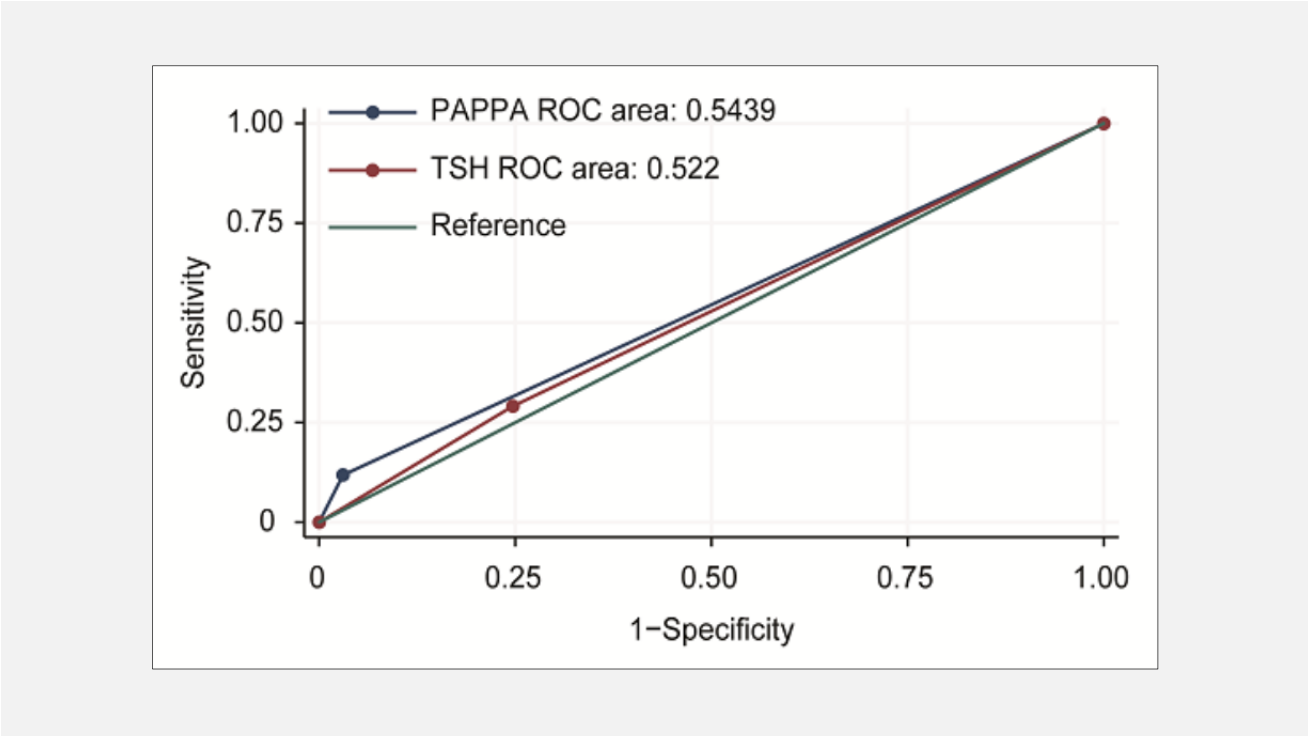

Figure 1. ROC curves of low PAPP-A and high TSH for predicting LBW.

stress-related biomarkers in early and late pregnancies for prediction of fetal growth disorders, such as homocysteine, dihydro-2-deoxyguanosine (8-OHdG), leptin, endothelial cell adhesion molecules or C-reactive protein [21-25]. Overall, the predictive accuracy of endothelial function/oxidative stress-related markers for FGR was minimal (median positive and negative LRs of 2.0, range 0.8 - 19.2, and 0.8, range 0.0 - 1.1, respectively).

The association between reduced PAPP-A at the first trimester and LBW has been properly proven in previous studies [26,27]. These studies declared similar associations and screening results as were found in our analysis. Although the ORs appear to be reasonable in most of the literature, sensitivities continue to be unsatisfactory for this method´s use as a screening test. It remains unknown whether women with low PAPP-A at early stages of gestation should be offered additional surveillance, because there is not much evidence that such a practice improves the outcome of the pregnancy [28,29].

PAPP-A lyses insulin-like growth factor-binding pro-

tein 4, a signalling protein that inhibits the action of receptor-bound insulin-like growth factor (IGF). Theoretically, proteolysis of IGF binding proteins elevates the availability of free IGF, which is responsible for placental growth and transfer of nutrients to the baby. Reduced levels of PAPP-A could cause low amounts of available IGF, potentially leading to impaired placental development, reduced fetal growth, and adverse perinatal outcomes [30,31].

Thyroid function may influence fetal development in two possible ways as follows: thyroid hormones regulate both proliferation and function of trophoblastic cells, and they are also important for the development of the infant. Thyroid hormones boost growth in the fetus by anabolic actions and their influence on many growth factors (i.e., insulin-like growth factors). So maternal thyroid dysfunction can cause LBW via both placental and maternal mechanisms [32].

A recent meta-analysis reported relevant data on the association between isolated hypothyroxinemia and LBW, showing an OR of 1.05 (95% CI 0.37 - 2.92), indicating that isolated hypothyroxinemia was not associated with growth disorders, but the combined OR for LBW of subclinical hypothyroidism (SCH) in pregnant women was 1.54 (95% CI, 1.06 - 2.25), indicating that SCH was associated with growth retardation [33].

Previous studies about the possible relationships between LBW infants and FT4 and TSH levels in euthyroid patients are scant showing quite different results [34]. A possible interpretation for these indeterminate results is the divergent moment in pregnancy of assessment of maternal thyroid function, which is relevant when relating it to LBW. Furthermore, separate definitions for LBW (weight percentiles must be corrected for gender, parity, and term of gestation) and different cutoffs have been defined (< 10th percentile or < 3rd percentile). Lastly, a possible correlation between thyroid function in the mother and LBW should be adjusted for other important variables, such as pre-eclampsia or smoking habits.

Several strengths were seen in this research. To our knowledge, this is the first study that combined both biochemical markers to contrast the screening effectiveness and provide accurate likelihood ratios. The sample size was large enough to draw adequate conclusions, and the effects of certain variables, such as smoking habits, parity, method of conception, and maternal weight, on fetal growth were adjusted for in our study. However, our study also has limitations. First, although we customized growth charts for our center, our findings could not be generalizable to other centers with a lower or higher prevalence of fetal growth disorders. We did not adjust percentiles specifically for newborns' gender. We might have underestimated LBW in male babies and overestimated it in female babies.

We used specific cutoffs for PAPP-A and TSH at the first trimester according to our literature review but did not try to assess others that may present better results as a screening test. Moreover, we found significant differences in TSH means when comparing cases to controls but did not manage to see a significant association with LBW when the TSH cutoff was established at 2.5 mU/L. A study by Alvarez et al. [35] demonstrated that reduced iodine in maternal urine was linked to a higher chance of LBW. However, the iodine intake in our city is accepted to be enough, so we did not check it in our cohort.

CONCLUSION

In conclusion, we observed that first-trimester PAPP-A < 5th percentile is associated with LBW, but it is not a sufficiently powerful predictor of this adverse outcome to be clinically useful as a screening test. The combination of PAPP-A < 5th percentile and TSH > 2.5 mU/L resulted in a very mild improved screening effectiveness for LBW compared with PAPP-A alone. More research should take place by combining these or other biochemical and biophysical markers with other predictors of growth abnormalities to check the possible amelioration in screening effectiveness for LBW at birth to adequate values. Our research suggests that pregnancy complications, such as LBW, may be due to irregularities that occur early in the pregnancy. Further studies are required to find potential strategies to improve outcomes in these high-risk populations.

Ethics Approval and Consent to Participate:
The study was approved by the Ethical Committee of Hospital General Universitario Gregorio Marañon de Madrid (Comité Ético de Investigacion Clínica, reference number OBS05042016). No consent to participate was required by this institution.

Consent for Publication:
Approved for publication by the Ethical Committee of Hospital General Universitario Gregorio Marañon de Madrid (Comité Ético de Investigacion Clínica, reference number OBS05042016).

Availability of Data and Material:
All data supporting the findings of this study are contained within the manuscript. Any additional information regarding the study will be shared upon request by the corresponding author.

Authors' Contributions:
SGTL designed and carried out the study, performed the statistical analysis, the literature review, and drafted the manuscript. SCS and GNP revised the final manuscript. All authors read and approved the final manuscript.

Acknowledgment:
We thank all the members in our department for their efforts in collecting some of the data, and especially Dr. Pilar Pintado Recarte for being an outstanding teacher in Obstetrics.

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Declaration of Interest:
The authors declare that there are no conflicts of interest.

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