

Evaluating Placental Thickness and Thickness of Uterine Muscle at Placenta Attachment in Prediction of Postpartum Blood Loss

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Received: 2 May 2025

Accepted: 22 June 2025

Abstract:

Objectives: To determine the significance of the placental thickness, the thickness of the uterine muscle layer at the placental attachment site and the placental myometrial ratio in the prediction of postpartum hemorrhage and to evaluate them as parameters for identifying high-risk patients. **Methods:** The present prospective observational cohort study involved 150 female patients with full-term pregnancy and single viable fetus. All patients were subjected to postpartum blood loss estimation and correlation between estimated blood loss (EBL) with placental thickness and myometrial ratio were done. **Results:** Placental thickness, placental-myometrial ratio (PMR) and time of delivery were significantly higher in high-EBL group compared to normal-EBL group. Myometrial thickness was significantly lower in high EBL group than normal EBL one. Multivariate analysis revealed that predictive significance of postpartum blood loss, PMR were the best independent predictors of postpartum blood loss ($P < 0.05$). The ROC curve analysis was conducted for placental thickness, myometrial thickness, and the PMR, revealing optimal cutoff values for predicting postpartum blood loss as >5.47 mm, <1.1 mm, and >5.66 mm respectively. These cutoffs corresponded to sensitivities of 75%, 81.25%, and 81.25%, and specificities of 67.16%, 93.28%, and 96.27%. Increased placental thickness and thinning of myometrium are significant predictors of PPH. **Conclusion:** This study indicates that lower myometrial thickness, larger

Placental thickness, increased PMR are significantly linked with prediction of PPH.

Keywords: placental thickness, Uterine Muscle Thickness, Postpartum Blood Loss, PPH, ultrasound

Introduction

Postpartum hemorrhage (PPH)—clinically recognized as blood loss exceeding 500 mL following a vaginal birth or over 1000 mL after a cesarean delivery (ACOG Practice Bulletin No. 183, 2017)—remains a formidable challenge in modern obstetrics, standing among the foremost causes of maternal mortality worldwide ⁽¹⁾.

PPH has an estimated incidence of 5% to 10% and a maternal mortality rate ranging from 0.5% to 1% ⁽²⁾. In addition to its acute life-threatening implications, PPH is associated with significant long-term sequelae. Approximately 12% of women who experience severe PPH develop postpartum anemia, which can impair physical recovery, limit functional capacity, and negatively affect maternal-infant bonding ⁽³⁾.

The etiology of PPH is typically categorized using the "Four T's" framework: tone (uterine atony), trauma (genital tract lacerations or uterine rupture), tissue (retained placental fragments or clots), and thrombin (coagulopathies). Uterine atony remains the most prevalent cause, accounting for approximately 70% of cases. This is followed by traumatic lesions (approximately 20%), retained placental tissue (approximately 10%), and coagulation disorders ^(4,5).

In light of the clinical burden imposed by PPH, there is a compelling need for reliable, non-invasive predictors to identify at-risk patients prior to delivery. This study investigates the relationship between the sonographically measured thickness of the placenta and myometrium at the site of placental attachment—referred to as the placental-myometrial ratio (PMR)—and the volume of blood loss in the immediate postpartum period.

The central hypothesis is that variations in PMR may serve as a surrogate marker for PPH risk. Establishing such a correlation could enable clinicians to stratify risk antenatally, allowing for the

implementation of individualized prophylactic strategies aimed at mitigating maternal blood loss. Ultimately, early identification and targeted intervention may contribute to a reduction in the incidence and severity of PPH and its associated complications.

Patients and Method

Study design and population

This prospective, observational, randomized cohort study was conducted on a total of 150 pregnant women attending the Department of Obstetrics and Gynecology, Faculty of Medicine, Benha University from December 2023 and end by January 2025. Ethical approval was obtained from the Institutional Review Board (IRB) of the Faculty of Medicine, Benha University (Approval No. Ms 9-12-2023). Informed written consent was obtained from all participants prior to their enrolment in the study.

The Inclusion criteria for the study are term pregnancy (37 weeks gestation or more), and a single viable fetus.

The exclusion criteria include severe hematological disorders that could result in abnormal coagulation, placental implantation abnormalities or placental abruption, and uterine anomalies—which encompass both congenital and acquired structural abnormalities—. Additionally, women with a previous history of postpartum hemorrhage (PPH), category 1 or category 2 cesarean sections (CS), multiple pregnancies, preterm labor, and intrauterine fetal death (IUFD) are excluded from the study.

Based on the predefined inclusion and exclusion criteria, all enrolled participants underwent a standardized clinical and diagnostic protocol.

Clinical evaluation:

All patients were subjected to comprehensive history-taking focused on clinically relevant obstetric, medical, and surgical information. General physical examination included the assessment of vital signs (pulse rate, blood pressure, and temperature), as well as anthropometric

measurements (weight and height), and evaluation for clinical pallor.

Obstetric examination:

A full obstetric examination was performed, which involved fundal height measurement and abdominal palpation using Leopold's maneuvers (fundal, umbilical, and first pelvic grip). For patients admitted in active labor, a vaginal examination was conducted to assess cervical dilatation, effacement, and fetal station.

Laboratory investigations:

All participants underwent a routine laboratory workup that included: complete blood count (CBC), liver and renal function tests, coagulation profile, screening for viral hepatitis markers, and blood grouping with Rh factor determination.

Antenatal Ultrasound Assessment:

Antenatal ultrasound was performed transabdominally within 24 hours before delivery under the supervision of qualified and experienced sonographers to ensure diagnostic accuracy. The primary equipment used was the GE Voluson P8 ultrasound system with a 3.5–5 MHz convex transabdominal probe. The patient was positioned in the supine position with left lateral tilt, and a standard obstetric scan was conducted, which included fetal biometry measurements: biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). In addition to routine parameters, a targeted ultrasound assessment was performed to evaluate placental characteristics including location, size, and thickness, along with measurement of myometrial thickness at the site of placental attachment.

Delivery and Blood Loss Estimation:

Delivery was conducted either vaginally or by cesarean section (CS), performed by skilled obstetricians trained in the management of PPH. The estimation of postpartum blood loss was performed using standardized methods:

- **For vaginal deliveries**, a calibrated blood collection basin was placed beneath the maternal pelvis immediately following placental delivery. The volume of blood collected in the basin was recorded, and additional blood loss was estimated from the weight of used nursing pads at 6 and 24 hours postpartum ± 2 hours.
- **For cesarean deliveries**, amniotic fluid was aspirated immediately after fetal delivery. Blood was collected via suction into a calibrated negative-pressure aspirator. Blood loss was further estimated based on the weight difference in surgical gauze and nursing pads used intraoperatively and during the first 24 hours postpartum.

Pre- and post-delivery hemoglobin and hematocrit levels were measured using venous blood samples collected 24 hours prior to delivery and within 24 hours after delivery to quantify blood loss physiologically.

Postoperative Management and Outcome Measures:

During the hospital stay, all patients received standardized preoperative and postoperative care. In cases where PPH occurred, prompt intervention was undertaken by senior obstetric staff in accordance with the institution's clinical protocol. The primary outcome was the amount of postpartum blood loss in milliliters. Secondary outcomes included maternal and neonatal status, need for blood transfusion or blood products, changes in hemoglobin level (g/dL), and any intraoperative or postoperative measures required to manage PPH.

Finally, the ultrasound-measured — particularly placental thickness and myometrial thickness at the site of placental attachment—were statistically correlated with the measured blood loss to evaluate their predictive value for postpartum hemorrhage.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Normality of quantitative variables was

tested using the Kolmogorov-Smirnov and Shapiro-Wilk methods. Parametric data were expressed as mean $\pm$ standard deviation with ranges, while non-parametric data were reported as medians with interquartile ranges. Categorical variables were presented as frequencies and percentages. Statistical tests were selected based on data distribution: independent-samples t-test for parametric comparisons, Mann-Whitney U test for non-parametric data, and Chi-square or Fisher's exact test for categorical variables. Pearson's correlation was used to assess relationships between continuous variables, with scatter plots and linear regression evaluating predictive associations between ultrasound parameters and postpartum blood loss. Diagnostic performance and optimal cut-off values were determined through ROC curve analysis. A 95% confidence interval was used, with P values < 0.05 considered statistically significant and < 0.001 highly significant.

Results

Normal-EBL and High-EBL group had comparable baseline characteristics, about age (years), BMI [wt/ (ht)²], GA "wks., mode of delivery and causes of delivery ", ($P>0.05$). (Table 1)

There was a significantly higher mean value of placental thickness (cm) in high EBL group was 5.83 ± 1.03 compared to

normal EBL group was 4.98 ± 1.12 , ($P = 0.004$). Also, there was a significantly lower mean value of myometrial Thickness (cm) in high-EBL group was 0.89 ± 0.27 compared to normal-EBL group was 1.35 ± 0.23 , ($P<0.001$). As for the placental myometrial ratio, there was a highly statistically significant highest mean value in high EBL group was 6.83 ± 1.55 compared to normal EBL group was 3.75 ± 1.02 , ($P<0.001$). (Table 2)

In VD patients, intra-partum hemorrhage occurred in 2(33.33%) patients and Post-partum hemorrhage in 4(66.66%) patients. Regarding procedures, bimanual compression was done in all patients, Bakri balloon in 4 (66.66%) patients, bilateral uterine artery ligation in 2 (33.33%) patients and B-lynch in 1 (16.66%) patient.

In CS patients, intra-partum hemorrhage occurred in 7(70%) patients and post-partum hemorrhage in 3(30%) patients. Regarding procedures for Intra-partum hemorrhage, bimanual compression was done in 3 (30%) patients and Bakri balloon in 2(20%) patients. For post-partum hemorrhage, bilateral uterine artery ligation in 7 (70%) patients, B-lynch in 3 (30%) patients, other uterine compression suture in 2 (20%) patients and CS hysterectomy in 1 (10%) patient. (Table 3)

Table 1: Demographic data, gravidity, Causes and mode of Delivery.

Baseline characteristics		Normal EBL (n=134)	High EBL (n=16)	Test value	p-value	Sig.
Age (years)	Mean $\pm$ SD	26.21 $\pm$ 3.43	27.75 $\pm$ 4.06	-1.215	0.345	NS
	Range	35 -19	22-35			
BMI [wt/ (ht) ²]	Mean $\pm$ SD	26.08 $\pm$ 2.66	27.51 $\pm$ 3.39	1.996	0.051	NS
	Range	20-29.4	22-32			
GA "wks."	Mean $\pm$ SD	38.01 $\pm$ 0.83	38.00 $\pm$ 0.63	0.035	0.972	NS
	Range	40 -37	-39 37			
Gravidity	Mean $\pm$ SD	2.30 $\pm$ 1.58	3.56 $\pm$ 1.26	-1.090	0.239	NS
	Median (IQR)	2 (2-3)	4 (2-5)			
	Range	0-6	2-5			
Mode of Delivery	CS	105 (78.4%)	10 (62.5%)	2.009	0.156	NS
	VD	29 (21.6%)	6 (37.5%)			
Causes of Delivery	Elective	102 (76.1%)	10 (62.5%)	2.254	0.324	NS
	Spontaneous	29 (21.6%)	6 (37.5%)			
	Scheduled	3 (2.2%)	0 (0.0%)			

Table 2: Comparison between Normal EBL and High EBL according to Placental Thickness (cm), Myometrial Thickness (cm) and Placental myometrial ratio.

		Normal EBL (n=134)	High EBL (n=16)	Test value	p-value	Sig.
Placental Thickness (cm)	Mean $\pm$ SD	4.98 $\pm$ 1.12	5.83 $\pm$ 1.03	-8.838	0.004*	S
	Range	2.08-7.21	4.2-7.76			
Myometrial Thickness (cm)	Mean $\pm$ SD	1.35 $\pm$ 0.23	0.89 $\pm$ 0.27	7.332	<0.001*	HS
	Range	1.1-2.64	0.65-1.45			
Placental myometrial ratio	Mean $\pm$ SD	3.75 $\pm$ 1.02	6.83 $\pm$ 1.55	10.707	<0.001*	HS
	Range	1.72-6.55	4.50-10.03			

Using: t-Independent Sample t-test for Mean $\pm$ SD , S: Significant; HS: Highly significant

Table 3: Additional measures descriptive (Intra-partum and post-partum haemorrhage) in VD and CS patients in High EBL group.

	VD (n=6)	CS (n=10)
Intra-partum hemorrhage	2(33.33%)	7(70%)
Post-partum hemorrhage	4(66.66%)	3(30%)
Procedures:		
– Bimanual compression	6/6 (100%)	3/10 (30%)
– Bakri balloon	4/6 (66.66%)	2/10 (20%)
– Bilateral uterine artery ligation	2/6 (33.33%)	7/10 (70%)
– B-lynch	1/6 (16.66%)	3/10 (30%)
– Other uterine compression suture	0/6 (0%)	2/10 (20%)
– CS hysterectomy	0/6 (0%)	1/10 1(0%)

A) ROC curve show relation between placental thickness and postpartum blood loss B) ROC curve show relation between myometrial thickness and postpartum blood loss C) ROC curve show relation between placental myometrial ratio and postpartum blood loss (**Figure 1**)

Scatter plot showing relation between estimated blood loss and placental thickness with Pearson correlation coefficient (r value) 0.304 and p value =0.002 (**Figure 2**)

Receiver Operating Characteristic (ROC) curve analysis was employed to evaluate the predictive value of placental thickness (cm) in estimating postpartum blood loss (PPBL). This parameter yielded an area under the curve (AUC) of 0.708 (95% CI: 0.628–0.779, P=0.001), with the optimal threshold identified as >5.47 cm, offering a sensitivity of 75.00% and a specificity of 67.16%. Similarly, the ROC analysis for myometrial thickness (cm) demonstrated a more robust predictive capacity, with an AUC of 0.916 (95% CI: 0.860–0.955, P < 0.001). A cutoff value of $\leq$ 1.1 cm was found to best predict PPBL, achieving a

sensitivity of 81.25% and a specificity of 93.28%. The PMR exhibited the highest diagnostic accuracy, with an AUC of 0.958 (95% CI: 0.912–0.984, P < 0.001). The optimal PMR cutoff for predicting PPBL was >5.66, with corresponding sensitivity and specificity values of 81.25% and 96.27%, respectively. Among all evaluated parameters, PMR emerged as the most statistically significant predictor of PPBL (P < 0.001). (**Table 4**)

Scatter plot showing relation between estimated blood loss and myometrial thickness with Pearson correlation coefficient (r value) 0.427 and p value <0.001(**Figure 3**)

Scatter plot showing relation between estimated blood loss and placental myometrial ratio with Pearson correlation coefficient (r value) 0.567 and p value <0.001 (**Figure 4**)

In Multiple linear regression analysis, placental thickness, myometrial thickness and placental myometrial ratio were independent predictors of postpartum blood loss (P<0.05). (**Table 5**)

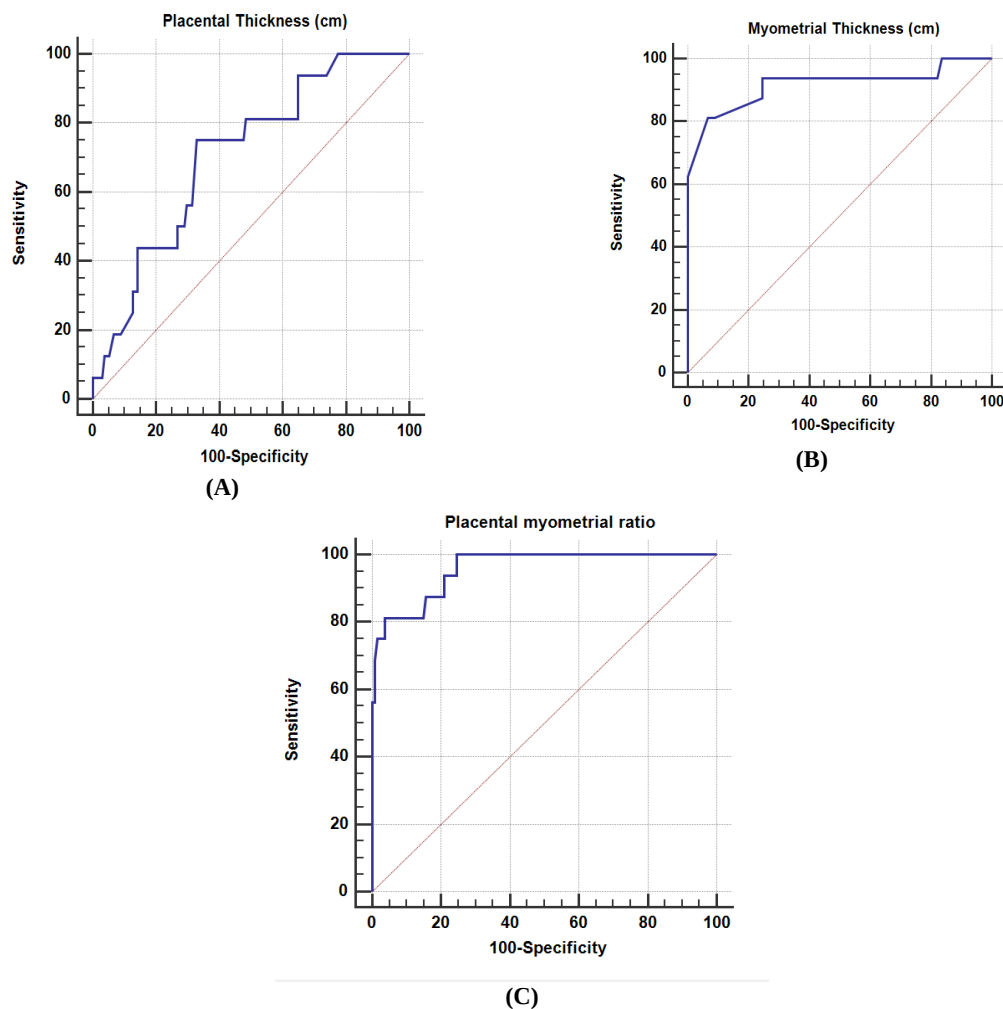
Table 4: Analysis of ROC curve.

Items	Cut-off	Sen.	Spe.	PPV	NPV	AUC (C.I.95%)	p-value
Placental Thickness (cm)	>5.47	75.00 %	67.16%	21.4 %	95.7 %	0.708 (0.628 to 0.779)	0.001*
Myometrial Thickness (cm)	≤1.1	81.25 %	93.28 %	59.1 %	97.7 %	0.916 (0.860 to 0.955)	<0.001*
Placental myometrial ratio	>5.66	81.25 %	96.27%	72.2 %	97.7 %	0.958 (0.912 to 0.984)	<0.001*

Table 5: Multiple linear regression analysis using placental thickness (cm), myometrial thickness (cm) and placental myometrial ratio as dependent variable in prediction of postpartum blood loss.

Parameters	Coefficient	±SE	Odds ratio	Sig.
Placental Thickness (cm)	0.689	0.255	1.993	0.006*
Myometrial Thickness (cm)	-13.911	3.889	0.001	0.003*
Placental myometrial ratio	2.24925	0.524	9.480	<0.001*

β: Regression coefficient, SE: Standard error

**Fig. (1):** Receiver-operating characteristic (ROC) curve for prediction of postpartum blood loss in correlation with

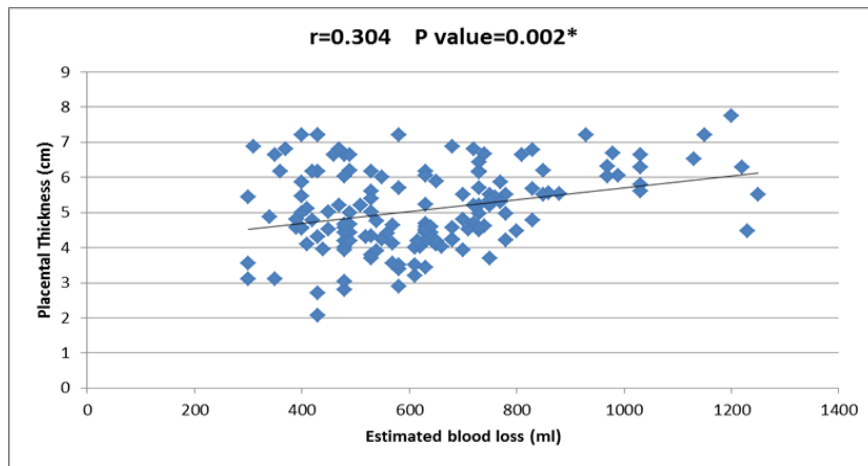


Fig. (2): Scatter plot between estimated blood loss “ml” and placental thickness (cm)

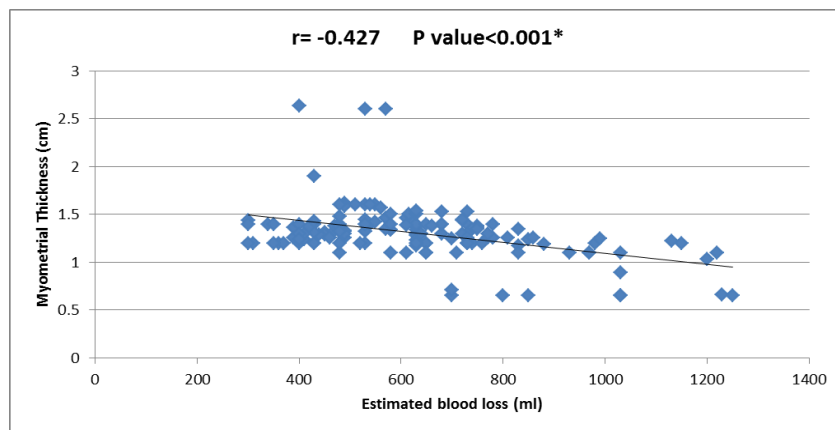


Fig. (3): Scatter plot between estimated blood loss “ml” and myometrial thickness (cm).

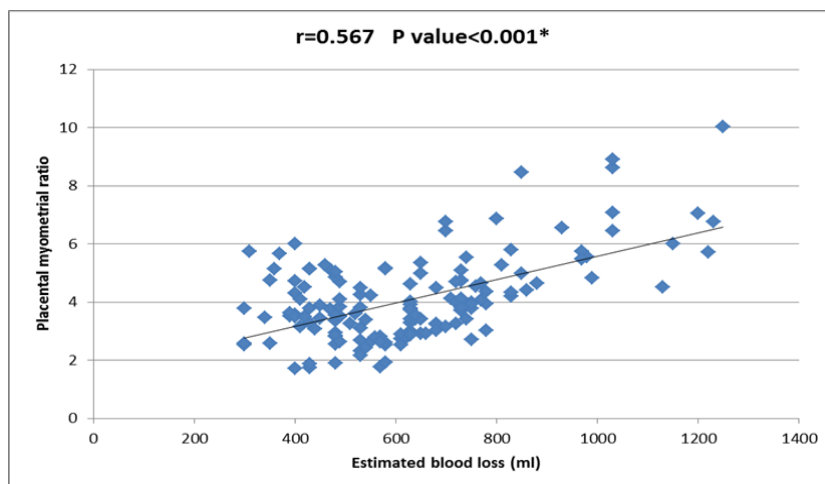


Fig. (4): Scatter plot between estimated blood loss “ml” and placental myometrial ratio.

Discussion

PPH is a serious complication of labor, with an incidence of approximately 5-10% and a mortality rate of 0.5-1%. Preventing and treating PPH is an unprecedented challenge ⁽⁶⁾.

In this investigation, the mean placental thickness measured (cm) 5.07 ± 1.14 , mean of myometrial thickness (cm) was 1.30 ± 0.27 and mean of placental myometrial ratio was 4.08 ± 1.44 . These outcomes are assured by ⁽⁷⁾ who found that increased placental thickness (≥ 4.35 cm) was independent risk factors for postpartum hemorrhage. The explanation of being increase of myometrial thickness causes postpartum hemorrhage is that the pathophysiology of myometrial involution may lead to uterine atony and postpartum hemorrhage. During pregnancy, the myometrium undergoes significant enlargement, primarily driven by hormonal stimulation from the fetal adrenal cortex and the placenta, leading to both hyperplasia and hypertrophy of uterine myocytes. Following childbirth, the uterus experiences a rapid reduction in weight, as physiologic involution sets in. This process involves extensive degradation and remodeling of myometrial tissue. Histopathological findings aligned with this transformation have been characterized in the literature as "postpartum uterine involution," a term that encapsulates the profound structural regression occurring in the postpartum uterus. This phenomenon may account for the delayed resumption of menstrual cycles until myometrial involution is complete ⁽⁸⁾.

Our study shows that the cesarean delivery predominated, with 115 cases (76.7%), followed by VD, with 35 cases (23.3%); while the most cases were elective delivery, 112 (74.7%). ⁽²⁾ confirmed what we found as they demonstrated that most of women with postpartum hemorrhage, had delivered by cesarean section. Added to that, ⁽⁹⁾ found matched results as they stated that women

with postpartum hemorrhage, most of them were delivered by cesarean section.

With respect to the findings, there was a statistically significant hemoglobin drop in postoperative hemoglobin (Hb) levels when compared to preoperative values ($P=0.002$). This decrease is consistent with the observations reported by ⁽¹⁰⁾, who noted that PPH is a leading contributor to maternal anemia and hemoglobin depletion. Furthermore, severe anemia may compromise uterine contractility and reduce resistance to infections, thereby exacerbating the risk and severity of PPH. Our results show that neonatal birth weight (gm) ranged 2500-3600 with mean 3141.60 ± 227.72 . Similarly, ⁽¹⁰⁾ who stated that neonatal birth weight was 3410 ± 434 gm in women presented postpartum hemorrhage. Moreover, ⁽¹¹⁾ supported what we found as they stated that neonatal birth weight was 2880.9 ± 471.3 gm in 317 women presented postpartum hemorrhage. Consistent with [12], we found no baseline differences between baseline characteristics (age, BMI, GA) and obstetric history (gravidity, term, preterm, abortion, living) ($P>0.05$) in normal and High EBL.

Alongside with our findings, ⁽¹²⁾ found that there was no statically significance between massive postpartum hemorrhage and non-massive postpartum hemorrhage regarding multiparity and BMI, nevertheless, the study disagreed to us as they noted significant difference between two group regarding maternal age ($P=0.007$).

In the current study, there was a highly statistically significant highest mean value of placental thickness (cm) in high EBL group was $5.83 \pm 0.1.03$ compared to normal EBL group was 4.98 ± 1.12 , ($P<0.004$). Also, there was a statistically significant lowest mean value of myometrial thickness (cm) in high EBL group was 0.89 ± 0.27 compared to normal EBL group was 1.35 ± 0.23 , ($P<0.001$). As for the placental myometrial ratio, there was a highly statistically significant

highest mean value in high EBL group was 6.83 ± 1.55 compared to normal EBL group was 3.75 ± 1.02 , ($P < 0.001$). Also, there was a statistically significant positive correlation between estimated blood loss “ml” with placental thickness (cm) and placental myometrial ratio, ($P < 0.05$). While, there was a statistically significant negative correlation between estimated blood loss “ml” with Myometrial Thickness (cm) and Post-operative Hb., ($P < 0.05$). Also, Multivariate analysis revealed that predictive significance of postpartum blood loss was placental myometrial ratio were the best independent predictors of postpartum blood loss, ($P < 0.05$).

In accordance with our results, ^(7,11) confirmed our findings as they found that increase thickness of placenta in a risk factor for postpartum hemorrhage. Furthermore, ⁽⁵⁾ supported our findings by reporting a significant negative correlation between myometrial thickness and estimated blood loss in a cohort of 75 patients ($p = -0.604$, $P < 0.001$). Similarly, ⁽¹³⁾ demonstrated through linear regression that reduced uterine muscle thickness at the site of placental attachment was significantly associated with increased postpartum blood loss at 2 hours ($t = -6.9848$, $P < 0.001$) and greater hemoglobin decline after delivery ($t = -2.242$, $P = 0.026$). Our work finds that there was a highly statistically significant increased time of delivery in high EBL group compared to normal EBL group, ($P < 0.001$); while there is no statistically significant difference normal EBL and High EBL according mode of delivery and causes of delivery ($P > 0.05$). These outcomes proved by ⁽¹⁴⁾ who reported a significant increase in delivery time among women with PPH, attributing this to prolonged labor—particularly an extended first stage—which may elevate the risk of PPH by leading to uterine atony, a condition where the myometrium fails to contract effectively, resulting in insufficient constriction of uterine blood vessels.

Concerning ROC curve, placental thickness (cm) demonstrated an AUC (area under the curve) of 0.708 (95% CI: 0.628–0.779; $P = 0.001$), indicating moderate predictive accuracy for postpartum blood loss (PPBL). The optimal cutoff value was >5.47 cm, with a sensitivity of 75% and a specificity of 67.16%. In comparison, myometrial thickness (cm) showed a higher predictive value, with an AUC of 0.916 (95% CI: 0.860–0.955; $P < 0.001$). The best cutoff point was <1.1 cm, achieving a sensitivity of 81.25% and a specificity of 93.28%. The PMR exhibited the strongest diagnostic performance, with an AUC of 0.958 (95% CI: 0.912–0.984; $P < 0.001$). The optimal cutoff value for predicting PPBL was >5.66 , with a sensitivity of 81.25% and specificity of 96.27%. Overall, PMR emerged as the most significant predictor of PPBL ($P < 0.001$).

In the same line of our results, ⁽¹³⁾ concluded that ultrasound measurement of myometrial thickness at placental attachment to predict postpartum hemorrhage. The ROC curve illustrates the sensitivity and specificity of using myometrial thickness at the placental attachment site as a predictor for postpartum hemorrhage. The AUC is 0.634, with an asymptotic significance of 0.002. A linear regression analysis was conducted to investigate the association between the amount of postpartum hemorrhage and the thickness of the uterine muscle at the site of placental attachment. The results indicated a significant effect of myometrial thickness at the placental attachment on postpartum hemorrhage, as evidenced by a t value of -6.9848 and a $P = 1.33E-11 < .05$.

Additionally, our findings are consistent with those of ⁽¹¹⁾ who demonstrated that increased placental thickness was significantly associated with the risk of massive intraoperative hemorrhage. Sensitivity analyses confirmed this association, showing consistent results across the minimally adjusted, crude, and fully adjusted models (p for trend = 0.001, 0.001, and 0.037, respectively). Notably,

individuals in the highest quartile of placental thickness (Q4) had a significantly elevated risk of major bleeding compared to those in the lowest quartile (Q1), with an odds ratio of 2.26 ($p=0.034$). A clear linear relationship was observed between placental thickness and the likelihood of experiencing massive hemorrhage.

Conclusion:

- Increase placental thickness and thinning of myometrium are major predictors of postpartum hemorrhage.
- The placental myometrial ratio is the most significant predictor of post partum blood loss.
 - The best cut off value of Placental thickness for prediction of post-partum blood loss is >5.47 cm with sensitivity 75% and specificity 67.16%.
 - The best cut off value of myometrial thickness for prediction of postpartum blood loss is was <1.1 cm with sensitivity 81.25% and specificity 93.28%.
 - The best cut off value of placental myometrial ratio for prediction of postpartum blood loss is $>>5.66$ with sensitivity 81.25% and specificity 96.27%.
- We can reduce the mortality rate linked to postpartum hemorrhage by addressing the potential association between a increased frequency of postpartum hemorrhage and a thinner myometrium and thicker placenta.
- **Author contributions**
A.A.Ahmed, O.K.Naser, W.M.Tawfik, A.I.Elmashad designed research; A.A.Ahmed, O.K.Naser, W.M.Tawfik, A.I.Elmashad performed research; A.A.Ahmed, O.K.Naser, W.M.Tawfik, A.I.Elmashad analyzed data; and A.A.Ahmed, O.K.Naser, W.M.Tawfik, A.I.Elmashad wrote the paper. All authors made a substantial contribution in the revision of the manuscript.
- **Funding:** Nil
- **Competing interests:** Nil

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To cite this article: Omar K. Naser, Ahmed A. Abdelhamid, Ashraf I. Elmashad, Waleed M. Tawfik. Evaluating Placental Thickness and Thickness of Uterine Muscle at Placenta Attachment in Prediction of Postpartum Blood Loss. *BMFJ* 2025;42(8):133-143.