

Maternal serum homocysteine and uterine artery pulsatility index as predictors of spontaneous preterm labor

Original
Article

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ABSTRACT

Objective: The aim of this cohort study was to investigate the uterine artery Doppler indices and the maternal serum homocysteine concentrations as predictors of spontaneous preterm delivery (sPTD).

Setting: Obstetrics and Gynecology Department, Benha University Hospital.

Materials and Methods: The study population included 200 singleton pregnant women with threatened preterm labor at 28-35 gestational weeks. The participants underwent uterine artery (UtA) Doppler examination, estimation of maternal serum homocysteine levels and routine management preterm labor. Participants were scheduled according to the gestational age at delivery into; preterm group (n= 45) and term group (n= 155). The diagnostic significance of both parameters for prediction of spontaneous preterm birth was calculated.

Results: Both maternal serum homocysteine (Hcy) and uterine artery pulsatility index (PI) were significantly higher in preterm group compared to term group (8.52 ± 2.36 vs. 4.62 ± 2.07 ; $p < 0.0001$ for Hcy and 1.18 ± 0.42 vs. 0.76 ± 0.29 ; $p < 0.0001$ for PI). For the prediction of sPTD, the maternal serum Hcy levels at a cut-off value 7.09 multiple of the median showed 55.56% sensitivity, 83.87% specificity, 50% PPV, and 86.67% NPV, and the UtA PI, the cut-off value of 0.99 showed 62.22% sensitivity, 83.87% specificity, 53.83% PPV, 88.44% NPV. However, both Hcy and UtA PI showed 71.11% sensitivity, 87.1% specificity, 61.54% PPV, 91.22% NPV for prediction of sPTD.

Conclusion: Maternal serum homocysteine levels and uterine artery pulsatility index are two promising predictors of spontaneous preterm delivery. The combination of the two parameters improves their diagnostic performance.

Key Words: Preterm labor, uterine artery, Doppler, homocysteine

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INTRODUCTION

Preterm delivery refers to fetal birth before completing 37 gestational weeks. It complicates 5-15% of pregnancies and is the major cause of neonatal morbidity and mortality. Only 50% of hospitalized women with threatened preterm labor actually achieve preterm delivery. Therefore, estimating the likelihood of spontaneous preterm delivery (sPTD) is essential to provide the best clinical interventions that may improve neonatal outcome and save valuable resources.^[1,2] The diagnostic significance of the available methods for assessing the likelihood of sPTD are limited, therefore; new markers are required to identify precisely pregnant women who have a high risk of preterm birth.^[3]

Spontaneous preterm labor is a syndrome of multifactorial etiology. Previous preterm labor, older mothers, tobacco smoking, black race, periodontal diseases, infections and low body mass index are supposed major risk factors for sPTD. However, these factors cannot clearly explain the exact pathophysiology underlying sPTD.^[4] Various reports indicate that the risk-factors of sPTD and cardiovascular diseases are similar, and the

risk of cardiovascular disease is doubled in women with previous preterm labor.^[5] The vascular placental pathology is reported in one-third of sPTD. These factors propose that maternal atherogenic lipid profile and increased homocysteine (Hcy) level, the potent and modifiable risk factors of vascular diseases, are debatable risk-factors of sPTD. This supports the possibility of vasculopathy role in initiation of preterm labor.^[6]

The current study was designed to investigate the uterine artery Doppler and the maternal serum Hcy concentrations as indirect predictors of sPTD among pregnant women hospitalized with threatened preterm labor.

PATIENTS AND MEDTHODS

This prospective observational cohort study was approved by the Local Ethics Committee of Benha University Hospital, and registered at Clinical Trials.gov: NCT03440281. The study included all pregnant women with threatened preterm labor admitted to the Department of Obstetrics and Gynecology, Benha University Hospital, from August 2015 to November 2017. Eligible women had a

viable singleton pregnancy at 28-35 gestational weeks with threatened preterm labor as they had 3 uterine contractions within 30 min. and the cervix less than 3 cm dilatation and less than 80% effacement. Pregnant women with multiple gestation, malpresentations, preterm premature rupture of membranes, active labor, uterine or fetal congenital abnormalities, diagnosed cervical insufficiency, placenta previa, intrauterine growth restriction, polyhydramnios, oligohydramnios, infectious or inflammatory disease, fetal distress as diagnosed by abnormal cardiotocography or fetal Doppler indices, medical problems (e.g., hypertensive disorders of pregnancy, anemia, pregestational diabetes, lung, heart, kidney disease, or Cushing syndrome), diagnosed vitamins B12, B6, folic acid deficiencies, or medically recommended preterm delivery were all the exclusion criteria. The participants received no medications apart from antenatal vitamins. All participants provided written informed consent.

On admission, the participants underwent a complete history taking and physical examination. The gestational age was calculated based upon the date of first day of last normal menstrual period. Gestational age was confirmed by the fetal crown-rump length measures obtained during first trimester ultrasonographic scan. Fetal heart rate and the existence of uterine contractions were monitored. Vaginal examination was done to assess the Bishop Scores. Venous blood sampled was obtained after overnight fasting and plasma Hcy levels were estimated using the RIA technique (LINCO Research, St. Charles, MO, USA). Serum Hcy level of 515- $\mu\text{mol/liter}$ was considered normal. Before the initiation of tocolytics, all participants underwent transabdominal ultrasonographic evaluation using a 3.5 MHz abdominal probe with Doppler facility (Voluson 730 PRO V, GE Healthcare, USA) by a single ultrasonographer. The uterine artery (UtA) Doppler velocimetry was done transabdominally with the patient in a flat supine position. The ultrasound probe was positioned in the inferior abdominal quadrant, inclined medially, and the Doppler color-flow mapping was used to identify the UtA flow at the obvious crossover with the external iliac artery. The UtA flow-velocity waveform was obtained by placing the Doppler pulsed gate on the target vessel 1cm distal to the crossover point, and the insonation angle was adjusted to be $<30^\circ$. When three consecutive waves were recorded, the uterine artery pulsatility index (UtA-PI), uterine artery resistance index (UtA-RI), Systolic/Diastolic ratio (S/D) and presence or absence of diastolic notch were estimated. The mean value of each measurement of right and left uterine arteries was calculated and used for the analysis. The obstetricians were blinded to the UtA Doppler results. Maternal corticosteroid was administered (four doses of 6 mg of intramuscular Dexamethasone at a 12-h interval) to any pregnant less than 34 completed gestational weeks to enhance the fetal lung maturation. The initial recommended management included bed rest and maternal hydration with 500mL of Dextrose

5% or lactated Ringer's solution. Tocolytic therapy was started if there was a documented persistent uterine contraction for at least 2 h. The first-line tocolytic therapy was the calcium channel blockers. In cases of persistent contractions, indomethacin was used before 32 weeks of gestation, however; ritodrine hydrochloride was used after 32 weeks of gestation. Tocolytics were stopped 48 h after the initiation of steroids. Combined tocolytic therapy was avoided. Patient discharge was considered if there was cessation of uterine contractions for at least 48 h. Patient readmission was considered if there was recurrence of uterine contractions, progressive cervical changes, or presence of any maternal or fetal complications. Patients were followed up regularly through antenatal visits till delivery, and contact information was used to follow the dropout cases.

The participants were classified into two groups according to the gestational age at delivery. The preterm group included those delivered before completed 37 gestational weeks, and the term group included those delivered after completed 37 gestational weeks. Both groups were compared regarding their demographic and characteristic data, serum Hcy level, and uterine artery Doppler indices. The accuracy of serum Hcy and UtA-PI for prediction of preterm labor was estimated.

STATISTICAL ANALYSIS

The collected data were statistically analyzed using SPSS (Statistical Package for Social Sciences Version 15.0, SPSS Inc.; Chicago, USA) program. Categorical data were presented as numbers and percentages and the Chi square test was used to test the difference. Continuous data were presented as mean \pm SD and compared using the unpaired student's *t* test. The statistical significance was considered at a *p* value <0.05 . The receiver operating characteristic (ROC) curves was used to estimate the predictive value and area under the curve was calculated. The specificity, sensitivity, positive predictive value (PPV), negative predictive values (NPV), and accuracy of UtA PI and/or maternal serum Hcy in the prediction of sPTD were calculated.

RESULTS

A total of 243 singleton pregnant women with threatened preterm labor were eligible for the study. Thirteen women refused to give written consent and thirty patients were lost to follow-up. Therefore, the study population included 200 participants. They were classified according to the gestational age at delivery into two groups; preterm group ($n=45$ cases) and term group ($n=155$ cases). Table 1 shows no significant differences between preterm delivery group and term delivery group regarding maternal age, body mass index, parity and gestational age at admission. However, the gestational age at delivery and birth weight

were significantly lower in the preterm delivery group compared to the term delivery group. Significantly higher total maternal serum Hcy levels (8.52 ± 2.36 vs. 4.62 ± 2.07 ; $p < 0.0001$) and mean UtA PI (1.02 ± 0.42 vs. 0.76 ± 0.29 ; $p < 0.0001$) were noted in the preterm delivery group compared to the term deliver group, respectively. However, no significant difference was noted between both groups regarding RI and the frequency of prediastolic notching of the uterine arteries waveforms. Table 2 shows the diagnostic significance of elevated maternal serum Hcy and UtA PI or both among studied population for the prediction of sPTD. Figure 1 represented the ROC curves.

Considering the cut-off value as a 7.09 multiple of the median (MoM), the maternal serum Hcy levels showed 55.56% sensitivity, 83.87% specificity, 50% PPV, 86.67% NPV, and AUC = 0.736 for the prediction of sPTD (Figure 1a). In the ROC curve analysis of the arithmetic mean of right and left UtA PI, the cut-off value was considered to be 0.99 with 62.22% sensitivity, 83.87% specificity, 53.83% PPV, 88.44% NPV, and AUC = 0.769 for the prediction of sPTD (Figure 1b). In the ROC curve analysis of the combination of the above two predictors, it showed 71.11% sensitivity, 87.1% specificity, 61.54% PPV, 91.22% NPV, and AUC = 0.809 for the prediction of sPTD (Figure 1c).

Table 1: Demographic and clinical characteristics of the study groups

	Preterm group (n=45)	Term group (n=155)	<i>p</i>
Age, y	25.8± 4.5	26.5± 2.1	0.144
BMI, Kg/m ²	24.2± 5.8	23.1± 3.7	0.129
Primigravida, n (%)	34 (75.6%)	112 (72.3%)	0.66
Gestational age, wk			
- At admission	31.1± 2.46	31.8± 2.4	0.09
-At delivery	33.3± 2.1	38.1± 1.8	< 0.0001
Birth weight (g)	2340.3± 430	3340.3± 230	< 0.0001
Homocysteine level (micromoles/liter)	8.52± 2.36	4.62± 2.07	< 0.0001
Uterine artery Doppler			
-UtA PI	1.18± 0.42	0.76± 0.29	< 0.0001
-UtA RI	0.61± 0.1	0.59± 0.15	0.401
-UtA S/D	3.6± 0.91	3.5± 1.01	0.55
-Diastolic notch (Unilateral/ Bilateral)	10 (22.2%)	40 (25.8%)	0.62

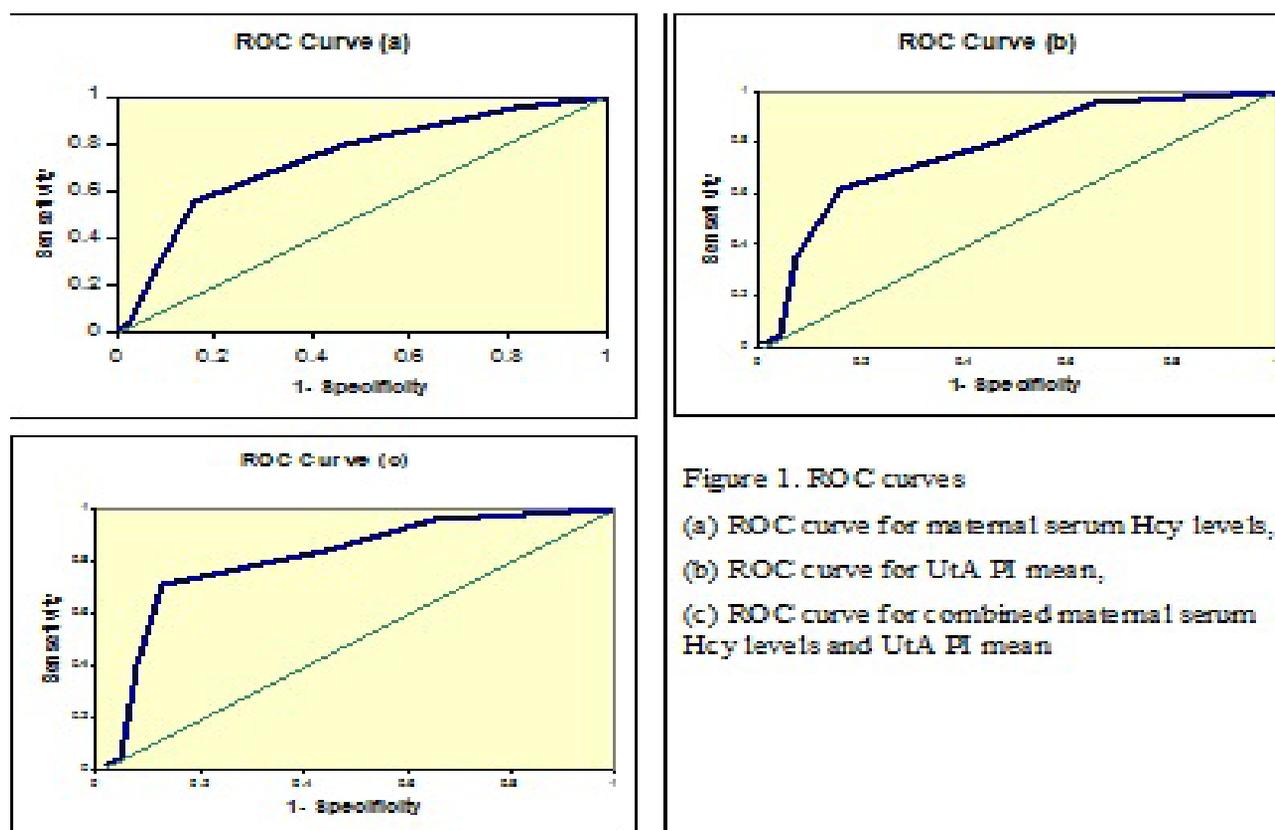
Data presented as number (percent) and mean ±standard deviation, BMI, body mass index; Kg/m², kilogram per squared meter; UtA PI: uterine artery pulsatility index, UtA RI: uterine artery resistance index, UtA S/D: uterine artery systolic diastolic ratio.

Table 2: Diagnostic significance of increased maternal homocysteine, uterine artery pulsatility index and combination of these parameters for prediction of preterm labor

	Increased Hcy	Increased UtA PI	Increased UtA PI+ Hcy
Cut-off	7.09	0.99	Both
Sensitivity	55.56% (40.00% to 70.36%)	62.22% (46.54% to 76.23%)	71.11% (55.69% to 83.63%)
Specificity	83.87 % (77.12% to 89.28%)	83.87 % (77.12% to 89.28%)	87.10 % (80.78% to 91.94%)
PPV	50.00% (39.08% to 60.92%)	52.83% (42.27% to 63.14%)	61.54% (50.51% to 71.49%)
NPV	86.67 % (82.32% to 90.08%)	88.44 % (83.93% to 91.80%)	91.22 % (86.74% to 94.28%)
Accuracy	77.50% (71.08% to 83.09%)	79.00% (72.69% to 84.43%)	83.50% (77.62% to 88.36%)
AUC	0.736	0.769	0.809

PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve, UtA PI: uterine artery pulsatility index, Hcy: homocysteine

Data represented as value (95%confidence interval)

**Fig.1:** ROC Curves

DISCUSSION

Homocysteine, a sulphur containing amino acid, is recognized as an indicator of the risk of cardiovascular disorders, neurodevelopmental conditions, pregnancy complications and adverse neonatal outcomes. Its serum level during normal pregnancy showed biphasic pattern as it declines during first trimester, achieved the minimum in the second trimester then showed slight increase toward the end of pregnancy.^[7] Lopez *et al.* reported significant lower levels of Hcy throughout pregnancy compared to those of non pregnant controls.^[8] The current study showed significant higher levels of maternal serum Hcy in preterm compared with the term delivery groups among those hospitalized with threatened preterm labor. These conclusions are supported by the findings of other authors although the evidence is weak. Kramer *et al.*^[9] and Qiu *et al.*^[10] proved that sPTD was correlated with elevated maternal serum Hcy level during the second and third trimesters, however; Knudtson *et al.*^[11] failed to prove such association. Vollset *et al.*^[12] in their large Norwegian study showed significantly higher maternal Hcy levels few years following conception in women with history of preterm labor. However, Ronnenberg *et al.*^[13] showed an association between elevated preconceptional Hcy level and preterm birth in a study conducted on 29 Chinese women with preterm birth. The increased Hcy level may be a direct consequence of folate deficiency. Although several trials have reported significant relation between low folate levels or supplementation and accidental hemorrhage that may lead to preterm birth^[14], few studies confirmed this association with global preterm birth.^[15] Unfortunately, randomized studies failed to show a decline in the risk of preterm birth following folate supplementation.^[16] These findings attract the attention to the possible role of increased Hcy level in the pathogenesis of preterm labor.

The increased Hcy level was suggested to cause preterm labor through induction of placental vascular endothelial dysfunction that induces inflammatory, hormonal or cellular (e.g., increased gap junctions) effects that instigate or hasten the sequence of events ending in preterm labor. This postulated mechanism is supported by increased prevalence of decidual vasculopathy and accelerated villous maturation among women with hyperhomocysteinemia. Also, an *in vitro* study demonstrated that Hcy enhanced the rate of spontaneous contraction of myometrium isolated from human pregnant uterus.^[6, 9, 17]

The current study showed that serum Hcy level is a supportive biomarker for prediction of preterm birth. However, there are no available persuasive findings derived from larger studies for comparison because Hcy has not been extensively evaluated in biomarker trials of complicated pregnancies other than preeclampsia.^[9, 13-]

In the preterm delivery, the uterine hyperactivity increases vascular resistance that causes non physiological decrease in the uterine perfusion; that is achieved even with

absence of painful uterine contraction. There is reduction or reversal of diastolic flow, however; systolic blood flow is preserved up to an intrauterine pressure of 130 mmHg to assure an adequate perfusion of the intervillous space. In complicated pregnancy, the increased vascular impedance of the uteroplacental circulation is a subsequent event to failure of the trophoblastic invasion of the spiral arteries preserving them as high resistant vessels under vasomotor control.^[18, 19] The study found a significant increase of UtA PI in the preterm compared with the term delivery groups, but no significant difference was noted regarding RI and the frequency of prediastolic notching of the uterine arteries waveforms. The combined use of UtA PI and Hcy as predictors of preterm birth improved the accuracy of their diagnostic performance.

To date, many authors evaluated the uterine artery Doppler indices in the preterm labor. Fonseca *et al.*^[20] and Spencer *et al.*^[21] in their studies included normal population which found that UtA PI was significantly higher in the preterm delivery group than the term delivery group; however, its diagnostic significance was poor either alone or in conjunction with other parameters. Agarwal *et al.*^[22] showed that preterm delivery group had significantly different PI, RI and S/D values compared with term delivery group, and RI had 87.7% specificity, 95.8% sensitivity with 70% positive predictive and 84% negative predictive value for the likelihood of preterm delivery. Axt-Fleider *et al.*^[23] in their study included high-risk population and reported that bilateral uterine artery notching had 79% specificity, 83% sensitivity, 97% negative and 33% positive predictive value, and 12.2 relative risk (RR) for the prediction of adverse pregnancy outcome as childbirth before 34 weeks. Park *et al.*^[24] found that RR for preterm delivery before 34 weeks in a low-risk cases was 2.67 (1.24–5.74) and 5.88 (2.46–14.7) in women with abnormal unilateral and bilateral uterine artery Doppler velocimetry, respectively. Olgan and Celiloglu^[25] in their pilot study concluded that the UtA PI during peak uterine contraction in pregnant women with threatened preterm labor might be a promising predictor of pregnant women at risk for sPTD.

The difference between our results and those of other authors may be attributed to different sample sizes, heterogeneity of population as regarding presence or absence of risk factors, different screening parameters, different time of screening during pregnancy, study design and research methodology.

The strengths of the study included being a pilot study to test the UtA PI and serum Hcy in cases of sPTD, prospective, strict criteria for inclusion help to identify those at risk of sPTD among those hospitalized for threatened preterm labor, highlighted the possible role of folate supplementation for prevention of preterm birth, no interobserver variability as single ultrasonographer performed all Doppler examinations and the obstetricians were blinded to the UtA Doppler results to avoid their impact on the subsequent treatment regimen.

STUDY LIMITATIONS:

- 1) The diagnostic performance of the study was limited by small sample size; however, these results are acceptable as our participants were not chosen from high-risk population, large prospective studies are required to justify the results.
- 2) We did not compare a high-risk population with normal population.
- 3) The data regarding the maternal preconceptional Hcy level and nutritional status that could affect that level were not available.
- 4) Unfortunately, some of the participants might have been using medications e.g., progesterone that may disturb the population uniformity.

CONCLUSION

Maternal serum homocysteine levels and uterine artery pulsatility indices are two promising predictors of spontaneous preterm delivery. The combination of the two parameters improves their diagnostic performance. Large scale prospective studies are recommended to confirm these results.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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