



# Association of combined second trimester maternal serum Homocysteine and Uterine Artery Doppler to predict adverse pregnancy outcome

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## Abstract

**Introduction** Disturbances in placentation increase the risk of maternal and fetal complications. Several biochemical and imaging modalities have been studied, but the hunt for a single effective screening test never became a reality as the causes of this complex condition are multifactorial and polygenetic, many of which we are only beginning to discover. Not many studies have been conducted in the developing countries like India and other low resource settings to consider whether it would be worthwhile to combine inexpensive and effective markers together for better prediction of adverse pregnancy outcome. This study primarily aims to investigate the predictability of combined screening with maternal serum homocysteine and second trimester uterine artery Doppler in diagnosis of adverse pregnancy outcome.

**Methodology** A prospective cohort study which involved 100 women with singleton gestation, meeting the inclusion criteria, attending the inpatient or outpatient of Obstetrics and Gynaecology in Amrita Institute of Medical Sciences, Kerala, a tertiary care centre in Southern India from July 2016 and September 2018 was conducted. Serum Homocysteine estimation (tHcy) was done between 18 and 28 weeks of gestation with informed consent, and uterine artery (UA) Doppler PI which is a non-invasive routine study was done along with targeted second trimester anomaly scan (18–24 weeks) in Fetal Medicine Department. Cutoff values of tHcy and UA PI were computed at 95th ( $>=9.7$  mmol/l) and 90th percentile, respectively as reported by Onalan et al. [9] and Nicholaides et al. [4]. Statistical analysis was performed using IBM SPSS version 20.0 software. Chi-square test and diagnostic measures were also used.

**Results** Of the 100 patients, 15% ( $n=15$ ) developed hypertensive disorder. 7% ( $n=7$ ) had FGR and 7% ( $n=7$ ) had spontaneous preterm birth. 6% ( $n=6$ ) neonates had an APGAR score  $<7$  and 8% neonates ( $n=8$ ) required immediate NICU admission. Statistically significant association was found when tHcy and UA PI were used together for the prediction of FGR ( $p=0.003$ ), preterm birth ( $p=0.002$ ) and low APGAR score at birth ( $p=0.009$ ) with a specificity of 83.4%. With regard to PIH, both parameters were found to be statistically significant only when used independently ( $p=0.001$ ) but not when used in combination ( $p=0.17$ ). Both elevated tHcy and abnormal UA PI used in combination predicted adverse pregnancy outcome like FGR but with a low sensitivity of 14.3% and high specificity of 98.9%. However, when used independently these markers predicted FGR with a better sensitivity (tHcy- 28.6% and UA PI- 44.4%)

**Conclusion** Findings from this study have been promising with potential clinical implications for the diagnosis and management of high-risk pregnancies. Though the independent role of the two markers in screening various adverse pregnancy

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The study was performed in Amrita Institute of Medical Science, Kochi, Kerala

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outcomes could be proved, their combined use to improve predictivity of more complications warrants further studies on a larger population with appropriate randomisation.

**Keywords** Homocysteine · Uterine Artery Doppler · Hypertension in pregnancy · Placental disorders

## Introduction

The primary goal of antenatal care is the identification and management of high-risk pregnancies and their complications. Any disturbance in placentation or implantation over an altered vascular territory increases the risk of early miscarriage and other complications like pre-eclampsia, abruptio placenta, intra-uterine fetal death (IUD) or fetal growth restriction (FGR). Ever since the publication of the landmark paper on pre-eclampsia by Roberts et al., this has been a focus of extensive research but unfortunately very few antenatal investigations have proven to be reliable [1]. These can broadly be divided into two categories—Biochemical markers and Doppler studies.

Amongst the biochemical markers, PAPP A, SFLT-2, PLGF, activin, inhibin A and homocysteine are a few that have proven associations with adverse pregnancy outcome [2]. Homocysteine is a thiol-containing amino acid produced by the intracellular demethylation of methionine. The levels in a normal pregnancy show a decreasing trend. Studies have reported a positive co-relation between maternal hyperhomocysteinemia and placenta mediated diseases that are attributable to vascular damage with consequent infarction and placental insufficiency [3].

Uterine artery Doppler (UA) is a simple, non-invasive and effective screening test in pregnancy that is widely used to study the pattern of utero-placental circulation that can help identify a range of clinical complications that may be attributed to placental disease. According to Fetal Medicine Foundation (FMF), Pulsatility index (PI) is currently the most commonly used Index for the evaluation of uterine artery Doppler waveform patterns. Uterine artery PI provides a measure of uteroplacental perfusion and high PI implies impaired placentation [4].

The role of early detection of adverse pregnancy outcomes and appropriate management is to reduce both maternal and neonatal morbidity and mortality. The hunt for a single screening test to diagnose the pathology of placental insufficiency has been going on for the past three decades. Over the years, several biochemical markers and imaging modalities have been tried, but a single effective screening test might never become a reality as the causes of this complex disorder are multifactorial and polygenetic, many of which we are only beginning to discover. The newer screening modalities are not only expensive but also difficult to avail for the general population in developing countries and other low resource settlements and not many studies have been

conducted in the Indian Sub-continent to consider whether it would be worthwhile combining two investigations together to assess the microvascular status of placental circulation which may hopefully increase the early detection rate of such high-risk outcomes [5]. There have been conflicting results amongst the various studies published over the years [6–8].

This study primarily aims to investigate the possible role of combined screening with two such investigations—maternal total serum homocysteine (tHcy) and second trimester uterine artery Doppler PI (UA PI) to predict adverse maternal and fetal outcomes.

## Methods

This was a prospective cohort study involving 100 singleton gestations between 18 and 28 weeks of gestation, without any known comorbidities or risk factors, attending the antenatal clinic of Department of Obstetrics and Gynaecology, in Amrita Institute of Medical Sciences, Kochi, a tertiary care centre in Kerala, Southern India from October 2016 and September 2018.

## Inclusion criteria

Women aged between 20 and 35 years with an uncomplicated pregnancy from spontaneous conception between gestational age of 18–28 weeks.

## Exclusion criteria

1. Multiple pregnancy.
2. Mothers on any form of thromboprophylaxis
3. Maternal medical illness such as chronic hypertension, multiple gestation, diabetes mellitus, SLE, thrombophilias or other bleeding disorders, severe anaemia, pre-existing cardiovascular or renal diseases
4. Fetal anomalies and chromosomal abnormalities.

Gestational age was calculated according to the date of last menstrual period or by an ultrasound scan at 11–13 weeks if the disparity in dates was  $\geq 7$  days. A second trimester scan was used to confirm/reassign the gestational age if the 11–13 weeks scan was not available.

Informed consent was obtained from study participants and under aseptic precautions 2 ml blood was drawn for total serum Homocysteine (tHcy) estimation between 18 and 28 weeks of gestation. Uterine artery Doppler Pulsatility Index (UA PI) a non-invasive routine study was done as a part of targeted second trimester anomaly scan (18–24 weeks) at the Department of Fetal Medicine in our institution. Since there is no standard nomogram for tHcy value in second trimester, we have taken 95th centile ( $>/=9.7$  mmol/l) as cutoff for this study, based on the earlier publication (Onalan et al. [9]). The uterine artery PI is considered to be abnormal if it is above the 90th centile for that gestational age (*Fetal Medicine Foundation* [4]). On the basis of the same reference study mentioned, the sensitivity for predicting the outcome using the combined method was found to be 61.3% with a 95% Confidence Interval (CI) and 10% allowable error, a sample size of 100 was considered [9].

The patients were followed up till their delivery in order to assess the main maternal and fetal outcome measures namely the development of hypertension, FGR and pre-term birth. Gestational age at delivery, birth weight of the baby at delivery, APGAR score at birth and NICU admissions were the neonatal outcomes considered. Data was obtained from hospital's electronic medical records and telephonic interview. The relationship between mid-trimester tHcy levels and UA PI with adverse pregnancy outcomes was assessed.

## Statistical analysis

Statistical analysis was performed using IBM SPSS version 20.0 software. Categorical variables are expressed using frequency and percentage. Continuous variables are presented using mean and standard deviation. To find the cutoff value of tHcy and UA PI, 95 ( $>/=9.7$  mmol/l) and 90th percentiles were computed, respectively. To test the statistical significance of the association of tHcy and UA PI cutoff value with pregnancy outcomes, Chi-square test was used. Diagnostic measures such as sensitivity and specificity were calculated wherever required.

## Results

Mean maternal age of the participants was found to be  $28 \pm 4$  years. Out of the 100 samples, 39% ( $n = 39$ ) were primi gravidas. During the course of pregnancy, of the 100 samples, 15% ( $n = 15$ ) were diagnosed with hypertensive disorder at some point while 7% ( $n = 7$ ) had FGR and 7% ( $n = 7$ ) had preterm delivery. Of the 15 pregnancies complicated by hypertensive disorders, 3 of them had FGR and 1 had preterm delivery (Table 1). Considering the neonatal outcome,

6% ( $n = 6$ ) of the neonates had an APGAR score of  $< 7$  and 8% ( $n = 8$ ) required immediate NICU admission.

On analysis of combined association of elevated tHcy and abnormal UA PI with pregnancy outcome, statistically significant results were found with regard to prediction of FGR ( $p$  Value = 0.003) with sensitivity of 14.3% and specificity of 98.9%, gestational age at delivery between 32 and 37 weeks ( $p = 0.002$ ), low APGAR score at birth ( $p = 0.009$ ) with a specificity of 83.3% (Figs. 1 and 2). No association could be established with regard to hypertensive disorders ( $p$  Value- 0.17), neonatal birth weight at birth ( $p$  Value-0.19) and NICU admission ( $p$  Value-0.11).

Independently, statistically significant association of elevated tHcy was established with Hypertensive Disorders ( $p$  value 0.001), FGR ( $p$  Value-0.03) and preterm birth ( $p$  Value-0.001) while UA PI was found to be associated with Hypertensive disorders ( $p$  value 0.001) and FGR ( $p$  Value = 0.05).

Both elevated tHcy and abnormal UA PI used in combination predicted adverse pregnancy outcome like FGR but with a low sensitivity of 14.3% and high specificity of 98.9%. However, when used independently these markers predicted FGR with a better sensitivity (tHcy-28.6% and UA PI-44.4%) (Table 2). With regard to PIH, both parameters—tHcy and UA PI, were found to be statistically significant only when used independently ( $p = 0.001$  for both) but not when used in combination ( $p = 0.17$ ) for prediction.

## Discussion

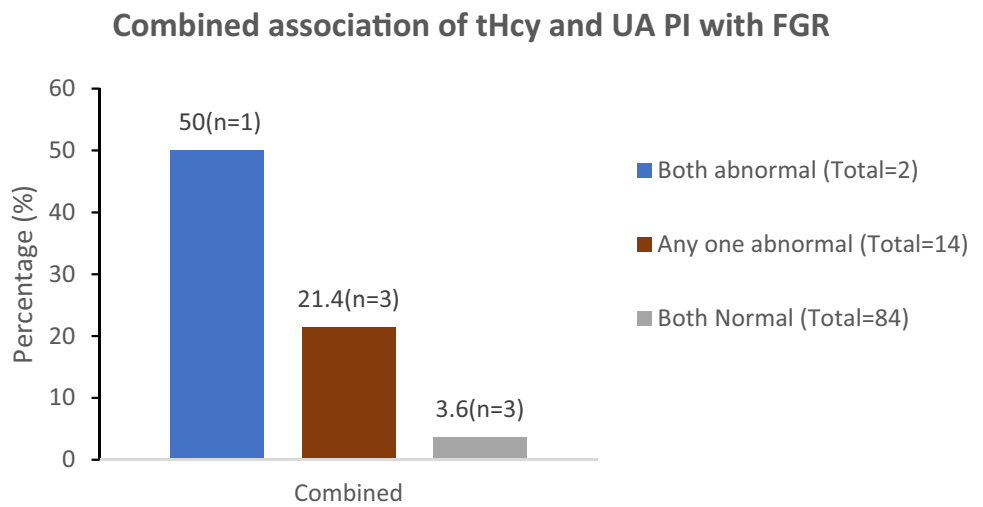
The disorders of utero-placental insufficiency are highly complex and poorly understood, albeit a very important cause of adverse maternal and fetal outcomes. These disorders include conditions such as gestational hypertension, pre-eclampsia and eclampsia in the mother and are often associated with low birth weight and FGR in the fetus.

In this study, the pregnant women with elevated tHcy and abnormal UA PI, independently were associated with adverse outcomes like Hypertensive disorders, FGR and

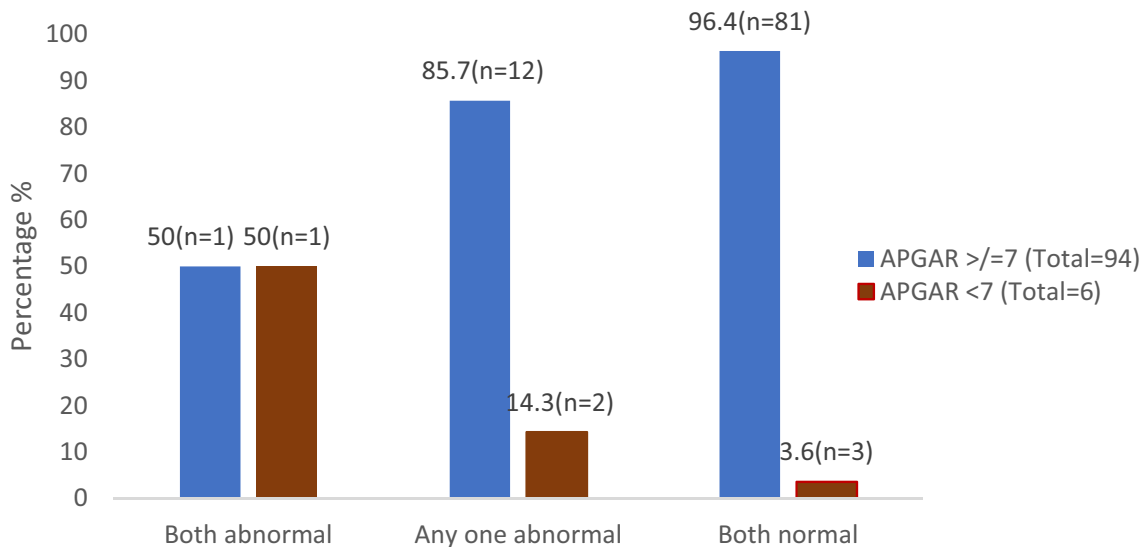
**Table 1** Distribution of Ante Partum complications

Complication	Frequency (n)	Percentage (%)
Hypertensive disorders only	11	11
Fetal growth restriction (FGR) only	4	4
Spontaneous pre-term labour only	6	6
Hypertensive disorders + Fetal growth restriction (FGR)	3	3
Hypertensive disorders + Spontaneous pre-term labour (PTL)	1	1

**Fig. 1** Graphical representation of combined association with FGR



**Combined association of tHcy and UA PI with APGAR score at birth**



**Fig. 2** Graphical representation of combined association with APGAR score at birth

**Table 2** Fetal growth restriction (FGR)

Variables	Sensitivity (%)	Specificity (%)
Homocysteine	28.6	98.6
Uterine Artery Doppler	44.4	90
Combined	14.3	98.9

preterm birth, mostly in view of termination of pregnancy due to worsening maternal and neonatal well-being secondary to utero-placental vascular insult. While combining both the modalities, significant improvement in the prediction of pregnancy outcome with respect to FGR, preterm

birth and low APGAR score at birth was found, but there was no statistical significance considering the prediction of hypertensive disorders including pre-eclampsia, neonatal birth weight and NICU admission. They predicted adverse pregnancy outcome like FGR and low APGAR score at birth but with a low sensitivity as compared to when used independently.

Also, those women who had elevated tHcy during the initial period of pregnancy were found to have a significantly higher risk of developing hypertensive disorders. However when the two investigations were used together for prediction they failed to predict the same.

Like the studies by Lopez-Quesada et al. [7] and Yu et al. [8], the present study did not predict disorders of uteroplacental insufficiency except FGR. However, a recent prospective study by Maged and colleagues [6] in 2017 and a Turkish study by Onalan et al. [9] found that there was significant improvement in prediction of disorders of not just utero-placental insufficiency but also preterm birth and neonatal birth weight when tHcy and UA PI were combined for prediction (Table 3).

On comparing other studies where mid-trimester tHcy was measured, the results were mixed. Consistent with the present study, similar positive correlation was found by Maru et al. who not only studied the association of tHcy with hypertensive disorders and NICU admission but also abruption and multi-organ dysfunction [10]. An important question which needs to be answered is whether serial testing of homocysteine is superior to a single measurement. Cotter's study involved serial measurement till delivery and found a positive correlation with pre-eclampsia [11]. Simply, testing for serum homocysteine levels in an unselected, low-risk population of pregnant women has repeatedly proven, not to be useful as a stand-alone screening test. Contrary to the present study, studies done by D'Anna et al. [12], Hietala et al. [13] and Hogg et al.

[14] suggested no association between elevated tHcy and hypertensive disorders and FGR.

In line with several other studies showing positive correlation using mid-trimester uterine artery Doppler measurements such as RI, diastolic notching as well as PI, the present study proved to be worthwhile in predicting hypertensive disorders and FGR. Consistent with the present study, Dhar et al. had obtained significant results of abnormal UA PI with pre-eclampsia and FGR, but no association was found with IUD and abruption [15]. Scandiuzzi and colleagues also associated elevated PI positively with hypertensive disorders and SGA but contrary to the present study, NICU admission showed a significant association [16].

### Limitations of the study

- Though the strength of this study is its prospective nature, the small sample size and the very small number of patients with elevated tHcy and abnormal second-trimester UA PI could have contributed to be insignificant in a few results when compared to other studies with significant results. Larger population studies are therefore required.

**Table 3** Similar studies evaluating the Association of Combined screening with Pregnancy Outcome

S.No	Study	Year	Outcome	
1	Present Study	2019	FGR, Gestational age at delivery, APGAR score at birth No association with hypertensive disorders, NICU admission	tHcy $\geq$ 9.7 (95th centile) and UA PI > 90th centile FGR-7%, ( $p=0.003$ ), Sn-14.3%, Sp-98.9%, Gestational age at Delivery (Preterm)—20%, ( $p=0.002$ ) Low APGAR score at birth-6%, ( $p=0.009$ ), Sn-1.1%, Sp-83.3%
2	Maged et al. [6]	2017	Hypertension, FGR, Birth weight, gestational age at delivery, Preterm Birth	(tHcy: > 4.701 ( $p < 0.001$ ) and Abnormal UA PI > mean $0.524 \pm 0.025$ , ( $p < 0.001$ ), Sn-85.2%, Sp-89.9%
3	Onalan et al. [9]	2006	Hypertension, FGR, abruption, stillbirth and Preterm Delivery	tHcy-6.3 (95th centile); UA-bilateral notches + mean RI 10.55 (50th centile), unilateral notches + mean RI 10.65 (80th centile), absence of notches + mean RI 10.7 (95th centile) Association with preeclampsia ( $p=0.001$ ), Sn-61.3%, isolated IUGR ( $p=0.01$ ), Sn- 54% and other complication ( $p=0.01$ ). Sn-56%
4	Lopez Quesada [7]	2004	No association with Hypertension, FGR	tHcy- 95 percentile (> 7.7) Abnormal uterine Doppler PI (median PI, > 1.2: mean/diastolic notch)—Sn-66.7%, Sp- 81.2%, in predicting obstetric complications Combining both- Sn-77.8%, Sp-77.6%
5	Yu et al [8]	2004	No association with hypertension	Abnormal PI > 95th centile (1.6) tHcy- Median ( $\mu\text{mol/l}$ ) Normal-5.5 Pre-eclampsia-5.1 No association with uterine artery Doppler or on combining the two



- Although the present study has obvious shortcomings such as inadequate randomization and matching, it may help to reinforce the concept of including tHcy as an adjunct in women with high risk of complications.
- In the present study, only UA PI was used as the parameter of choice, compared to many of the previous studies where RI and diastolic notching have also been used to denote abnormal Doppler patterns thereby improving the sensitivity of prediction of adverse outcome

## Conclusion

Incidentally, this is one of the first studies in an Indian population to assess the feasibility of using a combined modality testing (mid-trimester maternal tHcy and UA Doppler velocimetry) as a viable method of screening. It was found from this study that combining two investigations—tHcy and UA PI—has a good association with prediction of FGR, preterm birth and low APGAR score at birth. When both these markers were independently used, association of elevated tHcy was established with hypertensive disorders, FGR and preterm birth while abnormal UA PI was found to be associated with prediction of hypertensive disorders and FGR. Though these results were statistically significant, it was also established that the combined method had a lower sensitivity as compared to their independent sensitivity to predict these adverse outcomes that could have been attributed to by the limitations of this study.

Due to the myriad pathogenic factors, ethnic and genetic variations as well as the often-contradictory data in published literature, it is prudent to conduct more research until a valid consensus may be reached. The finding of this study certainly has been promising with potential clinical implications adding to the growing body of evidence, in diagnosis and management of utero-placental disorders.

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## Compliance with ethical standards

**Conflict of interest** I, Dr. Pooja Ramesh declare that I have no conflict of interest. Dr. Sudha S declares that she has no conflict of interest. Dr. Vivek Krishnan declares that he has no conflict of interest.

**Human participants** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Institutional and National) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

**Ethical approval** It is a prospective study and data was obtained from the medical records only and Institutional ethics committee clearance was obtained prior to the conduct of the Study (attached).

**Consent for publication** No patient identifiable images or data. Consent to participate and publish data were taken from all participants as per institutional policy.

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