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Full length Research Paper

Combination of First Trimester Maternal Plasma Homocysteine and Uterine artery Doppler Velocimetry for Perinatal Outcome

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Abstract
Prediction of adverse pregnancy outcome such as preeclampsia and intrauterine growth restriction is of utmost importance; and research about prediction markers is still continuous. To evaluate the predictive power of umbilical artery Doppler and homocysteine for adverse pregnancy outcome. This study was conducted at Damietta General Hospital. A total of 103 singletons, low risk pregnant women aged between 20 and 30 years were included in the study. All pregnant women took 400 mcg of folic acid. The ultrasound examinations were performed at 11 to 14 weeks of gestation. The blood for bio-chemical analysis was collected at the same time the ultrasonographic measurement performed. Total homocysteine levels ranged from 2.5 to 11 µmol/l with a mean 5.98±1.81 µmol/l; and there was statistically significant decrease of total homocysteine levels in non-complicated when compared to complicated group (5.29± 0.86 vs 7.87±2.32 respectively). In addition, there was significant moderate proportional correlation between ultrasound score and total homocysteine levels (i.e., with increased ultrasound score, there is significant increase in homocysteine levels). Combining both ultrasound score with hyperhomocysteinemia revealed negative for this combination The predictive power of hyperhomocysteinemia alone was 75.0%; while abnormal ultrasound alone had predictive power of 0.46 and finally both abnormal ultrasound with hyperhomocysteinemia had a predictive power of 75.0%.Maternal serum homocysteine level increases in 11 to 14 weeks of gestations that are complicated with pre-eclampsia and preterm birth. The addition of homocysteine determination to uterine artery Doppler in the first trimester adds advantage in predicting adverse perinatal outcome.

Keywords: *Homocysteine, uterine artery, Doppler ultrasound*

Introduction

The complex process of placentation begins during the first trimester. To reduce the vessel resistance and to increase utero-placental flow, the spiral arteries undergo a series of vascular transformations through two waves of trophoblastic invasion at 11 to 14 and 20 to 24 weeks of gestation (Tuuli et al., 2011). Any disturbance during this placentation process causes early pregnancy loss or complications that usually occur in the third trimester, such as placental abruption, pre-eclampsia, intrauterine growth restriction (IUGR), preterm birth or intrauterine fetal death (Whitley and Cartwright, 2010). Routine prenatal care focuses on the detection of women at increased risk, aiming at careful monitoring and appropriate intervention. Although these problems become manifest in the second half of pregnancy and a lot of research have been employed accordingly, there is evidence that they are associated with events taking place in the first trimester of pregnancy (Abdel Moety et al., 2016). Hyperhomocystenemia has been shown to be associated with placental abruption, pre-eclampsia, IUGR, preterm birth or intrauterine fetal death in several studies (Mierla et al., 2012).

Doppler velocimetry is a noninvasive method of evaluating blood flow characteristics in fetal and uteroplacental circulations. A study of Doppler waveforms of the uterine artery allows for the evaluation of blood flow received by the fetoplacental unit. Thus, increases in uterine artery resistance index (RI) or pulsatility index (PI) values and presence of unilateral or bilateral diastolic notch is associated with poor pregnancy outcome seen in pre-eclampsia and abnormal placentation (Carbillon, 2012).There are only a few first trimester screening studies and most of these have shown that, even in this early stage, impedance to flow in the uterine arteries was increased in pregnancies that subsequently developed pre-eclampsia and IUGR (Jamal et al., 2013).

To evaluate the predictive value of uterine Doppler velocimetry at 11 to 14 weeks of gestation for pregnancy complications. In addition, to study the relationship between abnormal uterine Doppler velocimetry and plasma homocysteine. Furthermore, to determine whether homocysteine measurement improves the predictive value of uterine Doppler screening.

Materials and Methods

Study area

This study was conducted at Damietta General Hospital. A total of 103 singletons, low risk pregnant women aged between 20 and

30 years were included in the study. All pregnant women took 400 mcg of folic acid. The ultrasound examinations were performed at 11 to 14 weeks of gestation. The blood for bio-chemical analysis was collected at the same time the ultrasonographic measurement performed. The local ethical committee approved the present study and an informed consent was obtained from all females before participation in the study. In addition, the right of withdrawal and confidentially were ascertained.

All included cases were submitted to full history taking, clinical examination and laboratory investigations including CBC, kidney and liver function tests, ABO and Rhesus groups. Then a Doppler study on umbilical artery was done. Maternal age, gravidity, parity, body mass index and gestational week at the examination were recorded at the first visit. Participants were followed until the end of their pregnancy and obstetric complications (pre-eclampsia, gestational hypertension, IUGR, placental abruption, intrauterine fetal death, preterm birth) encountered were recorded. Levels of homocysteine and Doppler scores were compared between pregnant women with and without obstetric complications.

Pre-eclampsia was diagnosed when a blood pressure higher than 140/90 mmHg was observed after the 20th week of gestation together with proteinuria (≥300 mg/24 h) and elevated arterial blood pressure (TA ≥ 140/90 mmHg) without proteinuria after the 20th week of gestation diagnosed as gestational hypertension (National High Blood Pressure Education Program, 2000). IUGR was defined as fetal growth lower than the 10th percentile of reference value for fetuses of the same age (Kandraju et al., 2012). Finally, preterm birth was defined as birth occurring before the 37th week of gestation (American College of Obstetricians and Gynecologists, 2001).

An 8-ml maternal blood sample was collected on the day the uterine artery (UA) Doppler examination was performed. The sample was centrifuged at 4000 cycles/min for 5 min to separate the serum. The sera were stored at -30oC until the time for the analysis of homocysteine level. Plasma homocysteine level was determined by solid phase competitive chemiluminescent enzyme immunoassay (DPC Immulite 2500 analyzer, China). Hyperhomocysteinemia is defined as homo-cysteine levels at > 7.46 µmol/l.

Statistical analysis: Comparison of maternal plasma concentrations of homocysteine with different Doppler scores was carried out using the Kruskal–Wallis test. The significance between the groups was evaluated by the Mann–Whitney U-test with Bonferroni correction. Spearman correlation analysis was performed for correlations of non-parametric variables.

Results

The present study included 103 pregnant females; 75 of them (72.8%) completed their pregnancy without complications, while 28 cases (27.2%) had complications. Prematurity was the most common complication, presented in 13 cases out of 28 cases (46.4%), and followed by pre-eclampsia in 10 cases (35.7%) and both conditions in 5 cases (20.8%). Female age ranged from 20 to 30 years and there was no statistically significant difference between non-complicated and complicated groups as regard to age (the mean age was 25.50±2.97 and 26.25±3.67 years respectively). Body weight ranged from 61 to 85 kg; the height ranged from 1.61 to 1.75m; while BMI ranged from 22.95 to 28.69 kg/m^2; and there was no statistically significant difference between complicated and non-complicated groups (table 1).

As regard to parity; 54 cases (52.4%) were primipara and 49 cases (47.6%) were multipara (47.6%); and there was no statistically significant difference between non-complicated and complicated cases as regard to parity (e.g., primipara represented 50.7% and 57.1% of non-complicated and complicated groups respectively). In addition, gestational age ranged from 33 to 40 weeks of gestation with a mean of 37.32±1.37 weeks; and there was statistically significant increase of gestational age in non-complicated group when compared to complicated group (37.90±0.79 vs 35.75±1.38 weeks respectively). Furthermore, fetal weight, it ranged from 1700 to 3350gm; and there was statistically significant increase of fetal weight in non-complicated when compared to complicated groups (3154.73±81.34 vs 2856.42±271.21g respectively) (table 1).

As regard uterine notch; it was absent in 64 cases out of 103 cases (62.1%); unilateral in 25 cases (24.3%) and bilateral in 14 cases (13.6%); and there was statistically significant increase of bilateral notch in complicated group when compared to non-complicated group (28.6% vs 8% respectively). In addition, pulsatility index of uterine artery was ranged from 0.87 to 1.60 on the right side and from 0.80 to 1.50 on the left side; and the mean PI ranged from 0.85 to 1.55; and there was significant decrease of uterine artery PI in non-complicated group when compared to complicated group on the right and left sides. Furthermore, ultrasound score was 0 in 38 cases (36.9%); 1 in 36 cases (35.0%); 2 in 14 cases (13.6%); 3 in 8 cases (7.8%) and 4 in 7 cases (6.8%), and there was statistically significant increase of higher score in complicated cases when compared to non-complicated cases (e.g., scores 3, 4 represented 25.0%, 25.0% of complicated group and 1.3%, 0.0% of non-complicated groups) (table 2).

Total homocysteine levels ranged from 2.5 to 11 µmol/l with a mean 5.98±1.81 µmol/l; and there was statistically significant decrease of total homocysteine levels in non-complicated when compared to complicated group (5.29± 0.86 vs 7.87±2.32 respectively) (Table 2).

Regarding relation between total homocysteine levels and ultrasound scores, there was statistically significant difference between different scores as regard to total homocysteine levels (the lowest level was observed in zero score (5.34±0.70) followed by score one (5.86±1.80); then score two (6.65±2.5); then score three (7.03±2.17) and finally score four (7.72±2.47). In addition, there was significant moderate proportional correlation between ultrasound score and total homocysteine levels (i.e., with increased ultrasound score, there is significant increase in homocysteine levels) (table 3).

As regard to relation between ultrasound score and complications, there was no complication in zero score; while there was significant increase of both prematurity and preeclampsia in score three and four when compared to lower scores (table 3).

As regard to relation between fetal body weight and ultrasound score, there was significant difference between different scores (the lowest weight was in score three (2911.25±189.09 g) followed by score four (2924.28± 182.92 g); then score two (3025.71±193.33); then score one (3055.83± 261.57 g) and finally score zero (3169.86±72.73g). In addition, there was inverse (negative), moderate and statistically significant correlation between ultrasound score and fetal body weight (table 3).

Combining both ultrasound score (any of scores 1, 2, 3 or 4) with hyper-homocysteinemia revealed that, 85 cases were negative for this combination and 18 cases were positive (i.e., 18 cases had ultrasound score extending from 1 to 4; and at the same time hyper-homocysteinemia).

Regarding relation between abnormal ultrasound with hyper-homocysteinemia and complications, all positive cases (18 cases) had complications; these complications were in the form of prematurity in 10 cases, preeclampsia in 4 cases and both in 4 cases; and there was statistically significant difference between negative and positive cases as regard to complications and type of complications. In negative cases, 10 cases (11.8%) had complications; complications were prematurity in 3 cases, preeclampsia in 6 cases and both in 1 case (table 4).

As regard to predictive power of hyperhomocysteinemia alone, it was 75.0%; while abnormal ultrasound alone had predictive power of 0.46 and finally both abnormal ultrasound with hyperhomocysteinemia had a predictive power of 75.0%.

Table (1): Comparison between complicated and non-complicated groups as regard to female characteristics, gestational age and fetal weight

		Not complicated	Complicated	Test	P value
Age (mean±SD)		25.50±2.97	26.50±3.67	0.23	0.82(ns)
Weight (mean±SD)		68.42±5.15	69.96±5.82	1.30	0.20(Ns)
Height (mean±SD)		1.6595±0.0299	1.6657±0.0310	0.93	0.35(ns)
BMI(mean±SD)		24.81±1.08	25.17±1.32	1.42	0.15(n)
Parity (n,%)	**Primipara**	38(50.7%)	16(57.1%)	0.34	0.55(ns)
	Multipara	37(49.3%)	12(42.9%)		
Gestational age (mean±SD)		37.90±0.79	35.75±1.38	9.90	<0.001*
Fetal weight (mean±SD)		3154.73±81.34	2856.42±271.21	8.60	<0.001*

Table (2): Comparison between complicated and non-complicated groups as regard to ultrasound findings and homocysteine levels

		Not complicated	Complicated	Test	P value
Uterine notch	**None**	49(65.3%)	15(53.6%)	7.45	0.024*
	Unilateral	20(26.7%)	5(17.9%)		
	Bilateral	6(8.0%)	8(28.6%)		
Right UA PI		1.07±0.13	1.21±0.21	3.95	<0.001*
Left UA PI		1.08±0.12	1.31±0.08	9.59	<0.001*
Mean PI		1.08±0.11	1.23±0.12	7.23	<0.001*
Ultrasound Score	**Zero**	38(50.7%)	0(0.0%)	49.82	<0.001*
	One	28(37.3%)	8(28.6%)		
	Two	8(10.7%)	6(21.4%)		
	Three	1(1.3%)	7(25.0%)		
	Four	0(0.0%)	7(25.0%)		
Homocysteine level		5.29±0.86	7.87±2.32	8.25	<0.001*

Table (3): Comparison between different ultrasound scores as regard to total homocysteine levels

		Zero	One	Two	Three	Four	Statistics		
							F	p	r
Homocysteine		5.34±0.70	5.86±1.80	6.65±2.50	7.03±2.17	7.72±2.47	2.12	0.002*	0.43*
Complications	**None**	38(100.0%)	28(77.8%)	8(57.1%)	1(12.5%)	0(0.0%)	61.40	<0.001*	-
	Prematurity	0(0.0%)	3(8.3%)	3(21.4%)	4(50.0%)	3(42.9%)			
	Pre-eclampsia	0(0.0%)	3(8.3%)	1(7.1%)	2(25.0%)	4(57.1%)			

Both	0(0.0%)	2(5.6%)	2(14.3%)	1(12.5%)	0(0.0%)			
Fetal body weight	3169.86± 72.73	3055.83± 261.57	3025.71± 193.33	2911.25± 189.09	2924.28± 182.92	5.29	<0.001*	0.40*

Table (4): Percentage of complications in cases with abnormal ultrasound with hyperhomocysteinemia

		Negative (85)		Positive (18)		Statistics	
		n	%	n	%	X^2	p
Complications	None	75	88.2%	0	0.0%	58.42	<0.001*
	Complicated	10	11.8%	18	100.0%		
Type of complications	None	75	88.2%	0	0.0%	64.81	
	Prematurity	3	3.5%	10	55.6%		<0.001*
	Preeclampsia	6	7.1%	4	22.2%		
	Both	1	1.2%	4	22.2%		

Discussion

Pre-eclampsia and fetal growth restrictions are major causes of maternal and fetal morbidity and mortality (Steegers et al., 2010). Early onsets of these conditions are associated with increased risk of complications (Crispi et al., 2008). Early-onset preeclampsia is associated with a 20-fold higher rate of maternal mortality than is late-onset disease and is one of the key contributors to early fetal growth restriction (von Dadelszen et al., 2009). Women with early-onset pre-eclampsia require admission to a tertiary care facility for treatment and one-third experience complications that may necessitate intensive care (Churchill and Duley, 2002). Infants are often delivered preterm, need prolonged intensive care and develop complications, including lifelong disability, giving rise to large healthcare costs (Tyson et al., 2008).

Early identification of women at risk is a key aim of antenatal care. The National Institute of Clinical Excellence (NICE) in the UK has prioritized the need for research to identify those at risk of pre-eclampsia. Clinical risk assessment for pre-eclampsia is carried out in the first trimester for early identification of women who may benefit from preventative treatment, such as aspirin. This includes women with at least one high-risk factor (a previous history of hypertension in pregnancy, chronic kidney Impaired placentation with abnormal blood-flow velocity and resistance in placental vessels is associated with pre-eclampsia and fetal growth restriction (National Institute for Health and Clinical Excellence, 2010).

Doppler of the uterine artery, a non-invasive method that can pick up these abnormalities, is currently not part of this assessment. Individual studies, owing to a lack of power, and existing systematic reviews with small numbers of included studies, have failed to produce robust guidance on first-trimester screening with uterine artery Doppler for adverse pregnancy outcome (Cnossen et al., 2008).

Routine prenatal care focuses on the detection of women at increased risk, aiming at careful monitoring and appropriate intervention. Although these problems become manifest in the second half of pregnancy and a lot of research have been employed accordingly, there is evidence that they are associated with events taking place in the first trimester of pregnancy (Abdel Moety et al., 2016). Hyperhomocystenemia has been shown to be associated with placental abruption, pre-eclampsia, IUGR, preterm birth or intrauterine fetal death in several studies (Dekker et al., 1995). Vitamin B12, B6 and folate play a role in the metabolism of homocysteine (Cotter et al., 2001).The aim of the present work was to analyze the relationship of maternal plasma homocysteine together with the first trimester uterine artery Doppler velocimetry on pregnancy outcome. We also aimed to find out whether the addition of homocysteine measurement to uterine artery Doppler screening improves the prediction of adverse pregnancy outcome. To reduce the false positive rate of Doppler velocimetry in predicting poor pregnancy outcome, some authors have proposed the addition of other parameters associated with placental functioning (Florio et al., 2003). Coining with this theory, in the present work, we analyzed the addition of homocysteine. It is well known that, hyperhomocystenemia is a risk factor for endothelial dysfunction and vascular diseases such as atherosclerosis and occlusive vascular disease (Vollset et al., 2000). In addition, hyperhomocysteinemia has also been shown to be associated with several pregnancy complications like recurrent pregnancy loss, placental abruption, intrauterine fetal demise, pre-eclampsia and IUGR (Kaymaz et al., 2011).

In Kaymaz et al. (2011) study, at 11 to 14 weeks of gestation, they also found no correlation between hyperhomocystenemia and the occurrence of obstetric complications. However, they found mean homocysteine levels to be significantly higher in different Doppler scores as presented in the current study. They added, as the Doppler scores increased, so did the mean plasma homocysteine levels. These results are in agreement with that of the present study. Mascarenhas et al. (2014) performed a cohort study compromising 100 antenatal women between 8-12 weeks of gestation. They found that, serum homocysteine level in late first trimester (8-12 weeks)of pregnancy are significantly associated with prior pregnancy losses, particularly in the second and third trimesters and with prior hypertensive disorders fo pregnancy. They added that increased serum homocysteine levels were also significantly associated with hypertensive disorders of pregnancy, pregnancy loss, oligohydramnios, meconium stained amniotic fluid and low birth weight.

Guven et al. (2007) performed a two-stage screening strategy by measuring uterine artery Doppler velocimetry at 20 to 22 and 24 to 26 weeks of gestation and searched for their possible association with midtrimester maternal plasma homocysteine levels. They found no difference in the homocysteine levels between pregnant women with abnormal Doppler findings and controls in both visits. They measured uterine artery RI instead of calculating Doppler scores as we did and defined abnormal blood flow as an average RI >0.58 and/or bilateral early diastolic notch and concluded that combining midtrimester maternal plasma homocysteine with uterine artery Doppler velocimetry was not a useful approach. These results are contradicted to that of the present work and it may be explained by the different time at registration of Doppler indices and measurement of plasma homocysteine levels.

In the present work, the predictive power of Doppler indices reached 57% and this had clinical implications especially in patients with preeclampsia. Initiation of aspirin treatment is recommended, at the earliest, at 12 weeks of gestation in women with risk factors. The meta-analysis by Bujold et al. (2010) showed that commencement of aspirin before 16 weeks of pregnancy halves the risk of pre-eclampsia, with no significant effect if commenced after that period.

Studies that commenced aspirin before 16 weeks in this meta-analysis included women who were at moderate or high risk for pre-eclampsia. However, an individual patient data meta-analysis did not identify any significant subgroup effect for aspirin commenced before or after 20 weeks of pregnancy (Askie et al., 2007). It is likely that early administration of aspirin reduces the risks by improving placentation, with a beneficial effect particularly on the risks of early- compared with late-onset pre-eclampsia (Velauthar et al., 2014).

A meta-analysis of five randomized trials demonstrated that commencement of low-dose aspirin before 16 weeks of pregnancy significantly reduces the risk of early-onset pre-eclampsia, with no effect on term pre-eclampsia. These findings reinforce the need for early identification of women at risk for pre-eclampsia (Roberge et al., 2012). Current recommendations (for commencing prophylactic aspirin for prevention of pre-eclampsia) target women with one clinical high-risk factor or two moderate-risk factors. Uterine artery Doppler in the first trimester will enable clinicians to identify women at risk of developing pre-eclampsia and fetal growth restriction and its complications, and initiate preventive measures such as aspirin and regular fetal monitoring to minimize adverse outcomes. There is no significant difference in the screening performance of uterine artery Doppler with the use of either lower or mean pulsatility indices (Napolitano et al., 2011).

In a population of 683 women, Yu et al. (2005) showed that maternal plasma homocysteine concentration at 22–24 weeks is not elevated in women who subsequently develop pre-eclampsia, even in cases defined as at high risk, by abnormal uterine artery Doppler velocimetry. Additionally, Lopez-Quesada et al. (2004) demonstrated no significant differences in plasma homocysteine concentrations of 94 pregnant women with respect to abnormal Doppler findings and the existence of obstetric complications. Moreover, the addition of homocysteine determination to uterine Doppler evaluation at 24 weeks of gestation did not improve its predictive value.

Finally, the current study has the limitations of having a relatively small number of subjects. The moderate positive correlation we found between the Doppler scores and occurrence of any pregnancy complication may be more powerful if analyzed with a greater number of subjects. However, the potential advantages of early screening of pregnancy complications may allow prophylactic interventions as soon as possible. Thus, it is necessary to identify women at risk at early stages of pregnancies. Developing new screening strategies to predict pregnancy complications by combination of Doppler parameters with other biochemical markers is an advantage for early detection of pregnancies at risk and these methods are further required in future studies.

Conclusion

Maternal serum homocysteine level increases in 11 to 14 weeks of gestations that are complicated with pre-eclampsia and preterm birth. The addition of homocysteine determination to uterine artery Doppler in the first trimester adds advantage in predicting adverse perinatal outcome.

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