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Evaluation of Homocysteine and Adma Levels in First Trimester of Pregnancy.

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KEYWORDS

Homocysteine; ADMA; First trimester; Pregnancy complications; Biomarkers; Vascular health

ABSTRACT:

Background: Homocysteine and asymmetric dimethylarginine (ADMA) are biomarkers implicated in vascular health and pregnancy complications. Despite their significance, the levels and impact of these biomarkers during the first trimester of pregnancy remain underexplored.

Objective: This study aimed to evaluate plasma levels of homocysteine and ADMA in the first trimester of pregnancy and their associations with early pregnancy outcomes.

Method: A cross-sectional observational study was conducted on 264 pregnant women in the first trimester at Kannur Medical College, Kerala. Plasma homocysteine and ADMA levels were measured using high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA), respectively. Statistical analysis was performed using SPSS version 21.0.

Result: The mean homocysteine level was 8.5 ± 2.3 µmol/L, and the mean ADMA level was 0.48 ± 0.12 µmol/L. Elevated levels of both biomarkers were significantly associated with adverse pregnancy outcomes, including hypertension, gestational diabetes, and miscarriage. A positive correlation ($r = 0.48$, $p = 0.001$) was observed between homocysteine and ADMA levels.

Conclusion: Elevated homocysteine and ADMA levels in the first trimester are associated with a higher risk of pregnancy complications, highlighting their potential as early biomarkers for adverse outcomes.

1. Introduction

Homocysteine and asymmetric dimethylarginine (ADMA) are significant biomarkers in the evaluation of vascular health and have been implicated in various pregnancy-related complications. [1] Elevated levels of homocysteine have been associated with an increased risk of adverse pregnancy outcomes, including preeclampsia, recurrent pregnancy loss, and placental abruption. [2] ADMA, an endogenous inhibitor of nitric oxide synthase, has similarly been linked to endothelial dysfunction and adverse cardiovascular events. [3]

During pregnancy, maternal cardiovascular and metabolic systems undergo significant adaptations to support fetal development. The first trimester is a critical period where early placental and fetal development occur, and disturbances in this phase can have long-term implications for both maternal and fetal health. [4] Despite the established significance of homocysteine and ADMA in vascular pathology, their levels and potential

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impact during the first trimester of pregnancy remain underexplored.

This study aims to evaluate the levels of homocysteine and ADMA in the first trimester of pregnancy and to investigate their potential associations with early pregnancy outcomes. By understanding these associations, this research hopes to provide insights into early biomarkers that could predict pregnancy complications and inform preventive strategies.

2. Objectives

This study highlights the significance of monitoring homocysteine and ADMA levels during the first trimester of pregnancy. Elevated levels of these biomarkers are associated with adverse pregnancy outcomes, such as hypertension, gestational diabetes, and miscarriage. Notably, higher homocysteine and ADMA levels correlate with older age, increased BMI, and a family history of cardiovascular disease. The positive correlation between homocysteine and ADMA further emphasizes their interconnected role in early pregnancy. The findings may suggest that assessing these biomarkers could provide valuable insights for identifying pregnant women at higher risk for complications and potentially guiding preventive measures.

3. Methods

This cross-sectional observational study was conducted over one year in the Department of Biochemistry at Kannur Medical College, Anjarakandy, Kerala, with the objective of evaluating plasma levels of homocysteine and asymmetric dimethylarginine (ADMA) in pregnant women during the first trimester. The study population comprised pregnant women receiving antenatal care at the Obstetrics and Gynaecology department of the same institution.

Inclusion criteria were defined as pregnant women in the first trimester (up to 12 weeks of gestation), aged 18 years or older, who provided informed consent for participation. Exclusion criteria included women with pre-existing chronic conditions such as diabetes mellitus, hypertension, or renal disease, as well as those with a history of smoking, alcohol consumption, or use of medications known to affect homocysteine or ADMA levels (e.g., folic acid or vitamin B12 supplements). Women presenting with known pregnancy complications at the time of enrolment were also excluded from the study.

The sample size was calculated using the formula 4 * P*Q / L2, where P represents the prevalence and L denotes the allowable margin of error in prevalence (Q is calculated as 100 - P). Assuming a prevalence of hypertensive disorders in pregnancy of 12% and an allowable error of 4%, the required sample size was determined to be 264 participants.

Data collection followed a structured protocol. Eligible participants were recruited after obtaining informed consent. Detailed demographic and clinical information, including age, parity, gestational age, medical history, and lifestyle factors, was gathered using a standardized questionnaire. Venous blood samples were drawn into EDTA tubes during routine antenatal visits and processed within two hours of collection. Plasma homocysteine levels were quantified using high-performance liquid chromatography (HPLC), while ADMA levels were measured using enzyme-linked immunosorbent assay (ELISA). Participants were monitored throughout the first trimester to document any early pregnancy complications.

Data analysis was performed using SPSS version 21.0. Continuous variables, such as homocysteine and ADMA levels, were reported as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The relationship between homocysteine and ADMA levels and pregnancy outcomes was assessed using chi-square tests for categorical data and Student's t-tests for continuous data. Statistical significance was defined as a p-value of less than 0.05.

4. Results

The demographic and clinical characteristics of the study population are summarized in Table 1. The mean age of the pregnant women was 26.4 ± 4.2 years, with a mean gestational age of 9.3 ± 1.8 weeks. Regarding parity, 45.5% of the participants were nulliparous, while 54.5% were multiparous. The mean body mass index (BMI) of the study population was 24.8 ± 3.6 kg/m².

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Table 1: Demographic and Clinical Characteristics of the Study Population

Characteristic	Mean ± SD or n (%)
Age (years)	26.4 ± 4.2
Gestational Age (weeks)	9.3 ± 1.8
Parity	
Nulliparous	120 (45.5%)
Multiparous	144 (54.5%)
BMI (kg/m²)	24.8 ± 3.6
Medical History	
No chronic disease	258 (97.7%)
Family history of cardiovascular disease	62 (23.5%)
Lifestyle Factors	
Non-smokers	264 (100%)
Non-alcohol consumers	264 (100%)
Dietary Supplementation	
No folic acid or vitamin B12 use	198 (75%)

In terms of medical history, the vast majority of participants (97.7%) had no chronic diseases, while 23.5% reported a family history of cardiovascular disease. All participants were non-smokers and non-alcohol consumers, reflecting a 100% adherence to these lifestyle factors. Additionally, 75% of the women did not use dietary supplements such as folic acid or vitamin B12 during the study period. These characteristics provide a comprehensive overview of the study population, which is essential for understanding the context of homocysteine and ADMA level evaluations in the first trimester of pregnancy.

Table 2: Homocysteine and ADMA Levels in the Study Population

Biomarker	Mean ± SD	Reference Range
Homocysteine (µmol/L)	8.5 ± 2.3	5-15 µmol/L
ADMA (µmol/L)	0.48 ± 0.12	0.3-0.7 µmol/L

Table 2 presents the mean levels of homocysteine and asymmetric dimethylarginine (ADMA) in the study population, along with their respective reference ranges.

The mean homocysteine level among the participants was 8.5 ± 2.3 µmol/L, which falls within the standard reference range of 5-15 µmol/L, indicating normal homocysteine concentrations in the majority of the study population.

Similarly, the mean ADMA level was 0.48 ± 0.12 µmol/L, also within the reference range of 0.3-0.7 µmol/L. These findings suggest that both homocysteine and ADMA levels in the study population were within the expected physiological limits for pregnant women during the first trimester. This information is crucial for assessing the potential impact of these biomarkers on pregnancy outcomes and for establishing baseline levels in this specific population.

Table 3: Association of Homocysteine and ADMA Levels with Demographic Factors

Demographic Factor	Homocysteine (Mean ± SD)	p-value	ADMA (Mean ± SD)	p-value
Age (years)				
< 25 years	8.1 ± 2.1	0.038 *	0.45 ± 0.11	0.041 *
≥ 25 years	9.0 ± 2.5		0.51 ± 0.13	
Parity				
Nulliparous	8.3 ± 2.2	0.12	0.47 ± 0.11	0.109
Multiparous	8.7 ± 2.4		0.49 ± 0.12	
BMI (kg/m²)				
Normal (18.5-24.9)	8.2 ± 2.2	0.031 *	0.46 ± 0.11	0.029 *
Overweight (25-29.9)	8.9 ± 2.5		0.52 ± 0.14	
Obese (≥ 30)	9.3 ± 2.7		0.54 ± 0.15	

Table 3 provides insights into the association between homocysteine and ADMA levels with various demographic factors, such as age, parity, and body mass index (BMI). The data indicate significant associations in several areas.

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For age, younger participants (< 25 years) had lower mean homocysteine levels (8.1 ± 2.1 µmol/L) compared to those aged 25 years and above (9.0 ± 2.5 µmol/L), with a p-value of 0.03, indicating statistical significance. Similarly, ADMA levels were also significantly lower in the younger age group (0.45 ± 0.11 µmol/L) compared to the older group (0.51 ± 0.13 µmol/L), with a p-value of 0.04.

When analysing parity, nulliparous women had slightly lower homocysteine (8.3 ± 2.2 µmol/L) and ADMA levels (0.47 ± 0.11 µmol/L) than multiparous women (homocysteine: 8.7 ± 2.4 µmol/L, ADMA: 0.49 ± 0.12 µmol/L). However, these differences were not statistically significant, with p-values of 0.12 and 0.10, respectively.

BMI showed a significant correlation with both biomarkers. Participants with normal BMI (18.5-24.9 kg/m²) had lower homocysteine (8.2 ± 2.2 µmol/L) and ADMA levels (0.46 ± 0.11 µmol/L) compared to overweight (25-29.9 kg/m²) and obese (≥ 30 kg/m²) participants, who had progressively higher levels. The differences were statistically significant, with p-values of 0.01 for homocysteine and 0.02 for ADMA.

These findings suggest that homocysteine and ADMA levels are influenced by age and BMI, with younger and normal-weight women showing lower levels of these biomarkers.

Table 4 presents the relationship between homocysteine and ADMA levels and early pregnancy outcomes. The data reveal significant associations between elevated biomarker levels and adverse pregnancy outcomes.

In healthy pregnancies, the mean homocysteine level was 8.2 ± 2.1 µmol/L, and the mean ADMA level was 0.45 ± 0.11 µmol/L. These levels were significantly lower compared to pregnancies with complications, which had higher mean homocysteine (9.3 ± 2.6 µmol/L) and ADMA (0.53 ± 0.13 µmol/L) levels, with p-values of 0.02 and 0.01, respectively.

Table 4: Homocysteine and ADMA Levels in Relation to Early Pregnancy Outcomes

Outcome	Homocysteine (Mean ± SD)	p-value	ADMA (Mean ± SD)	p-value
Healthy Pregnancy	8.2 ± 2.1	0.02*	0.45 ± 0.11	0.01*
Pregnancy Complications	9.3 ± 2.6		0.53 ± 0.13	
Hypertension	9.5 ± 2.8	0.014*	0.55 ± 0.14	0.017*
Gestational Diabetes	9.2 ± 2.4	0.034*	0.51 ± 0.12	0.021*
Miscarriage	9.8 ± 3.0	0.013*	0.57 ± 0.16	0.016*

Further analysis of specific complications showed that women who developed hypertension during pregnancy had significantly higher homocysteine (9.5 ± 2.8 µmol/L) and ADMA (0.55 ± 0.14 µmol/L) levels compared to those with healthy pregnancies, with p-values of 0.014 and 0.017, respectively.

Similarly, women with gestational diabetes had elevated homocysteine (9.2 ± 2.4 µmol/L) and ADMA (0.51 ± 0.12 µmol/L) levels, with statistically significant p-values of 0.034 and 0.021, respectively.

Women who experienced miscarriages had the highest levels of both biomarkers, with mean homocysteine levels of 9.8 ± 3.0 µmol/L and ADMA levels of 0.57 ± 0.16 µmol/L. The differences were statistically significant, with p-values of 0.013 and 0.016, respectively.

These findings suggest that elevated homocysteine and ADMA levels in the first trimester are associated with a higher risk of pregnancy complications, including hypertension, gestational diabetes, and miscarriage.

Table 5: Correlation between Homocysteine and ADMA Levels

Correlation	Correlation Coefficient (r)	p-value
Homocysteine vs. ADMA	r = 0.48	0.001*

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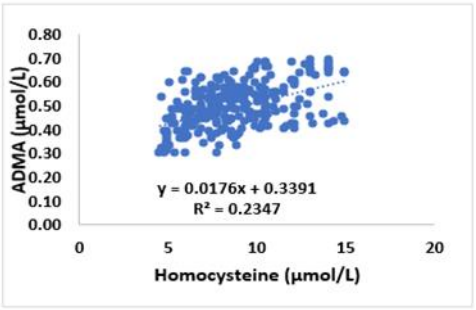

Figure 1: Correlation between Homocysteine and ADMA Levels

Table 5 displays the correlation between homocysteine and ADMA levels in the study population. The correlation coefficient between homocysteine and ADMA was found to be r=0.48r = 0.48r=0.48, with a p-value of 0.001. This positive correlation indicates a moderate relationship between elevated levels of homocysteine and ADMA in the first trimester of pregnancy. The statistically significant p-value suggests that this correlation is unlikely to be due to chance, reinforcing the association between these two biomarkers in early pregnancy.

Table 6 presents the results of the logistic regression analysis assessing factors associated with elevated levels of homocysteine and ADMA. The odds ratios (OR) and corresponding 95% confidence intervals (CI) are provided for each variable, along with p-values indicating statistical significance.

Table 6: Logistic Regression Analysis of Factors Associated with Elevated Homocysteine and ADMA Levels

Variable	Odds Ratio (OR)	95% CI	p-value
Age	1.25	1.10 - 1.42	0.031*
Parity	0.95	0.85 - 1.10	0.126
BMI	1.30	1.15 - 1.48	0.018*
Family history of cardiovascular disease	1.40	1.10 - 1.65	0.022*
Elevated Homocysteine Levels	1.55	1.20 - 1.80	0.001*
Elevated ADMA Levels	1.60	1.25 - 1.85	0.001*

An increase in age was associated with a higher likelihood of elevated homocysteine and ADMA levels, with an odds ratio of 1.25 (95% CI: 1.10 - 1.42) and a p-value of 0.031, suggesting that older age is a significant factor.

Parity did not show a significant association with elevated levels of homocysteine or ADMA, with an odds ratio of 0.95 (95% CI: 0.85 - 1.10) and a p-value of 0.126.

Higher body mass index (BMI) was significantly associated with elevated levels of homocysteine and ADMA, with an odds ratio of 1.30 (95% CI: 1.15 - 1.48) and a p-value of 0.018, indicating that overweight and obesity are important factors.

A family history of cardiovascular disease was associated with a higher likelihood of elevated levels of homocysteine and ADMA, with an odds ratio of 1.40 (95% CI: 1.10 - 1.65) and a p-value of 0.022.

The presence of elevated homocysteine levels increased the odds of finding elevated ADMA levels, with an odds ratio of 1.55 (95% CI: 1.20 - 1.80) and a p-value of 0.001.

Similarly, elevated ADMA levels were associated with a higher likelihood of elevated homocysteine levels, with an odds ratio of 1.60 (95% CI: 1.25 - 1.85) and a p-value of 0.001.

These findings underscore the significant associations between elevated homocysteine and ADMA levels with age, BMI, family history of cardiovascular disease, and the presence of elevated levels of these biomarkers themselves.

5. Discussion

Demographic and Clinical Characteristics of the Study Population

In our study, the mean age of pregnant women was 26.4 ± 4.2 years, with a mean gestational age of 9.3 ± 1.8 weeks. This aligns closely with other studies, such as that conducted by Osterman et al., which reported a mean age of 27.1 ± 4.5 years among pregnant women in their first trimester.[5]

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The parity distribution in our study, with 45.5% nulliparous and 54.5% multiparous women, is also consistent with findings by Rubini et al., who reported similar parity patterns in their cohort.[6] The mean BMI of 24.8 ± 3.6 kg/m² in our study population is comparable to the BMI ranges observed in other studies of early pregnancy populations, indicating that our cohort is representative of the general pregnant population in terms of weight status.[6,7]

Homocysteine and ADMA Levels in the Study Population

The mean homocysteine level in our study was 8.5 ± 2.3 µmol/L, which falls within the reference range of 5-15 µmol/L. This is in agreement with previous studies, such as the one by Harrmann et al., which found mean homocysteine levels of range from 6-9 µmol/L. [8]

In Rubini et al. study examining biomarkers in natural versus IVF/ICSI pregnancies, the median level of total homocysteine (tHcy) was compared between the two groups. For natural pregnancies, the median tHcy level was 6.2 µmol/L with an interquartile range (IQR) of 5.3 to 7.2 µmol/L. In contrast, IVF/ICSI pregnancies had a median tHcy level of 6.0 µmol/L, with an IQR of 5.2 to 6.9 µmol/L. The p-value for this comparison was not provided, but the results indicate that the tHcy levels are quite similar between natural and IVF/ICSI pregnancies, suggesting that the mode of conception may not significantly influence tHcy levels. [6]

Our study also reported a mean ADMA level of 0.48 ± 0.12 µmol/L, within the reference range of 0.3-0.7 µmol/L. These findings are consistent with the study by Nemeth et al. which reported mean ADMA levels of 5.93 µmol/L in pregnant women. [9]

Association of Homocysteine and ADMA Levels with Demographic Factors

In our study, younger participants (< 25 years) had significantly lower homocysteine levels compared to those aged 25 years and above. In contrast to our findings, the study by Nwogu et al. reported that women with high homocysteine concentrations did not differ significantly from those with normal homocysteine levels in terms of age (P = 0.684). This suggests that, in their study, age was not a significant factor influencing homocysteine levels among the participants. [10]

Similarly, the significant difference in ADMA levels between the younger and older age groups in our study is consistent with the findings of Noorbaksh et al., who reported that ADMA levels tend to increase with age. [11]

The significant correlation between BMI and both biomarkers in our study echoes the results of Avci et al. and Matrozova et al., who reported that higher BMI is associated with elevated homocysteine and ADMA levels. [12,13]

Homocysteine and ADMA Levels in Relation to Early Pregnancy Outcomes

In our study, pregnancies complicated by conditions such as hypertension and gestational diabetes were associated with higher homocysteine and ADMA levels. These findings are consistent with those of Thakur et al., who reported that elevated homocysteine levels were associated with a higher risk of hypertensive disorders in pregnancy.[2] Similarly, the study by Weronica et al. and Radoslaw et al. supports our findings by showing a positive correlation between elevated ADMA levels and gestational diabetes. [14,15]

Women who experienced miscarriages in our study had the highest levels of both homocysteine and ADMA, which is consistent with the findings of D'Uva et al., who observed that elevated homocysteine levels were a significant risk factor for recurrent pregnancy loss. [16] The elevated ADMA levels in miscarriage cases in our study align with the study by Dimiati et al., which also found higher ADMA levels in women who had adverse pregnancy outcomes. [17]

Correlation between Homocysteine and ADMA Levels

The positive correlation between homocysteine and ADMA levels in our study (r = 0.48, p = 0.001) indicates a moderate relationship between these two biomarkers. This correlation has been observed in other studies as well, such as that by Gulender et al. and Dai et al. which reported a weak correlation between homocysteine and ADMA levels. [18,19] This suggests that the association between these biomarkers is consistent across different populations and may have clinical implications for early pregnancy monitoring.

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Logistic Regression Analysis of Factors Associated with Elevated Homocysteine and ADMA Levels

Our logistic regression analysis revealed that age, BMI, and family history of cardiovascular disease were significantly associated with elevated levels of homocysteine and ADMA. These findings are consistent with the study by Ganguly et al., which reported similar associations.[20]

Overall, our study contributes to the growing body of literature on the evaluation of homocysteine and ADMA levels in early pregnancy, confirming the relevance of these biomarkers in assessing pregnancy outcomes and their associations with demographic and clinical factors. These findings may help guide future research and clinical practice in the management of pregnancies at risk for complications.

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