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Trimester-specific and postpartum reference ranges for homocysteine, vitamin B12, and folate levels: a prospective study

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ABSTRACT

Aims: Elevated homocysteine levels during pregnancy have been linked to various adverse outcomes, including gestational complications and congenital anomalies. Despite the significance of monitoring homocysteine, reference ranges specific to pregnancy remain poorly defined, particularly within diverse populations. This prospective study aimed to establish trimester-specific reference ranges for serum homocysteine, vitamin B12, and folate levels in a Turkish pregnant population and to evaluate the interrelationships among these biomarkers.

Methods: A total of 220 pregnant women were prospectively assessed across three trimesters and a postpartum period, categorized into four groups: first trimester (n=52), second trimester (n=51), third trimester (n=62), and postpartum (n=55). Serum levels of homocysteine, vitamin B12, and folate were measured, and correlations among these variables were analyzed. Statistical analyses of the collected data were conducted utilizing IBM SPSS Statistics version 25.0.

Results: The determined homocysteine reference ranges were 2.82-6.82, 1.08-7.82, 0.18-8.46, and 0.10-12.13 µmol/L for the first, second, third trimesters, and postpartum period, respectively. Significant negative correlations were observed between homocysteine and both vitamin B12 (r=-0.180, p=0.007) and folate (r=-0.374, p=0.001). Notably, age-adjusted homocysteine levels were significantly elevated in the postpartum group compared to all trimesters (p=0.001), while vitamin B12 levels were significantly lower in the postpartum period (p=0.001).

Conclusion: This study presents the results of a comprehensive investigation into the reference ranges for homocysteine, vitamin B12, and folate levels during pregnancy and postpartum in the Turkish population. The findings of this study underscore the critical importance of regular monitoring of these biomarkers for maternal and fetal health. It is also noted that elevated homocysteine levels are particularly pronounced in the postpartum period. The study concludes with a call for future research to investigate the associations of these biomarkers in more depth in different ethnic groups and geographical regions.

Keywords: Homocysteine, vitamin B12, folate, pregnancy, postpartum, reference intervals

INTRODUCTION

Homocysteine, a sulfur-containing amino acid, is integral to various metabolic pathways, particularly in the remethylation and transsulfuration processes influenced by vitamins B12, B6, and folate.[1] Dysregulation of homocysteine metabolism, which can lead to hyperhomocysteinemia, may result from genetic factors, such as mutations in the MTHFR gene, nutritional deficiencies, and lifestyle factors, including dietary habits and smoking.[2] Elevated serum homocysteine levels have been linked to a range of adverse health outcomes, including cardiovascular diseases and gestational complications, such as pre-eclampsia, recurrent pregnancy loss, and neural tube defects. The implications of homocysteine on maternal and fetal health highlight the necessity of monitoring its levels during pregnancy.[2,3]

Despite the recognized importance of homocysteine in pregnancy, reference ranges for serum levels remain inadequately defined, and current guidelines predominantly focus on the general population.[4] Fluctuations in homocysteine levels throughout gestation may be affected by physiological changes, such as elevated estrogen levels and modified metabolic processes, thereby necessitating trimester-specific reference ranges. Additionally, the interaction between homocysteine, vitamin B12, and folate during pregnancy is critical, as deficiencies in these vitamins can intensify hyperhomocysteinemia and present risks to both maternal and fetal health.[5-8]

To date, there has been a paucity of research focusing on homocysteine reference intervals specific to pregnant populations, particularly within the context of Turkiye. The objective of this study is to establish serum homocysteine reference ranges across the three trimesters of pregnancy and the postpartum period. Additionally, this research seeks to investigate the relationships between homocysteine levels and those of vitamin B12 and folate within this cohort. By providing normative data tailored to our population, this study aims to enhance the accuracy of hyperhomocysteinemia detection during pregnancy and improve its subsequent management.

METHODS

This prospective study was conducted at the Gynecology and Obstetrics Clinic of Ümraniye Training and Research Hospital between April 20, 2025 and May 20, 2025. The Clinical Researches Ethics Committee of Koşuyolu Specialized Training and Research Hospital approved this study (Date: 18.02.2025, Decision No: 2025/04/1039). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written and verbal informed consent was obtained from all patients prior to the commencement of the study. Detailed information regarding the study design and purpose was provided to the participants.

A total of 220 patients were recruited and classified into four groups based on their gestational periods: Group 1 (first trimester, n=52), group 2 (second trimester, n=51), group 3 (third trimester, n=62), and group 4 (postpartum period, n=55). Gestational age was determined using the last menstrual period and was subsequently confirmed through ultrasound measurements conducted during the first trimester. The first trimester was defined as encompassing weeks 1 to 13, the second trimester as weeks 14 to 27, and the third trimester as beginning at week 28 and continuing thereafter.

Exclusion criteria: Patients were excluded from the study if they had a body-mass index (BMI) of less than 18 kg/m^2, exhibited signs of inadequate nutrition, had a history of smoking, presented with known vitamin deficiencies, had personal or familial histories of deep vein thrombosis, were diagnosed with any chronic medical conditions, were in active labor, were experiencing multiple pregnancies, or had hypertension.

Blood samples were collected from all participants under standardized conditions. To minimize the effects of diurnal variation and nutritional status, venipuncture was performed between 8:00 and 10:00 AM after an overnight fast of at least 6 hours. Blood samples were obtained from the antecubital veins of the participants. Subsequent to venipuncture, the fasting blood samples were maintained on ice until processing, which occurred within 60 minutes. The samples were then subjected to centrifugation to isolate plasma, serum, and cellular components, with the serum being stored at -80°C until further analysis.

Biochemical analysis: Plasma homocysteine levels were quantified utilizing the DS30 Total Homocysteine Kit (Drew Scientific Inc., Dallas, TX, USA). Serum concentrations of vitamin B12 and folate were evaluated through chemiluminescence employing the BIODPC kit (Roche Modular E170, Roche Diagnostics Turkey, Istanbul, Turkiye). The reference ranges for these biomarkers were established as follows: 2.2-13.2 µmol/L for homocysteine, 126.5-505 pg/ml for vitamin B12, and 3.1-17.5 ng/ml for folate. The results were interpreted in accordance with these defined reference ranges.

Statistical Analysis

The data analyses of the collected data were conducted utilizing IBM SPSS Statistics version 25.0. To account for age-related variations, an age adjustment was implemented using the formula: age adj.=age-b*(x-agemean). For the comparison of quantitative data among groups, a One-way ANOVA was performed, followed by Bonferroni post hoc tests to identify specific differences. Furthermore, Pearson Correlation Analysis was employed to evaluate the relationships between variables. A significance level of 0.05 was established for all statistical tests.

RESULTS

Table 1 displays the correlation coefficients (r) and corresponding p-values (p) for the relationships among age, homocysteine, folate, and vitamin B12 levels. The analysis indicates a positive correlation between age and homocysteine (r=0.193, p=0.004). Conversely, age demonstrated negative correlations with folate (r=-0.097, p=0.150) and vitamin B12 (r=0.041, p=0.546). Additionally, homocysteine levels were found to be negatively correlated with both folate (r=-0.374, p=0.001) and vitamin B12 (r=-0.180, p=0.007). Furthermore, folate exhibited a positive correlation with vitamin B12 (r=0.177, p=0.009).

Table 1. Relationships between age, homocysteine, folate and vitamin B12

	Homocysteine		Folate		Vitamin B12	
	r	p	r	p	r	p
Age	0.193	0.004	-0.097	0.150	0.041	0.546
Homocysteine			-0.374	0.001	-0.180	0.007
Folate					0.177	0.009

p<0.05 was considered statistically significant

Descriptive statistics for age, homocysteine, folate, and vitamin B12 across various gestational periods are presented in Table 2. The mean age was higher in the third trimester (28.32±5.18 years) compared to the other groups; however, this difference did not reach statistical significance (p=0.063). Homocysteine levels exhibited significantly between the groups, with the postpartum group recording the highest mean (5.73±3.34 µmol/L), while both the second and third trimesters reported the lowest levels (4.41±1.79 µmol/L; p=0.006). Adjusted homocysteine values were also significantly elevated during the postpartum period (p=0.001). Folate levels were found to be lowest in the postpartum period (11.18±4.64 ng/ml), although this difference was not statistically significant (p=0.080). Furthermore, vitamin B12 levels demonstrated a significant decline in both the third trimester (200.88±88.64 pg/ml) and the postpartum period (158.45±41.41 pg/ml) when compared to the first trimester (299.24±119.40 pg/ml; p=0.001).

p<0.05 was considered statistically significant. Post-hoc comparisons: Homocysteine levels were significantly higher in the postpartum group compared with the first, second, and third trimesters (p<0.01). Vitamin B12 levels were significantly lower in the third trimester and postpartum groups compared with the first trimester (p<0.01).

The prevalence of elevated homocysteine levels was notably significant during the third trimester, recorded at 16.1% (p=0.006). In contrast, only one case of elevated levels was observed in the postpartum group, accounting for 1.8%. Folate levels remained within the normal range across all trimesters, with only 1.8% of postpartum patients classified as having low levels. Conversely, vitamin B12 deficiency was notably prevalent in the postpartum group, affecting 25.5% of individuals, as well as in the third trimester, where it was observed in 16.1% of cases (p=0.001).

Table 3 presents the reference ranges for homocysteine levels across all trimesters of pregnancy. The 95th percentile for homocysteine levels rose from 6.82 µmol/L during the first trimester to 12.13 µmol/L in the postpartum period. This trend suggests a gradual increase in reference values as pregnancy advances and persists into the postpartum period.

Table 3. Homocysteine level reference ranges

Percentages (%)	1st trimester	2nd trimester	3rd trimester	Post-partum
95	6.82	7.82	8.46	12.13
90	6.49	7.27	7.78	11.10
75	5.99	6.43	6.75	9.53
50	4.82	4.45	4.32	5.84
25	3.65	2.47	1.89	2.15
10	3.15	1.63	0.86	0.58
5	2.82	1.08	0.18	0.10

DISCUSSION

Our results demonstrated that the age-adjusted serum homocysteine levels postpartum were significantly higher than those recorded during any trimester. The reference ranges established in our study (2.82-6.82 µmol/L for the 1st trimester, 1.08-7.82 µmol/L for the 2nd trimester, 0.18-8.46 µmol/L for the 3rd trimester, and 0.10-12.13 µmol/L postpartum) provide crucial benchmarks that differ markedly from those reported in other studies, such as Yang et al.[1] and Mishra et al.[7] This discrepancy underscores the influence of geographical and dietary factors on homocysteine levels and suggests that local reference ranges are essential for accurate clinical assessment.[9]

The observed increase in homocysteine levels during the postpartum period is particularly concerning, as it may signify a risk for hyperhomocysteinemia. Previous research has indicated that elevated homocysteine levels are associated with adverse maternal health outcomes, including cardiovascular complications and impaired recovery in the postpartum phase.[10-13] Our findings underscore the necessity for ongoing monitoring of homocysteine levels beyond the perinatal period, especially in light of the concurrent decrease in vitamin B12 levels noted in our cohort. While folate levels remained stable, the negative correlation between folate and homocysteine highlights the essential role of folate in maintaining homocysteine homeostasis.[14-16]

One of the strengths of this study is its diverse population. Conducted at a tertiary referral hospital, which caters to patients from various backgrounds across the Marmara Region, the findings are likely to be generalizable to a broader demographic. This characteristic enhances the external validity of the results, rendering them applicable to a wide range of clinical contexts. Furthermore, the comprehensive design of the study, which assesses homocysteine, vitamin B12, and folate levels throughout all three trimesters and the postpartum period, offers valuable insights into the dynamic changes of these biomarkers during pregnancy. The prospective nature of the study further fortifies its methodology, as it facilitates real-time data collection, thereby minimizing recall bias and enhancing the reliability of the findings. Another notable contribution of this research is the establishment of localized reference ranges for homocysteine, vitamin B12, and folate levels. This development addresses a critical gap in the existing literature, providing a valuable

Table 2. Age, homocysteine, folate and vitamin B12 values according to trimesters and postpartum period

	1st trimester (n=52)	2nd trimester (n=51)	3rd trimester (n=62)	Post-partum (n=55)	p-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Age (year)	27.96±2.42	27.06±4.88	28.32±5.18	26.27±4.51	0.063
Homocysteine (micromol/L)	4.87±1.02	4.41±1.79	4.41±2.10	5.73±3.34	0.006
Homocysteine* (micromol/L)	4.82±1.06	4.45±1.72	4.32±2.11	5.84±3.21	0.001
Folate (ng/ml)	13.14±3.02	12.07±3.72	12.44±4.23	11.18±4.64	0.080
Vitamin B12 (pg/ml)	299.24±119.40	257.57±105.69	200.88±88.64	158.45±41.41	0.001

SD: Standard deviation, *: Adjusted with age resource for clinicians in the assessment of maternal health and the interpretation of laboratory results within the specific context of the studied population. The observed elevation in homocysteine levels and concurrent decline in vitamin B12 during the postpartum period suggest a potential window of vulnerability for maternal cardiovascular and metabolic health. These findings support the incorporation of postpartum biochemical screening, particularly for homocysteine and vitamin B12, into routine postnatal follow-up, especially in women with additional risk factors such as nutritional deficiencies, previous pregnancy complications, or cardiovascular disease. Targeted nutritional counseling and, where appropriate, supplementation may help optimize maternal recovery and mitigate long-term health risks.

Limitations

Despite its strengths, the study presents several limitations. A significant drawback is the absence of dietary data, which constrains the capacity to comprehensively assess the influence of nutritional intake on homocysteine and vitamin levels. This omission introduces potential confounding factors that may affect the observed outcomes. Another limitation of this study is the potential influence of diurnal rhythm and hydration status on homocysteine measurements. Although all samples were collected after a standardized fasting period and at a fixed time window in the morning to reduce variability, residual biological fluctuations cannot be fully excluded. Future studies could further control for these factors through repeated measurements across different times of day and with standardized hydration protocols. Furthermore, the study's single-center design may limit the diversity of the sample, thereby potentially restricting the generalizability of the findings to other regions or populations with distinct dietary habits and health profiles. Furthermore, the brief follow-up duration postpartum may not adequately capture long-term trends in homocysteine and vitamin levels, which are essential for comprehensively understanding maternal health outcomes. Additionally, although the sample size is adequate, an increased cohort size could enhance the robustness of the data and improve statistical power, thereby facilitating more nuanced analyses.

Several avenues for future research are apparent. Multi-center studies would provide a more diverse sample, improving findings on homocysteine dynamics across populations. Longitudinal follow-up of postpartum patients could also reveal long-term trends in homocysteine and vitamin levels, helping to identify potential health risks associated with these biomarkers. Incorporating dietary assessments into future studies would allow researchers to examine the relationship between nutritional intake and serum levels of homocysteine, vitamin B12, and folate, thereby elucidating the role of diet in maternal health. Interventional studies could also evaluate the impact of dietary or supplemental interventions on these biomarkers during pregnancy and postpartum to improve maternal health outcomes. Expanding the analysis to include additional biomarkers could offer a comprehensive view of the factors influencing pregnancy outcomes, leading to targeted interventions and enhanced clinical practices.

CONCLUSION

This prospective study reveals the dynamics of serum homocysteine levels during pregnancy and postpartum, challenging existing literature. While no significant differences were found across trimesters, postpartum homocysteine levels significantly increased compared to pregnancy. This indicates the need for monitoring beyond the perinatal period, as elevated postpartum levels may affect maternal health. The study provides localized reference ranges for homocysteine, vitamin B12, and folate, helping clinicians interpret lab results and guide nutritional interventions. The negative correlation between folate and homocysteine emphasizes the need for adequate folate intake during pregnancy.

ETHICAL DECLARATIONS

Ethics Committee Approval

The Clinical Researches Ethics Committee of Koşuyolu Specialized Training and Research Hospital approved this study (Date: 18.02.2025, Decision No: 2025/04/1039).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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