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Asthmatic Patients: Is Homocysteine an Issue?

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ABSTRACT

Objective: Understanding the causes of asthma is crucial for developing new therapeutic modalities. Homocysteine (HCY) is an intermediate in methionine metabolism. Elevated HCY levels may indicate folate and vitamin B12 deficiencies, which are cofactors for the methylation of HCY to methionine. The relationship between hyperHCY mia and atherosclerosis is well-documented, and it is considered a cause of cardiovascular, neurodegenerative, and ocular diseases. HyperHCY mia may also cause atopy and, consequently, asthma. We aimed to evaluate the levels of HCY, vitamin B12, and folic acid in asthmatic patients and healthy adults, as well as to determine whether correlations exist between these levels and lung function, eosinophil counts, total immunoglobulin E (IgE), and eosinophilic cationic protein (ECP) levels in asthmatic subjects.

Methods: A total of 142 asthmatic patients and 36 healthy controls were enrolled in the study. Folic acid, vitamin B12, total IgE, ECP, eosinophil percentage, eosinophil counts, and HCY levels were evaluated in both groups.

Results: HCY, vitamin B12, and folic acid levels did not significantly differ between patients with asthma and controls. There was a statistically significant positive correlation (at the 0.95 confidence level) between HCY values and forced vital capacity, peak expiratory flow (PEF), and eosinophil counts in patients with asthma. Folic acid levels correlated positively only with PEF%, whereas vitamin B12 levels did not correlate with any functional parameters or atopic markers like IgE and ECP.

Conclusion: Should large-scale randomized controlled trials conclusively establish HCY as a causative factor of asthma, metabolic interventions to lower HCY levels using methyl donors could be considered alongside conventional asthma treatments.

Keywords: Homocysteine, asthma, vitamin B12, folic acid, hyperhomocysteinemia, lung functions, spirometry

INTRODUCTION

Asthma is a common chronic disease, with a prevalence of 1-18% worldwide (1). Based on data primarily from studies using the European Community Respiratory Health Survey, the asthma prevalence in Türkiye is 1.2-9.4%, whereas asthma-like symptoms are estimated to be 9.8-27.3% among adults (2). According to the World Health Organization, in 2016, asthma was responsible for 24.8 million disability-adjusted life years worldwide and caused 417,918 deaths globally. These figures highlight asthma's significant burden as a non-communicable disease, particularly in low- and middle-income countries where over 80% of asthma deaths occur. Additionally, approximately 250,000 people die annually from asthma-related complications, emphasizing the need for better management and access to treatment (3).

Homocysteine (HCY) is an intermediate in methionine metabolism. Vitamin B_{12} and folate are cofactors of the conversion of HCY to methionine through methylation (4). Hyperhomocysteinemia can develop due to deficiencies in vitamins B_{12}, B_6, and folate, defects in enzymes involved in HCY metabolism, and lifestyle factors, such as smoking and alcohol consumption (5-7). Elevated HCY levels may be associated with atherogenesis, thrombosis, cardiovascular, neurodegenerative, and ocular diseases (5,8-11). Endothelial dysfunction is the primary cause of these conditions. Increased levels of reactive oxygen species and endothelial nitric oxide within the vascular structure trigger atherogenesis through this mechanism. Additionally, some studies have suggested that HCY may contribute to the pathogenesis of these diseases by affecting inflammatory and immune cells, particularly by impairing lymphocyte function (12,13).

Asthma is a chronic inflammatory disease in which oxidative stress plays a major role in its pathogenesis (14). During HCY methylation, S-adenosylmethionine and S-adenosylhomocysteine (SAH) are produced in balanced amounts. However, if this balance

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shifts in favor of SAH, it can lead to impaired lymphocyte DNA methylation and altered gene expression (15). The DNA of lung endothelial cells may be affected by hyperhomocysteinemia.

The present study aimed to determine whether elevated HCY levels or lower levels of folic acid and vitamin B_{12} are associated with asthma. We also investigated correlations between HCY, folic acid, and vitamin B_{12} levels and lung function test parameters, as well as total immunoglobulin E (IgE), eosinophil cationic protein (ECP), eosinophil counts/mm³, and eosinophil percentage, the latter four of which can be considered atopic markers.

METHODS

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Demiroğlu Bilim University Clinical Research Ethics Committee of Helsinki (number: 44140529/29489, date: 18.07.2023). This was a retrospective, observational, and cross-sectional study. Access to patient records has been granted with the permission of the relevant chief physician's office, and this information is included in the file submitted to the ethics committee.

All patients whose files were accessed were informed about the study content and provided consent to participate. The patients' personal information will not be published, and the details in the patient files will be carefully protected and will not be shared with third parties. All patients remain anonymous, and no personal data that could identify the patients is reported in this study.

Data related to the study topic were obtained using a retrospective case-control study design. A total of 142 asthmatic patients (Group I) and 36 healthy controls (Group II) who visited the outpatient clinic of the Internal Diseases Department at Fatih Sultan Mehmet Training and Research Hospital between December 2023 and May 2024 were enrolled in the study.

Inclusion criteria:

- Group I: Stable asthmatic patients, both male and female, aged 18-75 years, without additional metabolic or chronic diseases, with recorded levels of folic acid, vitamin B_{12}, total IgE, ECP, eosinophil percentage, eosinophil counts/mm³, HCY levels, and spirometric test results in their files.
- Group II: Non-asthmatic individuals without any additional metabolic or chronic diseases, with recorded levels of folic acid, vitamin B_{12}, total IgE, eosinophil percentage, and eosinophil counts/mm³.

Exclusion criteria:

- Individuals younger than 18 years or older than 75.
- Individuals with any additional metabolic or chronic diseases.
- Individuals with incomplete information in their files.
- Asthma exacerbation in Group I.
- Supplementary vitamin B_{12} or folic acid.

We evaluated folic acid, vitamin B_{12}, total IgE, ECP, eosinophil percentage, eosinophil counts/mm³, and HCY levels in patients with asthma (Group I). For control subjects (Group II), we evaluated folic acid, vitamin B_{12}, and HCY levels. All subjects with asthma had lung function test results. Spirometric analysis included the evaluation of forced vital capacity (FVC), FVC% predicted, forced expiratory volume in 1 second (FEV1), FEV1% predicted, FEV1/FVC ratio, (FEV1)/FVC% predicted, forced mid-expiratory flow (FEF) (FEF25-75%), FEF 25-75% predicted, peak expiratory flow (PEF), and PEF% predicted values.

Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics version 26.0. Quantitative (continuous) data were presented with descriptive statistics and prevalence measures. Kolmogorov-Smirnov test was used to assess normality.

For groups with a normal distribution, the Independent Sample t-test was used to compare the means of two independent groups (k=2). For groups that did not conform to a normal distribution, the Mann-Whitney U test was applied. Categorical data were presented using descriptive statistics, including frequency values and percentages. The chi-square test was used to examine relationships between variables, frequencies, or percentages when both variables were qualitative.

To investigate the strength and direction of the relationship between two variables, the Pearson correlation coefficient was used for variables that followed a normal distribution, whereas the Spearman's correlation coefficient was used for variables that deviated from linearity. Decisions were based on test statistics at a 95% confidence level. P values below the significance level of 0.05 were considered significant.

RESULTS

In Group I, 101 patients (56.7%) were female and 41 (23.0%) were male, with a mean age of 41±13 years. In Group II, 25 patients (14.0%) were female and 11 (6.2%) were male, with a mean age of 39.0±14.0 years. According to the Pearson chi-square test, there was no statistically significant difference in gender distribution between the two groups (p=0.843). Additionally, the independent sample t-test showed no significant difference in age distribution (p=0.371). The duration of asthma in Group I was 9.5±10.2 years. All patients in Group I had mild asthma according to the global strategy for asthma management and prevention guidelines (1), and they were on low-dose inhaled corticosteroid and beta-agonist combination treatment. None of the patients had taken oral steroids or experienced an asthma attack in the previous 3 months.

The data regarding age, gender, and smoking status of the participants are presented in Table 1.

The blood work results, including eosinophil counts/mm³, eosinophil percentage, total IgE, ECP, folic acid, vitamin B_{12}, and HCY levels, are presented in Table 2.

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In Group I, no significant differences were observed in HCY, folic acid, and vitamin B_{12} levels between smokers and nonsmokers with asthma (Table 3).

In Group I, folic acid levels did not correlate with eosinophil counts/mm³ (r=-0.067, p=0.451), eosinophil percentage (r=-0.034, p=0.713), total IgE levels (r=-0.034, p=0.713), or ECP levels (r=-0.034, p=0.850), according to Spearman's correlation test. However, among the lung function test parameters, only the PEF% correlated positively with the folic acid levels (r=0.196, p=0.025) (Figure 1).

In Group I, vitamin B_{12} levels were not correlated with eosinophil counts/mm³ (r=0.016, p=0.863), eosinophil percentage (r=-0.011, p=0.902), total IgE levels (r=0.046, p=0.620), or ECP levels (r=0.075, p=0.460) according to Spearman's correlation test. None of the lung function test parameters were correlated with vitamin B_{12} levels.

In Group I, HCY levels were not correlated with ECP levels (p=0.180) or total IgE levels (p=0.910). HCY levels were positively correlated with eosinophil counts/mm³ (r=0.175, p=0.042) and eosinophil percentage (r=0.198, p=0.022) (Figures 2 and 3).

HCY levels were positively correlated with FVC (r=0.182, p=0.034) and PEF (r=0.215, p=0.012) (Figures 4 and 5). Other lung function parameters were not correlated with HCY levels in Group I.

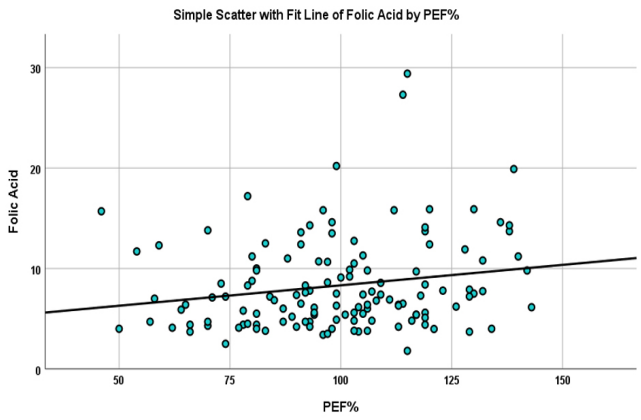

Figure 1. Correlation between folic acid levels with PEF%
PEF: peak expiratory flow

Table 1. Age, gender, and smoking status of the participants

	Group I (n=142)	Group II (n=36)	p-value
Mean age (years)	41±13	39±14	0.371*
Gender			0.843†
Female (n)	101 (56,7%)	25 (14%)	
Male (n)	41 (23%)	11 (6.2%)	
Smokers (n)	51 (28.2%)	11 (6.1%)	0.516†
Smoking duration (years)	4.8±7.9	3.7±7.1	0.381‡
Cigarettes/day	5±9	3±6	0.283‡

*Independent sample t-test, † Pearson's chi-square test, ‡ Mann-Whitney U test

Table 2. Laboratory test results of the groups

	Group I (n=142)	Group II (n=36)	p-value
Eosinophil count/mm³	246.9±204	181.4±108.4	0.108*
Eosinophil percentage (%)	3.73±2.58	2.33±1.28	0.001*‡
Total IgE (IU/mL)	139.75±186.38	9.15±5.89	0.002*‡
ECP (ng/mL)	27.29±23.95	N/A§	
Folic acid (ng/mL)	8.30±4.60	7.37±3.22	0.575*
Vitamin B12 (pg/mL)	413.5±160.6	386.7±178.7	0.256*
HCY (µmol/L)	10.16±3.04	11.08±3.38	0.115†

*Mann-Whitney U test, †Independent samples t-test, ‡statistically significant difference, § not available

Table 3. HCY, folic acid, and B_{12} levels in smokers and non-smokers with asthma

	Non-smoker asthmatic patients (n=88)	Smoker asthmatic patients (n=54)	p-value
Folic acid (ng/mL)	8.36±5.10	8.10±3.69	0.771*
Vitamin B_{12} (pg/mL)	410.2±150.8	435.4±174.4	0.435*
HCY (µmol/L)	9.83±2.99	10.86±3.52	0.064†

*Mann-Whitney U test, †Independent samples t-test

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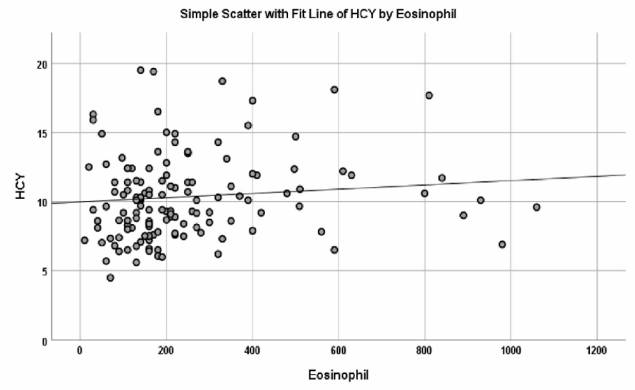

Figure 2. Correlation between HCY and eosinophil counts/mm^3
HCY: homocysteine, mm: milimetre

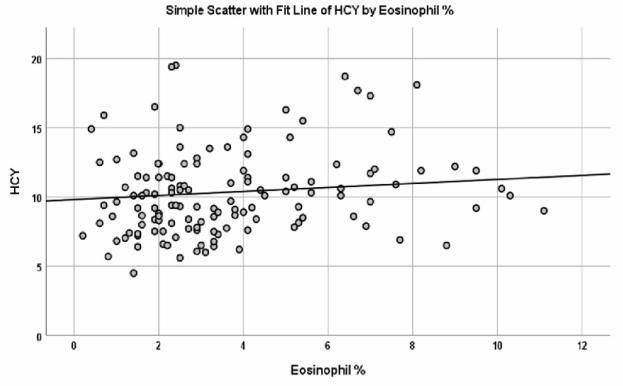

Figure 3. Correlation of HCY with eosinophil percentage
HCY: homocysteine

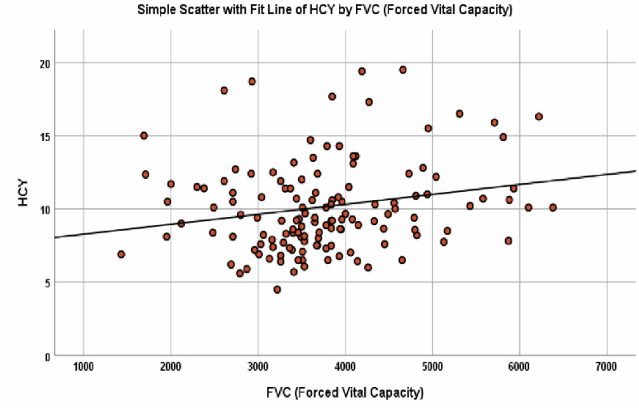

Figure 4. Correlation of HCY with FVC
HCY: homocysteine, FVC: forced vital capacity

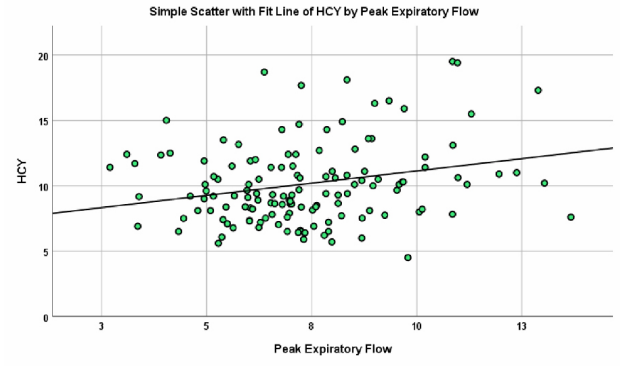

Figure 5. Correlation of HCY with PEF
HCY: homocysteine, PEF: peak expiratory flow

DISCUSSION

There are few studies in the literature that compare HCY levels and methyl donors, such as folic acid and vitamin B_{12}, in asthmatic patients with those in healthy individuals, and existing studies appear to yield conflicting results. In contrast to our findings, which indicate no difference in serum HCY levels between asthmatic and healthy individuals, Avci et al. (16) reported that HCY levels were significantly higher in asthmatic patients (10.01-30.70 µmol/L) compared with controls (7.22-12.39 µmol/L). Conversely, other studies suggest that HCY levels are significantly lower in patients with asthma compared with healthy non-asthmatic individuals (10). In their study, Abdel-Aziz et al. (15) evaluated asthmatic patients by dividing them into two groups: one with high airway bronchodilator reversibility (post-bronchodilator FEV1 ≥20%) and another with low bronchodilator reversibility. Patients in both asthmatic groups had significantly lower serum HCY levels than those in the healthy control group. Although not statistically significant, asthmatic patients with high airway bronchodilator reversibility had lower HCY levels than those with low bronchodilator reversibility. In our study, we did not subdivide the asthmatic group into high- or low-reversibility patients. Regarding IgE levels, Abdel-Aziz et al. (15) found similar serum IgE levels across all groups. Interestingly, they observed a negative correlation between HCY and IgE levels. In our cases, we did not detect a correlation between HCY levels and total IgE or ECP levels in patients with asthma. However, both eosinophil counts/mm^3 and eosinophil percentage were positively correlated with HCY levels. Additionally, we found no correlation between HCY levels and lung function parameters, except for PEF and FVC.

In a cross-sectional population-based study, Husemoen et al. (7) found no difference in plasma HCY levels between atopic and non-atopic individuals among 1,671 Danish participants aged 30-60 years. However, the methylene-tetrahydrofolate reductase (MTHFR) C677T polymorphism was more common among atopic

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individuals than among non-atopic individuals. Additionally, patients with the *TT* genotype appeared to be more atopic and had higher plasma HCY levels than those with the CT or *CC* genotypes. In the same study, researchers detected a correlation between asthma and the *TT* genotype, but not with pulmonary function tests. Similarly, Thuesen et al. (17) reported that the MTHFR C677T polymorphism was associated with asthma, but not with atopy or pulmonary function. Low serum folate levels were associated with an increased prevalence of self-reported physician-diagnosed asthma, but not with lung function or atopy. In a British cohort of mothers and their children, Granell et al. (18) found no correlation between the MTHFR C677T genotype and asthma or allergy.

Based on NHANES data, Matsui and Matsui. (19) demonstrated an inverse relationship between serum folate levels and IgE levels, as well as between serum folate levels and asthma or wheezing in the past year. In a study by Thuesen et al. (20), the MTHFR C677T polymorphism was not correlated with atopy, as defined by allergen-specific IgE or ECP levels, nor with pulmonary function. However, it was associated with asthma and dyspnea. In our asthmatic population, serum folate levels correlated only with the predicted PEF percentage and not with any other functional parameters.

Consistent with our findings, Thuesen et al. (17) found that serum vitamin B_{12} levels were not associated with asthma or atopy. We did not find any correlation between vitamin B_{12} levels and lung function parameters, eosinophil counts/mm³, eosinophil percentage, total IgE, or ECP levels. Skaaby et al. (21) also did not find evidence supporting a causal relationship between vitamin B_{12} and folic acid levels and asthma, atopic markers, or hay fever. However, they did detect a positive association between serum folic acid and IgE levels.

We discussed the role of vitamin B_{12} and folic acid as methyl donors in relation to HCY and noted that these vitamins can also influence the immune system. The mechanisms by which vitamin deficiencies affect immune function are described in great detail in a review by Wintergerst et al. (22). For example, vitamin B_6 affects lymphocyte maturation, antibody formation, and T-cell activity. In patients with vitamin B_6 deficiency, Th_1 cell activity is suppressed, whereas Th_2 type response emerges (23). Katunuma et al. (24) demonstrated that vitamin B_6 supplementation suppressed Th_2 responses and IgE production. In our study, we did not assess vitamin B_6 levels.

Conclusion

Although HCY appears to play a role in many inflammation-related diseases, its relationship with asthma and atopy is not fully understood. If randomized controlled trials with large patient populations can definitively establish HCY as a causative factor of asthma, metabolic interventions to reduce HCY levels using methyl donors may be considered alongside conventional asthma treatments.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Demiroğlu Bilim University Clinical Research Ethics Committee of Helsinki on (number: 44140529/29489, date: 18.07.2023).

Informed Consent: All patients whose files were accessed were informed about the study content and provided consent to participate.

Footnotes

Author Contributions: Surgical and Medical Practices - İ.K., A.G., M.E.; Concept - İ.K., A.G., M.E.; Design - İ.K., A.G., M.E.; Data Collection and/or Processing - İ.K., A.G., M.E.; Analysis and/or Interpretation - İ.K., A.G., M.E.; Literature Search - İ.K., A.G., M.E.; Writing - İ.K., A.G., M.E.

Conflict of Interest: The authors have no conflict of interest to declare.

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