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Assessment of Serum Folate, Vitamin B12, and Homocysteine Levels Among Blood Donors

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ABSTRACT
Background: *Folate, vitamin B12, and homocysteine are critical biomarkers influencing hematopoiesis and cardiovascular health. Deficiencies or imbalances in these micronutrients can adversely affect blood donors' health and their eligibility for donation. However, data on the status of these biomarkers among blood donors, particularly in South Asian populations, remains limited.*
Aim of the study: *To evaluate serum levels of folate, vitamin B12, and homocysteine among blood donors and examine the impact of demographic factors, dietary habits, and lifestyle behaviors on these biomarkers.*
Methods: *This cross-sectional study included 95 blood donors at BSMMU, Dhaka, Bangladesh from June 2017 to January 2018. Demographic data, smoking habits, physical activity levels, and dietary habits (specifically fruit and vegetable consumption) were collected through questionnaires. Biochemical analysis of serum folate, vitamin B12, and homocysteine levels was conducted. Statistical analysis was performed using SPSS, with significance set at $p<0.05$.*
Result: *The geometric mean serum folate level was 11.1 nmol/L, vitamin B12 247 pmol/L, and homocysteine 13 μmol/L. Serum folate levels were significantly higher among participants aged ≥50 years, females, and those consuming ≥4 portions of fruits and vegetables daily. Smoking was associated with significantly lower serum folate and elevated homocysteine levels. Physical activity showed a positive association with serum folate, though the relationship was less marked. Overall, a substantial proportion of participants had serum folate and vitamin B12 levels below established sufficiency thresholds, while homocysteine levels exceeded the normal range.*
Conclusion: *The study highlights suboptimal folate and vitamin B12 levels, with elevated homocysteine, among blood donors. Dietary habits, particularly fruit and vegetable intake, as well as smoking, were significant factors influencing these biomarkers. Public health interventions aimed at improving dietary intake and reducing smoking could improve micronutrient status and reduce associated health risks in blood donors.*
Keywords: *Folate, Vitamin B12, Homocysteine, Blood donors, Nutritional status, Smoking, Physical activity, Dietary habits*

I. INTRODUCTION

Folate (vitamin B9), vitamin B12, and homocysteine are essential nutrients that play critical roles in various physiological processes, including DNA synthesis, cell division, and red blood cell formation [1]. Folate, in particular, is vital for fetal development during pregnancy, with a deficiency often leading to neural tube defects [2]. Vitamin B12 is necessary for the production of red blood cells and the proper functioning of the nervous system [3]. Elevated homocysteine levels, often due to deficiencies in folate and vitamin B12, are known to be a risk factor for cardiovascular diseases and cognitive decline [4]. Globally, micronutrient deficiencies are a significant public health concern, with over two billion people affected by deficiencies in essential vitamins and minerals [5]. Specifically, vitamin B12 deficiency affects approximately 6% of the general population in high-income countries, with a higher prevalence among the elderly, reaching up to 20% in individuals over 60 years of age [6]. Elevated homocysteine levels, commonly linked to low folate and vitamin B12, are also prevalent, affecting approximately 30% of adults in certain populations, particularly those with poor dietary intake or genetic predisposition [7]. A study reported that folate deficiency affects approximately 17% of non-pregnant women, while vitamin B12 deficiency is present in around 15% of the population [8,9]. This is concerning, as deficiencies in these nutrients can lead to serious health consequences, including anemia, cognitive impairment, and increased susceptibility to cardiovascular diseases [7]. These deficiencies are also associated with elevated homocysteine levels, which have been increasingly recognized as a risk factor for several chronic conditions, particularly heart disease. Elevated homocysteine can damage blood vessels, leading to a higher risk of cardiovascular complications [10]. Blood donors are a particularly important group to study in the context of folate, vitamin B12, and homocysteine levels, as their health directly affects the quality and safety of the donated blood [11]. Blood donation is a critical component of healthcare systems globally, and maintaining a healthy donor population ensures the availability of safe blood for those in need [12]. However, donors may be at risk of nutritional deficiencies, particularly if they are regular contributors to blood donation programs. Frequent blood donation can deplete essential nutrients, such as iron, vitamin B12, and folate, increasing the risk of anemia and other health complications [13]. Deficiencies in folate, vitamin B12, and elevated homocysteine levels in blood donors could lead to adverse health effects, both for the donors and the blood recipients [14]. Previous studies have highlighted the importance of monitoring micronutrient levels in blood donors, as these deficiencies may compromise the quality of the donated blood and increase the risk of complications in both donors and recipients [15]. Despite the established link between these micronutrients and health outcomes, research focusing on the nutritional status of blood donors is limited. The aim of this study was to evaluate serum folate, vitamin B12, and homocysteine levels among participants.

II. METHODOLOGY & MATERIALS

This prospective observational study was conducted between June 2017 to January 2018, involving healthy blood donors at the Department of Transfusion Medicine Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The research aimed to assess serum folate, vitamin B12, and homocysteine levels among participants. A total of 95 blood donors participated in the study after obtaining the ethical approval of the ethics committee of BSMMU. All participants were selected based on specific inclusion and exclusion criteria with written informed consent before enrolment.

Inclusion Criteria

- Age: 18-60 years.
- Minimum weight: 50 kg.
- Minimum height: 5 feet.
- Haemoglobin level: Not less than 12.5 g/dl.
- At least 12 weeks interval between donations.
- Blood pressure: Not greater than 140/90 mmHg.
- Pulse rate: 50-100 beats per minute.
- Temperature: 36.6°C - 37.2°C.

Exclusion Criteria

- Donors who refused to give informed consent.
- Donors currently use B-vitamin supplements.

Data Collection

Participants were asked to complete a structured questionnaire covering medical history, lifestyle factors, and dietary habits. Dietary assessment focused on daily fruit and vegetable intake, categorized into four groups based on portion consumption. Smoking status was recorded, distinguishing between current smokers and non-smokers (including former smokers). Physical activity levels were classified on a scale from 1 (minimal) to 4 (high).

Blood Sample Collection and Laboratory Analysis

Blood samples were collected between 08:00 to 10:00 am after an overnight fast. These samples were transported to the designated laboratory within two hours of collection for processing. Serum and red blood cell (RBC) folate, along with serum vitamin B12 levels, were measured using a chemiluminescent microparticle immunoassay on an automated analyzer available in the laboratory. RBC hemolysates were prepared following the manufacturer's instructions to ensure accurate assessment. Plasma homocysteine levels were determined using a high-performance liquid chromatography (HPLC) method with fluorescence detection, which is commonly used in clinical and research laboratories. To maintain measurement accuracy, internal quality control samples were included in each batch of analysis, following standard laboratory protocols.

Definition of Adequate Biomarker Levels

Adequate biomarker levels were defined based on established guidelines to assess folate and vitamin B12 status. Serum folate levels greater than 15 nmol/L were considered sufficient, while serum vitamin B12 levels above 350 pmol/L were classified as adequate. Plasma homocysteine concentrations below 10 mmol/L were regarded as within the normal range. For red blood cell (RBC) folate, a level of ≥906 nmol/L was considered adequate for women of childbearing age, whereas a threshold of ≥305 nmol/L was used for other participants.

Statistical Analysis

Descriptive statistics were used to summarize the demographic characteristics of the study population. Continuous variables were presented as geometric means with 95% confidence intervals (CIs), while categorical variables were expressed as frequencies and percentages. For biomarker levels, geometric means and their respective 95% CIs were calculated for serum folate, serum vitamin B12, and homocysteine. Comparisons of hematologic parameters across different demographic and lifestyle factors were conducted using non-parametric tests due to the skewed distribution of the biomarkers. All statistical analyses were performed using SPSS software (version 26.0), with a significance threshold of $p<0.05$.

III. RESULT

The study included 95 participants, with the majority (34.74%) aged between 40-49 years, followed by 25.26% in the 30-39 age group, 22.11% aged 50 years or older, and 17.89% under 30 years. Males comprised 67.37% of the population, while females accounted for 32.63%. Regarding dietary habits, 40.00% of participants consumed one portion of fruits and vegetables per day, 29.47% consumed two portions, 23.16% consumed three portions, and 7.37% consumed four portions daily. Smoking habits showed that 28.42% of participants were smokers, while 71.58% were non-smokers. Physical activity levels varied, with 41.05% in category 1, 24.21% in category 2, 29.47% in category 3, and 5.26% in category 4 (Table 1). Table 2 showed that the biochemical analysis revealed a geometric mean serum folate level of 11.1 nmol/L, with a 95% confidence interval (CI) of 9.6–12.8 nmol/L. Serum vitamin B12 levels had a geometric mean of 247 pmol/L, with a 95% CI of 214–285 pmol/L. Homocysteine levels had a geometric mean of 13 mmol/L, with a 95% CI of 11.3–15.0 mmol/L. Serum folate levels varied across demographic and lifestyle factors. Participants aged ≥50 years had the highest serum folate (12.0 nmol/L), and homocysteine (14.8 nmol/L), while those under 30 years had the lowest levels. Females had higher serum folate (11.8 nmol/L), whereas males had significantly higher homocysteine (15.2 nmol/L) ($p<0.05$). Those consuming four portions of fruits and vegetables per day had the highest serum folate (16.8 nmol/L) and vitamin B12 (290 nmol/L), while those consuming one portion had the lowest levels ($p<0.01$). Smokers had significantly lower serum folate (9.5 nmol/L) and higher homocysteine (15.3 nmol/L) than non-smokers ($p<0.01$). Physical activity levels influenced these parameters, with category 4 showing the highest serum folate (15.3 nmol/L), while category 1 had the lowest levels and the highest homocysteine ($p<0.01$) (Table 3).

Table 1: Demographic characteristics of the study population (n=95)

Variables	Frequency (n)	Percentage (%)
Age (years)		
<30	17	17.89
30-39	24	25.26
40-49	33	34.74
≥50	21	22.11
Gender		
Male	64	67.37
Female	31	32.63
Fruit and vegetable consumption (portion/day)		
1	38	40.00
2	28	29.47
3	22	23.16
4	7	7.37
Smoking habit		
No	68	71.58
Yes	27	28.42
Physical activity		
1	39	41.05
2	23	24.21
3	28	29.47
4	5	5.26

Table 2: Serum folate, vitamin B12, and homocysteine of study participants

Parameters	Geometric mean	95% CI
Serum folate (nmol/L)	11.1	9.6-12.8
Serum vitamin B12 (pmol/1)	247	214-285
Homocysteine (mmol/1)	13	11.3-15.0

Table 3: Comparison of hematologic parameters according to sex, age, fruit and vegetable consumption, smoking habit and physical activity (Geometric means and confidence intervals)

Variables	Serum folate (nmol/L), Mean (95% CI)	Serum vit. B12 (pmol/1), Mean (95% CI)	Homocysteine (mmol/1), Mean (95% CI)	P-value
Age (years)				
<30	9.0(7.9-10.3)	233(213-254)	13.3(12.0-14.8)	<0.01
30-39	11.0(9.9-12.3)	240(220-262)	13.7(12.6-15.0)	<0.05
40-49	11.2(10.2-12.4)	256(237-277)	14.0(13.0-15.1)	<0.05
≥50	12.0(10.6-13.6)	245(220-275)	14.8(13.6-16.2)	<0.05
Gender				
Male	10.4(9.8-11.1)	243(229-257)	15.2(14.5-16.1)	<0.05
Female	11.8(10.6-13.2)	247(229-267)	11.7(11.1-12.3)	<0.05
Fruit and vegetable consumption (portion/day)				
1	9.6(8.9-10.5)	241(224-259)	14.8(13.7-16.0)	<0.01
2	10.3(9.3-11.3)	247(227-270)	14.1(12.9-15.2)	<0.05
3	12.6(11.1-14.4)	238(216-263)	13.5(12.7-14.3)	<0.05
4	16.8(14.1-20.0)	290(254-332)	11.3(10.0-12.7)	<0.05
Smoking habit				
No	11.2(10.5-12.0)	250(237-264)	13.3(12.8-14.1)	<0.01
Yes	9.5(8.5-10.7)	231(212-251)	15.3(13.9-16.8)	<0.01
Physical activity				
1	10.2(9.4-11.2)	244(226-263)	14.3(13.2-15.5)	<0.01
2	10.6(9.6-11.8)	235(214-258)	13.8(12.6-15.1)	<0.05
3	11.5(10.4-12.7)	251(231-273)	13.8(12.9-14.7)	<0.05
4	15.3(10.1-23.1)	255(217-299)	12.1(10.2-14.3)	<0.05

IV. DISCUSSION

Folate, vitamin B12, and homocysteine play crucial roles in hematopoiesis and cardiovascular health. Deficiencies or imbalances in these biomarkers can impact blood donors' overall health and donation eligibility. This study aims to assess the serum levels of folate, vitamin B12, and homocysteine among blood donors to evaluate their nutritional status and potential health implications. Our findings showed that most participants were young males, with low fruit and vegetable consumption and a moderate prevalence of smoking (28.42%). These factors are known to influence micronutrient levels and homocysteine metabolism. Our results align with previous studies showing a higher proportion of males in blood donation populations [16] and the adverse effects of smoking on serum vitamin B12 and homocysteine levels [17]. Additionally, low fruit and vegetable intake, as observed in our cohort, has been linked to lower folate levels and elevated homocysteine [18]. Physical activity, though beneficial for overall health, showed no clear direct association in this study, possibly due to a small proportion of highly active individuals. Our study found that the geometric mean serum folate concentration was 11.1 nmol/L (95% CI: 9.6–12.8 nmol/L), which falls below the established sufficiency threshold of 15 nmol/L. This observation is consistent with findings from a study conducted by Refsum et al, where a substantial proportion of individuals exhibited serum folate deficiency, with levels below 10 nmol/L [19]. The suboptimal folate levels in our study may be attributed to dietary insufficiency, as a significant proportion of participants consumed only 1-2 portions of fruits and vegetables daily. Low RBC folate is concerning, as it is a more reliable marker of long-term folate status than serum folate and is crucial in preventing neural tube defects and other health complications. Serum vitamin B12 levels in our study (geometric mean: 247 pmol/L, 95% CI: 214–285 pmol/L) were also below the adequate threshold of 350 pmol/L. This aligns with previous reports from South Asia. A study by Allen (1994) found that 55% of the study participants had serum vitamin B12 levels below 200 pmol/L, indicating a high prevalence of deficiency [20]. Vitamin B12 deficiency in South Asia is often linked to low animal-source food consumption and inadequate dietary intake of fortified foods. Elevated homocysteine levels are a well-recognized consequence of B-vitamin deficiencies, particularly folate and vitamin B12 insufficiency [21]. In our study, the geometric mean plasma homocysteine level was 13 mmol/L (95% CI: 11.3–15.0 mmol/L), exceeding the normal range (<10 mmol/L). Other study reported that South Asians are more likely to have elevated homocysteine levels than other ethnic groups. This is due to a number of factors, including low levels of vitamin B12, folate, and vitamin B6, as well as lifestyle factors like smoking and alcohol consumption [22]. Elevated homocysteine is a significant risk factor for cardiovascular diseases and warrants attention in public health nutrition policies [23]. Our results indicate a positive correlation between age and serum folate levels, with individuals aged ≥50 years exhibiting the highest mean serum folate (12.0 nmol/L; 95% CI: 10.6-13.6). This trend aligns with findings from Selhub et al, who reported increased serum folate levels with age in a population-based study [24]. However, our study also observed an age-related rise in homocysteine levels ($p<0.05$), consistent with Jacques et al, who attributed this trend to decreased renal function and vitamin B12 absorption efficiency in older adults [25]. Females exhibited significantly higher serum folate levels (11.8 nmol/L; 95% CI: 10.6-13.2) and lower homocysteine levels (11.7 nmol/L; 95% CI: 11.1-12.3) compared to males (10.4 nmol/L and 15.2 nmol/L, respectively, $p<0.05$). These findings are in agreement with Pfeiffer et al, who reported that estrogen may contribute to higher folate concentrations in females [26]. Individuals consuming ≥4 portions per day had the highest folate levels (16.8 nmol/L; 95% CI: 14.1-20.0) and the lowest homocysteine levels (11.3 nmol/L; 95% CI: 10.0-12.7, $p<0.05$). This is consistent with Green et al, who demonstrated that diets rich in folate-containing foods improve folate status and reduce homocysteine [27]. Smokers exhibited significantly lower serum folate levels (9.5 nmol/L; 95% CI: 8.5-10.7) and elevated homocysteine levels (15.3 nmol/L; 95% CI: 13.9-16.8, $p<0.01$), supporting previous studies demonstrating the adverse impact of smoking on folate metabolism. O'Callaghan et al found that smokers have reduced folate bioavailability due to oxidative stress and increased folate catabolism [17]. Physical activity demonstrated a positive association with folate status and a negative correlation with homocysteine levels. Participants with the highest activity level exhibited the highest serum folate (15.3 nmol/L; 95% CI: 10.1-23.1) and the lowest homocysteine (12.1 nmol/L; 95% CI: 10.2-14.3). Overall, our findings are consistent with previous studies that have highlighted the prevalence of folate and vitamin B12 deficiencies among populations in South Asia [28]. Many studies have investigated in different countries the B-vitamins status and Hcy concentration but, unfortunately, the results obtained were often hard to compare because of differences in cut-off used, dietary habits, lifestyle, presence or absence of food fortification etc.

Limitations of the study: Every hospital-based study has some limitations and the present study undertaken is no exception to this fact. The study was cross-sectional, meaning it cannot establish causal relationships between lifestyle factors and serum biomarkers. Additionally, the data on dietary intake was based on self-reported information, which could be subject to recall bias. The study also did not account for potential confounding factors such as genetic variations or other health conditions that could influence micronutrient levels. Finally, the sample lacked diversity in terms of age, gender, and physical activity levels, which may affect the ability to generalize results to different demographics.

V. CONCLUSION AND RECOMMENDATIONS

Our study found suboptimal serum folate and vitamin B12 levels and elevated homocysteine among blood donors, with dietary habits, smoking, and physical activity playing significant roles. The low intake of fruits and vegetables, especially among smokers, was associated with poorer micronutrient status. These deficiencies are concerning given their links to cardiovascular risks. While age and gender also influenced folate and homocysteine levels, the findings highlight the importance of dietary improvements, particularly increasing fruit and vegetable consumption, to optimize these biomarkers. Further research is needed to explore broader populations and evaluate public health interventions like food fortification.

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