"Comparative Analysis of Serum Vitamin B12 and RBC Folate in Relation to Gender among Healthy Individuals."

Running Head: Micronutrient in Relation To Gender in Healthy Individuals

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Author's contribution: IN and SN created the concept and design, while MW and MAA collected and analysed the data. MM and ZQ interpreted and wrote the manuscript. The manuscript was revised critically, and the final version was approved by all authors. All of the authors accept responsibility for the published article.

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Conflicts of interest:

There was no conflict of interest among the authors.

***ABSTRACT*:**

Background: Vitamin B12 and folate are important micronutrients whose deficiencies are linked to a variety of complications. Gender differences in these illnesses are evident in epidemiologic data**.**

Methodology: A cross-sectional study was conducted to investigate and compare the serum vitamin B_{12} and red cell folate levels and hematological parameters among healthy males and females. Data from medical charts were retrieved from healthy individuals aged 4–72 who received a routine medical evaluation in 2017–2018. Individuals with a history of illness, as well as those who had used medications or nutritional supplements, were barred from participating. The levels of vitamin B12 and red cell folate were measured using Cobas electrochemiluminescence e 411 analyzer and hematological parameters were measured using Sysmex XP100 Tokyo, Japan.

Results: In total, 240 people met the inclusion criteria. There was a statistically significant difference in serum vitamin B12 (pg/ml) levels between males (mean=239.48 pg/ml, SD=52.40) and females (mean=258.35 pg/ml, SD= 67.26); (p= 0.017). Mean RBC folate levels were significantly higher in males (549.11ng/ml±54.94) than females (528.77ng/ml±50.26); (p = 0.003).

Conclusions: Gender discrimination is observed in levels of vitamin B12 and red cell folate should be taken into consideration. Whereas men are more vulnerable to vitamin B12 deficiency as compared to women. It might be explained on basis of hormonal or nutritional effect. Genetic factors can account also for this.

***Index Terms*: RBC folate; serum vitamin B12; gender; healthy

INTRODUCTION:

Folate and vitamin B12 are both important micronutrients with quite similar functions, and their deficiency is a global health issue that affects individuals of all ages and genders. They both take part in one-carbon metabolism, including the remethylation of homocysteine to methionine, as well as DNA synthesis (1, 2). As a result, their deficiency can result in impaired one-carbon metabolism and elevated plasma homocysteine level, both of which have been linked to a variety of complications including cardiovascular disease, cancer, and cognitive decline. Decreased amounts of these vitamins have also been linked to congenital malformations and neurodegenerative problems (3). More serious deficiencies of these two micronutrients result in hematological disorders such as anemia, leukopenia, and thrombocytopenia (4). Because vitamin B12 and folate share a common metabolism, an imbalance in one affects the function of the other (5).

Vitamin B12 deficiency is more common in individuals with a reduced nutritional consumption of animal sources, as well as in individuals who have gastrointestinal conditions that cause vitamin B12 malabsorption (such as atrophic gastritis in the elderly) (6). On the other hand, low dietary intake, malabsorption (e.g., Crohn's disease, celiac disease), adverse effects from medications (e.g., methotrexate, anticonvulsants, etc.), increased demand without high intake (e.g., during pregnancy and breast-feeding), and vitamin B12 deficiency all contribute to folate deficiency (7). In addition, a few genetic polymorphisms have also been found to affect folate and Vitamin B12 levels (8).

Both vitamins are essential for central nervous system activity in individuals of different age and gender. They are needed for proper neurological development, and deficiencies increase the risk of cognitive decline, cognitive impairment, and aggravated behavioral disturbances, particularly in neurodegenerative dementia such as Alzheimer's disease and vascular dementia. Previous studies reported significant evidences of the contribution of folate and vitamin B12 in neurological function (9, 10). Therefore, use of folate and vitamin B12 dietary supplements may result in a slower rate of cognitive impairment and improved cognitive performance (11, 12).

The influence of gender in morbidity has been extensively debated, and it is presumed to result from both cultural and biological factors (13). Male have a higher risk of cardiovascular events, particularly acute coronary syndrome (14). However, women have been shown to be at a slightly higher risk of cognitive impairments and dementia (15-17). Low folate levels in the blood were linked to cardiovascular disease, neural tube defects, and certain cancers (18). Therefore, the relationship between vitamin B12 and folate deficiency and their role in both the genders is still debatable.

The goal of the cross-sectional study in the context mentioned above was to evaluate and compare RBC folate and vitamin B12 levels, as well as the impact of gender on these parameters.

MATERIAL AND METHODS:

It was a cross-sectional study that was conducted from May 2017 to April 2018. The research was carried out at Baqai Medical University Karachi. The samples were taken from 240 reportedly healthy participants from Gadap region of Karachi. The participants were divided into three age categories: children (n = 50), ages 4 to 12, adults (n = 118), ages 13 to 55, and the elderly (n = 72), ages 56 to 72. Each age group was then subdivided into two gender groups: male and female. Before signing the written consent, all of the participants were informed about the conduction of research in detail. The Ethics Committee of Baqai Medical University in Karachi approved the study (Reference No. BMU-EC/2017-04).

A detailed history was taken in order to determine the participant's health status. Age, gender, height, weight, medical history, smoking, use of multivitamin supplements, and gestational stage were all taken into consideration. Use of Supplements, alcohol, proton pump inhibitors, malabsorption syndrome, kidney diseases, smoking, metabolic disorders, liver disease, cancers, hemorrhage, recent transfusion, gastrointestinal surgery, any debilitating illness, pregnancy, lactation, and strict vegetarians were all excluded from the study. "A food frequency questionnaire (one pen-and-paper form) was used to assess dietary intake, including food and beverages."

Fasting venous blood samples of 10mL were taken following aseptic procedures. A similar volume of blood was transferred to yellow top vacutainers comprising of separating gel (for serum vitamin B12 assay) and purple top vacutainers comprising Ethylenediaminetetraacetic acid (for RBC folate and CBC). The specimens were placed cold and light-protected. Within 1 hour of collection, serum was derived by centrifugation (2000 rpm, 10 min) and transferred to aliquots, which were stored at – 20°C until analysed. Whole blood samples were kept at 4°C for 2 hours before being analysed. The automated cell analyzer Sysmex XP100 Tokyo, Japan, was used to assess the complete blood count (CBC) of all samples (19). The defined cut-off values of serum vitamin B12 for vitamin B12 deficiency were <150 pmol/L (<203 pg/mL) and for marginal vitamin B12 status (insufficiency) 150–221 pmol/L (203–299 pg/mL). The cut-off point of red blood cell folate level for folate deficiency was < 340 nmol/L (< 151 ng/mL) (20).

The statistical analysis was accomplished by using Statistical Package for Social Sciences (SPSS) version 20.0. Data was compiled as mean ± standard deviation. Independent t test and 1 way analysis of variance (ANOVA) with post hoc test was applied to examine the differences among the groups. Association of parameters was determined by Pearson correlation coefficient. P value of <0.05 was considered significant for all of the analyses.

RESULTS:

A total of 240 individuals were included. The study participants' characteristics were shown in Table 1. Mean age (years) of males (n=128) and females (n=112) was 40.5± 20.9 and 34.68± 20.96, respectively.

Table 1: Baseline characteristics of study population

Variables	Measure of the difference	Males (n = 128)	Female (n = 112)	p- value
Age (y)	Mean ± SD	40.5 ± 20.88	34.68 ± 20.96	0.03
Serum Vitamin B_{12} (pg/mL)	Mean ± SD	239.48 ±52.40	258.35 ± 67.26	0.017
Red Cell Folate (ng/mL)	Mean ± SD	549.11 ± 54.94	528.77 ± 50.26	0.003

SD, standard deviation; Differences between groups (male and female) were assessed by using an independent t test (p<0.05, significant).

Serum Vitamin B12:

- In terms of gender differences in serum vitamin B12 levels, there was a statistically significant difference in serum vitamin B12 (pg/ml) levels between males (mean=239.48 pg/ml, SD=52.40) and females (mean=258.35 pg/ml, SD= 67.26); (p= 0.017) as shown in **Table 1**.
- The participants' serum vitamin B12 and red cell folate levels comparison was done according to defined cut off values as shown in Table.2. The levels of vitamin B12 were significantly associated with gender of participants (p=<0.001) where participants with vitamin B12 deficiency (<203 pg/ml) were more likely to be male than females. Vitamin B12 deficiency was found to be in 33.3% of all participants, which was significantly higher in males (39.1%) than in females (26.8%).

- A significant negative correlation of serum vitamin B_{12} levels with age of female participants was demonstrated (r = - 0.356, p<0.001).
- Female participants (n=22) in the 4-12 year old age group had significantly lower serum vitamin B12 levels than females (n=60) in the 13-55 year old age group (p<0.001).
- Serum vitamin B12 concentrations in females aged 13-55 years were significantly higher than in females aged 56-72 years (n=30) and 4-12 years (p<0.001).
- Male populations in 4-12 years old (n=28) and 56-72 years old groups (n=42) had significantly lower levels of serum vitamin B_{12} levels than 13-55 years old male subjects (n=42) (p<0.001).
- A one-way analysis of variance (Post Hoc test; scheffe) was used to compare serum Vitamin B12 levels among male and females across all three age groups (p<0.05, significant).

Red Cell Folate:

- Gender differences in red cell folate levels revealed significant differences levels between males and females irrespective of the age (p-0.003).
- The red cell folate levels of the participants were compared using the cut off values shown in Table.2. Participants with red cell folate deficiency (151 ng/ml) were not found in either gender. However, when the other cut off value were used <450ng/ml and >450 mg/ml, both male and female results were nearly identical.
- Males (n=58) in the age group 13-55 years had significantly higher RBC folate concentrations than females (n=60) in the same age group (p<0.001).
- Similarly, males (56-72 years old, n=42) had significantly higher mean red cell folate levels than females (n=30) in the same age group (p<0.001).
- A one-way analysis of variance (Post Hoc test; scheffe) of Red cell folate levels revealed no significant difference between males of all three age groups and females of all ages.

Table 2. Comparison of Serum vitamin B12 and RBC folate according to cut off values among males and female

		Serum vitamin B12(pg/ml)			Red cell folate(ng/ml)		
Gender	**Values**	**Deficiency <203**	**Insufficiency 203-299**	**Normal >299**	**Deficiency <151**	**<450**	**>450**
Female (n=112)	**Count** **%**	30 26.8	42 37.5	40 35.7	Nil	5 4.5	107 95.5
Male (n=128)	**Count** **%**	50 39.1	60 46.9	18 14.1	Nil	6 4.7	122 95.3
Total (n= 240)	**Count** **%**	80 33.3	102 42.5	58 24.2	-	11 4.6	229 95.4

Table 3: Serum vitamin B12, Red cell folate and Hematological levels by gender and age group

Variable	Mean ± S.D	p-value
Serum Vitamin B12 (pg/ml)		
• 4-12 years		
Male	215.5 ± 39.1	
Female	224.0 ± 46.9	0.487
• 13-55 years		
Male	280.9 ± 44.7	0.028
Female	302.0 ± 57.5	
• 56-72 years		
Male	198.1 ± 11.5	
Female	196.1 ± 11.8	0.475
Red Cell folate		
• 4-12 years		
Male	520.0 ± 57.5	
Female	525.9 ± 47.5	0.319
• 13-55 years		
Male	559.5 ± 45.5	
Female	525.8 ± 47.2	<0.001
• 56-72 years		
Male	545.6 ± 53.7	
Female	520.1± 49.8	<0.001

DISCUSSION:

According to the findings, Vitamin B12 deficiency is relatively common among the healthy population in Gadap region of Karachi. The survey discovered that gender influences vitamin B12 metabolism, with males having a higher risk of deficiency, independent of anemia, folate, and homocysteine levels. The difference in the vitamin B12 deficiency between both the gender showed comparable results to that reported in a neighboring Middle Eastern country (21). High prevalence of Vitamin B12 deficiencies in our population could be due to poor dietary habits, and overcooked food. According to Barghouti et al, 44% of the individual were vitamin B12 deficient. They observed no significant difference between male and female (22).

The current study included the healthy population, therefore it is not likely that an underlying significant disease have facilitated the link between male and vitamin B12 deficiency (23). And in females menopause is nearly inevitable at the age of 55, therefore, estrogen may protect against vitamin B12 deficiency in young adults (24) as observed in our study.

This study did not find a gender difference in extreme age, which could be due to vitamin B12 deficiency increasing with age in both genders (2).

According to cut- off values for vitamin B_{12} deficiency (<203 pg/mL) and insufficiency (203–299 pg/mL), we observed 33.3% vitamin B12 deficiency in of all participants, which was significantly higher in males (39.1%) than in females (26.8%). The two major concerns of low Vitamin B12 levels, particularly in developing countries, are insufficient vitamin B12 absorption and reduced dietary intake (25). Food derived from animals is the primary source of vitamin B12. Many factors contribute to low meat, milk, and milk product consumption, including extreme ages (especially in children and the elderly), cost, inadequacy, socioeconomic status, moral and ethical values, and religious beliefs. On the other hands, conditions that impede vitamin B12 absorption, such as gastrectomy, gastric bypass, atrophic gastritis (26, 27), H. Pylori infections (26), and long-term use of antacids (28), also cause B12 deficiency. Because our study population was healthy, the aforementioned condition may not have explained the observed results

Our findings revealed a significant gender difference in RBC folate levels (Table 1); males had significantly higher folate levels than females (p=0.003). Other studies found similar results (2, 29). In our study, we also looked at folate levels in different age groups divided by gender. We discovered that adult and elderly males had higher folate levels (p 0.001, p= 0.028, respectively) than females of the same age groups. This could be due to increased folic acid requirements in females of reproductive age, such as during pregnancy and lactation. It has been proposed that approximately two-thirds of hyperhomocysteinema cases are caused by insufficient blood levels of one or more of these vitamin cofactors (8). Furthermore, Chambers et al. investigated the discrepancies in plasma hyperhomocysteinema (tHcy) levels between Europeans and Asians by comparing plasma vitamin B12 and folate concentrations. Thus, decreased folate and vitamin B12 levels in male participants may also make a contribution to the sex difference in plasma tHcy (30). However, contrary to our findings, Wahlin et al. and Beckett et al. found no gender discrimination in folate levels in their healthy population (31, 32).

Vitamins are required for cell development and growth. Their normal value within the body will aid in the maintenance and performance of the body. Because each process is interconnected, increased or decreased micronutrient intake disrupts the entire process (33). Furthermore, gender differences may be caused by differences in metabolic processes, hormonal effects, and genetic makeup (34).

CONCLUSION:

Vitamin B12 and red cell folate levels differed significantly by gender in the healthy population. Males were found to have more vitamin B12 deficiency, while females had more red cell folate deficiency. These values will be useful in interpreting change and gender-based influence in patient management, as well as in research.

LIMITATIONS:

There were some limitations to the study. The study participants were chosen based on their willingness to participation in the research, which is a significant limitation. Because the participants are mostly from one region of Karachi, the results obtained from this study may not be generalizable to the entire adult population. Furthermore, it was not possible to screen for all medical conditions that could have an impact on the results.

Data sharing statement:

The data sets generated or analyzed during the present study are available from the corresponding author on reasonable request.

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