ORIGINAL ARTICLE

Relation of Serum Vitamin B_{12} and Red Cell Folate Levels to Age and Cognition in Healthy Individuals of Different Age Groups: A Cross Sectional Study

IRAM NAZIR[1], MUHAMMAD USMAN[1], SADIA NAZIR[2], NADEEM AKHTAR[3], MAEESA WADOOD[1]
[1]Baqai Institute of Hematology; Baqai Medical University; Karachi, Pakistan
[2]Lahore Medical and Dental college, University of Health Sciences, Lahore, Pakistan
[3]Services Institute of Medical Sciences/Services Hospital Lahore College, Lahore, Pakistan
Correspondence to Dr. Iram Nazir, Email: iramnazir78@gmail.com

ABSTRACT

Background: Folate and vitamin B_{12} have important role in cognition. There is scarcity of globally established cut-off levels to demarcate deficiency of either vitamin among different age groups.
Aim: To determine age related differences in the levels of serum vitamin B_{12}, red blood cell folate and their association with cognition.
Methods: This cross sectional study was conducted at Baqai Medical University and 240 healthy volunteers (≤ 72 years) living in different areas of Karachi, fulfilling the inclusion-exclusion criteria, were included. The participants were grouped as: children (4-12 years); adults (13-55 years); elderly (56-72 years).
Results: The mean serum vitamin B_{12} and red blood cell folate levels for 4-12 years, 13-55 years, 56-72 years groups were 220.11±42.02pg/mL, 298.69±68.13pg/mL, and 197.33±11.65 pg/ml; 537.68±64.96ng/mL, 542.38±49.58ng/mL, and 536.44±52.05ng/mL, respectively. A significant strong positive correlation existed between serum vitamin B_{12} levels and mini mental state examination scores ($r = 0.770$, $p<.001$). MMSE scores were significantly negatively correlated with age ($r = -.717$, $p<.001$). A significant strong positive correlation between modified child MMSE scores and age was observed ($r = 0.784$, $p<.001$). Percent deficiency values of vitamin B_{12} was 41.3%, while 42.5% study population had marginal levels.
Conclusion: Increased prevalence of vitamin B_{12} deficiency and marginal status is a serious health issue. These findings should support dietary recommendations to enhance vitamin B_{12} status and to improve cognition, especially for children and elderly individuals. Vitamin B_{12} levels should be assessed in work up of cognitive decline and dementia.
Keywords: RBC folate, serum vitamin B_{12}, cognition, different age groups

INTRODUCTION

"Folate and vitamin B_{12} are micronutrients vital for cellular growth, differentiation and development. There is a connection in the metabolic pathways of both vitamins. The most important common metabolic pathway is homocysteine (Hcy) pathway, an intermediate, providing essential methyl groups for DNA and protein synthesis. Defects in these pathways may lead to low S-adenosyl methionine (SAM) and increase in total plasma homocysteine concentration"[1]. "Hyperhomocysteinemia is considered as vasotoxic and neurotoxic. Decreased SAM or increased Hcy causes sequence-specific DNA hypomethylation and "aberrant" gene activation which affect many brain functions, including cognition"[1,2]. "Both vitamins play vital roles in central nervous system functions in people of all age groups. They are required for healthy neurological development and deficiencies result in increased possibilities of cognitive impairment, cognitive decline and aggravated behavioral symptoms especially in the neurodegenerative dementias, Alzheimer's disease and vascular dementia"[2]. Several studies conducted in the past reported the substantial evidences of role of folate and vitamin B_{12} in brain functioning and use of folate and vitamin B_{12} supplementation may lead to slower rate of cognitive decline and enhanced cognitive functioning[2-4]. Cognitive impairment is a serious health issue. "World Health Organization (WHO) released a report which provided estimates that number of people suffering from dementia globally, is expected to rise to an alarming level"[5]. "Global prevalence of vitamin B_{12} and folate deficiency is uncertain due to lack of large scale data. Only a few countries have national or regional biochemical data on folate and vitamin B_{12} status among age groups. This leads to scarcity of globally established cut-off levels to demarcate deficiency of either vitamin"[6,7]. "Both vitamins levels fluctuate depending upon the methods used for detection. Variations are mostly confined in the lower range where it is deemed necessary to have accurate values. Red blood cell (RBC) folate is considered as indicator of long term folate status"[8]. "There is less data regarding RBC folate levels among different age groups for comparison"[6].

This study was designed to determine levels of serum vitamin B_{12}, RBC folate and cognitive scores in apparently healthy Pakistani population of different age groups. This research further intended to observe any age related difference in the values and its association with cognition.

MATERIALS AND METHODS

This study was conducted at Baqai Medical University, Karachi. Samples were collected from apparently healthy volunteers (n=240) from different areas of Karachi. The participants were segregated according to age into three

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groups: Children (n=50), 4-12 years; adult (n=118), 13-55 years; and elderly (n=72) in 56-72 years. All the participants were informed with details of study procedure before taking the written consents. The study was approved by the Ethics Committee of Baqai Medical University, Karachi. It was a cross sectional study started from May 2017 to April 2018.

For evaluation of health status, a detailed personal, medical, surgical, drug history and diet history was taken. The participants with history of supplements intake, alcohol, proton pump inhibitors, malabsorption syndrome, renal disease, smoking, diabetes, liver disease, malignancy, blood loss, recent transfusion, gastrointestinal surgery, any debilitating illness; pregnancy; lactation and strict vegetarians were excluded. “Assessment of dietary intake including food, beverages were recorded by using food frequency questionnaire (one pen-and-paper form)”[9]. This questionnaire contains a list of food and beverage items along with frequency and quantity for each items. Some food items were modified according to local eating habits. The participants were asked to recall the frequency of each food item consumed. “Cognitive functions were assessed in 13-72 years old participants by Mini Mental State Examination (MMSE). It is a simple, rapid and easy test for evaluation of cognitive impairment. It takes between 5-10 minutes for application. MMSE is a screening test consisting of 30 points questionnaire. It quickly assesses cognitive functions and covers different cognitive areas. A score ≥ 24 points designates a normal cognition, ≤ 9 points severe, 10-18 points show moderate and 19-23 points indicates mild cognitive impairment”[10]. In 4-12 years age cognition is assessed by “Modified Child Mini Mental Examination (mCMME). A score < 34 is considered abnormal (normal 34-37)”[11]. A brief general physical examination was conducted including blood pressure, pulse, temperature, height and weight. The body mass index (BMI) was also calculated (BMI= kg/m^2)

10ml fasting venous blood samples were collected. Equal amount of blood was transferred to yellow-top vacutainers containing separating gel (for serum vitamin B_{12} assay) and to purple-top vacutainers containing Ethylenediaminetetraacetic acid (EDTA). The specimens were kept cold and protected from light. Serum was extracted by centrifugation (2000 rpm, 10 min) and transferred to aliquots, stored at < – 20℃ until analyzed, within 1h of collection. Whole blood samples were kept at 4℃ and analyzed within 2h of sampling. Complete blood count (CBC) of all samples was assessed by automated cell analyzer Sysmex XP100 Tokyo, Japan. Peripheral smear morphology was done using Leishman’s stain. “Samples having hemoglobin levels according to WHO criteria”[11] and normocytic normochromic blood pictures with no hyper segmented neutrophils were accepted. Hemolysate was prepared to estimate the folate contents in red blood cells by mixing 3ml of 0.2% ascorbic acid solution and 100μl of whole blood. Then, incubated at 20-25 away from light for 90 minutes. Red blood cell folate and serum vitamin B_{12} levels were measured on Cobas Electrochemiluminescence e 411 analyzer of Roche Diagnostics, Mannheim, Germany.

“The defined cut-off values of serum vitamin B_{12} for vitamin B_{12} deficiency were <150 pmol/L (< 203 pg/mL) and for marginal vitamin B_{12} 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)”[7,8,13].

Statistical analysis: The statistical analysis was accomplished by using Statistical Package for Social Sciences (SPSS) version 23.0. Data was compiled as mean ± standard deviation, range and median. Independent t test was done to compare means of MMSE scores between adults and elderly group. one-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. The chi-squared test (χ^2 test) was performed to compare proportion among groups. The P value of <0.05 was considered significant for all of the analyses.

RESULTS

The detailed study characteristics were shown in Table 1. A total of 240 participants with mean age of 37.78 ± 21.07 years, ranging from 4.0 - 72.0 years were included. 21.25%, 45% and 33.75% of study subjects belonged to low income [<Rs. 13,000/- (US$120], lower middle (Rs. 13,000-25,000) and upper middle class (>Rs25,000), respectively. 61.66% individuals were college and university going.

Serum vitamin B_{12} levels: The serum vitamin B_{12} levels of the participants (n = 240) were given in Table 1. A significant negative correlation of serum vitamin B_{12} level was found with age (r = - 0.221, p = .001). No significant association of serum vitamin B_{12} levels with red cell folate levels was observed. There was statistically significant difference in levels of serum vitamin B12 for the three age groups [$F (2, 237) = 135.45$, $p<0.001$)]. The mean serum vitamin B_{12} levels in 4-12 years group were significantly lower than levels in 13-55 years age group (220.11 ± 42.02 pg/mL vs 298.69 ± 68.13 pg/mL, respectively; p<0.001) and comparatively higher than 56-72 years group (197.32 ± 11.64, p = 0.032). The serum vitamin B_{12} levels in 15-55 years group were significantly higher than in 4-12 years age group and 55-72 years age group (p<.001 and p<.001, respectively) as shown in Table 2. In children group serum vitamin B_{12} levels were found to be positively correlated with age (r = 0.353, p = 0.012). Serum vitamin B_{12} levels were strongly negatively correlated with age in 56-72 years group (r = - 0 .768, p< 0.001).

A group comparison was done according to defined cut –off values of serum vitamin B_{12} (vitamin B_{12} deficiency < 203 pg/ml and insufficiency 203–299 pg/ml) a significant association of age with serum vitamin B_{12} levels was found (p<.001), deficiency of vitamin B_{12} was present in 41.3% of all individuals, where participants with age 4-12 years (46%) and 56-72 years (55.6%) were more likely to have vitamin B_{12} deficiency than 13-55 years (30.5%) as shown in Table 2.

Red cell folate levels: The mean red cell folate levels (ng/mL) for entire study population (n=240) as given in Table 1. The RBC folate levels were not significantly correlated with age. The mean red cell folate concentrations for 4-12 years, 13-55 years, 56-72 years groups were shown in Table 2. Red cell folate levels showed no significant difference among three different age groups. All the participants had RBC folate levels above 150ng/ml.

Mini Mental State Examination Scores: For the study population aged 13-72 years (n=190), mean MMSE scores were shown in Table 1. MMSE scores were significantly negatively correlated with age (r= -.717, p<.001). A significant strong positive correlation existed between serum vitamin B_{12} levels and MMSE scores (r = 0.770, p<.001). There was a significant difference in the scores of MMSE for 13-55 years age group (M = 29.30, SD = 1.12) and 56-76 years age group (M = 24.97, SD = 1.60); (p < .001). When comparison was done in reference to cut –off values among adult and elderly group, MMSE score was significantly associated with age (p = .001). 8.4% of all participants (13-72 years) had score ≤ 23, which was more likely to be present in 56-72 years age group (16.7%) than 13-55 years age group (3.4%) (p = .001) as shown in Table 2. Analysis among groups revealed that adults in 13-55 years group, serum vitamin B_{12} level had significant positive association with MMSE (r = 0.611, p < .001).

Modified - Child Mini Mental State Examination Scores: The mean modified child mini mental state examination scores (m-CMMSE) for children aged 4-12 years are shown in Table 1. A significant strong positive correlation between modified child MMSE scores and age was observed (r = 0.784, p<.001). Serum vitamin B_{12} and RBC folate levels did not show significant correlation with m-CMMSE scores. 2% study participants had scores below the cutoff point (<34) as shown in Table 2.

{SD, standard deviation; MMSE, Mini Mental State Examination; mCMMSE, Modified Child Mini Mental State Examination; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; BMI, body mass index; Mean ± SD (n=190)* refers to score of MMSE in participants in 13-72 year}

Table 1: A detailed view of demographic and basic characteristics of study population (n=240)

Variables	Parameters	Total	Frequency%
Age (years)	Mean ± SD	37.78 ± 21.07	
	Median	36.50	
	Range	4.0 - 72.0	
	Percentile P(2.5-97.5)	6.0 - 70.0	
	Percentile P(5- 95)	8.0 - 68.0	
BMI(kg/m^2)	Mean ± SD	22 ± 2.2	
Income	Low income class	--	51 (21.25)
	Lower middle class	--	108(45.00)
	Upper middle class	--	81 (33.75)
Education level	No education	--	24(10.00)
	Primary (grade 5)	--	18 (7.50)
	Middle (grade 8)	--	20 (8.33)
	Matric (grade 10)	--	30 (12.50)
	College & University	--	148(61.66)
Serum Vitamin B_{12} (pg/mL)	Mean ± SD	254.86±68.13	
	Median	226.44	
	Range	185.12-462.95	
	Percentile P (2.5 - 97.5)	185.59-392.31	
	Percentile P (5 - 95)	186.31-379.92	
Red Cell Folate (ng/ml)	Mean ± SD	539.62±53.67	
	Median	540.48	
	Range	407.80-651.17	
	Percentile P (2.5 - 97.5)	450.96-623.47	
	Percentile P (5 - 95)	456.42-619.16	
MMSE	Mean ± SD (n=190)*	26.66±2.49	
	Median	28.00	
	Range	24.0-30.0	
	Percentile P (2.5 - 97.5)	24.0-30.0	
	Percentile P (5 - 95)	24.0-30.0	
mCMMSE	Mean ± SD (n=50)**	35.08±1.18	
	Median	36.00	
	Range	33.0 - 37.0	
	Percentile P (2.5- 97.5)	33.23 - 37.0	
	Percentile P (5 - 95)	34.0 -37.0	
Hb (g/dl)	Mean ± SD	13.8 ± 1.11	
Hct (%)	Mean ± SD	43.0 ± 3.26	
MCV (fl)	Mean ± SD	80.6 ± 5.09	

Table 2. Comparison of levels of RBC folate, serum vitamin B_{12} and cognitive score among different age groups.

Variables	Parameters	Age groups			Total count(%)	p-value
		4-12 years n=50 (%)	13 – 55 years n=118 (%)	55 – 72 years n=72 (%)		
Serum Vitamin B_{12} (pg/ml)	Mean ± SD $^{\alpha}$	220.11±42.02	298.69± 68.13	197.33±11.65		<.001‡
	95% CI$^{\upsilon}$ for mean Lower bound Upper bound	 208.17 232.05	 293.75 315.63	 194.59 200.07		
Vitamin B_{12} (pg/ml) (< 203)		23(46)	36(30.51)	40(55.56)	41.3	<.001†
Vitamin B_{12}(pg/ml)(203 – 299)		22(44)	50(42.37)	30(41.67)	42.5	
Vitamin B_{12}(pg/ml) (> 299)		5 (10)	32 (27.12)	2 (2.78)	16.3	
Red Cell Folate (ng/ml)	Mean ± SD	537.68±64.96	542.38±49.58	536.44±52.05		.731‡
	95% CI for mean Lower bound Upper bound	 519.22 556.14	 533.35 551.42	 524.21 548.67		
Folate deficiency (<151 ng/ml)		None	None	None		
MMSE$^{\infty}$ (max=30)	Mean ± SD	--	29.30±1.12	24.97±1.60		<.001¶
	95% CI for mean Lower bound Upper bound		 29.09 29.51	 24.59 25.34		
MMSE (max=30)≤ 23 points		--	4 (3.4)	12 (16.7)	8.4	.001†
mCMMSE* (max=37)	Mean ± SD	36.08±1.17	--	--		
	95% CI for mean Lower bound Upper bound	 35.74 36.41	--	--		
mCMMSE (max=37)<34 points		1 (2)	--	--		

$^{\alpha}$Standard deviation; $^{\upsilon}$ confidence interval; $^{\infty}$ mini mental state examination; *modified child mini mental state examination.
‡The one –way ANOVA (Post Hoc test; scheffe) (p<0.05, significant) was performed.† The chi square was done to compare proportion among groups. ¶Differences between groups were assessed by using an independent t test (p<0.05, significant).

Table 3: A brief summary of levels of serum vitamin B_{12} and RBC folate levels in different populations.

Area	Conducted by	Age y (n)	Serum vitamin B_{12}	RBC folate
Turkey	Akin et al[14]	0-24y (1,109)	$^{\mu}$127-590pg/ml	-
India	Yajnik et al. 2006[18]	30-50y (441) Rural Slum Urban-middle class	$^{@}$119 pmol/L 145pmol/L 89pmol/L	$^{@}$522nmol/L 461 nmol/L 525 nmol/L
European cities	González – Gross et al. 2012[23]	12.5-17.49y (1051)	$^{@}$319 pmol/L	$^{@}$721.9 nmol/L
Canada	Zinck et al.2015[24]	20-≥60y (3092)	∞301 pmol/L	1267 nmol/L
China	Ni et al.2017[25]	18-81y (330)	*431.55±203.9 pmol	706.15±398.76 nmol
United State	Pfeiffer et al. 2005[26]	≥3y (≈ 7300)	$^{\#}$179-738 pmol/L	$^{\#}$347-1167nmol/L

*mean±standard deviation; ∞geometric mean; $^{\mu}$ percentile ($P_{2.5}$-$P_{97.5}$); $^{\#}$ 5th – 95th percentile; $^{@}$median; n (number).

DISCUSSION

Our study provides a detailed analysis of folate and vitamin B_{12} status and cognitive scores in apparently healthy Pakistani individuals of different age groups. The serum vitamin B_{12} level of our study population were lower (254.86±68.13). Akin et al. reported similar findings[14]. We found 41.3% vitamin B_{12} deficiency (<203pg/ml) in all participants, of which 46%, 30.51% and 55.56% were present in 4-12 years, 13-55 years and 56-72 years age groups, respectively. 42.5% study participants had marginal vitamin B_{12} levels (203-299pg/ml) among them: 44% were 4-12 year; 42.37% were 13-52 years and 41.67% were 56-72 years. Comparison of serum vitamin B_{12} levels among 3 different age groups revealed that children and elderly age group have significant lower serum vitamin B_{12} levels as compared to adult. Studies conducted in the past reported inconsistency in the prevalence of vitamin B_{12} deficiency and insufficiency (marginal levels) according to age groups and across countries globally. “It is highly prevalent in Asian and Africans countries”[6,7,15-18]. Recent studies conducted in our country reported “52.4% prevalence of vitamin B_{12} deficiency in 15-49 years of age females”[13] and “9.7% in 18-60 years”[19] and “6.6% in 30-75 years individuals” [20]. We noted a significant negative correlation of serum vitamin B_{12} levels with age (r = - 0.221, p=0.001). In elderly group, a significantly negative correlation was found between serum vitamin B_{12} levels with age (r = - 0 .768, p< 0.001). Similar finding was also reported in other studies conducted in different countries [6]. In children group a positive correlation of serum vitamin B_{12} levels with age was seen (r=0.353, p=0.012). There could be various explanations for substantial increase in vitamin B_{12} deficiency and marginal insufficiency in our study. “Animal origin food are richest source of vitamin B_{12}. Dietary insufficiency, non-affordability, eating habits and malabsorption are important causes of vitamin B_{12} deficiency”[15,16,21,22]. A comparison of serum vitamin B_{12} levels in different countries (Table 3).

In our study results, we found good folate status and no significant differences in levels among 3 different age groups and none of study participants in different age group found to be folate deficient. The main power of our study is that we determined “RBC folate level instead of serum folate level, which is more reliable indicator of long term folate status” [6,8]. The recent studies conducted on healthy individuals in our region determined “serum folate” levels [13,19,20] making difficult to compare study results. “There is scarcity of data regarding RBC folate status of healthy individuals in different age groups at national and provincial levels. Studies conducted in different countries showed findings similar to our results” [6]. The possible reasons of higher folate levels in our study population might be good dietary folate intake.” Folate is rich in green leafy fresh vegetables and fruits. These foods are easily available and affordable as compare to animal derived foods”[1,8]. RBC folate reported in different studies was shown in Table 3.

Mean Mini Mental State Examination scores (MMSE) for our study individuals aged 13-72 years were 27.66±2.49 (max=30) as shown in Table 1. Cognitive scores below cut off point (≤23) were present in 8.4% of all participants (13-72 years) of which 3.4%, 16.67% participants were 13-55 years and 56-72 years groups, respectively. We observed a significant strong positive correlation between MMSE scores and serum vitamin B_{12} levels (r = 0.770, p < .001). While, no significant association was observed between RBC folate levels and MMSE scores. Smith also reported in a review, “the negative association between dietary intakes of vitamin B_{12} or serum levels with cognitive decline in six prospective studies conducted on 4607 subjects” [3]. Moreover, we observed significant positive correlation between adults MMSE scores and serum vitamin B_{12} levels (r = 0.611, p < .001). Our results showed age related decline in MMSE scores (r = -717, p < .001). Clarke and colleagues found similar association of low serum vitamin B_{12} levels with more rapid cognitive decline[27].

The mean modified child MMSE score for children was 36.08±1.18 (max=37). We found that 2% participants had scores below the cutoff point (< 34). A significant strong positive correlation between m-CMMSE scores and age was also observed (r= 0.784, p < .001). As in “infancy; poor weaning, infections and decreased vitamin B_{12} stores are main causes of vitamin B_{12} deficiency”[28]. Comparability with other studies was limited as most of the available studies reported the effect of vitamin B_{12} on cognitive function in elderly individuals. Few studies covered this aspect[29,30].

CONCLUSION

Our study provides comprehensive information about folate and vitamin B_{12} levels and cognitive scores in healthy Pakistani individuals of different age groups. Good RBC folate levels were seen in all age groups. Age seems to have an influence on blood levels of vitamin B_{12} and should be taken into account. Vitamin B12 deficiency is becoming an emerging health issue. Low blood levels of serum B_{12} were associated with decline in cognitive scores, suggested that B_{12} levels should be assessed in work up of cognitive decline and dementia. These findings should support dietary recommendations to enhance vitamin B_{12} status to improve cognition, especially for elderly individuals. However, early screening for vitamin B_{12} would be a cost effective approach in timely management of the disorders arising from their deficiency, especially cognitive decline. A comprehensive collaboration is required along with encouragement of population based studies to measure the RBC folate and vitamin B_{12} levels in the population and to define deficiency.

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