Original Article

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Biochemistry Section

Association of Vitamin B12 with Atherogenic Index of Plasma- A Cross-sectional Study

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ABSTRACT

Introduction: Vitamin B12 has a pivotal role in several metabolic processes and it's inadequacy can pose serious health implications.

Aim: To assess if vitamin B12 inadequacy is associated with increased risk of Cardiovascular Disease (CVD) based on the Atherogenic Index of Plasma (AIP).

Materials and Methods: A cross-sectional, retrospective study was conducted in a tertiary care hospital, Riyadh, Saudi Arabia from December 2019 to January 2021. Data of apparently 569 healthy individuals within the age range of 18-60 years, who were screened for vitamin B12 deficiency was collected. The variables were analysed by ANOVA under categories of vitamin B12 level; Deficient (<148 pmol/L), Marginal (148-221 pmol/L) and Sufficient (>221 pmol/L). Correlation and regression analysis were done to find the correlation of vitamin B12 with lipid profile and AIP. The level of significance was set at p-value <0.05.

Results: The prevalence of B12 deficiency was found to be 7.2%. B12 was negatively correlated with Triglycerides (TG) (r=-0.161, p-value <0.05). Total Cholesterol (TC) (r=-0.169, p-value <0.05) and AIP (r=-0.15, p-value <0.05). According to regression analysis for every unit increase in B12, the TG and AIP decreased by 0.001 unit and TC reduced by 0.002 unit even after model adjustment for age, gender and BMI.

Conclusion: Vitamin B12 is inversely related to AIP. The lower level of vitamin B12 is related to increased cardiovascular risk and poor lipid profile.

Keywords: Cardiovascular disease, Dyslipidemia, Micronutrient

INTRODUCTION

Micronutrients have gained much recognition globally in health research due to their essential impact on the pathophysiology of various metabolic disorders. Vitamin B12 (B12), a water soluble vitamin, has a key biological role in lipid, carbohydrate metabolism and DNA synthesis [1]. At a cellular level, it acts as a cofactor in two important enzymatic reactions one of which is Methionine synthase, essential for synthesis of purines and pyrimidines and other being the Methyl malonic coenzyme A mutase that converts methylmalonic coenzyme A to succinyl coenzyme A. Due to vitamin B12 deficiency, these two reactions get affected which leads to accumulation of substrates like homocystein and methymalonic acid, subsequently leading to clinical manifestations like megaloblastic anemia and neurological defects.

Vitamin B12 is usually assessed in serum or plasma with current definition of deficiency as <148 pmol/L and marginal status defined as 148-221 pmol/L. Though, the gold standard is elevated serum or urine Methylmalonic Acid (MMA), but it has limitations due to higher cost, need for mass spectrometry, and possibility of being falsely raised by bacterial overgrowth [2]. The worldwide prevalence of vitamin B12 deficiency based on serum level ranges widely, 4% in US population, 11% UK, while in Latin America it is estimated to be 40% whereas it is markedly high in Asian, African continents, for e.g., 47% in India [3,4], 4% in US [5], 39% in China [6], 70% in Kenya [7].

Role of vitamin B12 in dyslipidemia has raised curiosity among many researchers as dyslipidemia is an independent risk factor for CVD, though the underlying mechanism of vitamin B12 is not yet clearly explicated. Some studies have demonstrated the association of vitamin B12 with CVD [8,9] while some were inconclusive [10]. AIP is representative of small, dense Low Density Lipoprotein (sd LDL) which has higher tendency to cause atherosclerosis compared to other lipids and hence it is a good predictor of CVD [11].

In view of widespread prevalence of Vitamin B12 deficiency and it's association with metabolic disorders, the present study intends to evaluate the correlation between vitamin B12 and AIP to predict cardiovascular risk. The association of vitamin B12 and AIP has not been reported so far in previous studies to the best of author's knowledge. Additionally, it reports the prevalence of vitamin B12 deficiency in the study population.

The study mainly aims to highlight the importance of screening vitamin B12 status in the population and timely intervention so as to prevent future cardiovascular complications.

MATERIALS AND METHODS

The cross-sectional, retrospective study was conducted in a tertiary care hospital, Riyadh, Saudi Arabia from December 2019 to January 2021. Data was collected for 569 apparently healthy individuals who visited the hospital for routine health check up during the period December 2019 to January 2021. No animal or human experiments were conducted in the study and all procedures were in accordance with standards of the Institutional Ethics Committee. (Approval no. RC-21.08.10).

Inclusion criteria: Subjects were in the age range of 18-60 years who were screened for vitamin B12 deficiency based on clinical presentation in the OPD of Internal Medicine. Those who had concurrent lab tests for lipid profile were included in the study. Lab data was collected for serum vitamin B12, TG, TC, Low Density Lipoprotein-Cholesterol (LDL-C) and High Density Lipoprotein-Cholesterol (HDL-C). AIP was calculated as logarithm to the base 10 of the ratio TG/HDL-C and value >0.1 was considered to be associated with increased cardiovascular risk [11].

Exclusion criteria: Pregnant patients, patients with chronic diseases of kidney, liver, diabetes, celiac disease, history of gastrectomy and pancreatic deficiency were excluded. Individuals who were taking vitamin supplements or with incomplete lab data were also excluded.

Sample size calculation: Based on a previous study, with effect size-0.2 [12], confidence interval 95%, power 95%, the estimated sample size was 262. The study included 569 subjects who fulfilled all inclusion and exclusion criteria.

Information related to age (years), gender, weight (kg), height (meters), systolic and diastolic blood pressures (mmHg) were also retrieved and Body Mass Index (BMI) was calculated as Weight/(Height)^2. Vitamin B12 and all lipid parameters were directly measured on Alinity ci series, Abbott, Germany in the hospital laboratory which is accredited by the College of American Pathologists. Subjects were categorised based on vitamin B12 level: Deficient (<148 pmol/L), Marginal (148-221 pmol/L) and Sufficient (>221 pmol/L) [2].

STATISTICAL ANALYSIS

Statistical software SPSS version 20.0 was used for analysis. For descriptive statistics, variables were presented as mean with standard deviation, proportions. ANOVA was used to compare continuous data in three vitamin B12 categories. Correlation and regression analysis were done to study relationship of vitamin B12 with lipid parameters and AIP. Statistical significance was considered at p<0.05.

RESULTS

[Table/Fig-1] depicts baseline features of study population. With almost equal gender distribution, the mean age was 40.1±9.98 years. Mean vitamin B12 was 254.92±84.50 while mean AIP was 0.035±0.28. [Table/Fig-2] shows that the prevalence of vitamin B12 deficiency and marginal status in the study population was 7.2% and 30.2%, respectively. The mean vitamin B12 in the deficient category was 127.65±14.73 pmol/L, while the mean in sufficient category was 297.24±77.28 pmol/L. More females had deficient vitamin B12 levels compared to males (26/15). Mean BMI was highest in the vitamin B12 deficient group (26.26±3.61), systolic, diastolic blood pressures were also highest in the same group; 135.97±11.92 mm Hg and 80.07±7.48 mm Hg, respectively. Among the lipid parameters TG (2.00±0.69 mmol/L), TC (5.55+0.79), LDL-C (2.76+0.90) were highest while HDL-C (1.00+0.33 mmol/L) was lowest in the vitamin B12 deficient class. AIP was also found to be 0.29+0.29 in the vitamin B12 deficient group indicating increased cardiovascular risk. The TG, TC and AIP showed a distinct, statistically significant rising shift from sufficient towards deficient vitamin B12 categories while HDL showed a significant downward trend. The BMI, TG, TC and AIP showed a weak negative correlation with vitamin B12 [Table/Fig-3]. According to regression analysis for every unit increase in vitamin B12 measure, TG and AIP reduced by 0.001 unit and TC reduced by 0.002 unit even after adjustment for age, gender and BMI with statistical significance [Table/Fig-4].

Age (years)	40.1±9.98
Vitamin B12	254.92±84.50
Gender (Female/Male)	276/293
BMI (kg/m^2)	24.09±2.79
Systolic BP (mmHg)	133.04±9.97
Diastolic BP (mmHg)	78.5±7.90
Triglycerides (mmol/L)	1.49±0.63
Total cholesterol (mmol/L)	4.95±0.83
LDL-C (mmol/L)	2.50±0.89
HDL-C (mmol/L)	1.31±0.39
AIP	0.035±0.28

[Table/Fig-1]: Baseline characteristics of the study population (N= 569).
Data presented as Mean+Standard deviation; proportion

Variables	Deficient N=41 (<148 pmol/L)	Marginal N=172 (148-220.9)	Sufficient N=356 (>=221)	p-value
B12 mean (pmol/L)	127.65±14.73	197.66±18.95	297.24±77.28	<0.001*
Age (years)	40.39±9.91	38.86±9.28	40.70±10.35	0.181
Gender (Female/Male)	26/15	82/90	168/188	0.139#
BMI (kg/m^2)	26.26±3.61	24.04±2.97	23.87±2.48	<0.001*
Systolic BP (mmHg)	135.97±11.92	133.92±10.31	132.28±9.46	0.099
Diastolic BP (mmHg)	80.07±7.48	78.73±7.94	78.28±7.92	0.386
Triglycerides (mmol/L)	2.00±0.69	1.54±0.63	1.42±0.60	<0.001*
Total cholesterol (mmol/L)	5.55±0.79	5.06±0.80	4.83±0.81	<0.001*
LDL-C (mmol/L)	2.76±0.90	2.56±0.91	2.43±0.88	0.041*
HDL-C (mmol/L)	1.00±0.33	1.32±0.46	1.33±0.34	<0.001*
AIP	0.29±0.29	0.05±0.30	-0.001±0.26	<0.001*

[Table/Fig-2]: Characteristics of subjects under vitamin B12 categories.
Test applied- ANOVA (on all variables except Gender); continuous data presented as Mean±standard deviation and categorical data as frequency (%)/proportion; N: number; B12: Vitamin B12; BMI: Body mass index; BP: Blood pressure; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol; AIP: Atherogenic index of plasma; #chi-square test; *p-value <0.05 significant

Variables	r-value	p-value
Age	0.055	0.186
BMI	-0.107	0.01*
Triglycerides	-0.161	<0.05*
Total cholesterol	-0.169	<0.05*
LDL-C	-0.007	0.87
HDL-C	0.052	0.216
AIP	-0.15	<0.05*

[Table/Fig-3]: Correlation between variables and B12.
Test applied: Pearson's correlation test; BMI: Body mass index; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein-cholesterol; AIP: Atherogenic index of plasma; r: Pearson's correlation coefficient; *p-value <0.05 significant

Dependent variables	Unadjusted model			Adjusted model		
	B+ SE	B(S)/ 95%CI	p-value	B+ SE	B(S)/ 95%CI	p-value
Triglycerides	-0.001±0.00	-0.113	0.002*	-0.001±0.00	-0.161	<0.05*
Total cholesterol	-0.002±0.00	-0.131	0.001*	-0.002±0.00	-0.169	<0.05*
LDL-C	-0.000±0.00	-0.011	0.789	-0.000±0.00	-0.007	0.870
HDL-C	0..000±0.00	0.016	0.682	0.000±0.00	0.052	0.216
AIP	-0.0005±0.00	-0.100	0.006*	-0.001±0.00	-0.150	<0.05*

[Table/Fig-4]: Regression analysis between lipid profile and vitamin B12 (for unit change in Vit B12).
Model adjusted for age, gender, BMI; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein-cholesterol; AIP: Atherogenic index of plasma; *p-value <0.05 significant

DISCUSSION

The present retrospective study had some key findings. Firstly, the prevalence of vitamin B12 deficiency in the study population was 7.2%. Secondly, vitamin B12 was negatively associated with BMI, TG and cholesterol. Thirdly and most importantly, it showed a statistically weak negative correlation between vitamin B12 and AIP which is indicative of increased cardiovascular risk due to lower vitamin B12 level.

The reported prevalence of vitamin B12 deficiency in the present study population was nearly same as that reported in a similar study in neighbouring region, Jordan as 6% [13]. Other studies in different parts of world have reported variable prevalence, for example in US it is much lower (4%), whereas a study in India has reported 47%. The reason explained for higher prevalence is mainly food habits and diet deficient in vitamin B12 [4,5]. Other studies were focused on specific cohort for example, one study in Saudi Arabia reported prevalence of 30% in diabetic patients on metformin therapy [14] and another in Turkey in elderly reported 64% [15]. Old age has been demonstrated as

an important determinant of vitamin B12 deficiency since ageing affects absorption and metformin drug is known to reduce serum vitamin B12 levels. On the other hand, the present study excluded diabetic patients and those aged above 60 years which could be the reason for lower prevalence.

The study showed negative correlation between vitamin B12 and BMI as reported in a recent US based population study [16]. Vitamin B12 levels were found to be lowest in obese individuals compared to those who overweighed or had normal weight, according to a latest systematic review [17]. This is possibly due to block in conversion of homocystein to methionine and therefore reduced protein synthesis and lesser lean tissue mass or could also be due to vitamin B12 deficiency associated chronic inflammation leading to adipocyte dysfunction. Obesity could also be causal factor for vitamin B12 deficiency by sequestration in adipose tissue or possibly by delaying vitamin B12 absorption as a result of changes in gut flora [18].

As for the lipid parameters, the current study showed a significant association between vitamin B12, TG and TC which corroborated with findings of a similar study in African population [19]. A study on European diabetic cohort showed independent association of vitamin B12 with TG, as also in the present study [12]. A polish study showed similar negative correlation between vitamin B12, TG and TC as in present study. However, in regression analysis the relationship was lost [20] but in current study both were associated with vitamin B12 with statistical significance even after adjusting confounders which could be due to larger sample size. There is evidence from previous study that hypertriglyceridemia improved effectively with treatment by vitamin B12 which supports our finding [21]. Vitamin B12 is a cofactor in the enzymatic reactions involving fatty acid catabolism and so presumably modulates lipid profile. It is suggested that accumulation of methymalonyl CoA due to vitamin B12 deficiency inhibits the principal fatty acid oxidation enzyme, Carnitine Palmitoyl Transferase (CPT1) to induce lipogenesis. Another possible mechanism is deficiency induced hyperhomocysteinemia which affects phospholipid metabolism leading to more synthesis of VLDL. Furthermore, it is proposed that low vitamin B12 increases expression of genes regulating lipogenesis like SREBF1 and LDLR [22].

The AIP as marker of cardiovascular risk worsened with lower vitamin B12 status in the current study with statistical significance. Most previous studies demonstrated the association of vitamin B12 with CVD indirectly through raised homocystein levels as a result of vitamin B12 deficiency. Homocystein is a byproduct of enzymatic reaction for which vitamin B12 is the key coenzyme and is known to cause direct damage to blood vessels. It is prothrombotic and proatherosclerotic hence, contributes to hypertension and cardiovascular events [23]. The present study is unique as it reported association between lower vitamin B12 level and unfavorable AIP to assess the risk for CVD. Additionally, deficient vitamin B12 subjects were found to have highest mean systolic and diastolic blood pressures which may also be explained by the effect of hyperhomocystenemia. Corroborating with the findings of the present study, a recent study on elderly population evinced higher risk of CVD deaths in those with lower vitamin B12 levels [24]. Furthermore, a meta-analysis of prospective studies has given a compelling evidence of correlation between vitamin B12 and CVD by showing notable decrease in incidence of stroke after vitamin B12 supplementation [25]. The present study further adds to the existing evidence of association of vitamin B12 deficiency with increased cardiovascular risk.

Limitation(s)

The current study had certain limitations. Due to retrospective nature of study the selection bias could not be avoided. The study cannot be representative of general population since it was single centered. Serum homocystein and methyl malonic acid were not taken into consideration, which are recommended indicators of vitamin B12 status in combination with serum vitamin B12 measurement. Yet the study showed a negative correlation between vitamin B12 and AIP showing that vitamin B12 deficiency can be a potential cardiovascular risk factor.

CONCLUSION(S)

The prevalence of vitamin B12 deficiency in the study population was 7.2% whereas that of marginal status was 30.2%. The lower vitamin B12 level is related to adverse lipid profile and increased cardiovascular risk lays grounds for future research. Longitudinal studies might be helpful to vindicate the findings of current study.

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