Evaluation of Serum B12 Levels among Patients with Type 2 Diabetes Mellitus

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Abstract

Background and objectives: Vitamin B12 insufficiency is widespread among type 2 diabetic people. Advanced age of patients, malnutrition, and malabsorption are the causative factors. Vitamin B12 deficiency causes raised levels of homocysteine & estimation of homocysteine will represent the metabolic status of B12. The aim of this study was to assess the incidence of B12 in relation with homocysteine levels and Insulin resistance in patients with type 2 diabetes mellitus regardless type of treatment used in treating diabetes.

Materials and methods: This is a cross-sectional study at Dohuk Azadi teaching hospital (biochemistry department)/Dohuk city from (February 2022. - August 2022), offering questionnaire randomly we collected 138 subjects and biochemical tests include (B12 assay, fasting glucose, $HbA_{1c,}$ fasting insulin, homocysteine) with measuring of Body Mass Index. Exclusion criteria: Age < 18 years old, using vitamin B/multi vitamins, using non-steroidal anti-inflammatory drug, debilitating diseases, alcohol intake.

Results: the sample (138) was divided into 2 groups: Type 2 Diabetes Miletus (70) patients & apparently healthy subjects (68). The difference was significant in age (51.24±9.27 vs29.57±7.2 years, p<.05), B12 (252.05±83.7vs337.03±121.6 pg/ml, p<.01), Homocysteine (13.493±4.8 vs 10.041±2.2 μmol/L, p<.01), insulin resistance (6.82±2.0 vs 1.47±0.56, p<.001).), there was a significant negative correlation between serum B12 and age, BMI, insulin resistance and serum homocysteine p value <0.01.

Conclusion: Vitamin B12 deficiency is frequent in people with type 2 diabetes mellitus. Yearly screening for vitamin B12 deficit using serum homocysteine and supplement should be adopted among diabetes individuals with risk factors of vitamin B12 insufficiency**.**

Keywords: Homocysteine, Insulin resistance, Type2 diabetes mellitus, Vitamin B12.

Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which there is hyperglycemia resulting of insulin resistance or inefficient insulin activity or both.

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Type 2 Diabetes mellitus (T2DM) is the main cause of morbidity and death worldwide in the recent years.[1] An assessment of 537 million persons between 20–79 years old age globally (10.5% of all adults in this age group) have diabetes. By 2030, 643 million, and by 2045, 783 million of people aged 20–79 years are believed to be living with diabetes. Thus, whilst the world's population is anticipated to rise 20% over this time, the number with diabetes is expected to grow by 46%.[2.] The most typical sequelae of diabetes mellitus include nephropathy, peripheral neuropathy, retinopathy, coronary artery disease, cerebrovascular disease etc. Vitamin B12 is a water-soluble vitamin. It exerts its physiological effects by facilitating two principal enzymatic pathways i.e., the methylation process of homocysteine to methionine and the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. For the methionine which is subsequently activated into S-adenosyl-methionine that transfers its methyl group to methyl acceptors such as membrane phospholipids, neurotransmitters and myelin. Significant shortage of B12 biologically therefore will result in disruption of the methylation process and increase of intracellular and serum homocysteine. So, estimation of homocysteine level in the serum will reflect the metabolic status of B12 in histological level because the prevalence of B12 deficiency in the population is estimated to be between (28-41%) with association of Hyperhomocysteinemia especially in the diabetic patients.[1] Vitamin B12 insufficiency is widespread among people with T2DM. Advanced age of patients with T2DM, nutritional deficiency, and malabsorption are the other contributing factors.[3] Vitamin B12 deficiency also causes elevated levels of homocysteine, which is a risk factor for cardiovascular disease.[4,5] Estimation of homocysteine level in the blood would represent the metabolic state of B12 in cellular level.[6] The aim of this study was to assess the incidence of B12 deficiency in relation with homocysteine levels and Insulin resistance in T2DM patients in comparison with apparently healthy subjects not taking in consideration type of treatment used in treatment of diabetes mellitus.

Materials and methods

The present cross-sectional research was done at Dohuk teaching center/biochemistry department / Azadi Teaching hospital/Dohuk city/Kurdistan region /Iraq over the period from February 2022 till August 2022.

A well-prepared questionnaire was randomly distributed to subjects attending the biochemistry laboratory and a group of 138 of them were selected to be enrolled in this study. Then a set of biochemical tests was performed including (B12 assay, fasting blood glucose, HbA1c, fasting insulin and homocysteine) with measurements of Body Mass Index (BMI Kg/m2). The participants were then divided into two groups, patients with type 2 diabetes mellitus, were 70 participants and 68 apparently healthy subjects. Putting more emphasis on the significance of B12 levels and Homocysteine in relation to insulin resistance and glycemic control.

Exclusion criteria involved age less than 18 years old, individuals consuming vitamin B complex supplements or multi vitamins, participants using NSAIDs, debilitating disorders (cerebrovascular accident, chronic renal diseases, and neoplasm) and alcoholics. A conventional weight and height scale was used to estimate the Body mass index BMI (weight in kilograms divided by height in square meters Kg/M2) and WHO criteria (identified as BMI > 30kg/m2 and 24.9 < BMI < 30.0 kg/m2 according to the WHO guidelines),[7] were applied to define obesity . For every participant venous blood sample was taken into a tube containing ethylene diamin tetraacetic acid (EDTA) for HbA1c, and a standard biochemical tube (gel tube) for (B12, fasting insulin, FBG and Homocysteine), in the morning time, at least, 8 hours overnight fasting. The tubes were gently shaken and separated by centrifugation at 3200 rpm for 10–15 minutes, and then analyzed using cobass 6000 chemistry analyzer. Serum B12 levels were assessed using a chemiluminescent immunoassay technique, normal values were 200-1200 pg/mL. Patients with blood vitamin B12 level <200 pg/mL (148 pmol/L) were identified as having vitamin B12 insufficiency. Based on the literature.[8] Serum insulin levels were measured by chemiluminescent immunoassay, normal range 2.6–24.9 µU/mL.[9] Serum glucose levels were determined by enzymatic colorimetric test. HbA1c was measured using a turbidimetric test technique. To quantify insulin resistance, we utilized the HOMA-IR (Homeostasis Model of Assessment-Insulin Resistance) score [fasting glucose (mg/dL) x fasting insulin (µU/mL) / 405], and patients with a HOMA-IR score ≥2.7 were considered to have insulin resistance (IR)].[10]

Ethical permission was gained from the Research Committee of Ministry of Health. For each participant she/he was given the right to know the contents of research and investigations to be done and written permission was collected from each one and had the right to withdraw at any time. Statistical analysis was done using Statistical Package for the Social Sciences SPSS (IBM Corp. Released 2017. IBM SPSS for Windows, Version 25.0. Armonk, NY: IBM Corp.). Independent two sample t test was performed. P-value of <0.05 was considered significant.

Results

Demographic and Biochemical parameters of study population are elucidated in (Table 1). Patients with T2DM presented significantly with higher mean values of BMI, FBG, fasting insulin, and HOMA-IR, HbA1c and serum homocysteine, in comparison to the apparently healthy subjects. Except serum B12 concentration which were significantly inferior in patients with T2DM in relation to the apparently healthy group. (Table 1). Key: BMI = Body Mass Index,, FBG = Fasting Blood Glucose, hemoglobin A1c=HbA1c, serum B12, Serum homocysteine and HOMA-IR= Homeostatic Model Assessment for Insulin Resistance. Table (2) presents the comparison of laboratory and demographic characteristics between T2DM patients and healthy adults under 45 years of age. The results show similar trends as seen in Table 1, with T2DM patients having higher mean values of BMI, FBG, insulin, HOMA-IR, HbA1c, and serum homocysteine, and lower mean values of serum B12 levels. Association of B12 concentration with demographic and biochemical parameters in study population of young age (<45 years) which were chosen to exclude the age gap: According to Pearson correlation coefficient (r), there was a significant negative correlation between serum B12 and BMI, FBG, Fasting Insulin, HOMA-IR, HbA1c and serum homocysteine in the current study, these results were arranged and shown in the table below (Table 3). Concentration of serum B12 and serum homocysteine in patients with T2DM according to diabetes control status **:** When T2DM patient with good and poor glycemic status are comped by serum B12 levels and serum homocysteine levels there was a statistical significance between poor-control group and good-control group, (p<.05) (Table 4) Vitamin B12 and study variables According to serum concentration of B12 levels, T2DM subjects were divided into two groups T2DM with Normal and Low B12 levels. The demographic characteristics and biochemical parameters per category are listed in (Table 5). Parameters as age, FBG, BMI, serum homocysteine, HbA1c and fasting insulin are increased in group with low B12 levels.

Table (1). The laboratory and demographic characteristics comparison in the study population.

Variables	Mean ± SD	
	T2DM patients (n=70)	Apparently Healthy adults (n=68)
Age (years)	51.24±9.27	29.57±7.2
Gender (%)	Female 25(35.71%) Male 45(64.29%)	Female 45(66.18%) Male 23(33.82%)
BMI (kg/m2)	31.01±4.7	23.4±2.77
FBG (mg/dl)	224.1±50.5	90.96±5.05
Insulin (µU/mL)	12.6±3.8	6.57±2.46
HOMA-IR	6.82±2.0	1.47±0.56
HbA1c (%)	9.70±1.17	5.07±0.22
Serum B12 levels pg/ml	252.05±83.7	337.03±121.6
Serum homocysteine (µmol/L)	13.493±4.8	10.041±2.2

Table (2). The laboratory and demographic characteristics comparison between T2DM patients and apparently healthy adults of young age group (age < 45 years).

Variables	Mean ± SD	
	T2DM patients (n=20)	Apparently Healthy adults (n=66)
Age (years)	39.80±5.01	28.98±6.49

Gender (%)	Female 8(40%) Male 12(60%)	Female 45(68.18%) Male 21(31.82%)
BMI (kg/m2)	31.03±5.46	23.3±2.79
FBG (mg/dl)	233.0±50.8	90.77±5.01
Insulin (µU/mL)	12.1±2.9	6.56±2.48
HOMA-IR	6.86±1.69	1.47±0.56
HbA1c (%)	9.70±1.01	5.06±0.21
Serum B12 levels pg/ml	255.28±74.66	339.67±122.4
Serum homocysteine (µmol/L)	12.860±4.8	10.039±2.3

Table (3). Pearson correlation analysis between serum B12 concentrations and possible metabolic risk factors in the study population of young age group (<45 years).

Variables	Study young age population (n=86)	
	r(84)	p value
BMI (kg/m2)	-.341**	.01
FBG (mg/dl)	-.286**	.008
Insulin (µU/ml)	-.371*	.02
HOMA-IR	-.333**	<.01
HbA1c (%)	-.313*	.03
Serum Homocysteine(µmol/L)	-.350*	.012

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

Table (4). Distribution of serum B12 concentrations and serum homocysteine levels in the patient with T2DM according to control status by HbA1c level.

Variables	T2DM patients with HbA1c ≤7% Good-control group (n=28)	T2DM patients with HbA1c >7% poor-control group (n=42)	p value (2-tailed)
Serum B12 (pg/ml)	349.76±120.8	249.19±83.9	<*.05*
Serum Homocysteine (µmol/L)	10.13±2.27	14.47±4.87	<*.05*

Table (5). Distribution of the variables between two groups of T2DM according to the level of B12.

	T2DM patients with Normal Vitamin B12 (n=49)	T2DM patients with Low Vitamin B12 (n=21)	p value
Age (years)	50.38±10.17	51.61±8.95	>0.05*
BI (kg/m2)	30.51±4.24	32.20±5.60	>0.05*
FBG (mg/dl)	221.69±49.2	229.76±54.2	< 0.05**
Insulin (µU/mL)	12.7±4.16	12.2±3.15	< 0.05**
HOMA-IR	6.78±1.84	6.90±2.38	<0.01***
HbA1c (%)	9.46± 1.04	9.80±1.22	<0.05**
Serum homocysteine (µmol/L)	10.85±3.37	18.97± 2.97	<0.01***

(*) non-significant as p-value >0.05.(**) significant as p-value < 0.05 level).(***) high significant as p-value <0.01

Discussion

Vitamin B12 is a vital micronutrient for maintaining DNA methylation and lipid metabolism. A lack of B12 can lead to endothelial dysfunction. Homocysteine is generated during the metabolic breakdown of dietary methionine (found mainly in animal protein). Vitamin B12 is necessary for homocysteine metabolism, and its deficiency results in elevated homocysteine levels.

The current study included 138 participants with an age range of 20-69 years (mean 40.57±13.68 years) and a roughly equal gender ratio of female to male. The diabetic group had a mean age of 51.24±9.27 years and a higher proportion of males (1:1.8). This finding is in line with studies done by Miyan et al,[11] Ekpenyong et al,[12] Wilson et al,[13] and Ko et al.[14] However, it contradicts the results of Nervo et al[15], who found a higher proportion of females in the diabetic group. This discrepancy can be attributed to the different sample populations used in each study, as Nervo et al's study included data from various clinics including prenatal care clinics that covered both T2DM and gestational diabetes. The body mass index (BMI) of the diabetic group was significantly higher than that of the non-diabetic group in this study (31.01 ± 4.7 vs 23.4 ± 2.77). This is consistent with the known association between obesity and type-2 diabetes mellitus. The difference in means was 7.61 with a (p-value<0.01), indicating statistical significance. These findings are similar to those of a study done by Pflepsen et al.[16] which supports the correlation between BMI and hyperglycemia. However, they contrast with the results of a study by Negrabetsky et al.[17] which found no correlation between BMI and glycemic control due to the inclusion of insulin therapy in the study population. The prevalence of the low B12 level was 31% (between the diabetic and non-diabetic groups which is near to the prevalence of B12 which is 29% in a study done by Alvarez et. Al,[18] but contradicted with other study by Yajnik CS, et al[19] which was remarkably high 67% it was due to the vegetarian eating habits of the India peoples. The correlation between vitamin B12 levels and demographic and biochemical variables in the study population revealed a mild but statistically significant negative correlation between vitamin B12 and age, as well as fasting blood glucose (FBG) and HbA_{1c} (p-value < 0.01). This suggests that as age, FBG and HbA_1c levels increase, vitamin B12 levels decrease. These findings are consistent with those of a study by Appold et al.[20] which attributed the correlation to malnutrition in older adults. However, they contrast with the results of a study by Alsaeed[21] which found a positive correlation, likely due to the inclusion of patients receiving vitamin B12 supplements. The correlation of B12 levels with other parameters (BMI, serum Insulin, Insulin resistance and Homocysteine) showed negative correlation also (p-value <0.01), that's mean whenever the B12 level decreases the homocysteine increases. There was a significant difference between the good glycemic control group (n=28) and poor glycemic control group (n=42) from the total diabetic group (n=70) in the B12 levels and Homocysteine levels in which the cutoff point is HbA1c≤ 7% which give us a view on the effect of glycemic control on the level of B12 and its outcomes and these results are similar to study done by Kang et al[22]. In which it was based on effects of metformin usage on B12 levels, in contrary to a study done by Shivaprasad CH, et al; [23] it shows the same results and that give us an idea about glycemic control and its effects on B12 levels.The current study found a significant association between low vitamin B12 levels and insulin resistance. This association has been previously reported in studies such as Baltuci et al.[24] and Setola et al.[25] which found that low vitamin B12 levels are associated with higher levels of insulin resistance and that B12 treatment can improve insulin resistance. However, other research such as Gammon et al.[26] did not find a correlation between vitamin B12 levels and insulin resistance. These conflicting findings suggest that there may be multiple factors, such as glycemic control, body mass index (BMI), and metformin treatment, which contribute to both low vitamin B12 levels and insulin resistance. Additionally, therapy with vitamin B12 has found to be promising in improving this association.[27] The current study found a significant difference between T2DM patients with normal vitamin B12 levels and those with low vitamin B12 levels, as the latter group exhibited a one-fold increase in homocysteine concentrations (18.97 ± 2.97 vs 10.85 ± 3.37, p-value < 0.01), which further strengthened when the effect of glycemic control was considered (p-value < 0.001). These findings are consistent with a study by Esmaeilzadeh et al[28] and it is supported by previous research that has established the relationship between insulin resistance and B12 levels.

Conclusions

The current study adds to the existing literature by highlighting the importance of considering vitamin B12 levels in the management of T2DM as Regular screening for deficiency using sensitive methods like serum homocysteine, as well as vitamin B12 supplementation and the need for further research to fully understand the complex relationship between vitamin B12, insulin resistance and other metabolic parameters.

Conflicts of interest: There were no conflicts of interest.

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