Prevalence of vitamin B12 deficiency among diabetic patients in Benha City, Egypt, a hospital based: A cross-section study.

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Introduction:

Vitamin B12 (Cyanocobalamin), a water-soluble vitamin, has a vital function in the metabolism of the body as it plays an important role in the synthesis of DNA, and myelin (1). The clinical picture of vitamin B12 deficiency, is based mainly on hematological and neuro-cognitive dysfunction (2). In general, vitamin B12 deficiency is related to certain conditions such as obesity, gastrointestinal disease, bariatric surgery, and renal insufficiency (3), (4), (5). Further studies reported an association between vitamin B12 deficiency and old age (6), low income (7) certain ethnic groups (8), and lifestyle factors, such as caffeine, or tobacco (9) consumption of alcohol, and a sedentary lifestyle (4). The regular use of medications, such as metformin (10) and proton pump inhibitors (11), may be a reason of low vitamin B12. In type 2 diabetes mellitus (DM), several cross-sectional studies have reported an increased frequency of vitamin B12 deficiency (12). Metformin therapy has been established as a primary factor of vitamin B12 deficiency among patients with type 2 DM (2), resulting from malabsorption of the vitamin in this situation (13). Vitamin B12 deficiency in metformin users has been related to the duration, dose of therapy (14), and older age group (15). Type 1 DM is an

auto-immune disorder resulting from auto-immune destruction of beta cells of the pancreas (16). It is well known that pernicious anemia, resulting from chronic autoimmune gastritis, is highly frequent among patients with type 1 DM with presence of auto antibodies to intrinsic factor (17) and parietal cell (18). Therefore, vitamin B12 deficiency occurs frequently among patients with type 1 DM (16). Moreover, primary autoimmune hypothyroidism, and celiac disease (19) are common comorbidities in type 1 diabetes that affect vitamin B 12 level. This study was undertaken to establish the prevalence of B12 deficiency in patients with DM in Benha Univeristy Hospital, Egypt, and to evaluate the accompanying factors of vitamin B12 deficiency in those patients.

Subjects and methods:

This is a cross sectional, hospital based study of patients with diabetes;100 patients with known history of type 2 DM, and 30 patients with type 1 DM who were attending internal medicine outpatient clinic in Benha University Hospital. Diagnosis of DM was based on American Diabetes Association (ADA) criteria 2019 (20). Twenty age and sex matched healthy volunteers were recruited as control in the study. Data were approved by the Ethics Committee of Benha Faculty of Medicine, Benha University (MS; 12-2-2020). When the study was done; exclusion criteria were; [1] Age < 18; [2] Patients with stage 3, 4 or 5 CKD; [3] Patients with decompensated liver disease, [4] Malabsorption syndromes such as prior bariatric surgery, ileum resection, gastrectomy or Crohn's disease ; [5] Patients on certain medications like B_{12} supplementation, acid blockers (H_2 blockers and/or proton pump inhibitors), herbal supplements, or multivitamins in the last 3 months before the study; [6] Pure vegetarians ; [7] Pregnancy. Demographic data, age, anthropometric details, history of metformin therapy, and duration of intake were

included in the study. Laboratory investigations were examined in Clinical Pathology Department, in Benha Univeristy Hospital according to the routine methods in the laboratory in the form of complete blood count (CBC) fasting blood glucose (FBG), hemoglobinA1c, lipid profile, Kidney function tests, and Liver function test. Assay of Vitamin B12 was estimated by Electrochemiluminescence methodology.

Statistical analysis:

The collected data were statistically analyzed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The normality of distribution for the analyzed variables were tested using Shapiro-Wilk's test. Quantitative data were collected and summarized in terms of Mean ± Standard Deviation (SD), while qualitative data were presented as number and percentage. Comparisons between the different study groups were carried out using student t-test for Mean ± Standard Deviation of quantitative data, Chi-square ($\chi 2$) to compare (number and percentage) of qualitative data as appropriate. The level of significance in this study was ($p \leq 0.05$). While $p \leq 0.001$ was considered highly statistically significant (HS).

Results:

Our study constituted 3 groups; group A (type 2 DM) was 100 patients (49 males, and 51 females), group B (type 2 DM) was 30 (17 males, and 13 females), and group C (control group) was 20 (12 males, and 8 females. There was nonsignificant difference among the three groups regarding age, sex, residence (table 1). The duration of diabetes is insignificantly different (P =0.424) between type 1 and type 2 DM with mean ± SD of 9.60±1.133 and 9.91±1.450 years respectively. Table (2) showed that hemoglobin(

HB), High-density lipoprotein cholesterol (HDL-C), and estimated glomerular filtration rate (e-GFR) was statistically higher in the control group, while mean corpuscular volume (MCV), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), albumin excretion ratio (AER), serum creatinine, FBG, HemoglobinA1c, Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST), were significantly higher in group A and B in comparison to the control group. Table (3) revealed that serum B 12 level was statistically lower in groups A (mean ± SD = 344.83 ± 108.204 pg/mL) and B (mean ± SD = 338.87 ± 96.919 pg/mL) when we compare with the control group (mean ± SD = 447.55 ± 35.259 pg/mL). However, there was a non significant difference in the level of vitamin B 12 between type 2 and type 1 diabetic patients (P =0.774). In type 2 DM, we identified 10 % of patients had vitamin B 12 deficiency as well as 56 % had insufficient level of the vitamin. In type 1 DM, 6.7 % of patients had vitamin B 12 deficiency and 60 % had insufficient level. Sex and residence were insignificantly related to vitamin B 12 deficiency in both type 1 &2 DM. Metformin therapy users in type 2 DM had lower vitamin B 12 level than non-users (p = 0.048). In patient with type 1 DM, we found non significant difference in serum vitamin B 12 level between metformin treated and untreated patients (p = 0.155). In our study the dose of metformin therapy was not significantly related to deficiency of vitamin B 12 in both type 1 & 2 DM (table 4).

Table (1): Demographic data of the study population

	Group (A) (n=100)		Group (B) (n=30)		Group (C) (n=20)		P Value
	No.	%	No.	%	No.	%	
Age (years) (Mean± S.D)	60.21±8.286		58.83±8.840		62.10±8.207		0.432
Sex: Males	49	49.0	17	56.7	12	60.0	0.567
females	51	51.0	13	43.3	8	40.0	
Residence: Urban	46	46.0	14	46.7	9	45.0	0.993
Rural	54	54.0	16	53.3	11	55.0	

Statistically significant at P <0.05.

Table (2): laboratory characteristics of the study population.

	Group (A) (n=100)	Group (B) (n=30)	Group (C) (n=20)	P Value
HB (g/dL)	11.84±1.196	12.29±1.216	13.31±0.826	<0.001*
MCV (fl)	106.56 10.764	103.86 6.684	85.32 9.456	<0.001*
Platelet ($x10^5/\mu L$)	297.39±66.487	289.90±83.101	329.35±82.830	0.105
WBCs ($x10^3/\mu L$)	5.48±1.443	6.04±1.249	5.88±1.344	0.117
Total cholesterol (mg/dl)	191.33±27.602	191.43±22.048	183.90±27.826	0.509
LDL-c (mg/dl)	134.06±13.002	115.50±11.691	100.50±12.796	<0.001*
TG (mg/dl)	162.63±21.717	166.00±14.704	130.75±12.892	<0.001*
HDL (mg/dl)	29.10±4.541	35.07±7.995	63.61±14.451	<0.001*
Serum creatinine (mg/dl)	0.79±0.204	0.73±0.114	0.64±0.114	0.004*
e-GFR (mL/min/1/.73m2)	72.63±6.542	79.40±8.771	92.65±12.654	<0.001*
AER (mcg/min)	5.52±0.347	4.91±0.637	3.81±0.447	<0.001*
FBG (mg/dl)	129.56±9.312	121.37±7.788	92.10±9.941	<0.001*
HemoglobinA1c	7.85±0.911	7.50±0.742	4.78±0.247	<0.001*
ALT (IU/L)	40.28±3.696	35.13±4.240	25.65±2.720	<0.001*
AST (IU/L)	28.66±6.178	25.97±7.924	22.15±8.821	0.005*
Albumin (g/dL)	4.38±0.666	4.19±0.644	4.28±0.511	0.376

Statistically significant at P <0.05
Highly significant at P <0.001

Table (3): levels of B 12 in different study groups.

Vitamin B12 levels	Group (A) (n=100)		Group (B) (n=30)		Group (C) (n=20)		P Value
	No.	%	No.	%	No.	%	
Deficiency (<200 pg/mL)	10	10.0	2	6.7	0	0	<0.001*
Insufficiency (200–400 pg/mL)	56	56.0	18	60.0	0	0	
Normal (>400 pg/mL)	34	34.0	10	33.3	20	100	
Vitamin B12 levels (Mean ±SD)	344.83±108.204		338.87±96.919		447.55±35.259		<0.001*
Comparing A &B groups							0.774
Comparing A&C groups							<0.001*
Comparing B&C groups							<0.001*

Table (4): Relation of the levels of vitamin B 12 and sex, residence, metformin therapy & dose among the study population.

		Deficiency (<200)		Insufficiency (200–400)		Normal (>400)		P Value
		No.	%	No.	%	No.	%	
Sex Group (A)	Male Female	4 6	40.0 60.0	29 27	51.8 48.2	16 18	47.1 52.9	0.760
Group (B)	Male Female	0 2	0 100	9 9	50.0 50.0	8 2	80.0 20.0	0.076
Group (C)	Male Female	0 0	0 0	0 0	0 0	12 8	60.0 40.0	------
Residence Group (A)	Urban	5	50.0	25	44.6	16	47.1	0.941
	Rural	5	50.0	31	55.4	18	52.9	
Group (B)	Urban	1	50.0	7	38.9	6	60.0	0.560
	Rural	1	50.0	11	61.1	4	40.0	
Group (C)	Urban	0	0	0	0	9	45.0	-------
	Rural	0	0	0	0	11	55.0	
Metformin therapy: Group (A)	No Yes	5 5	50.0 50.0	21 35	37.5 62.5	13 21	38.2 61.7	*0.048
Group (B)	No Yes	2 0	100 0	9 9	50.0 50.0	8 2	80.0 20.0	0.155
Metformin dose Group (A)	<1000 mg 1000-1999 mg ≥2000 mg	1 1 3	10.0 10.0 30.0	7 8 11	12.5 14.3 19.6	5 2 11	14.7 5.9 32.4	0.786
Group (B)	<1000 mg 1000-1999 mg ≥2000 mg	0 0 0	0 0 0	1 1 7	5.6 5.6 38.9	0 1 1	0 10.0 10.0	0.550

Discussion:

This is a cross sectional study for the prevalence of vitamin B 12 deficiency in patients with diabetes in Benha University Hospital. In this study, vitamin B 12 is significantly lower in type 1 and type 2 DM than the control group. Ten percent of type 2 DM patients were found to have vitamin B 12 deficiency (a level of vitamin B 12 <200 pg/mL) and 56.0 % of them had insufficient vitamin B 12 levels (200–400 pg/mL). On the other side, 6.7 % of type 1 DM had vitamin B 12 deficiency and 60.0 % had insufficient vitamin B 12 level. One study found that 22% of type 2 DM patients identified with metabolic B12 deficiency (21). Another study evaluated the level of vitamin B 12 in type 1 DM, with 28.5% of them were deficient (22). There was non significant difference in the level of vitamin B 12 between type 2 and type 1 DM patients (P =0.774). To our knowledge, there is no studies comparing vitamin B 12 deficiency in both type 1 and type diabetic patients. In our study, hemoglobin was significantly lower with higher MCV values in both type 1 and 2 DM compared to the control groups. In contrast to another study that found normal MCV among diabetic with vitamin B 12 deficiency (23). In the present study, sex was not significantly associated with vitamin B 12 deficiency in both type 1 & 2 DM. Previous studies revealed no significant difference in sex for the plasma level of vitamin B12 in type 2 DM (23), (24) & (25). On the contrary, one study demonstrated that males had lower values of vitamin B 12 than females (26). In the current study, residence in rural or urban areas did not significantly associated with vitamin B 12 deficiency. Certain ethnic groups, low income, and lifestyle factors, such as higher consumption of alcohol, caffeine, or tobacco, and a sedentary lifestyle were the variable factors from rural to

urban areas. It was previously highlighted that lifestyle, and low income were associated with low vitamin B 12 (3) & (7). Concerning type 2 diabetic group, our results recognized that a low vitamin B 12 level (either deficiency or insufficiently) was seen in 40 patients (65.6 %) among metformin users. Low serum B12 level was significantly associated with the use of metformin which is consistent with several studies (27) & (28). Furthermore, the prevalence of low level of B12 in type 2 diabetic using metformin is estimated to be from 5.8% to 33% (21)& (29).This variable prevalence of metformin induced B 12 deficiency may be related to inconstant definitions of cut point for the deficiency. Metformin related vitamin B12 deficiency may be explained by: Firstly, alterations in small bowel motility, stimulating bacterial overgrowth which is competitive inhibition or inactivation of vitamin B12 absorption. Secondly, alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor (30). Thirdly, metformin inhibits the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum (31). Our results detected that metformin dose did not appear to affect vitamin B 12 levels in both type 1 & type 2. While other studies reported that metformin dose and longer duration were risk factors for B12 deficiency (14). The matter of discussion is the time needed to deplete body stores of B12 which depends on the initial amount stored, and the efficiency of absorption from the diet and reabsorption from bile (32). It would also be interesting to know that normal body stores are about 1 to 3 mg; the turnover of the vitamin in healthy persons is about 0.1% per day; whereas signs of deficiency appear if the pool drops below 300 μg. If there is no intake from food or supplements, and absorption is normal, a 1-mg store would be enough for the body’s needs for 3

years, 2 mg for 5 years, and 3mg for 6 years. However, in people with less efficient absorption of the vitamin from food because of gastric atrophy, these values would be decreased to 2, 3.6, and 4 years, respectively (33). In the current study in type 1 diabetic patients, 9 out of 11 had low B 12 level while on metformin therapy and only 2 patients were deficient in vitamin B 12 but not on metformin therapy. Moreover, low vitamin B12 level did not associated with metformin use. Vitamin B12 deficiency due to pernicious anemia observed occasionally among patients with T1DM (16). Celiac disease, affecting vitamin B 12 level (19), occurs in 1-16% of type 1 diabetic patients (34). In our results, neither pernicious anemia nor celiac disease has been tested. Therefore, the estimation of vitamin B 12 level in patients with type 1 DM requires measurements of intrinsic factor (AIF), parietal cell antibodies (PCA), and celiac screening in larger groups of patients. It is important to add that the use of metformin as adjuvant to insulin therapy in type 1 DM is still under argument (35).

Conclusion:

We found 66 % and 66.6 % with low vitamin B 12 in type 2 and type 1 DM respectively who attended internal medicine clinic of Benha University Hospital. The study also confirmed that metformin therapy is associated with vitamin B12 deficiency in type 2 DM and hence to follow the recommendations of ADA of periodic monitoring of vitamin B12 levels in patients with type 2 diabetes on metformin, particularly in those with a diagnosis of peripheral neuropathy and/or anaemia.

Limitation of the study:

This is a cross-sectional study and therefore a true cause of B12 deficiency in metformin user cannot be established. Accordingly, a larger placebo-controlled studies are needed to determine the long-term effects of metformin on serum B 12 level in both type 1 & type 2 DM. We did not assess the presence of pernicious anemia or celiac disease in type 1 DM that affect the level of vitamin B 12 level. We need a comprehensive assessment of vitamin B 12 deficiency through measurement of methylmalonic acid and homocysteine blood levels. Our patients were not on any vitamin B 12, multivitamins, or herbal supplements in the last 3months of the study, so, we did not estimate empiric treatment with multivitamin, absorption or stores of vitamin B 12 before the beginning of the study.

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