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Effect of Metformin on Serum Vitamin B_{12} Levels in Type 2 Libyan Diabetic Patients

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ABSTRACT

Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes. The aim of this work is to investigate the effect of metformin on serum vitamin B_{12} levels in Libyan diabetic patients of type 2. This study was carried out in Diabetes and Endocrinology Hospital, Tripoli, Libya.

The study investigates 101 blood samples from volunteers; 81 blood samples were collected from patients suffered from type 2 diabetes and 20 healthy (10 males, 10 females). Therapy for 40 patients was metformin (19 males, 21 females), while the other 41 patients receiving other oral hypoglycemic and/or insulin treatment (22 males, 19 females). Serum vitamin B_{12} evaluation was carried out by electrochemiluminescence immunoassay (ECLIA).

Gender has shown no influence on serum vitamin B_{12} levels in diabetic patients or healthy people. There is no major difference in serum vitamin B_{12} levels between diabetics who received metformin and diabetics who received non-metformin medicine (oral hypoglycemic and/or insulin). This study has found that HbA1c level was improved slightly over long duration of treatment. Moreover, this study found that people of 40 to 60 years old (60% of the total sample) were the most valunerable to diabetes. The highest percentages of the diabetics have suffered from hypertension followed by another cardiac disease.

The present study has revealed that, there is no significant changes in serum vitamin B_{12} levels in diabetic patients received metformin only compared to those who received other hypoglycemic and/or insulin. Hypertension and cardiac disease are the most abundant concomitant diseases that diabetic patients suffered. Nutrition in Libyan culture may have effective role in maintaining vitamin B_{12} in normal level.

Keywords - *Vitamin B_{12}; Metformin; Type 2 diabetes*

INTRODUCTION

Type 2 diabetes usually appears in people over the age of 40, though in South Asian and black people, who are at greater risk, it often appears from the age of 25. It is also increasingly becoming more common in children, adolescents and young people of all ethnicities.[1]

The etiology of type 2 diabetes is associated with obesity which is the main reason of increasing the incidence of the disease over last 50 years[2], also decreased physical activity and unhealthy diets. It involves insulin resistance in nearly all cases. Diabetes type 2 occurs more frequently in individuals with hypertension, dyslipidemia, and is a component of "metabolic syndrome". It is often runs in families, it is a complex disease caused by mutations in more than one gene, as well as by environmental factors.[3]

Managing type 2 diabetes involves a journey of therapy, which begins with changes in diet and pattern of exercise. This continues as a way of life; patient may start taking hypoglycemic tablets, eventually take insulin injection. Good diabetes care includes both medical treatment and education.[4]

Overall aim of treatment is symptom relief and prevention or delay of complications by targeting normal blood glucose levels.[5] Patients treated with several classes of anti-diabetic medications available. Metformin (500 mg) tablet is generally recommended as a first line treatment, as there is some evidence that it decreases mortality. A second oral agent may be used if metformin is not sufficient as: sulfonylureas, nonsulfonylurea, secretagogues inhibitors, thiazolidinediones, glucagon-like peptide-1 analog, or dipeptidyl peptidase-4 inhibitors. Metformin should not be used in those with severe kidney or liver problems. Injections of insulin may either be added to oral medication or used alone.[6]

Metformin is an oral anti-diabetic drug of biguanide class.[7] The principal underlying mechanism remains unclear; several anti-diabetic mechanisms have been implicated, including suppression of hepatic glucose output via inhibition of gluconeogenesis. This is believed to be the main mechanism in reducing plasma glucose levels.[8] Metformin enhances tissue sensitivity (muscles) to insulin. Several studies have shown increases in insulin-mediated glucose uptake and oxidative metabolism in muscle, and delayed gastrointestinal absorption of glucose.[9-12] Metformin has other beneficial effects as lowering triglyceride levels and body weight, and decreasing

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plasminogen-activator inhibitor levels.[13]

Vitamin B_{12} (cobalamin) is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system; it is important for the formation of blood. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, also fatty acid synthesis and energy production. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.[14,15]

Vitamin B_{12} is used to treat vitamin B_{12} deficiency, cyanide poisoning, and hereditary deficiency of transcobalamin II. It is also given as part of the Schilling test for detecting pernicious anemia.[16] High vitamin B_{12} level in elderly individuals may protect against brain atrophy or shrinkage, associated with Alzheimer's disease and impaired cognitive function. Administration of high-dose of Vitamin B_{12} has been validated to stimulate the activity of the body's (TH1) T helper cells type1; which are subpopulation of T cells (sub-group of lymphocytes), suppressor T-Cells; which are a subpopulation of T cells (sub-group of lymphocytes), which then down-regulates the over-production of the allergen antibody IgE in allergic individuals.[17]

Vitamin B_{12} is used to regenerate folate in the body.[18] Most vitamin B_{12} deficiency symptoms are actually folate deficiency symptoms; since both B_{12} and folate deficiency cause pernicious anemia and megaloblastosis. The two diseases are due to poor synthesis of DNA when the body does not have a proper supply of folic acid for the production of thymine; if sufficient folic acid is available, all known B-12 related deficiency syndromes normalize.[19]

In recent years, a number of studies have concluded that metformin can cause vitamin B_{12} deficiency in some diabetic patients, although the precise mechanism by which this occurs remains unknown. A study in Pakistan (2013) demonstrated significantly high prevalence of vitamin B12 deficiency in patients treated with metformin with significant effect of dose amount and duration of metformin use on B12 levels.[21] Another study reported in Archives of Internal Medicine found an increased risk of vitamin B_{12} deficiency associated with current dose and duration of metformin use[20], while other study found that in patients taking metformin, the drug was not always the cause of vitamin B_{12} deficiency. [22] However, one possible factor is interference with vitamin B_{12} absorption created by the B_{12}-intrinsic factor complex[23], however, this can be reversed quickly with vitamin B_{12} supplementation (or with discontinuing the use of metformin). Metformin may interfere with calcium metabolism, this may indirectly reduce vitamin B_{12} absorption, and since vitamin B_{12} absorption requires calcium.[24] Long term treatment with metformin increases the risk of vitamin B_{12} deficiency, which results in raised homocysteine concentrations.[25]

The aim of the study is to investigate the effect of metformin in Libyan diabetic patients. A screening was accomplished to measuring serum vitamin B_{12} levels in 81 Libyan diabetic patients, with or without metformin use. Also study the influence of diet on serum vitamin B_{12} levels. A comparison was made between impact of metformin, or oral hypoglycemic and/or insulin on serum vitamin B_{12} levels.

MATERIALS AND METHODS

The prospective clinical study was undertaken on 81 Libyan diabetic patients at Endocrinology Hospital in Tripoli, with or without metformin use to assess serum vitamin B_{12} levels. Serum levels of vitamin B_{12} were evaluated in adults on 81 diabetic patients (41 males and 40 females). Healthy subjects (10 males and 10 females) were used as control group. 40 patients are on metformin treatment, while 41 patients are using insulin and/or oral hypoglycemic treatments.

General information regarding the age, duration of treatment were taken from the diabetes booklet of each patient, in addition to HbA1c test result. Moreover, information about other diseases, diet and lifestyle of these patients were gathered by interviewing the subjects.

Blood samples were taken by vacuum blood collection plane tube (glass, no additive) from the patients, separated by centrifuge for 5 min at 3,000 rpm to eliminate cell debris using Heraeus centrifuge from Germany. Serum samples were stored at -20°C and kept until assay for vitamin B_{12}. Kits used, for evaluation of vitamin B_{12}, is COBSE and ELECSYS from Roche (Roche Diagnostics GmbH, D-68298 Mannheim - USA).

Measurement of serum vitamin B_{12} levels

Binding assay was used for the in-vitro quantitative determination of vitamin B_{12} in human serum. The electrochemiluminescence immunoassay (ECLIA) is intended for use on Elecsys and cobas e immunoassay analyzers. The samples were run automatically in these systems.

Reagent – working solutions: PT1 Pretreatment reagent 1, 4 L: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5. PT2 pretreatment reagent 2, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L. M streptavidin-coated microparticles, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative. R1 intrinsic factor~Ru (bpy) ⅔, 10 mL: Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative. R2 vitamin B_{12} ~biotin, 8.5 L: biotinylated vitamin B_{12} 25 µg/L; phosphate buffer, pH 7.0; preservative.

Test principle

The Elecsys Vitamin B_{12} assay employs a competitive test principle using intrinsic factor specific for vitamin B_{12}. Vitamin B_{12} in the sample competes with the added vitamin B_{12} labelled with biotin for the binding sites on the ruthenium-labelled intrinsic factor complex. Total duration of assay: 27 minutes.

1. Incubation: By incubating the sample (15 µL) with the vitamin B_{12} pre-treatment 1 and pre-treatment 2; bound vitamin B_{12} is released.

2. Incubation: By incubating the pre-treated sample with the ruthenium labelled intrinsic factor, a vitamin B_{12} binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

3. Incubation: After addition of streptavidin-coated microparticles and vitamin B_{12} labelled with biotin, the still-vacant sites of the ruthenium labelled intrinsic factor become occupied, with formation of a ruthenium labelled intrinsic factor-vitamin B_{12} biotin complex. The entire

complex becomes bound to the solid phase via interaction of biotin and streptavidin.

4. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell.

5. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

6. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Statistical analysis

Statistical Package for the Social Sciences software (SPSS version 13) was used for data analysis. Descriptive analysis including the estimation of mean values and standard error of the mean for continuous variables were calculated. One-Sample Kolmogorov-Smirnov Test is applied for each treatment to find out if the group's behavior is parametric or non-parametric. For comparison of parametric groups, analysis of variance (ANOVA) was used to determine the differences between treatments; LSD was applied as a Post Hoc test for comparison of each two groups together. For non-parametric groups comparison was applied using Mann-Whitney Test. *P* value ≤ 0.05 was considered as statistically significant.

RESULTS

In this study, the number of patients of age 41-60 was higher than patients of 60-80 years old; while the number of patients of age 60-80 is more than patients of 21-40 years old. The number of patients with duration of treatment groups 1-8 years > 9-16 years; while the number of patients with the duration of treatment groups 9-16 years > years (Table 1).

Comparing the mean of vitamin B_{12} levels in healthy male and female adults and diabetic patients; it was found that there is no significant difference ($P > 0.05$) between vitamin B_{12} levels in male and female in each group (Table 2). Serum vitamin B_{12} levels were not changed in diabetic patients compared to healthy adults ($P > 0.05$). Metformin did not change the levels of vitamin B_{12} compared to patients received non-metformin therapy as oral hypoglycemic and/or insulin (Table 3).

Comparing the mean of HbA1c values with regard to duration of treatment {(1-8), (9-16), (17-32) years}, it can be noted that there is no significant difference ($P > 0.05$) between all diabetic patients or between patients treated with metformin only and patients treated with non-metformin (Table 4).

For the diabetic patients who have other diseases, it was found that the number of diabetic patients with Hypertension > Cardiac > Thyroid > Ulcer > Arthritis > Abnormal lipid profile > Kidney disease > Allergy (Table 5). Patients serum vitamin B_{12} levels were not changed ($P > 0.05$) during metformin treatment for up to 32 years {(1-8), (9-16), (17-32) years}. This observation was the same in patients treated with non-metformin therapy for up to 32 years of treatment ($P > 0.05$) (Table 6).

Consuming food rich in vitamin B_{12}, it was found that around 90% of patients regularly eat tuna, while only 18.5% eat fortified cereals and 22.2% eat liver; in addition, approximately 85% their diet involve milk and 72.8% include chicken (Table 7; Figure 7).

Table 1: The number of diabetic patients in different age groups, and the number of diabetic patients with different duration of treatment.

	Years	Number of patients	Percentage
Age group (years)	21- 40	6	7.4
	41-60	47	58.02
	61-80	28	34.56
Duration of treatment (years)	1-8	46	56.79
	9-16	21	25.92
	17-32	14	17.28

Table 2: Serum vitamin B_{12} levels in male and female healthy adults and diabetic patients.

Gender	Vitamin B_{12} levels (pg/ml) in diabetic patients	Vitamin B_{12} levels (pg/ml) in healthy adults
Male	481.07 ± 38.287 (n=41)	431.84 ± 48.592 (n=10)
Female	567.08 ± 42.15 (n=40)	418.16 ± 33.956 (n=10)

The scale is the mean ± SE; Normal range of vitamin B_{12} (176 – 1100 pg/ml)

Table 3: Serum vitamin B_{12} levels in diabetic patients and healthy adults

Treatment	Serum vitamin B_{12} levels (pg/ml)
Healthy adults (n=20)	425 ± 28.892
Diabetic patients (n=81)	523.54 ± 28.667
Diabetic patients with metformin (n=41)	510.51 ± 47.046
Diabetic patients with oral hypoglycemic and/or insulin (n=40)	536.25 ± 33.67

The scale is the mean ± SE

Table 4: HbA1c values in all diabetic patients with different duration of treatment after treatment with metformin or non- metformin therapy

Duration of treatment (years)	HbA1c % in all diabetic pts.	HbA1c % in pts with metformin use	HbA1c % in pts with non-metformin use
1-8	9.22 ± 0.454	8.86 ± 0.482	9.15 ± 0.929
9-16	8.34 ± 0.787	8.75 ± 2.250	8.14 ± 0.713
17-32	8.04 ± 0.617	8.04 ± 0.617	7.16 ± 0.375

The scale is the mean ± SE

Table 5: Concomitant diseases associated with diabetes

Disease	Number of patients	Percentage %
Hypertension	27	33.33
Cardiac	6	7.4
Thyroid	4	4.93
Ulcer	3	3.7
Arthritis	2	2.46
Abnormal lipid profile	2	2.46
Kidney disease	1	1.23
Allergy	1	1.23
Total	46	100

Table 6: Effect of the duration of treatment on serum vitamin B12 levels in diabetic patients treated with metformin or other oral hypoglycemic medication.

Duration of treatment (years)	Vitamin B_{12} levels (pg/ml) with metformin therapy	Vitamin B_{12} levels (pg/ml) with non-metformin therapy
1-8	506.68 ± 59.888	476.77 ± 41.906
9-16	550.11 ± 110.458	541.45 ± 55.519
17-32	485.18 ± 89.842	630.11 ± 86.407

The scale is the mean ± SE

Table 7: Vitamin B_{12} food sources consumed by diabetic patients

Vitamin B_{12} source	Fish (tuna)	Milk	Dairy product	Chicken	Meat	Egg	Fruit	Liver	Fortified cereals
Number of patients	73	69	64	59	46	39	21	18	15

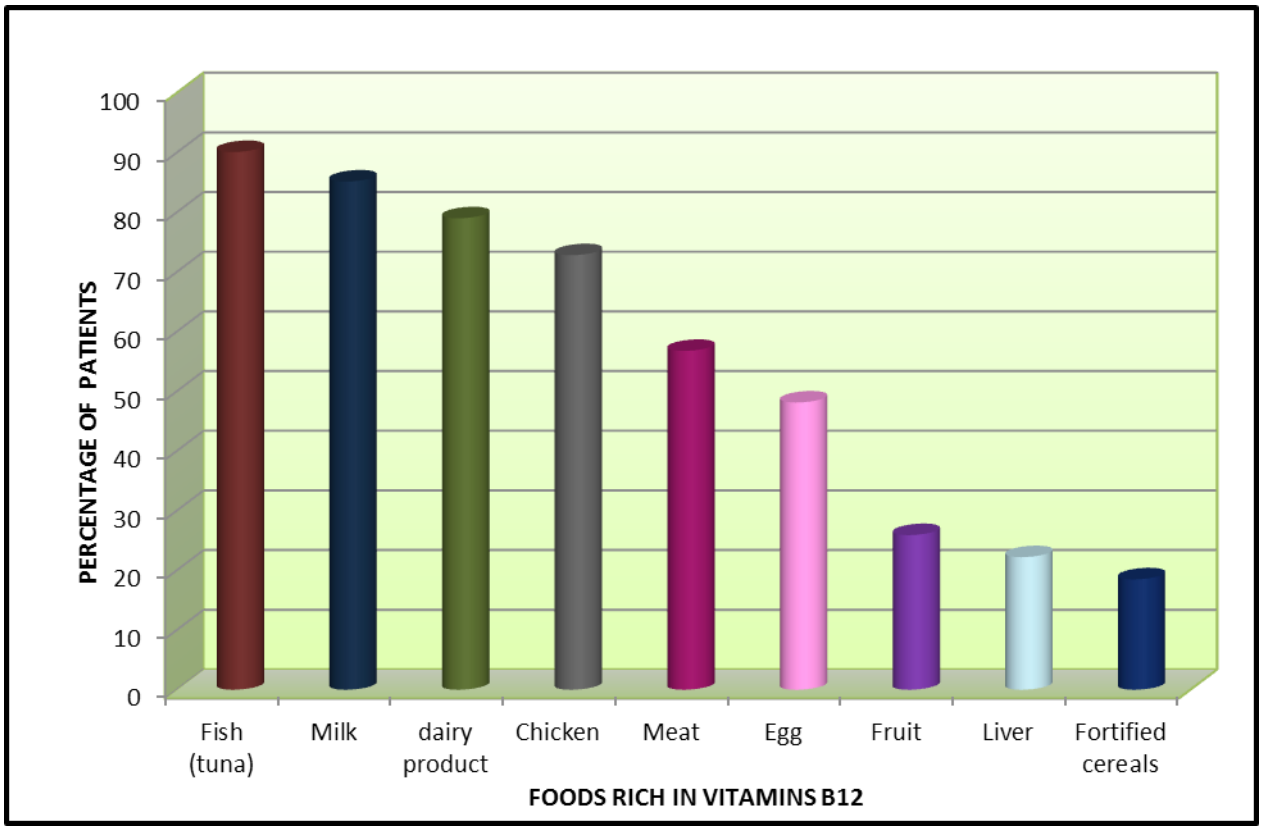

Figure 1: Vitamin B_{12} food sources consumed by diabetic patients.

DISCUSSION

There is no major difference between serum vitamin B_{12} levels in healthy male and female volunteers. This work indicates that the difference between serum vitamin B_{12} levels in male and female diabetic patients is not significant. Therefore, it can be stated that gender may not be considered as a factor that affect the level of vitamin B_{12} in healthy or diabetic volunteers. This finding confirms previous studies[26,27], that indicated there was no significant difference in serum vitamin B_{12} levels according to the gender. One study[28] has shown that the behavior of vitamin B_{12} levels in diabetic males and females were the same, although their results show deficiency in both; which may be related to culture nutrition.

The levels of serum vitamin B_{12} levels in healthy and diabetic volunteers were within the normal range, indicating that the levels of vitamin B_{12} is not affected by diabetic disease either with metformin treatment or not; it may be related to nutrition in Libyan society.

The levels of serum vitamin B_{12} were within the normal range in diabetic patients with metformin or with other oral hypoglycemic and/or insulin. In Egypt, cultured study has shown that there were vitamin B_{12} deficiency in diabetics with metformin and non-metformin therapy[29], this finding may prove that metformin has nothing to do with vitamin B_{12} deficiency. However, a recent study showed that diabetic patients with type 1 tend to have vitamin B_{12} deficiency[30], it may be related to the pathophysiology of diabetes.

The importance of routine screening for B_{12} deficiency in diabetic patient is still unidentified, and the ideal supervision of such patients is still uncertain; although some clinicians withdraw metformin and fully investigate the patient, but others take more pragmatic approach to continue the metformin, a valuable drug which may not be the cause of the deficiency.[22]

In diabetic patients treated with metformin at different durations, vitamin B_{12} level was not changed, which is confirmed by a previous study regardless the presence of vitamin B_{12} deficiency.[31] Other studies have shown that metformin produces vitamin B_{12} deficiency in diabetic patients.[25,32] However, the mechanism is not clear yet.[33]

Vitamin B_{12} deficiency could be masked by lack of hematological changes or by concomitant causes of microcytosis and that serum vitamin B_{12} levels as well as other ancillary tests should be considered in all suspected cases.[34] Our findings suggest that regular measurement of vitamin B_{12} concentrations during long term metformin treatment should be considered.

The total number of diabetic patients who were included in this study was 81. It was clear that the lowest percentage of diabetic patients was in the age of 21 to 40 years (7.4%), while the largest percentage was in the age group of 41 to 60 years (58.04%), and (34.56%) of patients were in the group of 61 to 80 years old. Type 2 diabetes starts usually after the age of 40 that is why it used to be called "adult - onset diabetes". However, as in type 1, type 2 diabetes can develop at any age and in recent years, has been seen in children.[35]

The main group (56.79%) of diabetic volunteers screened was patients with 1 to 8 years of duration of treatment;

while the lowest (17.28%) were patients with 17 – 32 years of treatment. It was noticed that later group of patients found difficulty in visiting the medical center regularly.

In the present study hypertension was the most abundant concomitant disease that diabetic patients suffer (33.33%). Diabetes and high blood pressure tend to occur together because they share certain physiological traits. Diabetes increases the total amount of fluid in the body, which tends to raise blood pressure; also it can reduce the ability of the blood vessels to stretch, thus, increasing average blood pressure. Diabetes changes the way that body produces and handles insulin, leading to an increase in blood pressure. Though these common biological traits partially explain why diabetes and high blood pressure is such a common pair.[36, 37]

Cardiac disease was the second most abundant co-morbidity in the diabetic patients in this study (7.4%). Intensive blood-glucose control substantially decreases the risk of microvascular complications, but not macrovascular disease, in patients with type 2 diabetes.[37]

Epidemiological study of the general population have shown an increased risk of cardiovascular disease with fasting glucose or haemoglobin A1c (HbA1c) levels just above the normal range. Improving glucose control, by any therapy, has not been found to reduce the risk of cardiovascular endpoints.[38]

The values of HbA1c in diabetic patients improve slightly over long duration of treatment, either in metformin or non-metformin patients; previous study concludes that metformin lower HbA1c after long duration of treatment compared to other diabetes type2 drugs.[39]

CONCLUSION

The levels of vitamin B_{12} in Libyan diabetic patients (type2) were found within the normal range. There are no significant changes between vitamin B_{12} levels in males and females diabetic patients. Diabetic patients treated more than 8 years showed slightly insignificant decrease in HbA1c levels. Future work should be conducted with larger number of patients.

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