Original Research Article

Assessment of Serum Vitamin B12 among Diabetic Patients under Treatment with Metformin

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Abstract

Introduction: Metformin is the most widely administered anti-diabetic medication among type 2 diabetes mellitus (T2DM) patients. However, metformin induces vitamin B12 mal-absorption which may increase the risk of vitamin B12 deficiency among T2DM patients. *Aim:* To determine the prevalence of vitamin B12 deficiency among diabetic patients on metformin therapy. *Materials & Methods:* This is a case-control hospital-based study conducted in Khartoum state. A hundred subjects were involved, fifty were with type 2 diabetes patients underuse of metformin, and fifty were diabetic patients not underuse of metformin. Serum Vitamin B12 levels were estimated by the ELIZA method in a fully Automated Biochemistry Analyzer (Cobas). *Results:* Table 3.2 shows the mean serum level of vitamin B12 in diabetes underuse of metformin and control group respectively were (252.6 ± 101.3, 339.4 ± 112.3), there was a significant decrease of serum level of vitamin B12 in patients under treatment with metformin compared to patients not underuse of metformin (p value=0.000). *Conclusion:* Significant decrease of serum vitamin B12 in the diabetic patients under treatment with metformin compared to diabetic patients not use of metformin. Vitamin B12 deficient subjects presented with significantly longer duration of T2DM, duration of metformin use, and use of higher metformin doses compared to non-deficient subjects.
Keywords: Metformin, diabetic, deficiency, vitamin B12.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease associated with hyperglycemia owing to impaired insulin secretion, insulin activity, or both [1]. High blood glucose levels can cause damage to all parts of the body including the cardiovascular system, eyes, kidneys, and nervous system [2]. American Diabetes Association 2019 recommended Metformin for the treatment of type 2 DM and should be continued as long as it is tolerated [3]. Metformin is one of the most widely used oral hypoglycemic agents [4]. Most of the current global clinical practice recommendations, including those of the American Diabetes Association, the European Association for the Study of Diabetes, and the Korean Diabetes Association, propose that metformin if there are no contraindications, should be initiated with concurrent lifestyle modifications at initial diabetes diagnosis [5-7]. Most of the side effects due to metformin are mild and usually include gastrointestinal symptoms, such as abdominal distress, soft stools, and diarrhea [8]. Generally, these side effects appear shortly after the initiation of metformin and promptly disappear after discontinuation. However,

insidious or asymptomatic side effects resulting from long-term treatment, such as vitamin B12 deficiency, may not be easily detected without close attention. Serum vitamin B12 levels have been reported to be inversely associated with the dose and duration of metformin use [9-11]. These studies reported that an average of 10 to 30% of patients exhibited mal-absorptive vitamin B12 deficiency [9, 12]. Although the clinical significance of vitamin B12 deficiency related to metformin treatment is debatable, monitoring for vitamin B12 has been recommended for patients with type 2 diabetes, especially those on long-term metformin treatment [10]. Clinically, vitamin B12 deficiency could lead to altered mental status, megaloblastic anemia, and neurological damage [10, 13]. Unfortunately, diabetic neuropathy symptoms can overlap with paresthesias, impaired vibration sensation, and proprioception [15]. Therefore, peripheral neuropathy due to vitamin B12 deficiency may be confused with diabetic peripheral neuropathy or may contribute to the aggravation of diabetic peripheral neuropathy [13, 15]. The progression of neurologic damage due to vitamin B12 deficiency can be stopped by early detection and treatment with cobalamin

supplementation [14]. However, if this occurrence is misdiagnosed as diabetic neuropathy, permanent neurological damage may occur [15]. As metformin has been prescribed worldwide and treatment periods increase, the prevalence of metformin-induced vitamin B12 deficiency may have also significantly increased. However, the relationship between metformin use and vitamin B12 deficiency in the African population has not been widely investigated. This study focused on the prevalence of vitamin B12 deficiency and the factors associated with it in Sudanese patients with type 2 diabetes who were treated with metformin.

MATERIALS & METHODOLOGY

A hundred subjects including fifty patients known diagnosed with Diabetes mellitus undertreatment with metformin and others fifty also known diagnosed with Diabetic patients but not treated with metformin as control groups in different hospitals from March 2020to March 2021 were enrolled into this case-control study.

Baseline characteristics of populations consisted of 26 females/24males and their ages ranged between 40 and 70 years with average age of 54 years. Under aseptic and antiseptic precaution, blood specimens were collected. The blood was collected in a heparinized tube at room temperature and centrifuged for 10 minutes at 3500rpm. All the precautions were taken in accordance with the Clinical and Laboratory Standards Institute criteria. Serum vitamin B12 was estimated by the ELIZA method in a fully Automated Biochemistry Analyzer (Cobas). Ethical approval was obtained from the local research ethics committee and present all subjects gave their informed written consent. Data were analyzed using SPSS version 25. The results expressed as mean, standards deviation, frequency, and percentage. An Independent t-test was obtained to

compare the mean of B12 in the case and control. Chi-square was used to compare the proportion of B12 deficient and measure the risk in case and control. ANOVA was done to compare the mean of B12 across the duration of treatment and treatment doses. Pearson correlation was obtained to study the relationship between the age and B12 level. A P-value less than 0.05 is considered as significant or association.

RESULTS

A total of hundred (100) subjects with D.M were included in the study. All subjects categorized into two groups as described in material and method (case and control group)in this study majority of patients 23(46%) in case group (diabetic patients treated with metformin) were used metformin 5-10 years, 22(44%) of patients have used metformin less than 5 years and 5(10%) of patients were used metformin more than 5 years presented in Figure-1. The age of population ranged between 40-70 years with a mean age was 54 years with an average dose of metformin Dose (mg) 627.0 mg and the mean age of control was 53 years presented in Table-1.
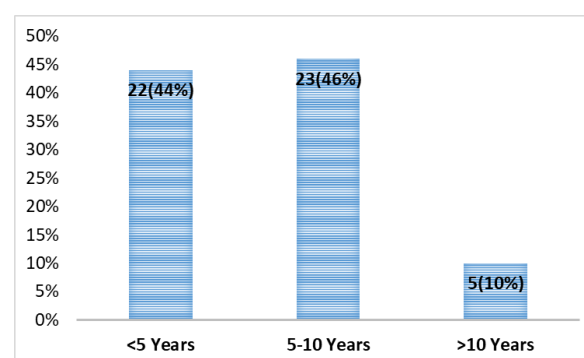

Fig-1: Distribution of study group according to the duration of used metformin

Table-1: Mean age and dose of the study group

Variables	Case (Mean±SD)	Control (Mean±SD)	P-value
Age (Years)	54.00±9.23	53.84±9.03	0.930
Dose (mg)	627.0±191.2	-	-

This study was carried to measure serum vitamin B12 levels in adults with diabetes who used metformin as treatment compared with diabetes patients who not used metformin. Table-2 Shows the mean serum level of vitamin B12 in diabetes used metformin

and control group respectively were (252.6±101.3, 339.4±112.3), was a significant decrease of serum level of vitamin B12 in the diabetic patients used metformin compared to diabetic patients not used metformin (p value=0.000).

Table-2: Mean of B12 comparison in case versus control group

Parameters	Group	N	Mean±SD	P-value
B12 (mol/L)	Case	50	252.6±101.3	0.000
	Control	50	339.4±112.3	

In Table-3 in this set of data, the subjects were categorized according to level of serum vitamin B12 [Deficient and normal], 9 (18.0%) patients of case

group had serum vitamin B12 deficiency compared to one patient (2.0%) in control group.

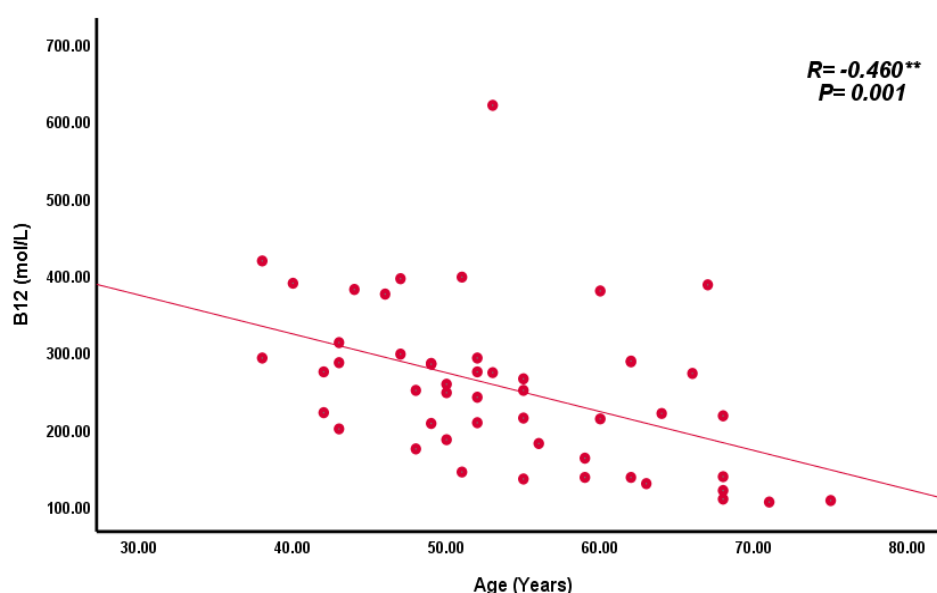**Table-3: B12 Deficient in case versus control group**

B12	Group		P-value	OR (CI Lower-CI Upper)
	Case	Control		
Deficient	9 (18.0%)	1 (2.0%)		
Normal	41 (82.0%)	49 (98.0%)	0.008	10.756 (1.308-88.473)
Total	50 (100.0%)	50 (100.0%)		

In Table-4 demonstrates the association of various risk factors with serum vitamin B12 deficiency [Duration of treatment and dose], the mean serum level of vitamin B12 in diabetes used metformin less than 5 years, 5-10 years and more than 5 years respectively were (307.3±93.92, 220.1±85.91&162.0±77.79), was a significant decrease of serum level of vitamin B12 in the diabetic patients used metformin with long duration of treatment (p value=0.002&0.001). the mean serum level of vitamin B12 in diabetes used metformin 500 mg, 850 mg and 1000 mg respectively were (277.8±95.87, 222.5±93.80&147.4±78.63), was a significant decrease of serum level of vitamin B12 in the diabetic patients used metformin with high dose of treatment (p value=0.044 & 0.006).

Table-4: Mean of B12 comparison across duration

Factor		Mean±SD	P-value
Duration	<5 Years	307.3±93.92	
	5-10 Years	220.1±85.91	0.002
	>10 Years	162.0±77.79	0.001
Dose	500 mg	277.8±95.87	
	850 mg	222.5±93.80	0.044
	1000 mg	147.4±78.63	0.006

Figure-2 Shows Correlation between the serum levels of sodium and Patient's age. There was insignificant Correlation between the serum levels ofvitamin B12 and Patient's age among diabetic patients used metformin, was a significant decrease of serum level of vitamin B12 in the diabetic patients used metformin with older age (p value=0.0091).

Fig-2: Correlation between age and B12 level

DISCUSSION

This study reports a high prevalence of vitamin B12 deficiency among Diabetic patients used metformin.

Vitamin B12 deficient subjects presented with significantly longer duration of T2DM, duration of metformin use and used higher metformin doses compared to non-deficient subjects. We found longer duration of metformin use (≥5 years) and higher daily dose of metformin (≥1000 mg/day) to be significantly associated with increased risk of vitamin B12 deficiency. Varying prevalence rates of vitamin B12 deficiency among diabetic patients on metformin have been reported in diverse populations. Report from an early clinical observational study by Tomkin et al., [15] among Irish diabetic patients on long-term metformin therapy with concomitant dietary management indicated a prevalence of 30% for vitamin B12 mal-absorption. DeFronzo et al., also reported a prevalence of 29% among American T2DM patients using metformin [16]. Another study by Sparre Hermann et al., reported a 26.7% prevalence of vitamin B12 deficiency in metformin-treated T2DM patients in Sweden [17]. Additionally, a study by Damião et al., [18] and Pflipsen et al., [19] found a 22.5% and 22% prevalence of B12 deficiency among Brazilian and American

T2DM patients using metformin. Another study by Ko et al., reported a 9.5% prevalence of vitamin B12 deficiency in metformin-treated T2DM patients in Korea [20]. Furthermore, a study by Reinstatler et al., among adults ≥50 years of age from NHANES 1999–2006 found that biochemical vitamin B12 deficiency was present in 5.8% of T2DM patients using metformin and 2.4% among those not using metformin [21].The association of duration of metformin use and vitamin B12 deficiency found in this study is expected as similar findings have been reported by previous studies. A study by Bauman et al., found that 12 out of 14 T2DM patients presented with reduced serum total vitamin B12 levels after 3 months of metformin therapy [22]. A study by Ting et al., also found increased duration of metformin use to be associated with more than two-fold increased risk of developing vitamin B12 deficiency [23]. Another study by De Jager et al., found that the absolute risk of vitamin B12 deficiency after a period of 4 years was 7.2% higher in T2DM patients on the metformin [24]. A similar finding has also been reported by Wile and Toth [25], Wulffelé et al., [26], and Andrès et al., [27]. Despite the coherence with previous studies, metformin-induced vitamin B12 deficiency in this study appeared to be duration of use- and dose-dependent similar to the findings of Beulens et al., [28]. Ko et al., [29] found that T2DM patients who had taken metformin >1,000 mg/day were approximately 10 times as likely to have vitamin B12 deficiency as patients taking metformin ≤1,000 mg/day which is in harmony with this present study. However, Ko et al., also found that this dose (>1,000 mg/day) was associated with vitamin B12 deficiency after ≥45.5 months (≈12 years) of therapy which is a longer period compared to the cutoff predictive of vitamin B12 deficiency in this study (≥6 years). The longer duration of metformin use may be due to dissimilarities in the cutoff values of B12 used.

RECOMMENDATIONS
- Routine screening of type 2 diabetic patients on metformin for vitamin B12 inadequacy is highly recommended due to its high prevalence and the significant clinical impacts
- Besides, we suggest, beginning treating patients with B12 once a borderline or low level is recognized.

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