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Evaluation of Vitamin B_{12} Serum Level in a Group of Jordanian Patients with Type 2 Diabetes Mellitus

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Abstract: Diabetes Mellitus is one of the most prevalent metabolic disorders affecting humans and metformin is commonly used in its treatment protocol. However, this hypoglycemic agent is known to cause vitamin B_{12} deficiency, studies released from Jordan indicated the lack of relationship between both diabetes mellitus or metformin use and vitamin B_{12} deficiency. This investigation was under taken to further clarify this association among Jordanian patients with type 2 diabetes mellitus. A total number of 110 type 2 diabetes patients were enrolled in this study. The mean age of patients was 56.3 years (SD of 11.02) and female constituted 61% of subjects. The percentage of patients with vitamin B_{12} deficiency was established at 39.1% and those receiving metformin treatments amounted to 76.4% of the studied population. Duration of diabetes had no effect on vitamin B_{12} deficiency ($p<0.52$) whereas; duration of metformin use by the patients had ($p<0.01$). This is the first report which indicates a significant inverse relationship between blood vitamin B_{12} level and duration of diabetes among Jordanian patients treated with metformin.

Key words: Vitamin B_{12} deficiency, Diabetes mellitus, treatment with Metformin, HBA1c, Jordan

INTRODUCTION

Prevalence of type 2 diabetes mellitus (T2DM) among Jordanian population has significantly increased over the years. In a cross sectional study, Ajlouni *et al.* (1998) determined the prevalence of (T2DM) in the country to be 13.0%, whereas 10 years later this percentage was increased to 17.1% (Ajlounia *et al.*, 2008). It is now documented that this disease is driven primarily by a sedentary lifestyle, increasing obesity, lack of physical exercise and increased life expectancy (Marar *et al.*, 2011).

Metformin is the first-line treatment for patients with T2DM due to its proven hypoglycaemic effect (Liu *et al.*, 2006). The capacity to use metformin with other anti-diabetic agents is an added value to this therapeutic agent. However, recent studies have shown that metformin modulation of calcium-dependent membrane channels can reduce vitamin B_{12} level in blood circulation through hindering its absorption by the intestine. This phenomenon of mal-absorption during biguanide therapy was reported in the early eighties of the last century (Adams *et al.*, 1983). The inverse relationship between the dose and duration of metformin intake on vitamin B_{12} level was established by Ting *et al.* (2006).

Studies to assess type 2 diabetic patients on metformin have been published from different parts of the world. Reported literature indicated a great variation in the prevalence rate of vitamin B_{12} deficiency among patients with type 2 diabetes receiving metformin. Reinstatler *et al.* (2012) demonstrated that B_{12} deficiency was present in 5.8% of diabetics using metformin, while Pflipsen *et al.* (2009) demonstrated that 22% of their T2DM patients were with vitamin B_{12} deficiency. Qureshi *et al.* (2011) found that 33.0% of T2DM patients on high doses of metformin for more than four years exhibited reduced level of vitamin B_{12}. Kibirige and Mwebaze (2013) emphasized the importance of cultural and religious beliefs as possible reasons for the variation in reported prevalence rates of vitamin deficiency in T2DM patients. In Jordan, the prevalence rate of vitamin B_{12} deficiency among T2DM patients was not properly investigated. Barghouti *et al.* (2009) found that patients with diabetes and hyperlipidaemia had significantly higher B_{12} levels than non-diabetic patients or patients with normal lipid profile. The same authors did not specify wither their patients were on metformin or not. On the other hand, Oteer (2012) did not find any significant correlation between vitamin B_{12} levels and doses as well as duration of metformin treatment among Jordanian diabetics. Similar findings were also reported by Aljabra *et al.* (2015).

A part from the few publications given above, literature regarding the relationship between diabetes and metformin treatment on vitamin B_{12} serum levels in Jordanian subjects remains inconclusive. Due to the wide variation in results reported from Jordan and those released from other countries regarding the prevalence of vitamin B_{12} deficiency among diabetic patients particularly those receiving metformin therapy this investigation was undertaken.

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MATERIALS AND METHODS

Blood collection and sample preparation: Patients with T2DM who attended the Medical Diagnostic Laboratory over a period of 4 months; from the beginning of January to the end of May/2015 were candidates for enrollment in this investigation. Blood samples were drawn by trained technicians and then divided into 2 portions. One portion was placed in a plain tube for serum collection after centrifugation and the other in a tube containing anticoagulant. Serum was used for vitamin B_{12} testing and whole blood for HBA1c determination.

Selection criteria: Patients were excluded from this study if they had history of anemia, prior transfusion, renal insufficiency, prior gastric surgery and were on B_{12} supplements. When fitted the inclusion criteria, patients were asked about their age, duration of diabetes, duration and dose of metformin if at all used for their treatment; answers were recorded. A written consent was obtained from each patient that his or her results will be published with names being anonymous.

Analytical procedure: HBA1c was performed using i-Chamber which is a thermostatic accessory for i-Chroma HbA1c test (boditech Med Inc- Korea). Ichroma™ is an immunofluorescence-based diagnostic system. Vitamin B_{12} testing was carried out in Sultan Medical Laboratory in Amman- Jordan within less than 24 h of collection. Method of determination relied on Chemiluminescent emission which is then measured by a photomultiplier using COBAS 600 (Roche Diagnostics-USA). Vitamin B_{12} deficiency was defined at a concentration below <200 pg/ml (El-Khateeb *et al*., 2014).

Data analysis: For statistical calculations, a package of SPSS was used. Data was presented as percentage, mean and standard deviation for numerical variables. The association between variables was tested using Chi-Square test and significance was established whenever a p-value was <0.05.

RESULTS AND DISCUSSION

Diabetes is a major health problem worldwide and Jordan is no exception. Complications associated with this disease may affect several organs of the body including kidneys, eyes, nervous system, heart and blood vessels. The use of metformin for a long time as a hypoglycemic agent is questionable due to its ability to cause vitamin B_{12} deficiency. This work reports on the relationship between T2DM and vitamin B_{12} deficiency among Jordanian patients. Out of 110 T2DM patients included in this study, female constituted 61% of patients (Table 1). This observation merely indicates that the majority of patients who attended to the laboratory during the study period happened to be females.

Table 1: Number and percentages of patients enrolled according to gender and age group

	No. of patients in age groups (Years)				
Gender	<35	35->50	50->65	<65	Total (%)
Female	5	20	23	19	67 (61)
Male	2	11	16	14	43 (39
Total	7	31	39	33	110 (100)

Table 2: Demographic characteristics of diabetic patients included in the study

Demographic characters	Calculated values
Age (years) mean (SD)	56.3 (±11.02)
Male n (%)	43 (39%)
Female n (%)	67 (61%)
Duration of diabetes (years) mean (SD)	9.3 (±6.3)
Mean HbA1c readings (%) mean (SD)	8.1% (±1.1)
Vitamin B_{12} readings pg/ml mean (SD)	228.7 (±117.4)
Number of Vitamin B_{12} deficiency (%)	43 (39.1%)

Although number of patients in the stratified age groups does not need elucidation, it is consistent with the generally accepted literature as T2DM usually affects people above 30 years of age.

The mean age of patients included herein was 56.3 years with SD of 11.02. These values are almost similar to those reported by Iftikhar *et al.* (2013) and close to those reported by others (Sato *et al*., 2013; Marar *et al*., 2011). Demographic characters of patients studied are illustrated in Table 2. The overall mean value obtained for HBA1c readings was 8.1% (SD±1.1), while the duration of diabetes had a mean value of 9.3 years (SD±6.3 years). In this context it should be indicated that different authors have reported variable mean values for HBA1c and duration of the illness (Iftikhar *et al*., 2013; Reinstatler *et al*., 2012; Marar *et al*., 2011). This is absolutely dependent on the populations studied and such a variation has never been reported to influence vitamin B_{12} readings.

The mean value for vitamin B_{12} reading as determined herein was 228.7 pg/ml (SD±117.4) and this figure is lower than those reported for healthy individuals. Readings of vitamin B_{12} determined in surveillance studies in Jordan varied: it was quoted by Al-Fararjeh *et al.* (2011) to be 265.79 pg/ml while in another investigation this value was established at 273.26 pg/ml (Qutob *et al*., 2011). The mean duration of diabetes in the overall population studied was 9.3 years (SD 6.3) but as seen in Table 3 the duration of diabetes did not significantly affect the vitamin B_{12} level. In fact, Barghouti *et al.* (2009) demonstrated that diabetes in Jordan is not a cause of vitamin B_{12} deficiency; in the contrary they found that patients with diabetes were less likely to have B_{12} deficiency.

The percentage of vitamin B_{12} deficiency among the patients studied was 39.1% and this percentage is close to the 38% deficiency reported from Jordan by Al-Amoush and Abu Shaqra (2015). Studies conducted on healthy population in the kingdom of Jordan reported 16

Table 3: Demographic variables between metformin and non metformin treated diabetic patients

Variables	Metformin treated	Non-metfotmin treated	p-value
Total number of males	31	12	0.02
Total number of female	53	14	0.03
Mean age in year	57.0	54	0.85
Mean duration of diabetes in years	9.8	7.6	0.36
Mean HbA1c readings	8.2	7.9	0.71
Mean vitamin B_{12} readings	214.7	274	0.04

Table 4: Demographic characteristics of patient on metformin

	---------- Vitamin B_{12} levels ----------		
Demographics	Deficient≤200 pg/ml	Normal>200 pg/ml	p-value
Number of patient's n (%)	36 (43%)	48 (57%)	0.41
Female's n (%)	22 (41.5%)	31 (58.5%)	0.25
Male's n (%)	14 (45%)	17 (55%)	0.38
Age years (mean, SD)	59.4 (±8.6)	55.3 (±7.4)	0.52
Vitamin B_{12} levels (mean, SD)	153 (± 95)	261 (±132)	0.04
Duration of metformin use in years (mean, SD)	11.1 (±1.3)	6.4 (±2.2)	0.01
Duration of Diabetes years (mean, SD)	10.3 (±5.1)	9.5 (±4.83)	0.52
Glycosylated Hemoglobin HbA1c (%) SD	8.4 (±0.90)	8.1 (±0.87)	0.6

to 50% variation in the prevalence rate of vitamin B_{12} deficiency (Barghouti *et al*., 2009; Abu-Samak *et al*., 2008; El-Qudah *et al*., 2013; Fora and Mohammad, 2005). Therefore, our finding regarding the prevalence of vitamin B_{12} deficiency in diabetics is still within the already established range.

Table 3 provides a comparison between the demographic characters of metformin and non-metfotmin treated diabetic patients. Although this table is self explanatory, few characters are worth some emphasis. The majority of patients (76.4%) were on metformin therapy and this percentage is not far away from the 84% that was reported from Prince Rashid Hospital-Jordan by Aljabra *et al*. (2015). In fact this reading suggests that the majority of T2DM patients in Jordan are treated with this hypoglycemic agent. The same table shows that patients on metformin treatment have a longer duration of diabetes, relatively higher mean of HBA1c value and a lower mean vitamin B_{12} reading as compared to those who were not treated with metformin. These observations are in agreement with those reported from Japan and Korea by Sato *et al*. (2013) and Kos *et al*. (2012), respectively.

Other parameters presented in the same table include; duration of diabetes in years, mean HBA1c reading and Mean vitamin B_{12} reading. The former two parameters showed no significant association with metformin treatment while the later was the only character which showed significant difference between metformin and non-metformin treated patients. However, these findings are comparable with those reported by Iftikhar *et al*. (2013) and Sato *et al*. (2013); they are in disagreement with those given by Aljabra *et al*. (2015) who did not find any correlation between neither diabetes nor metformin use and vitamin B_{12} deficiency. Nevertheless, it should be noted here that the calculated p-value was 0.04 and this is just inside the significant range which was assigned at 0.05.

Table 4 is a comparative illustration to the demographic characters of normal and deficient vitamin B_{12} in T2DM patients treated with metformin. It is clear from this table that only two of the parameters tested were significantly associated with vitamin B_{12} deficiency. The first is perhaps the key finding of this investigation and is related to the duration of metformin use and the second was already anticipated as it indicated the obvious. In brief, mere presence of diabetes does not mean that patients will have vitamin B_{12} deficiency but long term use of metformin is most likely to do so.

Despite the fact that several investigations and case reports (Hermann *et al*., 2004; Liu *et al*., 2006) have established an increased frequency of vitamin B_{12} deficiency among diabetic patients, other reports have denied the existence of a significant association between diabetes duration and vitamin B_{12} deficiency (Kos *et al*., 2012; Ting *et al*., 2006). In fact, data presented in Table 4 especially for the duration of diabetes indicated that this parameter has no significant relationship with the presence or absence of vitamin B_{12} deficiency.

Metformin use has been proposed as the prime factor associated with vitamin B_{12} deficiency in patients with T2DM. The risk of developing metformin associated vitamin B_{12} deficiency is greatly influenced by increasing age, metformin dose and duration of its use (Kibirige and Mwebaze, 2013). Table 4 shows that duration of metformin use is inversely related to serum vitamin B_{12} level in Jordanian diabetic patients. This observation is contrary to that made by Aljabra *et al*. (2015) and Oteer, (2012) but consistent with generally accepted literature.

Conclusion: This investigation is the first from Jordan which links metformine duration of use with reduced vitamin B_{12} among Jordania diabetics. The limitation of this work is multi-factorial; first the use of small study population, second it was not possible to comment on

the dose of metformin taken by the patients included as about 40% of the patients enrolled failed to provide the exact daily concentration of mitformin they were prescribed and third; none metformine users registered in this work were almost one fourth of the total number of patients. Hence, a more comprehensive research that takes the above 3 mentioned factors into consideration should be conducted in the kingdom before a solid conclusion could be reached regarding the effect of metformin treatment on vitamin B_{12} deficiency in Jordanian T2DM patients.

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