



Assessment of vitamin B12 deficiency and associated risk factors among type 2 diabetic patients in Palestine

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HIGHLIGHTS

- High prevalence of low serum vitamin B12 was observed in the study.
- Significant association between low serum level of vitamin B12 with T2DM.
- Low serum level of vitamin B12 associated with hyperthyroidism and metformin treatment.
- A significant correlation was observed with Insulin usage.

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ABSTRACT

Metformin usage can lead to Vitamin B12 deficiency which can have an impact on neuropathy and lead to further complications. The current study aims to address the prevalence of low serum vitamin B12 level among type 2 diabetic patients in Ramallah district and to determine the factors associated with it. The study comprised a total sample size of 400 patients and all the participants gave their informed consent. Relevant medical history and laboratory data were obtained from the medical records of the patients. A questionnaire was taken from the participants directly by the researcher. Statistical analysis was done by SPSS, version 23. The results show that (60.5%) of the diabetic patients were obese. The majority of patients (62.8%) had dyslipidemia, (59.8%) hypertension and (12%) hyperthyroidism. (36.5%) complained of numbness, (13.8%) memory changes, and (6.5%) of mood changes. Only (21.5%) had diabetic retinopathy, (5.3%) had diabetic nephropathy and nearly (10.3%) had diabetic neuropathy. It was observed that (39.5%) of participants had a low serum Vitamin B12 level.

The majority of participants with low serum Vitamin B12 (38.4%) were using Metformin followed by (29.5%) who were using Insulin. There was a negative significance ($p = 0.198$) between low serum level of vitamin B12 and metformin treatment while a significant correlation was observed with Insulin usage ($p = 0.039$) and these results can be attributed to different factors. A negative significant effect was observed on the prevalence of low serum vitamin B12 level among patients who encountered different diabetic complications.

1. Introduction

Diabetes mellitus (DM) is one of the most common metabolic chronic diseases affecting a large number of populations worldwide and it is associated with several systemic complications such as Peripheral neuropathy (PN) [1]. It is known for years that according to the American Diabetes Association (ADA), metformin is the treatment line of choice for type 2 Diabetes mellitus (T2DM) patients and it works by improving peripheral insulin sensitivity and blocking hepatic

gluconeogenesis and glucose uptake from the intestines [2].

According to the latest statements released in January 2019 by the American Association of Clinical Endocrinologists and the American college of Endocrinology, has good antihyperglycemic efficacy at doses of 1,000 to 2,000 mg/day and in up to 16% of users, metformin is responsible for vitamin B12 malabsorption and/or deficiency [3,4]. Vitamin B12 malabsorption may lead to deficiency which could be a causal factor in the development of peripheral neuropathy and anemia [5].

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Vitamin B12 (Cyanocobalamin), is a water-soluble vitamin that plays an important role in the metabolism in the body as well as in the neurologic function as it contributes to the synthesis of myelin and DNA synthesis and other functions as well. Deficiency of this vitamin can lead to a large spectrum of neurologic, hematologic and neuropsychiatric disorders [6,7]. In addition to the above-mentioned disorders, vitamin B12 deficiency may exert indirect cardiovascular effects as well [8]. Vitamin B12 deficiency may take many years (5–10 years) to develop and the levels of this vitamin can start decreasing from the fourth month of using Metformin due to the malabsorption [9,10].

Metformin can decline vitamin B12 level in the blood through reducing its absorption by the intestine and some hypothesis state that metformin competes with calcium causing the vitamin malabsorption and reducing its uptake by the intestine [11]. As using Metformin can lead to vitamin B12 deficiency which can have an impact on neuropathy, the monitoring of type 2 diabetic patients and this vitamin level is very important. Therefore, this study aimed to address the prevalence of low serum vitamin B12 level among type 2 diabetic patients in Ramallah district and to determine the factors associated with low serum levels of vitamin B12.

2. Materials and method

The study comprised a total sample size of 400 patients. The study was approved by the health and ethics committee of the health center in Palestine, and all the participants gave their informed consent in accordance with the Declaration of Helsinki [12]. Relevant laboratory data, sociodemographic and medical history data were obtained from the medical records of the patients including height, age, weight, current medical issues, gender and antidiabetic medications if the patient was diabetic. A general questionnaire was distributed to the participants. Measurement of the last recorded serum vitamin B12 level was considered in the current study and Complete Blood Count (CBC) was taken as well.

2.1. Inclusion criteria

All patients aged 20 years or older who were being treated for T2DM at the outpatient clinic were eligible for inclusion, regardless of metformin use.

2.2. Exclusion criteria

We excluded all participants with diabetes after necrotic pancreatitis, late-onset autoimmune diabetes of adults, and pure vegetarians had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine 3.0, prior bariatric surgery, gastrectomy, B12 supplementation with B12 shots or an oral Vitamin B12 dose of 500 mcg/day, prior ileum resection, or Crohn's disease.

2.3. Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, version 23). Chi-square tests for categorical variable and multivariate logistic regression was used to assess the correlation between low serum B12 level and other variables. P. value < 0.05 was considered statistically significant.

3. Results

This study included a total of 400 patients with type 2 diabetes and the Mean age \pm SD was 53.3 \pm 9.7 years and (72.8%) of them were females compared to (27.2%). Most of patients (70.5%) had an educational level which is less than high school and most of them (96.8%) are married. About 29.5% of the diabetics had a high level of education. The majority of patients (79.5%) were non-smokers followed by (13%)

Table 1
Socio-demographic characteristics of 400 patients with type 2DM.

Variable	Frequency (N)	Percent (%)
Age (years)	Mean \pm SD = 53.3 \pm 9.7	
20–40	40	10.1
41–60	264	66.0
> 60	96	23.9
Gender		
Males	109	27.2
Females	291	72.8
Education		
< High school	282	70.5
> High school	134	29.5
Marital status		
Single	13	3.2
Married	387	96.8
Smoking		
No	318	79.5
Yes	53	13.2
Past-smoker	29	7.3

of the population who were current smokers and (7.3%) who were past smokers (Table 1).

Among the study population: (28.2%) of the diabetic patients were overweight and (60.5%) were obese. The majority of patients (59.8%) had hypertension, (11.3%) had ischemic heart disease, (7.8%) had hypothyroidism, (12%) had hyperthyroidism and the majority (62.8%) had dyslipidemia. Regarding the subjective complaints, (13.8%) of the patients were complaining of memory changes, (36.5%) had numbness in their feet and (6.5%) were complaining of mood changes. It was revealed that (24.5%) of the diabetics had history of vitamin B12 deficiency and the same percentage had received treatment (Table 2).

About half of the diabetic population (50.5%) had diabetes for more than five years of duration. Only (40.5%) of them had HBA1c < 7% and the majority of them (59.5%) had it equal or above 7. Regarding the diabetic complications, only (21.5%) had diabetic retinopathy, about (5.3%) had diabetic nephropathy and nearly (10.3%) had diabetic neuropathy. It was observed that only (39.5%) of diabetic patients had a low serum Vitamin B12 level (Table 3). Regarding medications used for diabetes, the majority (94.5%) of the diabetics were using Metformin therapy, (28%) were on insulin and (42%) were on

Table 2
Clinical and anthropometric characteristics of the study population (400 patients with type 2DM).

Variable	N (%)	Variable	N (%)
BMI (kg/m ²)	Mean \pm SD = 31.8 \pm 4.8	Dyslipidaemia	
Underweight	0 (0)	Yes	251 (62.8)
Normal	45 (11.3)	No	149 (37.2)
Overweight	113 (28.2)		
Obese	242 (60.5)	Mood changes	
Hypertension		Yes	26 (6.5)
Yes	239 (59.8)	No	374 (93.5)
No	161 (40.2)	Memory Changes	
Ischemic heart disease		Yes	52 (13.8)
Yes	45 (11.3)	No	348 (87.0)
No	355 (88.7)	Numbness	
Hypothyroidism		Yes	146 (36.5)
Yes	31 (7.8)	No	254 (63.5)
No	369 (92.2)	Mean corpuscular volume (MCV) > 100 fL	
Hyperthyroidism		Yes	0 (0)
Yes	8 (2.0)	No	400 (100)
No	392 (98.0)		
History of Vitamin B12 deficiency		History of received treatment for vitamin B12 deficiency	
Yes	98 (24.5)	Yes	98 (24.5)
No	302 (75.5)	No	302 (75.5)

Table 3
Clinical characteristics for type 2 diabetic patients (400 patients).

Variable	Frequency (N)	Percent (%)
Duration of DM in (years)		
0–5	198	49.5
> 5	202	50.5
HBA1c		
< 7%	162	40.5
> 7%	238	59.5
Diabetic Retinopathy		
Yes	86	21.5
No	314	78.5
Diabetic Nephropathy		
Yes	21	5.3
No	379	94.7
Diabetic Neuropathy		
Yes	41	10.3
No	359	89.7
Low serum Vitamin B12 level		
Yes	158	39.5
No	242	60.5
P-value	0.061	

Table 4
Type of treatment for patients with T2DM (400 patients).

Variable	Frequency (N)	Percent (%)
Metformin		
Yes	378	94.5
No	22	5.5
Insulin		
Yes	112	28.0
No	328	82.0
Sulphonylurea		
Yes	168	42.0
No	232	58.0
Combination of oral hypoglycaemic agents and insulin		
Yes	23	5.7
No	377	94.3
Duration of use of Metformin (years)		
0–5	260	65.0
5.1–10	120	30.0
10.1–15	15	3.7
15.1–20	5	1.3
> 20	0	0.0

Sulphonylurea and (5.7%) of the diabetics were on combined oral hypoglycemic agents (Metformin and Sulphonylurea) and insulin (Table 4).

The hematological parameters for patients with low levels of serum vitamin B12 revealed that the mean hemoglobin (Hb) was 13.20 g%, mean red blood corpuscle (RBC) was 4.72 million/cmm, mean corpuscular volume (MCV) was 82.01 fl, mean corpuscular hemoglobin concentration (MCH) was 28.2 pg, mean platelets was 251.2 k/ML and white blood cells (WBC) was 7.1 million/cmm (Table 5).

The majority of patients who encountered a low serum Vitamin B12 (42.7%) were elderly and were above 60 years and most of them were males (41.3%) and most of them (41.8%) had a high school degree or higher. The majority (40%) were married and most of them were past smokers (65.5%). The results showed that there is no significant correlation between low serum vitamin B12 level and the above-mentioned socio-demographic characteristics (Table 6).

Most of patients who encountered a low serum Vitamin B12 had normal body mass index (BMI) (42.2%) and the majority (41.4%) were hypertensive and most of them had ischemic heart disease (46.6%), and Dyslipidemia (38.6%). (32.3%) of patients had hypothyroidism. The results showed that there is no significant correlation among the previous variables while patients with hyperthyroidism (62.5%) showed a significant correlation ($p = 0.0438$). Regarding the subjective

Table 5
Mean hematological parameters for patients with low levels of serum vitamin B12.

Parameter	Mean \pm SD	Range
Hb	13.20 \pm 1.4 (g %)	13.0–13.3
RBC	4.72 \pm 0.52	4.7–4.8
MCV	82.01 \pm 3.4 (fl)	80.1–82.9
MCH	28.2 \pm 1.8 (pg)	27.2–28.7
Platelets	251.2 \pm 45.1 (k/ML)	246.1–259.4
WBC	7.1 \pm 1.5 (million/cmm)	7.0–7.3

SD-standard deviation, Hb-hemoglobin, -RBC-red blood corpuscle(million/cmm), MCV-mean corpuscular volume (fl), MCH-mean corpuscular haemoglobin concentration, MCHC-mean corpuscular haemoglobin concentration, platelets, WBC-white blood cells.

Table 6
Correlation between the prevalence of low serum vitamin B12 level with the Socio-demographic characteristics of 400 T2DM patients.

Variable	Yes	No	p-value
	N (%)	N (%)	
Age (years)			
20–40	16 (40)	24 (60)	
41–60	81 (30.6)	151 (69.4)	0.129
> 60	41 (42.7)	40 (57.3)	
Gender			
Males	45 (41.3)	64 (58.7)	0.067
Females	93 (31.2)	198 (68.8)	
Education			
< High school	90 (31.9)	192 (68.1)	0.385
> High school	56 (41.8)	78 (58.2)	
Marital status			
Single	5 (38.5)	8 (61.5)	0.402
Married	155 (40.0)	232 (60.0)	
Smoking			
Non	260 (38.4)	196 (61.6)	
Yes	19 (35.8)	34 (64.2)	0.064
Past-smoker	19 (65.5)	10 (34.5)	

complaints most of patients with low serum level of vitamin B12 reported Mood changes (61.5%) and (67.3%) with Memory changes and both were observed to be significantly correlated ($p = 0.036$ and $p = 0.001$). Those who reported Numbness represented (32.2%) with no significant correlation ($p = 0.083$) (Table 7).

The majority of patients who encountered a low serum Vitamin B12 (38.4%) were using Metformin ($p = 0.198$), (29.5%) of them were using Insulin ($p = 0.039$) which is significantly correlated and (32.1%) used Sulphonylurea ($p = 0.336$). Regarding the diabetic complications and low serum vitamin B12, the majority of patients with low level vitamin B12 (57.1%) had Nephropathy, (26.8%) had Neuropathy and (36%) had Retinopathy, but no significant correlation was observed. Most of them (43.8%) had an HBA1c level below < 7% and a duration of diabetes of 5 years or less (Table 8).

A multivariate analysis of factors associated with low serum vitamin B12 level among type 2 diabetics and non-diabetic patients is shown in (Table 9), the only two variables that remained significantly associated with low serum vitamin B12 level were the type 2 diabetes mellitus and hyperthyroidism after adjusting for the other variables in the multivariate analysis. Those who were diabetics had higher odds for low serum vitamin B12 showed clear statistical significance (p-value: 0.041). Patients with hyperthyroidism were found to be 3.91 times more likely to have low serum vitamin B12 level than those who did not have hyperthyroidism, with a confidence interval of (1.21, 13.9); p value = 0.032 (Table 9).

Table 7

The prevalence of low serum vitamin B12 level by the clinical and anthropometric characteristics of 400 patients with T2DM.

Variable	Yes	No	Variable	Yes	No
	N (%)	N (%)		N (%)	N (%)
BMI (kg/m ²)			Numbness		
Underweight	0 (0)	(0)	Yes	47 (32.2)	99 (67.8)
Normal	19 (42.2)	26 (57.8)	No	89 (35.0)	156 (65.0)
Overweight	38 (33.6)	75 (66.4)			
Obese	85 (35.1)	157 (64.9)			
p-value	0.620		p-value	0.083	
Hypertension			Mood changes		
Yes	99 (41.4)	140 (58.6)	Yes	16 (61.5)	10 (38.5)
No	54 (33.5)	107 (66.5)	No	136 (36.1)	239 (63.9)
p-value	0.183		p-value	0.036	
Ischemic heart disease			Hyperlipidaemia		
Yes	21 (46.6)	24 (53.4)	Yes	97 (38.6)	154 (61.4)
No	128 (36.1)	227 (63.9)	No	56 (37.6)	93 (62.4)
p-value	0.261		p-value	0.836	
Hypothyroidism			Hyperthyroidism		
Yes	10 (32.3)	21 (67.7)	Yes	5 (62.5)	3 (37.5)
No	132 (33.7)	237 (64.3)	No	109 (37.3)	183 (62.7)
p-value	0.582		p-value	0.0438	
Memory changes			MCV > 100		
Yes	35 (67.3)	17 (32.7)	Yes	0 (0)	0 (0)
No	117 (33.6)	231 (66.4)	No	148 (37.0)	252 (63.0)
p-value	0.001		p-value	-	
History of low serum vitamin B12			History of received treatment for low serum vitamin B12		
Yes	98 (100)	0 (0)	Yes	98 (100)	0 (0)
No	67 (22.2)	235 (77.8)	No	67 (22.2)	235 (77.8)
p-value	0.001		p-value	0.001	

Table 8

The prevalence of low serum vitamin B12 level by Specific clinical characteristics for type 2 diabetic patients (400 patients).

Variable	Low serum vitamin B12 level		
	Yes	No	P-Value
	N (%)	N (%)	
Metformin			
Yes	145 (38.4)	233 (61.6)	0.198
No	6 (28.6)	16 (71.3)	
Insulin			
Yes	33 (29.5)	79 (70.5)	0.039
No	135 (41.2)	193 (58.8)	
Sulphonylurea			
Yes	54 (32.1)	114 (67.9)	0.336
No	90 (38.8)	142 (61.2)	
Diabetic Retinopathy			
Yes	31 (36.0)	55 (64.0)	0.242
No	126 (40.1)	188 (59.9)	
Diabetic Nephropathy			
Yes	12 (57.1)	9 (42.9)	0.339
No	146 (38.5)	232 (61.5)	
Diabetic Neuropathy			
Yes	11 (26.8)	30 (73.2)	0.201
No	138 (38.4)	221 (61.6)	
HbA1c			
< 7%	71 (43.8)	91 (56.2)	0.166
> 7%	85 (35.7)	153 (64.3)	
Duration of DM (years)			
0-5	79 (39.9)	119 (60.1)	0.586
> 5	74 (36.6)	128 (62.4)	

4. Discussion

Reviewing the literature there was a link between Metformin usage and Vitamin B12 deficiency and 10–30% of patients who took

Table 9

Logistic regression analysis of factors associated with low serum vitamin B12 level.

Variables	OR (95% confidence interval)	P-Value
T2DM	1.61 (1.01, 1.89)	0.041
Hyperthyroidism	3.91 (1.21, 13.9)	0.019

T2DM: Diabetes mellitus type 2, OR: odds ratio.

metformin had declined vitamin B12 absorption [13,14]. Some studies mentioned that long term therapy of metformin is associated with decreased absorption of this vitamin [15].

In the present study, serum B12 lower than 208 pg/ml among type 2 diabetic population was found to be among (39.5%) of these patients and these findings were lower than results reported in other studies in the middle east countries, where they found low serum levels of vitamin B12 in about (48%) of all participants which was similar to other studies as well that showed almost similar findings to our results regarding the prevalence of low serum vitamin B12 levels [16,17].

Previous studies have demonstrated that supplemental Vitamin B12 improved somatic and autonomic symptoms of diabetic neuropathy [18]. Despite low levels of serum vitamin B12 found in almost one third of the population in the current study, Mean Corpuscular Volume (< 100 fL) results of the study sample was found to be normal. This may indicate that the low serum vitamin B12 in this population's sample is of stage 1 and 2 according to Herbert's classification [19].

The current study showed diabetics aged above 60 years in whom low serum vitamin B12 level was observed represented 42.7%. This finding agrees with some other previously held studies [20,21]. There are several reasons why elderly people especially the diabetics, are at high risk for the development of vitamin B12 deficiency, including age associated physiologic changes, chronic diseases especially diabetes Mellitus, and high prevalence of drug intake including the oral hypoglycemic agents, metformin and insulin.

In the current study no significant differences in the plasma vitamin B12 were found (P > 0.05) in relation to the gender among type 2 diabetics. This finding is similar to what was reported in other studies [16,22]. However, some studies reported that plasma vitamin B12 level for males were lower than those obtained for females [8].

This study revealed no significance in association of the educational status, marital status, body mass index (BMI), hypertension, Ischemic heart disease, hypothyroidism and dyslipidemia with the low vitamin B12. Many previous studies have reported both significant and non-significant correlation between the educational status, marital status, body mass index (BMI), hypertension, Ischemic heart disease, hypothyroidism and dyslipidemia with the low vitamin B12 and low vitamin B12 [17].

The present study reported 67.3% of those who had low serum vitamin B12 level among the study sample, have complained of partial memory impairment which was significant (P < 0.05), this is in line with our findings but were among the diabetic patients from United Arab Emirates identified B12 deficiency in 21.9% of the people and were associated with memory impairment [16,17,23]. As expected, this study showed that the prevalence of B12 deficiency among secondary care treated T2DM patients who were using metformin is 38.4%, though it was statistically not significant (P-value 0.198).

In the current study, 29.5% of type 2 diabetics who received insulin therapy showed low serum vitamin B12 level with a significant relationship (P-value 0.039). The explanation could be due to the chronic status of the disease itself and the effect of multiple drug especially metformin therapy, because the majority of our diabetic population are already on metformin therapy (94.5%), before starting them on insulin as a combined therapy unless there is contraindication for its use. This is of importance due to the fact that hyperhomocysteinemia is an

independent risk factor for cardiovascular disease, considering the fact that uncontrolled DM patient are seemingly at high risk of cardiovascular morbidity [16,24]. Our study revealed that the majority of patients who encountered a low serum Vitamin B12 (38.4%) were using Metformin but the correlation was found to be insignificant ($p = 0.198$) followed by (29.5%) of participants who were using Insulin which was found significant ($p = 0.039$). The duration for Metformin intake by the majority of our participants was between (0–5) years and some of them have just initiated it and that could explain the negative significance with the low serum B12 levels although most of them were using it.

Diabetes control and the duration of diabetes mellitus showed no significant effect ($P > 0.05$) on the prevalence of low serum vitamin B12 level among patients of T2DM this result is in consistence with others who found significant correlation for metformin duration and low serum vitamin B12 level among patients of T2DM [25].

In the current study Logistic regression analysis demonstrated a significant association of vitamin B12 deficiency and hyperthyroidism in this population of T2DM patients. This interesting coincidence of the two diseases was also reported by Emamifar and Jensen Hansen in a cohort study that comes in line with our study and supports the prevalence of thyroid diseases and B12 deficiency in DM patients [26].

Regarding nephropathy and Metformin usage, the latest statement in January 2019 by the American Association of Clinical Endocrinologists and the American college of Endocrinology stated that the FDA recently changed the package label for Metformin use in chronic kidney disease (CKD) patients, lifting the previous contra-indication in males with serum creatinine > 1.5 mg/dL and females with serum creatinine > 1.4 mg/dL (187,188) and newer CKD guidelines are based on estimated glomerular filtration rate (eGFR), not on serum creatinine [3]. Further research needs to be conducted in large and well-designed studies on screening vitamin B12 deficiency and also to look at the potential risk or protective factors for vitamin B12 deficiency.

In summary, this study demonstrates significant association between low serum level of vitamin B12 with T2DM, hyperthyroidism and metformin treatment. The high prevalence of low serum vitamin B12 obtained in the study and the absence of megaloblastic anemia among the patients necessitates that broader research for more specific diagnostic tools of vitamin B12 deficiency is needed, and supports the need to promote and implement the utilization of vitamin B12 screening at the national level, to detect those who have low serum vitamin B12 level at an early stage and to supplement vitamin B12 accordingly.

5. Limitations

The current study associated the duration of diabetes with the Vitamin B12 serum level deficiency, but did not associate the duration of intake of antidiabetic drugs with the vitamin B12 serum levels as it assessed only the intake itself in general regardless of duration and it is planned to be extended and further studied in future work.

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Declaration of competing interest

None.

Conflicts of interest

The authors state that they have no conflict of interest.

References

- [1] American Diabetes Association, Diagnosis and classification of diabetes mellitus, *Diabetes Care* 37 (2014) 81–90.
- [2] S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, et al., Management of hyperglycemia in type 2 diabetes: a patient-centered approach, *Diabetes Care* 35 (2012) 1364–1379.
- [3] J.G. Alan, J.A. Martin, I.B. Joshua, et al., Consensus statement by the American association of Clinical endocrinologists and American college of Endocrinology on the comprehensive type 2 diabetes Management, *Endocr Pract* 25 (2019) 1.
- [4] K. Leishear, R.M. Boudreau, S.A. Studenski, et al., Relationship between vitamin B12 and sensory and motor peripheral nerve function in older adults, *J Am Geriatr Soc* 60 (2012) 1057–1063.
- [5] A.K. Singh, A. Kumar, D. Karmakar, R.K. Jha, Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients, *J Postgrad Med* 59 (2013) 253–257.
- [6] C. Robert, L.B. David, Vitamin B12 deficiency, *Am Fam Physician* 67 (2003) 979–986.
- [7] R. Green, Physiology, dietary sources, and requirements, vol. 4, Academic Press, 2013, pp. 351–356.
- [8] R. Carmel, R. Green, D.S. Rosenblatt, D. Watkins, Update on cobalamin, folate and homocysteine, *Hematology (N Y)* 1 (2003) 62–81.
- [9] K. Neeraj, Nutritional neuropathies, *Neurol Clin* 25 (2007) 209–255.
- [10] E. Andrés, E. Noel, G. Kaltenbach, Usefulness of oral vitamin B12 therapy in vitamin B12 deficiency related to food-cobalamin malabsorption, *Eur J Intern Med* 16 (2005) 218.
- [11] J.F. Adams, J.S. Clark, J.T. Ireland, C.M. Kesson, et al., Malabsorption of vitamin B12 and intrinsic factor secretion during Biguanide therapy, *Diabetologia* 24 (1983) 16–18.
- [12] World Medical Association, Declaration of helsinki – ethical principles for medical research involving human subjects, Available at: <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects> (Accessed January 2019).
- [13] A. Dareen, A. Abduelmula, S. Moyad, S. Monzer, Evaluation of factors associated with inadequate glycemic control and some other health care indicators among patients with Type 2 diabetes in Ramallah, Palestine, *Res J Pharmaceut Biol Chem Sci* 4 (2013) 445–451.
- [14] W.A. Bauman, S. Spencer, J. Elizabeth, et al., Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin, *Diabetes Care* 23 (2000) 1227–1231.
- [15] J.C. Bailey, R.C. Turner, Metformin, *NEJM* 34 (1996) 574–579.
- [16] M. Shahwan, N. Hassan, A. Noshi, N. Banu, Prevalence and risk factors of vitamin B12 deficiency among patients with type 2 diabetes on metformin: a study from northern region of United Arab Emirates, *Asian J Pharmaceut Clin Res* 11 (2018) 225.
- [17] Y. Gielchinsky, D. Elstein, R. Green, et al., High prevalence of low serum vitamin B12 in multi-ethnic Israeli population, *Br. J. Haematol* 115 (2001) 707–709.
- [18] H. Fakhrzaddeh, Total plasma homocysteine, folate, and vitamin B12 status in healthy Iranian adults, *BMC Publ Health* 13 (2006) 629–631.
- [19] V. Herbert, Nutrition science as a continually unfolding story: the folate and vitamin B12 paradigm, *Am J Clin Nutr* 46 (1987) 387–402.
- [20] S. Moyad, S. Monzer, A. Abduelmula, et al., Prevalence and risk factors of vitamin D deficiency among type 2 diabetics and non-diabetic female patients in Jordan, *Res J Pharmaceut Biol Chem Sci* 4 (2013) 278–292.
- [21] A. McCaddon, C.L. Kelly, Familial Alzheimer's disease and vitamin B12 deficiency, *Age Ageing* 23 (1994) 334–337.
- [22] A. Wahlin, L. Bqchman, J. Hultdin, et al., Reference values for serum levels of vitamin B12 and folic acid in a population-based sample of adults between 35–80 years of age, *Publ Health Nutr* 5 (2002) 505–511.
- [23] M.J. Shahwan, S.K. Zaidi, S.A. Gacem, Prevalence of diabetic nephropathy and associated risk factors among type 2 diabetes mellitus patients in Ramallah, Palestine, *Diabetes & Metabolic Syndrome: Clin Res Rev* 13 (2019) 1491–1496.
- [24] J. Zalaket, T. Wehbe, E.A. Jaoude, Vitamin B12 deficiency in diabetic subjects taking metformin: a cross sectional study in a Lebanese cohort, *Journal of Nutrition & Intermediary Metabolism* 11 (2018) 9–13.
- [25] S. Akinlade, O. Agbebaku, K. Rahamon, O. Balogun, Vitamin B 12 levels in patients with type 2 diabetes mellitus on metformin, *Ann Ib Postgrad Med* 2 (2015) 79–83.
- [26] A. Emamifar, I.M. Jensen, The influence of thyroid diseases, diabetes mellitus, primary hyperparathyroidism, vitamin B12 deficiency and other comorbid autoimmune diseases on treatment outcome in patients with rheumatoid arthritis, *Medicine (Baltim)* 97 (2018) 10865.