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Evaluation of correlation of serum vitamin B12 with proteinuria in type 2 Diabetes Mellitus patients

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ABSTRACT

Background and Aims: Diabetic nephropathy is one of the leading causes of end stage renal disease (ESRD) in the world. This study was conducted to investigate the correlation of B12 deficiency with presence of proteinuria in patients with Diabetes Mellitus.

Materials and Methods: This study was performed on patients with type 2 Diabetes Mellitus. The inclusion criteria were: HbA1C ≤8%, absence of hypertension or controlled blood pressure (BP<140/90) in hypertensive patients, no smoking, absence of any recent stressful illness such as MI, CVA and causes of proteinuria other than diabetic nephropathy. The serum level of B12 and the amount of urine protein–to-creatinine ratio (UPCR) were measured, and correlation between b12 deficiency and presence of proteinuria was assessed using Spearman correlation test.

Results: In this study 78 patients (44.87% women and 55.13% men) were examined. There was an inverse and weak correlation between the serum B12 deficiency and presence of proteinuria (r=-0.104), which was not statistically significant (p=0.254). Due to the statistically significant difference between two groups with and without proteinuria in antiproteinuric drugs, as well as Metformin use and chronic kidney disease (CKD) stage, a subgroup analysis was performed in each of these subgroups. There was no correlation between the B12 deficiency and presence of proteinuria.

Conclusion: The findings revealed no statistically significant correlation between the serum B12 deficiency and presence of proteinuria.

INTRODUCTION

Diabetes is known as a common non-communicable disease in various societies. This disease affects various organs. One of the complications of diabetes is nephropathy [1]. Diabetic nephropathy is the cause of 30-50% of the ESRD cases in the United States. The clinical presentation of diabetic nephropathy is defined as proteinuria, hypertension, and progressive decline in renal function. The growth factors, hemodynamic and hormonal changes cause the release of inflammatory factors and reactive oxygen species which cause glomerular hyperfiltration, renal hypertrophy and altered glomerular composition that presents clinically with albuminuria and hypertension [1]. The care and treatment suggested so far (including controlling blood sugar, lipids, blood pressure, and prescribing drugs such as angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) were inadequate to treat diabetic nephropathy and other unknown factors seem to be effective in causing diabetic nephropathy [1, 2].

In diabetic patients, the serum level of B vitamins is re-

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duced, but the mechanism of reduction is not entirely clear; nevertheless, one reason for the decrease can be long-term use of Metformin that reduces the absorption of vitamin B12 and folic acid from the gastrointestinal tract [3, 4]. In a cross-sectional study, 22% of patients with diabetes lacked B12 [2]. However, in another study of diabetic patients with or without nephropathy, vitamin B1 and B6 deficiency were common and vitamin B12 deficiency was rare [5]. Many patients with type 2 diabetes are treated with Metformin and the association between long-term use of Metformin and the B12 deficiency has been established. Although, there have been differences in the extent of this relationship in different studies; in one study, nearly 30% of diabetic patients treated with Metformin were deficient in vitamin B12 [6]. The decrease in the level of B vitamins, including B12, causes hyperhomocysteinemia, consequently, it causes increasing oxidative stress and decreasing antioxidant function [7-9]. Therefore, considering the importance of recognizing the causes of diabetic nephropathy other than the known factors for the prevention of ESRD, we decided to investigate the link between vitamin B12 deficiency and proteinuria in patients with Diabetes Mellitus.

MATERIALS and METHODS

This study was approved by the ethical committee of Shahid Beheshti medical university (IR.SBMU.RETECH.REC.1398.424).This study was performed as a descriptive correlation study in patients with type 2 Diabetes Mellitus referred to Loghman Hakim Hospital. The patients were first explained about the study and its goals; the patients were included in the study with personal consent. The inclusion criteria were: HbA1C ≤8%,absence of hypertension or controlled blood pressure (BP<140/90)in hypertensive patients, no smoking, absence of any recent stressful illness such as MI, CVA and causes of proteinuria other than diabetic nephropathy such as acute febrile illness and kidney disease (glomerular or tubular). At first, a complete history of the patients was taken and a physical examination was performed. After 12 hours of fasting, 7 cc of blood was taken from the patients on an outpatient basis and collected in three test tubes. 1 cc in test tube containing EDTA anticoagulant for blood cell count, 1 cc in test tube containing EDTA anticoagulant for HbA1C, 5 cc in third test tube that was centrifuged and the serum was removed. 0.5 cc of the serum was frozen at minus 20 ° C and was maintained to measure the serum B12 level and the remaining 4.5 cc was used to measure triglycerides, cholesterol, HDL, LDL, fasting blood sugar (FBS) urea and creatinine. Biochemical assays were used to measure triglycerides, cholesterol, HDL, LDL, FBS, urea, creatinine. The B12 level was measured by COBAS E411 and Electrochemiluminescence (ECL) method. The patients were instructed not to do too much physical activity before preparing a urine sample and rest for 10 minutes in a supine position. Random urine was taken from a patient to measure the level of creatinine and protein in the urine, urine protein, and creatinine were measured by biochemical tests. The amount of proteinuria was calculated by measuring the ratio of protein to creatinine in random urine. GFR was calculated using the MDRD formula.

The data were analyzed utilizing SPSS software version 24 by spearman correlation coefficient, independent t and chi-square tests. $p < 0.05$ is considered as a statistically significant level in this study.

RESULTS

IIn this study, 300 patients with type 2 Diabetes Mellitus were studied, of which 78 patients were eligible for the study. The data of these 78 patients (35 females and 43 males) with a mean age of 8/14±59years (minimum 26 and maximum 87 years) were analyzed. 33(42.3%) patients had proteinuria and 45 (57.7%) patients had no proteinuria. There were 48 (61.5%) patients with hypertension, but all of them had controlled blood pressure at the time of the study. 51(65.4%) patients were treated with ACEI and ARB. 61(78.2%) patients also took other protein-lowering drugs including Pentoxifylline, Atorvastatin, Gliclazide, Allopurinol, Indapamide, Dipyridamole, and Spironolactone.

Table 1. Demographic and clinical characteristics of patients (BMI= Body mass index, ARB= Angiotensin receptor blockers, ACEI= Angiotensin-converting enzyme inhibitors, GFR= Estimated glomerular filtration rate)

Characteristics	All patients	Subgroups		p-value
		With proteinuria N=33	No proteinuria N=45)	
Age (mean± SD)	78	62.82±10.9	56.20±16.6	0.038
Male	43 (55.1)	22 (66.7)	21 (46.7)	0.110
BMI (kg/m²) (mean± SD)	78	28.78±5.65	34.26±9.35	0.002
Hypertension	48 (61.5)	22 (66.7)	26 (57.8)	0.480
ARB/ACEI Consumption	51 (65.4)	27 (81.8)	24 (53.3)	0.015
Taking proteinuria lowering drugs	61 (78.2)	30 (90.9)	31 (68.9)	0.026
Taking multivitamins	14 (17.9)	7 (21.2)	7 (15.6)	0.561
Metformin consumption	36 (46.2)	9 (27.3)	27 (60)	0.006
eGFR 90≤	2 (2.6)	0 (0)	2 (4.4)	
60-89	22 (28.2)	4 (12.1)	18 (40)	
30-59	40 (51.3)	18 (54.4)	22 (48.9)	0.002
15-29	9 (11.5)	6 (18.2)	3 (7/6)	
<15	5 (6.4)	5 (15.2)	0 (0)	
B12 deficiency	24 (30.77)	12 (36.4)	12 (26.7)	0.254

Table 2. Laboratory findings of patients (FBS= Fasting blood sugar, eGFR= Estimated glomerular filtration rate)

Variables	Subgroups		p-value
	With proteinuria N=33 (Mean±SD)	No proteinuria N=45 (Mean±SD)	
Serum Vitamin B12 (pg/ml)	377.12±332.53	294.63±283.082	0.254
Hemoglobin (g/dl)	12.712±1.965	13.309±1.44	0.333
Triglyceride (mg/dl)	139.06±111.85	141.49±59.14	0.910
Cholestrole (mg/dl)	135.52±33.46	150.27±36.84	0.069
HDL (mg/dl)	41.69±10.77	40.08±8.87	0.483
LDL (mg/dl)	73.78±26.67	85.84±31.45	0.071
Creatinin (mg/dl)	2.25±1.62	1.25±0.415	0.001
FBS (mg/dl)	118.27±45.08	131.3±34.75	0.172
eGFR (ml/min/1.73 m²)	37.74±18.47	57.12±17.23	0.000

The percentage of ACEI, ARB and other proteinuria-lowering drugs were higher in patients with proteinuria. There were 36(46.2%) patients treated with Metformin, of which 15 (41.7%) patients had B12 deficiency (P = 0.054).Most patients had stage 2 (28.2%) and 3 (51.3%) CKD. More details about the demographic and clinical information of patients with comparison between the two groups with and without proteinuria are given in Table 1.

55.1% of patients (n = 43) had HbA1C below 7% and 44.9% (n = 35) had 7-8 HbA1C. There was no significant difference between taking multivitamins containing B vitamins and the serum lipid level of the two groups with and without proteinuria (P> 0.05).

In this study, the serum B12 level was measured by ECL method with a normal level of 197-771 pg/ml. The values below 197 were considered as B12 deficiency. 30.8% of patients (n = 24) had B12 deficiency. 36.4% of patients with proteinuria and 26.7% of patients without proteinuria had B12 deficiency. The mean serum B12 level in patients with proteinuria was 377.2 ± 332.53 and in patients without proteinuria was082/283 ±63/294 . Using Spearman correlation test, it was found that there is a weak inverse correlation between the serum B12 level and proteinuria (r = -0.104) which was not statistically significant (p = 0.254). Due to the statistically significant difference between the two groups with and without proteinuria in the use of ACEI and ARB and other proteinuria reducing drugs as well as the use of metformin and CKD stage, subgroup analysis was performed and Spearman test indicated that the correlation between proteinuria and the B12 level in each of the above subgroups was not statistically significant.

Other laboratory information of patients is given in detail in Table 2.

DISCUSSION

Diabetic nephropathy is one of the leading causes of ESRD in the world [1]. B12 deficiency has been suggested as one of the possible effective causes in the development and acceleration of diabetic nephropathy [10]. Therefore, considering the importance of recognizing the factors affecting diabetic nephropathy for prevention and treatment, the aim of this study was to determine the correlation between the serum B12 level and proteinuria in patients with Diabetes Mellitus which according to the results of this study, the correlation between the two was inverse, weak, and was not statistically significant. The consideration for the role of vitamin B12 stems from the fact that the lack of this vitamin can increase oxidative stress by increasing homocysteine and cause toxic effects [7,8]. But the question that needs to be answered first is whether there is a correlation between the serum B12 level and proteinuria that in the next step, if this correlation is proven, the effects of this vitamin in the prevention and treatment of diabetic nephropathy should be investigated. In a study of 100 Indian patients with type 2 diabetes, the B12 level was significantly lower in the group with nephropathy than in the group without nephropathy [10], yet in a study conducted in the United States on 2965 patients, there was no correlation between the serum B12 level and albuminuria or decreased renal function [11]. However, this study has not been performed on diabetic patients; also, in another study performed on diabetic patients in Germany, there was no significant relationship between the serum B12 level and albuminuria [5]. A study was conducted in 2010 to evaluate the effect of high doses of B vitamins (including folic acid, B6, and B12) on patients with nephropathy that at the end of treatment, not only proteinuria did not change significantly, but these patients had a greater decrease in GFR and a greater increase in MI and stroke than the control group [12]. Therefore, lowering homocysteine by administering high doses of vitamin B12 was not necessarily associated with reducing the effects of hyperhomocysteinemia [13]. Therefore, there is currently no strong evidence to support the administration of vitamin B12, to reduce diabetic nephropathy.

One of the significant results in this study is the rate of B12 deficiency in patients treated with Metformin (41.7%), which is higher than similar previous studies [2, 4]. This indicates the need to pay more attention to the evaluation of patients treated with metformin for B12 deficiency.

LIMITATIONS and SUGGESTIONS

One of the limitations of this study is the small number of samples and another limitation is the lack of measurement of the serum levels of methylmalonic acid and homocysteine. Thus, our suggestion is to conduct a study with a large number of sample volumes in this field, and in addition to the serum B12 level, the serum level of Methylmalonic Acid and homocysteine should also be measured.

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CONFLICT OF INTERESTS

The authors declared no competing interests.

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