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Original Article

The study of association of Vitamin B₁₂ deficiency in type 2 diabetes mellitus with and without diabetic nephropathy in North Indian Population

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ABSTRACT

Aim: Diabetic Mellitus is the chronic metabolic disorder associated with various complications of heart, eyes, nerves, kidney etc. Diabetic Nephropathy is one of the leading causes of death in diabetic patient. We hypothesized that decrease Vitamin B₁₂ levels is associated with Diabetic Nephropathy. Aim of our study is to study the serum Vitamin B₁₂ levels in type 2 diabetes mellitus patients with and without nephropathy.

Methods: Our study population consist of 100 subjects out of which 50 cases of Diabetes Mellitus without Diabetic Nephropathy and 50 cases of Diabetes Mellitus with Diabetic Nephropathy. We measured various routine lab parameters, apart from it, we measured spot urinary albumin to creatinine ratio to assess diabetic nephropathy and in special investigation we measured serum Vitamin B₁₂ by chemiluminescence based immunoassay.

Result: Serum Vitamin B₁₂ level in the group with nephropathy (181.6 ± 17.6 pg/dl) was significantly lower than in the group without nephropathy (286 ± 30.1 pg/dl) ($p = 0.03$).

Conclusion: Our study points towards the decrease levels of serum Vitamin B₁₂ levels associated with the complication of diabetic mellitus such as diabetic nephropathy. So treatment of Vitamin B₁₂ deficiency by supplementing could prevent the development and progression of diabetic nephropathy and improves the overall management of diabetic patient.

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1. Introduction

Diabetes mellitus (DM) is the group of metabolic diseases characterised by hyperglycemia and its chronic exposure leads to

generation of free radicals and advanced glycation end products which lead to damage of many organs such as heart, kidney, eyes, nerves, blood vessels etc [1]. Diabetes mellitus has been evolved as a global health problem and responsible for significant morbidity and mortality [2]. India has already been declared as a world capital of diabetes according to WHO [3].

Diabetic Nephropathy (DN) is one of the chronic complications associated with diabetes mellitus, which is often one of the common cause of death of diabetic individual [4]. Hence it causes enormous medical, social and economic impact on health care system and society. Although the therapies for DM and its complication such as DN has been improved over the couple of decades, still the mortality and morbidity is very high which

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highlight the need for some innovative approaches for the management of DM and its associated complications.

Diabetic Nephropathy is a clinical syndrome characterised by persistent albuminuria, hypertension, progressive decline in GFR and is responsible for End Stage Renal Disease (ESRD) [5]. DN progression could be slow down by early detection and treatment that's why it is important to discuss the strategies to prevent the progression in the development of DN.

In various studies it has been reported that vitamin deficiencies such as Vitamin B₁₂ has been involved in the development of DN as chronic complication in DM [6]. So we hypothesized that vitamin B₁₂ deficiency is involved in the development of DN among the diabetic patient. The data regarding the status of vitamin B₁₂ levels in diabetic patient is lacking especially in Indian subcontinent so we undertook this study to determine the Vitamin B₁₂ status in DM with and without Nephropathy.

2. Materials and method

Our study was hospital based cross sectional, case control comparative study. It was done in Department of Medicine and Department of Biochemistry, Smt. Sucheta Kriplani Hospital, Lady Hardinge Medical College, New Delhi from the period of November 2011 to March 2015.

Our study population consisted of 100 patients out of which 50 were of type 2 diabetes mellitus without nephropathy and 50 were of type 2 diabetes mellitus with nephropathy. Inclusion criteria include known cases of type 2 diabetes mellitus patients of minimum 5 years duration of disease and exclusion criteria include any recent stressful events like acute MI, cardiovascular accident (CVA) etc and causes of proteinuria other than diabetic nephropathy like acute febrile illness, hypertension, renal (glomerular and tubular) disease as per clinical profile and investigations including ultrasound abdomen.

This study was carried out on 50 diabetics with nephropathy and 50 diabetics without nephropathy. Patients who attended the medicine and diabetes OPD in Smt. Sucheta Kriplani Hospital were approached, explained about the study and requested for enrolment. Only those patient who agreed for participation, were included in the study after applying inclusion and exclusion criteria.

After obtaining a written informed consent from the patients and their household contacts, a detailed history was taken. Relevant physical examination of all the participants was done and their samples were obtained for analysis. Anthropometric measurement was done.

After the patients had been at rest for at least 10 min in the supine position, 6 ml of blood sample was collected using a sterile aseptic condition. Blood was divided in different vacutainer for hemogram (EDTA vacutainer), for sugar (fluoride) and plain vacutainer for serum. Samples were allowed to clot at room temperature. The clotted blood sample was then centrifuged for 5 min. Serum was then collected, routine investigation done such

as complete blood count, Fasting Blood sugar, LFT, KFT etc and serum also stored at -20°C for further batch analysis to estimate Vitamin B₁₂ levels. We also examined urine sample for routine and microscopic and spot urinary albumin creatinine ratio for assessing the presence or absence of diabetic nephropathy.

Later batch analysis to measure Vitamin B₁₂ in the preserved samples were done using chemiluminescence assays on ACCESS 2 BECKMAN ANALYSER using their proprietary system packs as per the procedure protocol provided by BECKMAN. (Access Immunoassay System, Beckman Coulter). The vitamin B₁₂ assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of Vitamin B₁₂ levels in human serum, using the Access Immunoassay System.

Vitamin B₁₂ assay is a competitive binding immunoenzymatic assay. The Ag-Ab complexes and chemiluminescence substrate Lumi-phos 530 is used for light generation. The amount of analyte in sample is determined by multipoint calibration curve. Reagent cartridge and control of Lot no: 225396 from Beckman was used. The normal reference value of serum Vitamin B₁₂ is 200–835 pg/dl [7].

Spot Urinary albumin to creatinine ratio was used to detect the presence of diabetic nephropathy in diabetic patient. Its value include Normoalbuminuria: <30 mg/g of creatinine, Microalbuminuria: 30–300 mg/g of creatinine and Macroalbuminuria: >300 mg/g of creatinine. GFR was calculated by using Cockcroft – Gault equation [8].

3. Statistical analysis

Data for variables were calculated by mean \pm SD (standard deviation) or SEM (standard error of mean), whereas for comparison between normally distributed groups unpaired student *t*-test was used. All the analysis done using Microsoft excel 2010 and SPSS version 19 was used.

4. Result

Our study shows that study population was obese as shown in Table 1, which shows that obesity could be important key factor in development of diabetes mellitus and its related complication [9,10].

Table 2 shows that mean level of serum urea, creatinine were higher in diabetic patient who had nephropathy as compare to diabetic patient without nephropathy. Table 3 shows that first group had diabetic nephropathy as compare to second group.

In special investigation, the mean level of serum Vitamin B₁₂ were significantly lower (181.6 ± 17.6 pg/dl) in higher in diabetic patient who had nephropathy as compare to diabetic patient without nephropathy (286 ± 30.1 pg/dl) ($p = 0.03$) (Table 4).

Table 1
Anthropometry data of study population.

Parameters	Diabetic patient with nephropathy (n = 50)		Diabetic patient without nephropathy (n = 50)		p value
	MEAN	SD	MEAN	SD	
Age (years)	57.88	8.67	59.12	8.42	0.470
Weight (Kg)	69.36	10.43	71.08	9.64	0.394
BMI (Kg/m ²)	29.43	4.05	29.66	3.87	0.762

*p value ≤ 0.05 is considered statistically significant. SD = Standard deviation.

Table 2

Biochemical parameters of the study population.

Parameters	Diabetic patient with nephropathy (n = 50)		Diabetic patient without nephropathy (n = 50)		p value
	MEAN	SD	MEAN	SD	
Urea (mg/dl)	33.56	8.98	29.66	9.69	0.040
Creatinine(mg/dl)	1.43	0.64	0.72	0.154	<0.001
T.BIL (mg/dl)	0.72	0.278	0.726	0.266	0.942
ALT (IU/ml)	39.02	5.79	39.76	5.92	0.529
AST (IU/ml)	23.72	6.51	23.24	5.71	0.696
ALP (IU/ml)	55.66	12.47	60.44	16.31	0.103
BS-F(mg/dl)	131.22	15.99	133.46	16.27	0.489
BS-PP(mg/dl)	179.58	20.14	184.28	26.77	0.324
Total Cholesterol(mg/dl)	178.16	35.32	183.14	32.07	0.462
Triglyceride(mg/dl)	98.1	22.82	104.84	22.87	0.143

*p value ≤ 0.05 is considered statistically significant.**Table 3**

Renal profile of study population.

Parameters	Diabetic patient with nephropathy (n = 50)		Diabetic patient without nephropathy (n = 50)		p value
	MEAN	SD	MEAN	SD	
U-A/C (mg/g)	130.7	46.72	18.37	4.99	<0.001*
GFR (ml/min)	62.16	24.74	108.44	7.96	<0.001*

* p value ≤ 0.05 is considered statistically significant.**Table 4**Vitamin B₁₂ status of study population.

Parameters	Diabetic patient with nephropathy (n = 50)		Diabetic patient without nephropathy (n = 50)		p value
	MEAN	SEM	MEAN	SEM	
Vitamin B ₁₂ (pg/dl)	181.6	17.6	286	30.1	0.03*

* p value ≤ 0.05 is considered statistically significant. SEM = Standard Error of Mean.

5. Discussion

Diabetes contributes to large amount of morbidity and mortality worldwide, with the largest proportion of patients in India only. Even after a lot of knowledge and various treatment options it is difficult to control. We need to find a newer modifiable risk factor involved in the pathogenesis of diabetes. Diabetes has been shown to be associated with abnormalities in the metabolism of Vitamin B₁₂. Deficiency of Vitamin B₁₂ status has been reported as aggravating factors in the progression of diabetes [11]. A lot of studies have been done to find association between diabetes with Vitamin B₁₂ deficiency, over the last few years. This study is unique in its study composition and comparison between diabetics with nephropathy and without nephropathy. In India, this study supplements to our previous knowledge and one of the first of its type.

Our study points toward the deficiency of Vitamin B₁₂ in diabetic patient who has developed its complication such as diabetic nephropathy. Our study results coincide with the study done by Pflipsen MC et al. [6].

The drug of choice for uncomplicated diabetes is metformin which causes malabsorption of Vitamin B₁₂. It could be one of the reasons for vitamin B₁₂ deficiency in diabetic patient apart from dietary deficiency [12].

In human, there are only two enzymatic reactions known where Vitamin B₁₂ acts as a cofactor. In the first reaction homocysteine is converted into methionine by the enzyme methionine synthase which utilises Vitamin B₁₂ and folic acid as a cofactor. In the second

reaction Methylmalonic acid is converted into succinyl CoA by enzyme Methylmalonyl CoA mutase by using Vitamin B₁₂ as a cofactor [13]. Hence if there is deficiency of vitamin B₁₂, then it can lead to increased amount of methylmalonic acid and homocysteine, which are known to have toxic [14] effect on various tissue such as renal tissue lead to development of DN.

6. Conclusion

Hence if we supplement the patient of diabetes mellitus with Vitamin B₁₂, then it could prevent the excess of production of methylmalonic acid and homocystein and damage to various organs, one of them is kidney. That's why deficiency of Vitamin B₁₂ could influence the incidence of diabetic nephropathy and other complication in diabetic patient and help in overall management of diabetic patient.

Our Strength of the study were 1) well characterised and diverse study population, 2) measurement of spot urinary albumin to creatinine ratio and serum Vitamin B₁₂ was done by standardized technique. 3) the study population were well matched for age, sex and BMI.

7. Limitations

We had smaller sample size and it was cross sectional study so that cause effect relationship cannot be established. Dietary survey was not conducted.

8. Suggestions

We should go for the follow-up studies with the vitamin B₁₂ levels and complication associated with diabetes mellitus such as nephropathy and we should also follow the effect of Vitamin B₁₂ supplementation on various complications and their development in type 2 diabetes mellitus.

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