Original Article

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Comparison of Serum Vitamin B12 Levels in Type 2 Diabetes Mellitus Patients with and without Diabetic Retinopathy: A Case-control Study

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ABSTRACT

Introduction: Diabetes mellitus is characterised by hyperglycaemia. Chronic elevation of hyperglycaemia lead to generation of free radicals and Advanced Glycation End products (AGEs) which results in damage of many organs such as heart, kidney, eyes, nerves and blood vessels. Diabetic Retinopathy (DR) is the leading cause of blindness among diabetic patients. Vitamin B12 is a vital micronutrient that is essential for the proper functioning of the central nervous, cardiovascular and haemopoetic systems. It is also found that its deficiency is more prevalent in Type 2 Diabetes Mellitus (T2DM) and evident clinically.

Aim: To compare the serum vitamin B12 levels and Glycated Haemoglobin (HbA1c) levels in patients with and without diabetic retinopathy.

Materials and Methods: This was hospital based observational case-control study conducted in Biochemistry Department in collaboration with Ophthalmology Department at the Oxford Medical College, Hospital and Research Centre, Yadavanahalli, Bengaluru, Karnataka, India, from January 2018 to June 2018 with total of 90 subjects. Among 90 patients, 30 patients were with diabetes mellitus with Retinopathy (DR), 30 were with diabetes mellitus without retinopathy (NDR) and 30 were also recruited as control (healthy individuals). The mean and standard deviation were used to describe continuous data. Analysis of Variance (ANOVA) was used to statistically compare the mean difference between more than two sets of quantitative data.

Results: The mean plasma blood glucose levels were higher in T2D patients with DR, Fasting Blood Glucose (FBG) (270.4±94.2 mg/dL) and Postprandial Blood Glucose (PPBG) (425.6±131.8 mg/dL) compared to control subjects FBG (95.4±10.7 mg/dL). Among the T2D patients the plasma HbA1c concentration of DR group was found to be higher (11.0±2.3%) compared to the NDR group (p-value <0.001). While, the blood levels of vitamin B12 were comparable between the groups, serum vitamin B12 levels were significantly lower (p-value <0.001) in T2DM group with DR (200.7±201.9 pg/mL) compared to the control group (1004.8±304 pg/mL).

Conclusion: The patients with diabetic retinopathy showed that low serum vitamin B12 is associated with elevated Glycated haemoglobin (HbA1c) levels, as a result of poor glycaemic control, endothelial dysfunction and oxidative stress leading to development and progression of DR.

Keywords: Endothelial dysfunction, Fasting blood glucose, Glycated haemoglobin, Microvascular complication, Postprandial blood glucose

INTRODUCTION

Diabetes Mellitus is a chronic disorder caused due to insulin deficiency or resistance, resulting in hyperglycaemia. There is an estimated 451 million people with diabetes worldwide, as of 2017; these figures are expected to increase to 693 million by 2045 [1,2]. The patients suffering from long term uncontrolled diabetes mellitus are prone to develop life threatening complications like microvascular complications (stroke, peripheral artery disease, ischaemic heart disease) and macrovascular complications (retinopathy, neuropathy and nephropathy) [3].

Diabetic Retinopathy (DR) is considered as one of the most common microvascular complications of diabetes and listed first cause of blindness across the world [4]. Both the duration of diabetes and its metabolic control have been predicted as the risk factors for the development of DR [5]. DR occurs in 70% individuals having diabetes for more than 15 years. Diabetic retinopathy is characterised by the appearance of altered vascular lesions of increasing severity. Early or Non Proliferative DR (NPDR) is marked by retinal vascular microaneurysms, blot haemorrhages, cotton-wool spots, loss of retinal pericytes, increased vascular retinal permeability, alterations in regional blood flow and abnormal retinal microvasculature, all of which lead to retinal ischaemia macular oedema and vision damage [6]. Proliferative DR (PDR), the more severe state, leads to formation of abnormal, fragile new blood vessels that are susceptible to haemorrhage leading to neovascularization, vitreous haemorrhage, retinal detachment and early blindness [7,8].

Although genetic susceptibility appears to be the primary predisposing factor for DR, the role of environmental factors like nutrition and dietary factors are not to be forgotten. Concentrations of folic acid and other B12 vitamins are associated with increased risk of vascular damage through homocysteine. It is a by-product of transmethylation reactions and detoxified by methionine synthetase, which is dependent on vitamin B12 and folate as co-enzymes for its proper function [9-12]. It is proved that the development of DR is due to poor glycaemic control, hypercoagulability, ischaemic and anoxia of the retina leading to oxidative stress, increases Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase activity promotes uncoupling of endothelial nitric oxide synthase [13,14], and functional inhibition of Glutathione peroxidase and superoxide dismutase, the most common intracellular antioxidant enzymes [15]. Increased endothelial cell production of adhesion molecules occurs as a result of these inflammatory processes, contributing to leucocyte accumulation and attachment to retinal capillaries (leucostasis) [16]. Leucostasis, which is thought to be a precursor to DR, can lead to a breach of the blood-retinal barrier, as well as persistent leucocyte-mediated cell damage and death.

Very few studies have been done regarding the association between vitamin B12 and DR. Hence, the aim of the present study was to compare the serum vitamin B12 levels and Glycated haemoglobin (HbA1c) levels in patients with and without diabetic retinopathy.

MATERIALS AND METHODS

This was hospital based case-control study conducted in Biochemistry Department in collaboration with Ophthalmology Department at the Oxford Medical College, Hospital and Research Centre, Yadavanahalli, Bengaluru, Karnataka, India, from January 2018 to June 2018. Ethical Clearance was taken from the Institutional Ethical Committee with reference no (IEC/TOMCH&RC/055/17-18 dated 15/02/2018).

Sample size calculation: Sample size was determined assuming 95% Confidence Interval (CI) and 80% power, using SD of respective vitamins.

A total of 90 subjects were included in the study and divided into three groups; each group had 30 subjects. The subjects were grouped as- Type 2 Diabetes Mellitus (T2DM) patients with retinopathy (n=30), Type 2 Diabetes Mellitus patients without retinopathy (n=30) and healthy individuals (n=30).

Inclusion criteria:

1. All patients of type 2 diabetes mellitus having DR irrespective of their control level and duration of disease;
2. Patients who did not know the duration and did not follow the treatment;
3. Patients who were willing to participate in the study.

Exclusion criteria:

1. Patients with a history of vascular disease (myocardial infarct or angina, stroke, peripheral arterial disease, and deep-venous thrombosis), renal, hepatic, chronic gastroenterologic, thyroid or blood disease, dementia, and neoplasm;
2. Patients receiving vitamin supplementation.
3. Patients on drugs such as theophylline, statins, fibrates, levodopa, protons pump inhibitors, anticonvulsives, and contraceptives;
4. Chronic smoker and alchoholics;
5. Patient with indefinite duration non compliant with treatment were excluded.

Detailed medical history, physical examination and ophthalmic examination was carried out for all the study subjects. Self-declared diabetic condition was encouraged. The diagnosis of diabetes mellitus was made, based on current World Health Organisation (WHO) diagnostic criteria for diabetes [14]. All subjects underwent a thorough ophthalmic examination, which included visual acuity measurement using Snellen's chart, slit lamp evaluation of the anterior segment and fundus examination using indirect ophthalmoscopy, slit lamp microscopy, fluoroscein angiography and optical coherence tomography when indicated. Retinopathy was graded as the presence of any of such characteristic lesions such as microaneurysms, haemorrhage, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, new vessels [15].

Diagnostic Criteria for DR Non Proliferative Diabetic Retinopathy (NPDR) [16,17]

Atleast one micro aneurysm indicates a mild condition. Moderate condition was characterised by haemorrhages, micro aneurysms, and hard exudates. Severe condition included haemorrhages, micro aneurysms, and hard exudates present in all four quadrants with definite venous beading in two or more quadrants or Intra Retinal Microvascular Abnormality (IRMA) in one or more quadrants.

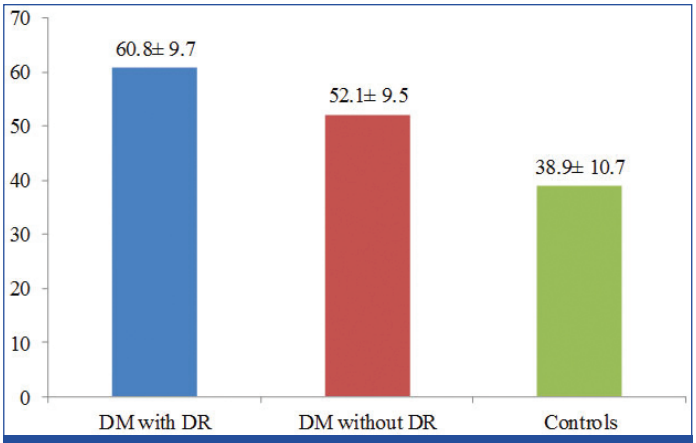
Blood samples were collected from the three groups for determination of Fasting Blood Glucose (FPG), Postprandial Blood Glucose (PPBG), HbA1c and vitamin B12 levels. FBG and PPBG were analysed by fully auto-analyser by Glucose Oxidase (GOD) and Peroxidase (POD) method. HbA1c was assessed by fully automated Immunoturbidometric method. Vitamin B12 concentrations >300 pg/dL were considered as normal. Mild vitamin B12 deficiency was defined as serum concentration of <200 pg/dL and borderline deficiency as 200-300 pg/dL [18,19].

STATISTICAL ANALYSIS

The data was put into a Microsoft Excel data sheet and analysed with Statistical Package for the Social Sciences (SPSS) version 22.0 software. Frequencies and proportions were used to represent categorical data. For qualitative data, the Chi-square test was employed as a measure of significance. The mean and standard deviation were used to describe continuous data. The Analysis of Variance (ANOVA) was a statistical test used to determine the statistical difference between more than two sets of quantitative data. Data Graphical Representation like MS Excel and MS Word were used to generate several types of graphs, including the bar diagrams. After assuming that all the variables were equal, a p-value (probability that the result is true) of 0.05 was considered statistically significant.

RESULTS

[Table/Fig-1] shows the age distribution of the study population. Mean age of subjects in DM with DR group was 60.8±9.7 years, DM without DR group was 52.1±9.5 years and controls was 38.9±10.7 years.



[Table/Fig-1]: Age distribution comparison between three groups.

In DM with DR group, 53.3% were males and 46.7% were females. In DM without DR group, 56.7% were males and 43.3% were females. In control group, 76.7% were females and 23.3% were males. There was significant difference in sex distribution between three groups [Table/Fig-2]. [Table/Fig-3] shows that there was no significant difference in smoking, alcohol consumption between the three groups. There was significant difference in diet pattern between three groups. There was significant difference in duration of DM between the groups and mean duration of DM was 7.4±4.1 years in DM with DR [Table/Fig-4].

Gender		Groups					
		DM with DR		DM without DR		Controls	
		Count	%	Count	%	Count	%
Sex	Females	14	46.7%	13	43.3%	23	76.7%
	Males	16	53.3%	17	56.7%	7	23.3%

[Table/Fig-2]: Sex distribution comparison between three groups.
χ^2=8.190; df=2; p=0.017*; p-value <0.05 considered significant

Most of the diabetics were on Oral Hypoglycaemic Agents (OHA) as compared to insulin. A 66.7% of DM with DR group and and 70% of DM without DR group were on OHA. A 33.3% of diabetics with DR were on Insulin. There was no significant difference in treatment between DM groups [Table/Fig-5].

Higher mean HbA1c was noted in DM with DR group (11.0±2.3%). DM without DR had a mean HbA1c level as 9.8±2.0%. There was significant difference in mean HbA1c between three the groups [Table/Fig-6].

Personal habits		Groups						p-value
		DM with DR		DM without DR		Controls		
		Count	%	Count	%	Count	%	
Smoking	No	28	93.3%	26	86.7%	29	96.7%	0.338
	Yes	2	6.7%	4	13.3%	1	3.3%	
Alcohol	No	27	90.0%	26	86.7%	29	96.7%	0.383
	Yes	3	10.0%	4	13.3%	1	3.3%	
Diet	Non vegetarian	13	43.3%	16	53.3%	24	80.0%	0.012*
	Vegetarian	17	56.7%	14	46.7%	6	20.0%	

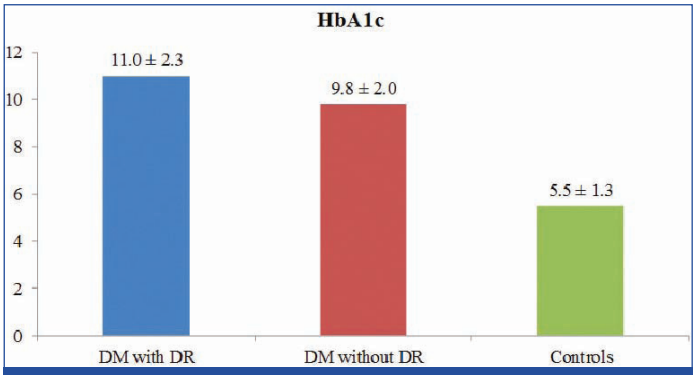
[Table/Fig-3]: Personal habits between three groups.
Chi-square test; p-value <0.05 considered significant

Groups	Duration of DM (years)	
	Mean	SD
DM with DR	7.4	4.1
DM without DR	5.0	2.9
p-value	0.011*	

[Table/Fig-4]: Comparison of duration of DM between the groups.
*p-value <0.05 is significant; Independent t-test

Variables	Groups			
	DM with DR		DM without DR	
	Count	%	Count	%
Insulin	10	33.3%	9	30.0%
OHA	20	66.7%	21	70.0%

[Table/Fig-5]: OHA/Insulin comparison between DM groups.
χ^2=0.077; df=1; p-value=0.7814; OHA: Oral hypoglycaemic agents



[Table/Fig-6]: HbA1c comparison between three groups.
p<0.001 *statistically significant; ANOVA test

The mean Fasting Blood Glucose (FBG) was 270.4±94.2 mg/dL and mean Postprandial Blood Glucose (PPBG) was 425.6±131.8 mg/dL in DM with DR group. In DM without DR group, mean FBG was 240.2±72.1 mg/dL and mean PPBG was 313.3±79.8 mg/dL. In control group, mean FBG was 95.4±10.7 mg/dL and mean PPBG was 116.1±12.2 mg/dL. There was significant difference in mean FBG and PPBG between three groups. In DM with DR group, mean vitamin B12 was 200.7±201.9. There was a significant difference in mean vitamin B12 between three groups [Table/Fig-6,7].

Variables	Group						p-value
	DM with DR		DM without DR		Controls		
	Mean	SD	Mean	SD	Mean	SD	
Vitamin B12 (pg/mL)	200.7	201.9	634.2	342.6	1004.8	304.0	<0.001*
FBG (mg/dL)	270.4	94.2	240.2	72.1	95.4	10.7	<0.001*
PPBG (mg/dL)	425.6	131.8	313.3	79.8	116.1	12.2	<0.001*

[Table/Fig-7]: Vitamin B12, FBG and PPBG comparison between three groups.
*p-value <0.05 is significant; ANOVA test

Out of 30 patients with Diabetic Retinopathy (DR), six patients belonged to mild NPDR. Among the mild NDPR patients, five patients had vitamin serum B12 levels below 200 pg/mL and one had serum vitamin B12 levels above 200 pg/mL. Five patients belonged to moderate NPDR. Out of five patients, three patients had serum vitamin B12 levels below 200 pg/mL and two patients had serum vitamin B12 levels higher than 200 pg/mL. Eight patients belonged to severe NPDR. Out of eight patients, seven patients had serum vitamin B12 levels below 200 pg/mL while one had serum vitamin B12 levels over 200 pg/mL. 11 patients belonged to PDR. Eight patients with PDR had a serum vitamin B12 level less than 200 pg/mL, while three had a serum vitamin B12 level greater than 200 pg/ mL [Table/Fig-8].

	Patients with DR	<200 (pg/mL)	>200 (pg/mL)
Mild NPDR	6	5	1
Moderate NPDR	5	3	2
Sever NPDR	8	7	1
PDR	11	8	3

[Table/Fig-8]: Distribution of serum Vitamin B12 levels among DR patients.

DISCUSSION

This study aimed to compare the serum vitamin B12 levels and Glycated haemoglobin (HbA1c) levels in patients with and without diabetic retinopathy. Approximately, 5% of the global prevalence of blindness is considered to be due to DR, with estimates of 15%-17% in developed countries [1]. In the present study, mean age of subjects in DM with DR group was 60.8±9.7 years, DM without DR group was 52.1±9.5 years and controls were 38.9±10.7 years. Satyanarayana A et al., conducted a study that was almost identical to that of the present study population (55.3±5.4 years and 54.8±6.1 years in Poliferative DR and no DR group respectively) [19]. In a study conducted by Brazionis L et al., (median age of 66.5 years in DR and 65 years in No DR) and Fotiou P et al., (median age of 68 years in DR and 61 years in No DR group), the research populations were considerably older than our study population [20,21].

In the study conducted by Satyanarayana A et al., (10.3±2.9% vs. 9±2.5%; p-value <0.01), they found that the mean/median HbA1c levels were significantly higher in patients with DR compared to patients without DR [19]. The result was comparable to our study. However, it is assumed that perfect glycemic control is impossible for most diabetic patients and glucose control has a tendency to worsen over time. The decreased serum vitamin B12 concentration is related with elevated fasting and postpandrial blood glucose level, which was a major observation in our research. This shows the known detrimental effect of hyperglycemia on the development and progression of DR.

In DM with DR group, mean vitamin B12 was 200.7±201.9 pg/mL. There was a significant difference in mean vitamin B12 between three groups. Out of 30 patients with DR, six patients belonged to mild NPDR, five patients belonged to moderate NPDR and eight patients belonged to severe NPDR. The result was very much consistent with study published by Patel Z et .al., [22]. In our study we also found that 42.7% elderly patient of age above 60 years, their serum vitamin B12 was low and 56.7% of them were males.

In a study conducted by Qureshi S et al., they considered vitamin B12 deficiency to be less than 150 pg/mL in serum and showed that 33% of diabetic patients had vitamin B12 deficiency [18]. Although there is no published guideline on routine screening for vitamin B12, it is still important that T2DM patients should be assessed for deficiency.

Vitamin B12 is an enzyme co-factor which assists the cytoplasmic regeneration of methionine from homocysteine and facilitates the conversion of methylmalonic MMA-coenzyme A (CoA) to succinyl-CoA in the mitochondria. These processes help in DNA regulation, Hcy (homocysteine) metabolism, myelin synthesis, nerve growth, and neuron maintenance; all of which impact vision and DR. Active forms of methylcobalamin easily transfers a methyl group to lower Hcy, converting it to methionine. Therefore reduced B12

level in serum indirectly increases Hcy metabolism which leads to decreased cerebral blood flow, lower retinal blood flow, reduced calibre of the central retinal artery, vascular endothelial growth factor (VEGF) expression and DR [9,10].

In the present study, serum vitamin B12 levels were lower in men compared to women and men had higher risk for vitamin B12 deficiency. Shahwan M et al., have reported that serum vitamin B12 levels were lower in women than men, which is contradictory to our findings [23].

People who eat vegetarian diet are more likely to have vitamin B12 deficiency than non vegetarians [24]. In addition, vegetarian are more likely to suffer vitamin B12 deficiency. According to our findings.

Most of the Diabetics were on Oral Hypoglycaemic Agents (OHA) as compared to insulin. A 66.7% of DM with DR group and 70% of DM without DR group were in OHA. A 33.3% of Diabetics with DR were on Insulin. There was no significant difference in treatment between DM groups [Table/Fig-5]. The mean duation of diabetes in DM with DR group was 7.4 years, whereas the mean duation of diabetes in DM without DR was 5 years. Similarly, Fotiou P et al., and Brazionis L et al., found that the duration of diabetes in the retinopathy group was considerably greater as compared to the no retinopathy category [20,21].

The mechanism by which metformin affects the uptake of vitamin B12 is unknown. The most likely explanation is that the drug acts by interfering with the calcium-dependent membrane action of the vitamin B12 intrinsic factor, albeit without direct evidence as showed in study by Bauman WA et al., [24].

Limitation(s)

The study was conducted on patients with type 2 diabetes. There was a major difference between two groups that used oral hypoglycemic medications such as metformin and also on a vegetarian diet, both of which were known to affect vitamin B12 levels. This may have impaired the finding between groups and may have contributed in false positive results. Authors also did not assess the serum levels of homocysteine and folate in the study subjects as vitamin B12 metabolism is finally linked with homocysteine. Therefore, the relationship whether serum vitamin B12 levels were influenced by the homocysteine and folate levels were not studied. Also, the cases and control were not age matched.

CONCLUSION(S)

The vitamin B12 levels were significantly low in the T2DM patients with DR. The low serum vitamin B12 levels and elevated Glycated haemoglobin (HbA1c) levels may be due to the result of poor glycaemic control, endothelial dysfunction and oxidative stress. Monitoring serum vitamin B12 concentration, as well as HbA1c status in T2DM patients may help in assessing microvascular risk in DM. Treatment with vitamin B12 and vitamin B6 may be useful in reducing the risk of microvascular complications in T2DM.

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