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MCV, 25-OH Vitamin D and Homocysteine to Monitor Progression in Diabetic Retinopathy: Don't Neglect Common Parameters

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Abstract

Purpose: Diabetic retinopathy is the leading cause of blindness and many factors accelerate its progression. In the present study, we elucidate the relation of Homocysteine, Mean corpuscular volume (MCV), 25-OH Vitamin D level in Type 2 diabetes mellites (DM) patients with retinopathy.

Materials and Methods: We enrolled 140 cases of Type 2 DM patients. A complete ophthalmological evaluation was done in all patients. MCV, glycosylated hemoglobin (HbA1c), homocysteine, 25-OH Vitamin D levels were compared within the diabetic retinopathy (DR) patients and in patients with and without DR also. Variables were compared using the Independent Sample T-test and One-Way ANOVA. Pearson's correlation to access correlation between the variables.

Results: 25-OH vitamin D level was significantly decreased with increasing severity of proliferative DR ($p=0.001$). There was no significant difference in homocysteine between DR and without DR while MCV ($p<0.001$) was significantly low in DR than that of without DR. There was a significant negative correlation between 25-OH vitamin D and HbA1c levels ($r= - 0.701$; $p<0.001$).

Conclusion: MCV is useful to monitor retinopathy while homocysteine is not related to retinopathy in type 2 diabetes. 25-OH vitamin D deficiency with poor blood sugar control with raised MCV is associated with the severity of DR in type 2 diabetes.

Keywords: Mean corpuscular volume, homocysteine, diabetic retinopathy, HbA1c, 25-OH Vitamin D

Introduction

Diabetic retinopathy is a common is cause of blindness. There are multiple factors that accelerate the progression of retinopathy. With progress in the management of complications of diabetes there is a lot of interest to find the association of conditions that can be modified or corrected such as vitamin d deficiency, hyperhomocysteinemia and uncontrolled blood sugar levels, so that increased the progression of retinopathy can be delayed.

Homocysteine has been as a risk factor for cardiovascular [1, 2] and vaso-occlusive diseases in the eye[3,4]. Homocysteine is the demethylated derivate of methionine[5] and can be metabolized by two pathways, either catabolized by the transulfuration pathway to cysteine or remethylated to methionine, mainly by the folate and vitamin B12 dependent enzyme methionine synthase hyperhomocysteniemia damages endothelium due to free radical increasing hypercoagulable state[6]. Multiple factors affect its level like renal function [7],

vitamin B12, folate, drug intake,[8] gender and age,[9] alcohol, coffee and smoking.[10] Still there is no clear consensus on hyperhomocystenimia, many studies don't support this view [11-13] few studies [14] quote relation of DR with hyperhomocyteniamia.

Although the treatment of DR has greatly improved but the management of progressive changes still a challenge. Now a days vitamin D deficiency is common which can be corrected. Determining a relation between 25-OH vitamin D deficiency with diabetic retinopathy and can help us to establish a preventive and a corrective measure that have a beneficial effect on the prognosis of diabetic retinopathy leading to improvement in the quality of life.

Diabetes patient with or without retinopathy are on drugs that have effect on folate levels such as OHA like metformin, diuretics like hydrochlorothiazide, chlorthalidone for blood pressure, lipid lowering agent atorvastatin and supplementation of vitamins most commonly folic acid. MCV is also helpful to know type of anemia in diabetics. However, studies on subjects having diabetics with normal hemoglobin suggests diabetics on treatment had higher MCV.[15] So patients having diabetes with or without retinopathy MCV is useful tool to monitor retinopathy and should be further evaluated for cause of increased MCV levels. We took MCV to find relation between retinopathy in diabetics as that impaired glucose tolerance (IGT) may be associated with increased MCV.[16,17] Since MCV is easily available and common investigation as compare to B12, folate levels , GBP, MTHFR and homocysteine.

Material And Method

Study Population

There were 643 type 2 diabetic patients attending our ophthalmology department of medical college. We enrolled 140 patients (42 without DR and 98 DR) after their consent. Study was approved by BRD Medical College Ethics Committee(BRD Medical College Ethics Committee (BRD/IEC/23/18-2017)). Only patients between 20 to 60 years of either gender, not receiving 25-OH vitamin D or calcium. Patients with macroangiopathy, proteinuria 300 mg/24h, pregnancy, recent history of acute illness, cardiovascular diseases, lactation, type 1 diabetes, history of cancer, liver disease, renal disease, malnutrition, hyperparathyroidism or hypoparathyroidism and patients on drugs known to influence on 25-hydroxyvitaminD level such as anticonvulsants, steroids and Rifampicin were excluded.

Ophthalmic Examinations

Routine ophthalmic examination including Fundoscopy, refraction, tonometry, slit lamp examination. Direct and indirect ophthalmoscopy was carried out. Retinopathy was assessed by ophthalmoscopy and fundus photography. Thereafter, two 45° standard field, 35-mm coloured fundus photographs were taken of each eye. Photographs were taken with a green filter, centred on the macular area and the optic disc. Diabetic retinopathy was clinically graded in accordance with the International Clinical Diabetic Retinopathy guidelines.

Biochemical Parameters

Once enrolled, completed a medical history questionnaire along with blood tests for HbA1c, serum creatinine, MCV, CBC, homocysteine and 25-hydroxy vitamin D, LFT, PTH and fasting and post prandial blood glucose level, phosphate and Serum calcium levels done were. Urine complete microscopy and 24hour urinary protein was evaluated. Fasting sample was used to measure homocysteine in all patients. Hyperhomocysteinemia was defined when homocysteine levels were higher than 15 μM/L. Vitamin D deficiency was considered when 25 hydroxyvitamin D level less than 20ng/ml. [18]

Statistical Considerations

The continuous variable was expressed as mean±SD. Continuous variables were compared between the group using the Independent Sample T-test and One-Way ANOVA was applied to compare among the groups. Pearson's correlation was applied to access correlation between the variables. Statistical calculations were performed using SPSS 17.0 (SPSS, Chicago, IL, USA) Statistical significance was considered as p-value less than 0.05.

Results

The mean age was comparable between retinopathy (57.6±10.4 years) and without retinopathy (56.1±9.4 years) patients. There were 52 males out of 98 in DR

Volume 5, Issue 1; January-February 2022; Page No 1288-1296

and 26 males out of 42 in without DR group, the difference was not significant. Duration of diabetes was significantly longer in DR (11.15±5.6) than that of without DR (4.5±3.1, p=0.043) patients. (Table 1)

Biochemical parameter in diabetic retinopathy and without retinopathy There was no significant difference in homocysteine, LDL, FBG, calcium, phosphate and PTH levels between DR and without DR. However 25-OH vitamin D (p=0.001) was significantly low and HbA1c (p<0.001) and MCV (p<0.001) were significantly high in DR than that of without DR patients. (Table 1, Figure 1) 3.2 25-OH vitamin D, HbA1c, MCV and homocysteine in different grades of proliferative diabetic retinopathy

25-OH vitamin D level was decreased with increasing severity of proliferative DR. We found significantly low 25-OH vitamin D in mild NPDR (29.21±6.96) as compared to low-risk PDR (18.19±1.94; p=0.003) and high-risk PDR (14.64±3.52; p=0.041).

Glycosylated hemoglobin level was increased with increasing severity of proliferative DR. HbA1c was significantly high in high-risk PDR (12.48±2.29) than that of mild NPDR (8.13±2.12; p<0.001) and moderate NPDR (8.74±2.07; p=0.006).

The MCV values were increased with increasing severity of proliferative DR. There were significant higher values of MCV in moderate NPDR (87.25±4.42; p=0.003), low-risk PDR (88.40±5.10; p=0.009) and high-risk PDR (90.40±4.50; p=0.004) than that of in without DR (83.76±3.64). There was no significant difference among different proliferative DR groups.

Interestingly we found increasing trend of homocysteine levels with increasing severity of proliferative DR, however there was no significant changes among any of the DR groups. (Figure 2)

Correlation between variables

We applied Pearson's Correlation to know the relation between variables. We found significant negative correlation between 25-OH vitamin D and HbA1c levels (r= -0.701; p<0.001) and significant positive correlation between MCV and HbA1c values (r=0.266; p=0.001). There was no correlation between homocysteine with MCV and HbA1c. (Figure 3).

Discussion

Correctable or treatable conditions should be kept on priority as at right time intervention may help to retard the progression of diabetic retinopathy which is common cause of blindness. As we have taken these factors in our study and we found low vitamin D levels and higher values of mcv was related to severity DR while there is no significant relation between homocysteine levels and diabetic retinopathy.

As homocysteine is associated with metabolic pathways and increased plasma homocysteine levels have been recognized as a risk indicator for macrovascular disease, it has also been discussed its role in microangiopathy in diabetes mellitus. Possible mechanisms by alteration of endothelial cell function,[6] oxidative stress, impaired generation of nitric oxide, increased proliferation of smooth muscle cells. Increased plasma homocysteine is common in renal failure, indicating that kidney function is important for homocysteine elimination. [7] Studies shown higher levels of homocysteine in patients with nephropathy.[11] The development and progression of retinopathy is closely related to the degree of metabolic control. As found in homocysteine has no relation in retinopathy with homocysteine until nephropathy[12, 19] develops which supports our results as ,as our population is having no renal complications, since we excluded renal failure which has confounding effect on results.

Also, studies shown there was a strong correlation between urinary albumin excretion rate and plasma levels of homocysteine [19]. As many patients of diabetes are supplemented with B12 or folate or multivitamin or on OHA or other medications and other comorbid conditions like hypertension it is not wise to investigate folate and B12 levels in all patients however MCV which routinely done in CBC is helpful to diagnose macrocytosis and also prevents unnecessary investigations. In our study, we found significant higher values of MCV in moderate NPDR (87.25±4.42; p=0.003), low-risk PDR (p=0.009) and high-risk PDR (p=0.004) than that of in without DR. As our patient profile is having controlled hypertension, no renal involvement and were having normal range hemoglobin, mcv is useful to monitor retinopathy as studies reported increased value of

Volume 5, Issue 1; January-February 2022; Page No 1288-1296

MCV is in patients with diabetes with retinopathy as compare to without DR.[17,18]

In our study 25-OH vitamin D level was decreased with increasing severity of proliferative DR and the mean vitamin D levels are significantly more in subjects with lesser HbA1c levels than those with higher HbA1c in our study suggesting inverse relation of Hba1c with vitamin D levels. Few studies also found that Glycated Hemoglobin is inversely related to serum vitamin D Levels in diabetics however they not provided details of type of diabetes [20]

Vitamin D is proposed to have a role in ocular health as anti-angiogenic and anti-inflammatory. Studies have also shown that giving vitamin D supplements have helped in improving insulin resistance present in the diabetics.[21] Further, the enzyme 1-α-hydroxylase, responsible for synthesis of 1,25(OH)2D is expressed in the retina suggesting a local action of the hormone calcitriol (1,25(OH)2D) in the eye and potent inhibitor of neovascularisation.[22. 23] So 25-OH vitamin D level must be assessed at the point of diagnosis and supplemented appropriately to possibly prevent or even retard the progression of DR.

In our study we found MCV values were increased with increasing severity of proliferative DR and there is significant positive correlation between MCV and HbA1c values, as chronic hyprglycemia causes thickening of the basal membrane and accumulation of oxidative products[24, 25] products causing disturbance to the blood flow[26] leading ischaemia which is a critical component of DR and progression, is potentially induced by glycated erythrocyte membrane which then have limited blood oxygen delivery capacity which might explain our study results. Few studies also demonstrated that RBC's characteristics might represent a risk factor for DR development and progression.[27]

Limitations of our study was small sample size and no follow-up .As many studies are inconsistent with results with homocysteine, a follow-up study is required to get useful conclusion .However we found MCV significantly related to retinopathy and gives better idea of any abnormalities in RBC size as chronic hyperglycemia causes changes in the RBCs characteristics, it can be used as routine investigation as it will help in further investigation management along with fundus examination.

Concluision

MCV is useful to monitor retinopathy while homocysteine is not related to retinopathy in type 2 diabetes and timely intervention will help to halt progression which may prevent blindness. Simple and common markers like MCV with vitamin d levels should be investigated along with fundus and HbA1c levels in diabetics. There should be a follow-up study of large sample size to know association of homocysteine with risk retinopathy in type 2 diabetes mellitus.

Abbreviations: MCV- Mean corpuscular volume; DM- diabetes mellites; HbA1c- glycosylated hemoglobin

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Volume 5, Issue 1; January-February 2022; Page No 1288-1296

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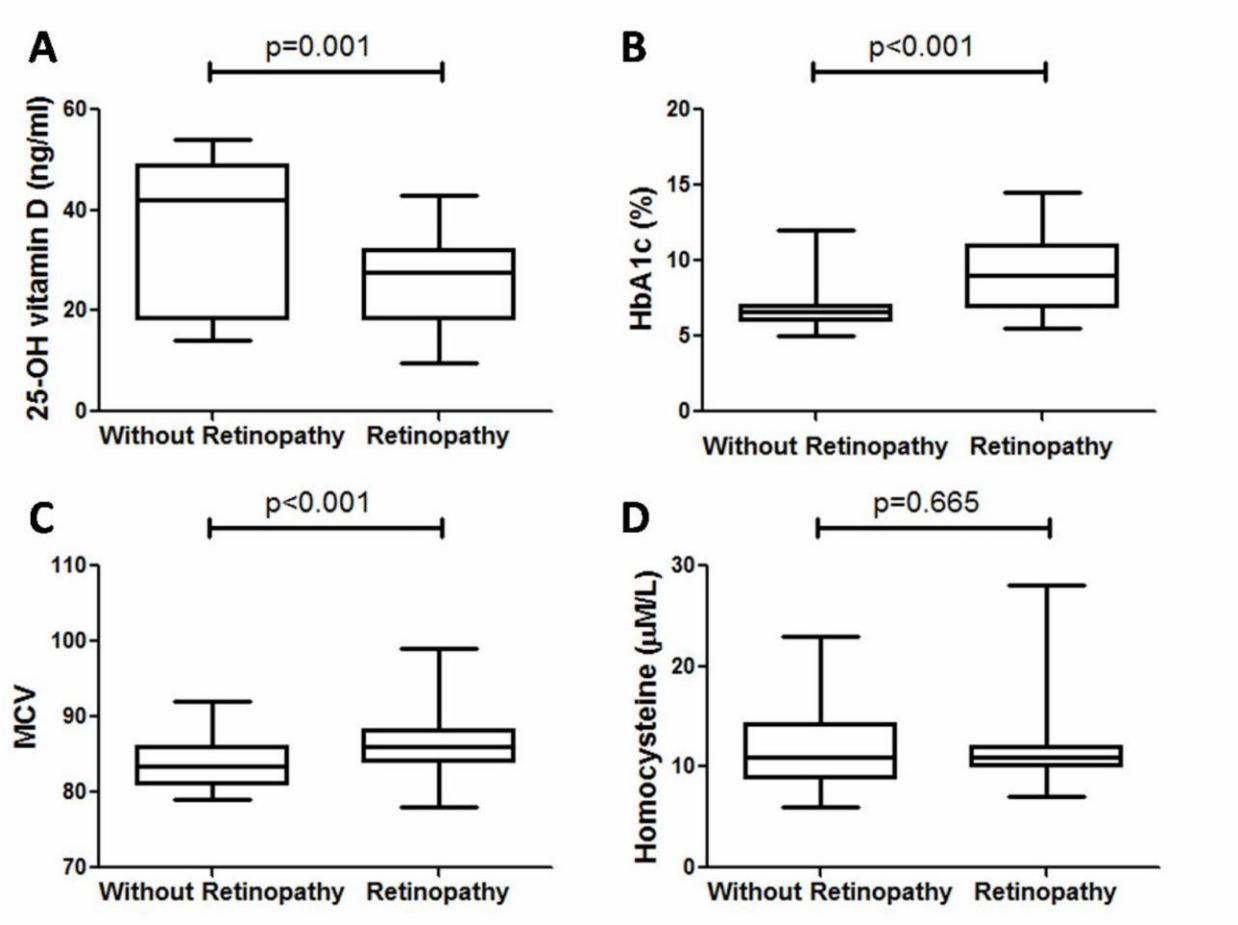
Table 1: Demographic and biochemical characteristics of patients with type 2 diabetes with and without retinopathy

Patients	Retinopathy (98)	Without Retinopathy (42)	p-value
Age (years)	57.6±10.4	56.1±9.4	NS
Male (n)	52	26	NS
Duration (years)	11.15±5.6	4.5±3.1	0.043
MCV (fL)	86.76±3.93	83.76±3.64	<0.001
25-OH Vitamin D (ng/ml)	25.91±7.83	34.74±15.43	0.001
HbA1c (%)	8.84±2.37	7.12±2.00	<0.001
Homocysteine (µM/L)	12.26±4.61	11.89±4.32	NS
HDL (mg/dL)	41.84± 9.87	44.84 ± 9.87	NS
Calcium (mg/dL)	8.98±0.49	9.01±50	NS
LDL (mg/dL)	120.66 ± 36.53	116.66 ± 36.53	NS
FBG (mg/dL)	192.50 ± 66.86	170.77 ± 58.63	NS
PTH (pg/ml)	51.9±14.23	52.6±16.18	NS
Phosphate (mg/dL)	3.4±0.51	3.3±0.48	NS

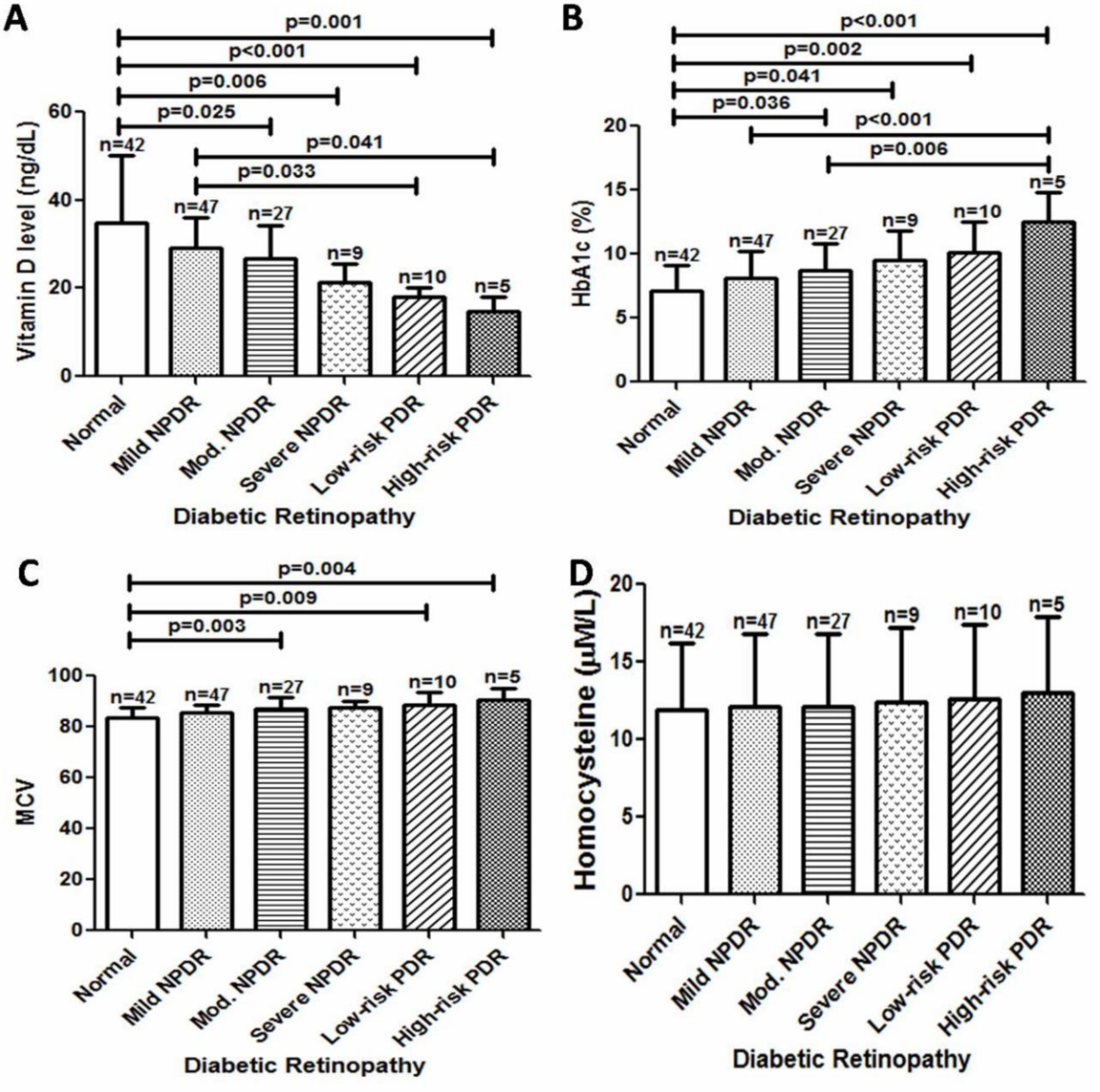
Volume 5, Issue 1; January-February 2022; Page No 1288-1296

Figure 1: Different parameters in DR and without DR patients. Significantly low level of 25-OH vitamin D in DR patients (A), HbA1c and MCV was significantly high in DR patients (B,C) and difference in Homocysteine level between DR and without DR was non-significant (D)



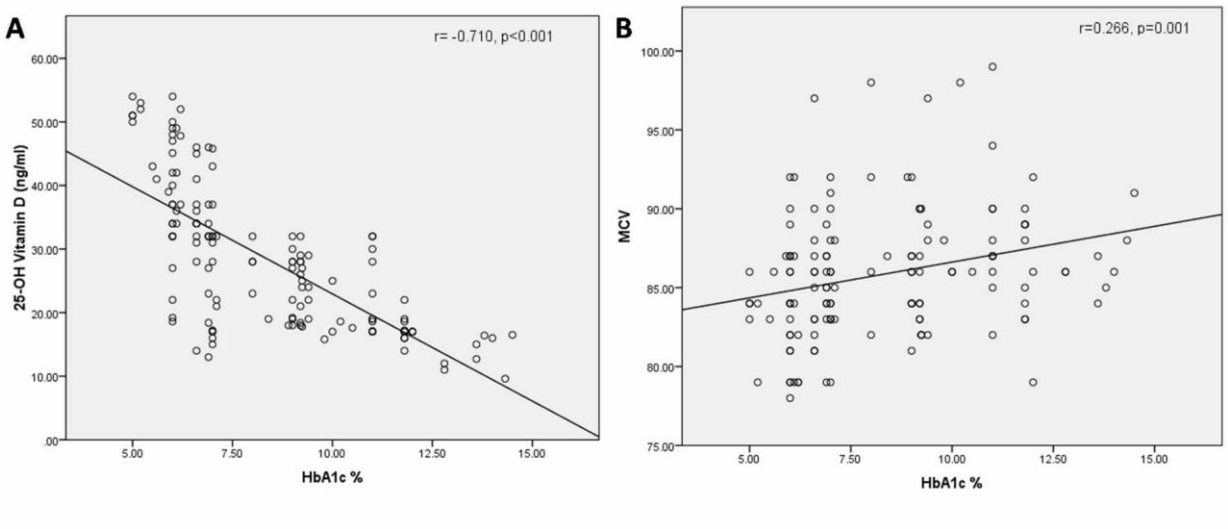
Volume 5, Issue 1; January-February 2022; Page No 1288-1296

Figure 2: Biochemical parameters in different stages of proliferative DR. 25-OH vitamin D level was significantly decreased with increasing severity of DR (A), HbA1c and MCV values were significantly increased with increasing severity of DR (B,C) Homocysteine was also increased with increasing severity of DR but difference was not significant (D).



Volume 5, Issue 1; January-February 2022; Page No 1288-1296

Figure 3: Correlation among biochemical parameters. 25-OH vitamin D was significantly correlate with HbA1c negatively (A), however MCV and HbA1c was significantly correlate each other positively (B).



Volume 5, Issue 1; January-February 2022; Page No 1288-1296