Original Article

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Association of Age and Sex with Different Status of Serum Vitamin D Level among Different Grades of Diabetic Retinopathy: A Cross-sectional Study

Physiology Section

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is rapidly escalating globally as well as in India, affecting all age and sex groups. One of the dreaded microvascular complications of DM is Diabetic Retinopathy (DR). In parallel to increase in prevalence of DM and its complications, several reports of serum 25 hydroxy (OH) Vitamin D deficiencies have been documented in India.

Aim: To establish the relation of different age and sex groups with different status of serum 25 (OH) Vitamin D level among different grading of Diabetic Retinopathy in patients of Type 2 Diabetes Mellitus.

Materials and Methods: This cross-sectional and observational study was conducted in Calcutta National Medical College and Hospital, Kolkata, West Bengal, India, from May 2019 to May 2020. Total 107 type 2 DM patients aged 40 years and above including both males and females were taken. Direct ophthalmoscopy was done for examination of retina and venous blood was taken for Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Glycated Haemoglobin (HbA1c) and serum 25 (OH) Vitamin D level estimation aseptically. Number of patients and percentage of patients were compared across the groups using Fisher's-exact test/Pearson's Chi-square test for independence of attributes as appropriate. Mean, median and standard deviation were compared across the groups using Mann-Whitney's U-Test/Kruskal-Wallis's Test as appropriate. Spearman's test was applied for assessing the correlation between age of diabetic patients and vitamin D levels. The p-value <0.05 was considered as statistically significant.

Results: In this study, most of the participants were under the age group of 50-59 years. No significant relationship between the age and Vitamin D levels of the subjects was observed. The association between different status of serum Vitamin D level and different age groups among different grading of Diabetic Retinopathy is statistically significant out of entire sample size, not in individual grading. The association between different sex groups and different vitamin D status among different grading of DR was not statistically significant. There was no significant difference between serum Vitamin D level in males and females with DR. Correlation between serum Vitamin D level and age was linear and positive; but strength was low and p-value was not significant (correlation coefficient=0.100, p-value=0.306).

Conclusion: This present study showed that maximum subjects were under the age group of 6th decade. There was a significant association between different status of serum Vitamin D level and different age groups out of whole study population, but not in individual grading of DR. No association was observed between different sex groups and different Vitamin D status among different grading or severity of DR.

Keywords: Age related macular degeneration, Neovascularisation elsewhere, Neovascularisation on disc, Proliferative diabetic retinopathy, Retinal detachment

INTRODUCTION

The Diabetes Mellitus (DM) is a large public health problem which affects more than 300 million individuals worldwide with significant morbidity and mortality [1]. In parallel to increase in prevalence of DM, there has been resurgence of Vitamin D deficiency worldwide and it is seen across all ages, races and geographic regions [2,3]. In India, inspite of adequate sunlight exposure, several reports have documented the prevalence of Vitamin D deficiency in general population [4]. Uncontrolled diabetes increases risk of microvascular complications. Diabetic Retinopathy (DR) is most common complication among them [5]. Diabetic retinopathy is a microangiopathy primarily affecting the precapillary arterioles, capillaries and post capillary venules although larger vessels may also be involved. Diabetic retinopathy is characterised by features of both microvascular occlusion and leakage owing to elevated blood glucose level for long duration. In patients diagnosed with diabetes before the age of 30 years, the incidence of DR after 10 years is 50% and after 30 years is 90%. Diabetic retinopathy rarely develops within five years of the onset of diabetes or before puberty, but about 5% of Type 2 diabetic patients have DR at presentation [6]. The individuals, who have high level of blood glucose chronically, are very much prone to develop moderate to severe retinopathy in comparison to individuals having lower blood glucose level. Although there is no cut-off value of HbA1c to determine the retinopathy, it has been seen that usually patients with HbA1c < 6.5% have less chance of developing DR [7]. Besides its main action in mineral homeostasis and bone remodelling, Vitamin D plays a potential role in glucose homeostasis and in the pathogenesis of Type 2 Diabetes and its complications by directly stimulating insulin secretion from β cells as well as improving insulin sensitivity to peripheral tissues [8]. Experimental study revealed that Vitamin D also has protective effects on DR by inhibiting Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor-β (TGF-β) [9]. There are several conflicting reports about the association between DR and hypovitaminosis of Vitamin D. Serum 25 (OH) Vitamin D level is widely accepted as a good indicator of status of Vitamin D in a subject [10]. This study was done to evaluate the association between different age and sex groups and different serum Vitamin D status among different grading of DR in type 2 diabetic patients.

MATERIALS AND METHODS

This cross-sectional and observational study was done in the Department of Medicine (Diabetic Clinic), Ophthalmology, Physiology and Biochemistry of Calcutta National Medical College and Hospital, Kolkata, West Bengal, India, from May 2019 to April 2020 with type 2 diabetic patients attending diabetic clinic of Calcutta National Medical College and Hospital. All examinations were done after taking consent from patients and with due permission of Institutional Ethics Committee.

Sample size calculation: Sample size was determined by applying the formula $4pq/e^2$; where 'p' is the prevalence. The prevalence of DR among Type 2 diabetic patients attending Medicine Outpatient Department of a tertiary care hospital in India is presently 31.5% [11].

So, p=0.315; q=(1-p) i.e.,0.685 and e=allowable error (10% in this study)=0.1.

Thus, the final sample size (n) calculated was 86.31. To avoid bias, a total of 107 patients were included in the study.

Inclusion criteria: Males and females aged 40 years and above and clinically diagnosed Type 2 DM with unknown Vitamin D status were included in the study.

Exclusion criteria: Whereas, the subjects with the following conditions were excluded:

- History of recent Vitamin D supplementation within last six months.
- History of intake of any medication such as rifampicin, phenytoin, or phenobarbitone those alter the blood level of 25 OH Vitamin D.
- Subjects with prior diseases that suggest baseline alteration in serum 25 (OH) Vitamin D level and calcium metabolism like osteomalacia, Hyperparathyroidism etc.
- Any Cardiovascular, Hepatic disease or Renal Disease.
- Other causes of retinopathy like trauma, Central Serous Retinopathy (CSR), Age Related Macular Degeneration (ARMD), Retinal Detachment (RD), Hypertensive retinopathy etc.,
- Patients who were cognitively impaired or unable to provide informed written consent and also Type 1 DM.

Procedure

Sampling method was systematic random sampling. After collecting 107 type 2 diabetic patients, direct ophthalmoscopy (β Heine-200) was done to detect presence of DR and to perform grading of retinopathy, if present. Venous blood was taken aseptically for Fasting Blood Sugar Test (FBS), Postprandial Blood Sugar (PPBS), glycated haemoglobin (HbA1c) estimation by High Performance Liquid Chromatography (HPLC) and 25 (OH)vitamin D estimation by Enzyme Linked Immunosorbent Assay (ELISA) method. In the present study, the patients were divided into 3 groups according to serum 25(OH) Vitamin-D level [Table/Fig-1] [12]-

Levels	Range (ng/mL)
Sufficient Vitamin D level	>30
Insufficient Vitamin D level	20-30
Deficient Vitamin D level	< 20

[Table/Fig-1]: Different Serum 25-hydroxy (OH) Vitamin D status used in present study [12].

- Sufficient,
- Insufficient and
- Deficient

[Table/Fig-2] shows the International Clinical DR Disease Severity Scale [13].

Proposed disease severity scale	Findings observable on dilated ophthalmoscopy
No apparent retinopathy	No microaneurysm
Mild Non Proliferative Diabetic Retinopathy (NPDR)	Microaneurysm only
Moderate Non Proliferative Diabetic Retinopathy (NPDR)	More than just microaneurysm but less than severe NPDR
Severe Non Proliferative Diabetic Retinopathy (NPDR)	Any of the following: i) More than 20 intraretinal haemorrhage in each of 4 quadrants ii) Definite venous beadings in 2+quadrants iii) Prominent Intra Retinal Microvascular Abnormality (IRMA) in 1+ quadrant and no sign of PDR
Proliferative Diabetic Retinopathy (PDR)	One or more of the followings: i) Neovascularisation 1. Neovascularisation on Disc (NVD) 2. Neovascularisation Elsewhere (NVE); ii) Vitreous/Preretinal haemorrhage

[Table/Fig-2]: International Clinical Diabetic Retinopathy (DR) Disease Severity Scale [13].
*Diabetic maculopathy may or may not be present

STATISTICAL ANALYSIS

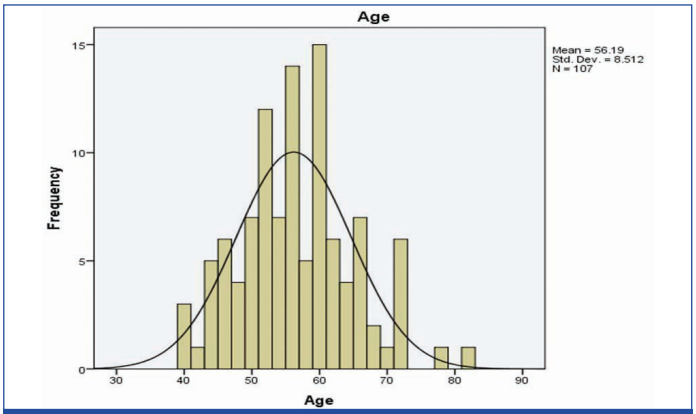
The statistical software- Statistical Package for the Social Sciences (SPSS; Version 20.0) was used for the analysis. Number and percentage of patients were compared across the groups using Fisher's-Exact test/Pearson's Chi-square test for Independence of Attributes as appropriate. Mean, median and standard deviation were compared across the groups using Mann-Whitney's U Test/ Kruskal-Walli's Test as appropriate. Spearman's test was applied for assessing the correlation between age of diabetic patients and Vitamin D levels. The p-value <0.05 was considered as statistically significant.

RESULTS

[Table/Fig-3] shows that maximum patients were in the age group of 50-59 years (44.9%) and minimum (0.9%) patients were in the age group of 80-89 years. Histogram of age [Table/Fig-4] shows that maximum patients were under the age group of 50-59 years.

Age (years)	Frequency	Percentage
40-49	22	20.6
50-59	48	44.9
60-69	28	26.2
70-79	8	7.5
80-89	1	0.9
Total	107	100

[Table/Fig-3]: Distribution of age in present study group.



[Table/Fig-4]: Histogram of age of patients.

Most of the patients in present study were females, 59.8% patients were females and 40.2% patients were males [Table/Fig-5].

About 43% had no apparent retinopathy, 11.2% had mild NPDR; 30.8% had moderate NPDR; 12.1% had Severe NPDR and only 2.8% had PDR [Table/Fig-6].

Sex	Frequency	Percentage
Female	64	59.8
Male	43	40.2
Total	107	100

[Table/Fig-5]: Gender frequency and percentage in study group.

Diabetic retinopathy grading	Frequency	Percentage
No apparent retinopathy	46	43.0
Mild NPDR	12	11.2
Moderate NPDR	33	30.8
Severe NPDR	13	12.1
Proliferative diabetic retinopathy	3	2.8
Total	107	100

[Table/Fig-6]: Distribution of subjects of different grades of Diabetic Retinopathy (DR).
NPDR: Non proliferative diabetic retinopathy

Most patients were Vitamin D deficient (51.4%); 41.1% patients had insufficient Vitamin D level in blood and only 7.5% Patients had sufficient Vitamin D level [Table/Fig-7].

Vitamin D	Frequency	Percentage
Deficient	55	51.4
Insufficient	44	41.1
Sufficient	8	7.5
Total	107	100

[Table/Fig-7]: Distribution of serum 25 (OH) vitamin D status of patients.

[Table/Fig-8] shows that the minimum age was 40 years, maximum age was 82 years; mean age was 56.19 years; minimum FBS was 72 mg/dL, maximum FBS was 351 mg/dL, mean FBS was 149.09 mg/dL; minimum PPBS was 88 mg/dL, maximum PPBS was 584 mg/dL, mean PPBS was 214.45 mg/dL; minimum vitamin D level 7.60 ng/mL, maximum vitamin D level was 98.83 ng/mL, mean value was 21.20 ng/mL, in respect to serum Vitamin D level; minimum level of HbA1c was 6%, maximum HbA1c was 15%, mean value was 8.30%.

Variables	Age (year)	FBS (mg/dL)	PPBS (mg/dL)	Vitamin D (mg/dL)	HbA1c (%)
Minimum	40	72	88	7.60	6
Maximum	82	351	584	98.83	15
Mean	56.19	149.09	214.45	21.20	8.30
Median	56	138	190	19.80	8.10
Standard deviation	8.51	54.38	95.59	11.40	1.77

[Table/Fig-8]: Comparison of Age, Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Vitamin D level and Glycated Haemoglobin (HbA1c) level.

Mean age was more in 'No Retinopathy' (56.41years) in comparison to 'Retinopathy' group (56.02 years), however the comparison was not statistically significant [Table/Fig-9].

Variables		Age (years)	FBS (mg/dL)	PPBS (mg/dL)	Vitamin D (mg/dL)	HbA1c (%)
No diabetic retinopathy	Mean	56.41	148.72	205.64	25.21	7.72
	Median	56.50	132.00	173.00	22.65	6.80
	Standard deviation	8.42	63.32	108.25	15.29	2.08
Diabetic retinopathy	Mean	56.02	149.38	221.10	18.18	8.73
	Median	56.00	146.00	207.00	18.20	8.20
	Standard deviation	8.65	47.07	85.14	5.71	1.36
p-value		0.897	0.483	0.060	**0.001**	**<0.001**

[Table/Fig-9]: Comparison of Age, Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Vitamin D level and Glycated Haemoglobin (HbA1c) level with presence or absence of Diabetic Retinopathy (DR) in present study group.
*Mann-Whitney U Test; p-value is not significant in case of comparison of mean age with presence or absence of Diabetic Retinopathy; p-value <0.05 was considered as statistically significant

[Table/Fig-10] showed that there was no statistically significant difference between mean age groups among different grading of DR. However, vitamin D and HbA1c showed significant difference among different grades of DR.

The association between different age groups and different grading of DR was not statistically significant [Table/Fig-11].

Grading of DR		Age (year)	FBS (mg/dL)	PPBS (mg/dL)	Vitamin D (ng/mL)	HbA1c (%)
No Retinopathy	Mean	56.41	148.72	205.64	25.21	7.72
	Median	56.50	132.00	173	22.65	6.80
	Standard deviation	8.42	63.32	108.25	15.29	2.08
Mild NPDR	Mean	51.83	176.25	265.08	20.45	8.84
	Median	52.50	170	248	20.45	7.90
	Standard deviation	7.92	73.23	118.11	6.36	2.12
Moderate NPDR	Mean	57.18	139.94	202.69	18.43	8.37
	Median	56	145	196	18.50	8.20
	Standard deviation	7.78	38.81	67.77	5.88	0.93
Severe NPDR	Mean	58.46	152.31	240.46	16.46	9.38
	Median	56	148.00	228	16.38	8.90
	Standard deviation	10.67	32.74	83.68	4.36	1.26
PDR	Mean	49.33	133	163.73	13.71	9.30
	Median	51	131	158.20	15.00	8.70
	Standard deviation	3.79	14.11	25.95	3.07	1.31
p-value		0.129	0.581	0.082	**0.004**	**<0.001**

[Table/Fig-10]: Comparison of Age, Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Vitamin D level and Glycated Haemoglobin (HbA1c) among Different grading or severity of Diabetic Retinopathy (DR).
*Kruskal Wallis Test; p-value is not significant in case of comparison mean age among different grading of Diabetic Retinopathy; p-value <0.05 was considered as statistically significant

Age (years)	Grading of DR					Total	p-value
	No retinopathy	Mild NPDR	Moderate NPDR	Severe NPDR	PDR		
40-49	9 (19.57)	5 (41.67)	5 (15.15)	2 (15.38)	1 (33.33)	22 (20.56)	0.748
50-59	20 (43.48)	5 (41.67)	15 (45.45)	6 (46.15)	2 (66.67)	48 (44.86)	
60-69	12 (26.09)	2 (16.67)	11 (33.33)	3 (23.08)	0	28 (26.17)	
70-79	5 (10.87)	0	2 (6.06)	1 (7.69)	0	8 (7.48)	
80-89	0	0	0	1 (7.69)	0	1 (0.93)	
Total	46 (100)	12 (100)	33 (100)	13 (100)	3 (100)	107 (100)	

[Table/Fig-11]: Association between different age groups with different grading of Diabetic Retinopathy (DR).
*Fisher's-Exact Test; p-value <0.05 was considered as statistically significant

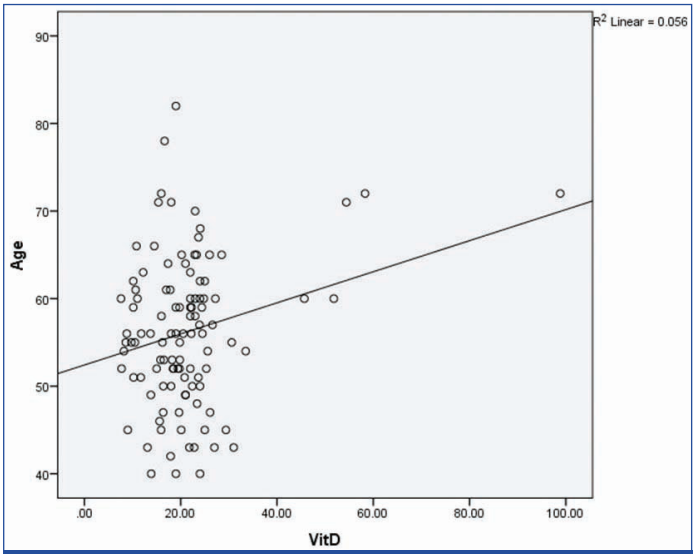
[Table/Fig-12] shows that the association between different status of Vitamin D level and different age groups was not statistically significant among different grades of DR. However, different serum Vitamin-D status showed a significant association with different age groups of diabetic patients out of whole sample size in current study. Correlation between serum Vitamin D level and age was linear and positive; but strength was low and p-value was not significant [Table/Fig-13,14]. [Table/Fig-15] shows that the association between different status of serum Vitamin D levels and different sex groups among different grading of DR patients was not statistically significant. There was no significant statistical difference in Vitamin D level of female diabetic patients with retinopathy and Vitamin D level of male diabetic patients with retinopathy [Table/Fig-16].

Grading of DR			Age (years) 40-49 (n, %)	50-59 (n, %)	60-69 (n, %)	70-79 (n, %)	80-89 (n, %)	Total	p-value
NO diabetic retinopathy	Vitamin D	Deficient	3 (33.33)	8 (40)	3 (25)	1 (20)	0	15 (32.61)	0.125
		Insufficient	5 (55.56)	11 (55)	7 (58.33)	1 (20)	0	24 (52.17)	
		Sufficient	1 (11.11)	1 (5)	2 (16.67)	3 (60)	0	7 (15.22)	
	Total		9 (100)	20 (100)	12 (100)	5 (100)	0	46 (100)	
Mild NPDR	Vitamin D	Deficient	2 (40)	2 (40)	1 (50)	0	0	5 (41.67)	0.802
		Insufficient	3 (60)	2 (40)	1 (50)	0	0	6 (50)	
		Sufficient	0	1 (20)	0	0	0	1 (8.33)	
	Total		5 (100)	5 (100)	2 (100)	0	0	12 (100)	
Moderate NPDR	Vitamin D	Deficient	2 (40)	12 (80)	4 (36.36)	2 (100)	0	20 (60.61)	0.064
		Insufficient	3 (60)	3 (20)	7 (63.64)	0	0	13 (39.39)	
	Total		5 (100)	15 (100)	11 (100)	2 (100)	0	33 (100)	
Severe NPDR	Vitamin D	Deficient	2 (100)	5 (83.33)	3 (100)	1 (100)	1 (100)	12 (92.31)	0.867
		Insufficient	0	1 (16.67)	0	0	0	1 (7.69)	
	Total		2 (100)	6 (100)	3 (100)	1 (100)	1 (100)	13 (100)	
PDR	Vitamin D	Deficient	1 (100)	2 (100)	0	0	0	3 (100)	NA
	Total		1 (100)	2 (100)	0	0	0	3 (100)	
Total	Vitamin D	Deficient	10 (45.45)	29 (60.42)	11 (39.29)	4 (50)	1 (100)	55 (51.40)	**0.033**
		Insufficient	11 (50)	17 (35.42)	15 (53.57)	1 (12.5)	0	44 (41.12)	
		Sufficient	1 (4.55)	2 (4.17)	2 (7.14)	3 (37.5)	0	8 (7.48)	
	Total		22 (100)	48 (100)	28 (100)	8 (100)	1 (100)	107 (100)	

[Table/Fig-12]: The association between different status of Vitamin D level and different age groups among different grading or severity of Diabetic Retinopathy (DR).
*Fisher's-Exact Test; p-value is significant in case of association between different status of serum Vitamin D level and different age groups among different grading of Diabetic Retinopathy out of entire sample size, not in individual grading; p-value <0.05 was considered as statistically significant

Spearman's rho		Vitamin D
Age	Correlation coefficient	0.100
	p-value	0.306

[Table/Fig-13]: Correlation between serum Vitamin D level and age of diabetic patients.



[Table/Fig-14]: Scatter plot diagram showing correlation between serum vitamin D level and age of diabetic patients.

DISCUSSION

In current study, an attempt was made to show the association of different age and sex groups with different status of Vitamin D level among severity of DR with type 2 diabetic patients. Most of the subjects (44.9%) belonged to 50-59 years of age group. The youngest patient enrolled was 40 years and the oldest was 82 years. Mean age was 56.19±8.51 years. Histogram of frequency of age also showed that maximum percentage of patients belonged between the age group 50-59 years. The present study is in accordance with the study done by Tan CSH et al., [14].

Grading of DR			Sex Female (n, %)	Male (n, %)	Total (n, %)	p-value
NO DR	Vitamin D	Deficient	12 (34.29)	3 (27.27)	15 (32.61)	0.079
		Insufficient	20 (57.14)	4 (36.36)	24 (52.17)	
		Sufficient	3 (8.57)	4 (36.36)	7 (15.22)	
	Total		35 (100)	11 (100)	46 (100)	
Mild NPDR	Vitamin D	Deficient	2 (33.33)	3 (50)	5 (41.67)	0.549
		Insufficient	3 (50)	3 (50)	6 (50)	
		Sufficient	1 (16.67)	0	1 (8.33)	
	Total		6 (100)	6 (100)	12 (100)	
Moderate NPDR	Vitamin D	Deficient	13 (68.42)	7 (50)	20 (60.61)	0.284
		Insufficient	6 (31.58)	7 (50)	13 (39.39)	
	Total		19 (100)	14 (100)	33 (100)	
Severe NPDR	Vitamin D	Deficient	2 (100)	10 (90.91)	12 (92.31)	0.657
		Insufficient	0	1 (9.09)	1 (7.69)	
	Total		2 (100)	11 (100)	13 (100)	
PDR	Vitamin D	Deficient	2 (100)	1 (100)	3 (100)	NA
	Total		2 (100)	1 (100)	3 (100)	
Total	Vitamin D	Deficient	31 (48.44)	24 (55.81)	55 (51.4)	0.529
		Insufficient	29 (45.31)	15 (34.88)	44 (41.12)	
		Sufficient	4 (6.25)	4 (9.3)	8 (7.48)	
	Total		64 (100)	43 (100)	107 (100)	

[Table/Fig-15]: The association between different status of Vitamin D level and sex groups among different grading or severity of Diabetic Retinopathy (DR) patients.
*Fisher's-Exact Test; p-value <0.05 was considered as statistically significant

The present study showed that age was not significantly associated with presence and severity of DR. The Correlation coefficient between age and Vitamin D level was also positive, however the strength of which was very low and p-value was not statistically significant. The distribution of different Vitamin D status in different

Vitamin D		Sex		Total (n, %)	p-value
		Female (n, %)	Male (n, %)		
Vitamin D	Deficient	19 (65.52)	21 (65.63)	40 (65.57)	0.561
	Insufficient	9 (31.03)	11 (34.38)	20 (32.79)	
	Sufficient	1 (3.45)	0	1 (1.64)	
Total		29 (100)	32 (100)	61 (100)	

[Table/Fig-16]: Comparison between Serum Vitamin D level in female diabetic patients with retinopathy and Vitamin D level in male diabetic patients with retinopathy. *Fisher's-Exact Test; p-value <0.05 was considered as statistically significant

age groups was statistically significant out of whole sample size, but not in individual grading of DR. In present study, the percentage of male patients was 40.2% in comparison to percentage of female patients (59.8%) out of total 107 patients. The association between sex groups and different Vitamin D status among different grading of DR was not statistically significant.

Kahn HA and Bradley RF found the positive association between retinopathy and age was limited to the group with diabetes of less than duration of 10 years [15]. Cahill M et al., concluded that the majority of elderly type 2 diabetics (greater than 70 years at diagnosis) will not develop significant DR [16].

Wei J et al., conclusively established that mean 25 (OH) Vitamin D concentration was lower in China than in the US (45.1 vs 83.5 nmol/L) with Chinese elderly lower than American elderly for different age groups. 70.3% in China and 17.4% in the US were considered as Vitamin D deficient. Older age, females, ethnic minorities, lower income, self-rated 'very bad' health and never drinkers were statistically significant in predicting lower serum 25 (OH) vitamin D levels in China. In the US, males, ethnic minorities, lower income, self-rated 'very bad' health, physically inactive, overweight and obese were related to lower serum 25 (OH) vitamin D levels [17].

Kader S et al., showed in their study that Vitamin D levels were found to be lower in both men and women as age progresses. Deficiency of Vitamin D (<10 ng/mL) was found in 83.8% of women and 18.2% of men, while insufficiency (10-30 ng/mL) of Vitamin D in 69.6% of women and 30.4% of men among admitted patients in Karapinar Public Hospital [18].

Muscogiuri G et al., found that serum Vitamin D level is lower in females in comparison to male group due to less sun exposure, higher Fat Mass percentage (FM%), lower intake of fish, which is the main dietary source of Vitamin D, extensive use of sunscreen etc., [19]. The results of the present study were not in accordance with the results of the study, done by Muscogiuri G et al., in respect to sex group. Nadri G et al., revealed that serum Vitamin D is a biomolecular biomarker for Proliferative Diabetic Retinopathy (PDR). They showed that a significant decrease in serum Vitamin D level is associated with severity of DR [20].

The most important factor leading to hyperglycaemia with increased age is deficiency of insulin secretion developing with age as well as growing insulin resistance caused by a change in body composition and sarcopenia. As age advances, decreased retinal blood flow, retinal thinning and microglial changes occur and these changes can render the retina more vulnerable to oxidative and ischaemic changes which lead to DR. As age progresses, serum Vitamin D level decreases owing to decreased concentration of 7-dehydrocholesterol in epidermis and a reduced response to ultraviolet ray and thereby decreases insulin secretion and insulin sensitivity as well as less inhibition of VEGF and other factors which lead to DR and its increased severity [8,9,21].

Limitation(s)

As this study is cross-sectional the study design allows only for the identification of the association between study variables at a time. Peripheral retinal lesions may be missed by direct ophthalmoscopy as field of vision is less in direct ophthalmoscopy in comparison to that in indirect ophthalmoscopy. The period of sun exposure of the participants was not determined. Though 1,25- OH Vitamin-D is active form of Vitamin D, serum 25(OH) Vitamin D is a better indicator of Vitamin D status because the hepatic 25 hydroxylase is constitutively expressed and unregulated and thereby circulating level of 25 hydroxy(OH)Vitamin D reflects the availability of precursor for 25- hydroxylation. In future, well designed prospective observational study should be conducted and the duration of sun exposure should be determined.

CONCLUSION(S)

No association exists between different status of serum Vitamin D level and different age and sex groups among different grades of DR. The study showed that maximum subjects were in the age group of 50-59 years. A low positive correlation was observed between the Vitamin D status and age of the diabetics which was not significant.

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