INTRODUCTION
Diabetes mellitus (DM) continues to be a tremendous health burden in the world affecting about 415 million adults which is likely to reach 642 million in 2040. It is estimated that 318 million people have impaired glucose tolerance. DM leads to significant amount of morbidity and mortality with a rate of one death every 6 seconds. [11] DM is the the second leading cause of new blindness as a result of diabetic retinopathy. [12] Though hyperglycemia, hypertension and dyslipidemia are established risk factors but pathophysiology of retinopathy progression is still not completely understood. There is increasing evidence that vitamin D deficiency (VDD) may play a role in the pathogenesis and progression of diabetic retinopathy. [13,14] VDD is now being recognized as a major global health problem Approximately 1 billion people worldwide have VDD. [15,16] It has been reported that patients with diabetes or impaired glucose tolerance have low concentrations of circulating 25-OH vitamin D in the blood than non diabetics. [7,8] Vitamin D(VD) plays important part in glucose metabolism from direct stimulation of insulin receptors to increased uptake of glucose by peripheral tissues and improving insulin resistance. It is also necessary for normal insulin release and maintenance of glucose tolerance. thus has a significant role in onset and progression of type 2 DM. [9,12] Longer duration of diabetes, poor glycemic control and hypertension are well established strong risk factors for the development and progression of DR [13] but many recent studies have suggested significant correlation between low VD levels and prevalence of diabetic microangiopathy. [7,14,15,16] Vitamin D plays a role in the pathogenesis of diabetic retinopathy by its immunomodulatory, down regulation of renin angiotensin system, anti-inflammatory and antiangiogenic effects. [17] VDD has been associated with development and progression of microvascular complications due to increased and uncontrolled angiogenesis and endothelial dysfunction. [18,19] VDD has also been implicated in the development of hypertension which is an established risk factor of DR. [20] Thus it can be anticipated that vitamin D supplementation would lead to an improvement in blood sugar levels and optimum control of blood pressure which ultimately would slow the progression of retinopathy.

Given these associations, we sought to determine the frequency of VDD in Type 2 DM and its relationship with prevalence and severity of diabetic retinopathy. 25-OH-D is the predominant circulating form of vitamin D in the normal population and is most commonly used to determine the Vitamin D status. Although there is no consensus on optimal levels of 25-OH-D, data primarily from bone metabolism suggest that levels >or = 30 ng/ml is sufficient (8) Thus Vitamin D deficiency was defined as levels <20 ng/ml and insufficiency 20-29 ng/ml, in accordance to WHO definition.

After taking clearance from the institutional ethical committee, the present study was conducted on 242 patients of type 2 diabetes, of both sexes, aged between 30-70 years, attending medical outpatient department of Government Medical College, Amritsar between September 2014 and January 2015. It was a prospective observational case control study. The control group comprised of age and sex matched 168 normal healthy volunteers with HbA1c <6.0 mmol/L. According to WHO guidelines in 2011, HbA1c >6.5 is suggestive of diabetes and values between 6 and 6.5 implies a high risk of diabetes.

Patients with HbA1c >6 were included for the study, irrespective of being hypertensive or not. Patients with significant renal, cardiac or hepatic disease or acute infection, thyroid or collagen dieases were excluded from the study.
Patients on antioxidants, taking multivitamins or minerals, Vitamin D as supplement or taking any other drug known to alter metabolism of vitamin D were also not included in the study.

Diabetic retinopathy (DR) was diagnosed by an experienced ophthalmologist by fundus examination with indirect ophthalmoscope and 90D lens on slit lamp.

Levels of retinopathy were classified according to early treatment diabetic retinopathy study (ETDRS) – No retinopathy (NR); Mild non proliferative diabetic retinopathy (mild NPDR); Moderate non proliferative diabetic retinopathy (moderate NPDR); Severe non proliferative diabetic retinopathy (severe NPDR) and Proliferative diabetic retinopathy (PDR).

Table 1: Classification of diabetic retinopathy.

-Non proliferative diabetic retinopathy (NPDR)
   A - Mild NPDR: at least one micro aneurysm
   B - Moderate NPDR
   -Hemorrhages or micro aneurysms, (H/Ma)
   -Soft exudates, venous beading (VB)and
   -intra retinal microvascular abnormalities (IRMA) definitely present.
   C - Severe NPDR
   -H/Ma in all 4 quadrants
   -VB in 2 or more quadrants
   -IRMA in at least 1 quadrant
   D - Very Severe NPDR
   - Any two or more of C
   E- Proliferative diabetic retinopathy (PDR)
   New vessels on the disc (NVD) or new vessels elsewhere on the retina(NVE)
   vitreous hemorrhage.

After taking informed consent, subjects enrolled in the study were then tested for serum 25-OH Vitamin D, FBS, HbA1c, fasting lipids, serum creatinine, liver function test, thyroid profile and urine for micro albuminuria .Vitamin D levels were measured from a single laboratory using same lab assay.

Estimated Vitamin D levels were correlated with grade of retinopathy, level of hyperglycemia, lipid levels, duration of diabetes and presence of co morbidities like hypertension. Chi-square test, Student's t-test and ANOVA test were used to study the, statistical significance of data obtained.

RESULTS
A total of 242 type 2 diabetics and 168 age and sex matched healthy volunteers were studied .There were 157 men and 85 women in the diabetic group and 114 men and 54 women in the control group.

![Fig 1 Male: Female distribution Mean age in the diabetic and control groups was 54.1+/−11.4 and 53.6+/−11.8 years respectively p=0.53059 which was not statistically significant The average duration of diabetes was 9.8 +/- 4.1 years Mean HbA1c level was 6.7+/− 0.8 in diabetics vs 5.7+/− 0.3 in controls.](image1)

![Fig 2: The mean vitamin D level was 18.043 ± 7.614 ng/ml in diabetics, while it was 27.173 ± 9.871 ng/ml in the control group. Out of the total 242 cases,129 (53.33%) had vitamin D <20 ng/ml and only 27 (11.15%) had above 30 ng/ml. On the contrary in the control group, 37 (22.02%) had vitamin D level <20 ng/ml and 63(37.5%) had levels more than 30 ng/ml. There was a significant difference in levels of vitamin D in diabetics and non diabetics p<0.00001](image2)
Fig 3: The mean vitamin D was 18.24 ± 7.231 in males and 17.36 ± 6.8103 in females in diabetics, and the difference between two genders was found to be insignificant p=0.638. Similarly, no significant difference was found in the vitamin D levels in males and females in the control group as well.

Fig 4: Among the diabetic patients 54.95% (133) had NDR, 12.8% (31) mild NPDR, 9.91% (24) moderate NPDR 15.28% (37) severe to very severe NPDR and 7.024% (17) had PDR

Fig 5: There was a downward trend in average 25(OH) vitamin D level with increasing severity of diabetic retinopathy

Fig 6: Co relation of Vitamin D deficiency and HbA1c level. Vitamin D was significantly lower in patients with higher HbA1c levels p<.00001. Out of 129 patients with vitamin D <20, 73 had HbA1c >7, 35 between 6.5 -7 and 21 between 6and 6.5.

Fig 7: Co relation of Vitamin D deficiency with severity of retinopathy. The patients with sight threatening proliferative diabetic retinopathy (PDR) not only had higher prevalence of vitamin D deficiency but also significantly lower levels of 25(OH) vitamin D level than other groups p<0.00001

Fig 8: Co relation of vitamin D deficiency with hypertension, nephropathy and duration of diabetes. There was no statistically significant difference in vitamin D deficiency with age and sex in two study groups. However, there was a statistically significant difference in the study groups with regards to hypertension p<.00494, level of nephropathy p<0.00001 and duration of diabetes p<0.00044
DISCUSSION

Vitamin D insufficiency or deficiency is being recognized as a growing epidemic worldwide. The risk for onset of diabetes and associated metabolic abnormalities increases with vitamin D deficiency. Similar to previous studies by Aksoy et al and Isaia G et al we also found significantly higher prevalence of vitamin deficiency or insufficiency (88.84%) in type 2 diabetes mellitus (T2DM) subjects as compared to age and sex matched controls (62.5%). Similar results were also reported by Hala Ahmadieh et al, Payne et al, Pittas et al, Liu et al. and Gagnon et al. Afsaneh T. et al also concluded and recommended that vitamin D supplementation should be included in treatment of type 2 diabetes to improve HbA1c level. However Suzuki A et al and Robinson JG et al. found no significant association between 25-hydroxy vitamin D levels and type 2 diabetes. Davidson MB et al also concluded that in pre diabetic and in hypovitaminosis D if doses of vitamin D supplementation is designed to raise serum 25-OHD levels into the upper-normal range for 1 year had no effect on insulin secretion, insulin sensitivity, or the development of diabetes compared with placebo administration.

In our study the mean vitamin D level was 18.043 ± 7.614 ng/mL in diabetics and 27.173 ± 9.871 ng/mL in the non diabetic control group. This difference was significant p<0.00001. Payne et al., Aksoy H et al. also concluded that diabetic subjects had lower vitamin D levels than non diabetic subjects. Afsaneh T. et al., Isaia G et al and Scragg et al. found significantly lower levels of vitamin D in females than in males where as in our study the difference was insignificant between both genders in diabetics (52.22% males and 55.29% females) as well as in controls (21.92% males and 22.22% females). Our study matched Suzuki et al. who in their observational study in T2DM subjects concluded that mean vitamin D level concentration in T2DM patients was not statistically different in both the sexes. The prevalence of Diabetic retinopathy in our study was 45.041%. Mean vitamin D level in patients with retinopathy was 15.425 ± 5.02 versus 22.13 ± 2.63 in patients without retinopathy(p==<0.00001). Haha Ahmadieh et al, Zoppini, Giacomo et al and Payne et al. in their respective studies also observed that level of vitamin D was significantly lower in those diabetics who had microvascular complications. Kaur et al. found that VDD is associated with a twofold increased risk of DR independent of diabetes duration and HbA1c. Bajaj S et al found that Vitamin D deficiency was significantly associated with microvascular complications and levels were lower when associated with more than one microvascular complication. We observed a significant negative correlation between the mean level of 1.25 (OH) D and the severity of retinopathy, being the lowest in PDR group. In our study 100% patients with PDR, 94.59% patients with severe NPDR, 54.16% with moderate NPDR and 48.38 % of mild NPDR patients had vitamin D levels <20 ng/mL. Our study is similar to study by Payne et al., Aksoy H et al. and Zoppini Giacomo et al who also observed that 1,25(OH)2D3 levels were inversely correlated with a higher grade of retinopathy. Suzuki A et al also concluded that lower 25-OHD levels were associated with proliferative retinopathy. He R et al found that Vitamin D deficiency is an independent risk factor for DR and sight-threatening DR. The prevalence of sight-threatening DR doubles when the serum 25-OH vitamin D level is <15.57 ng/mL. Patrick et al. reported an association between diabetic retinopathy and prevalence of vitamin D deficiency, but the findings were inconclusive about the existence of a relationship with the severity of retinopathy. Some studies have suggested that Vitamin D deficiency is responsible for the pathogenesis of neovascularization and angiogenesis in PDR and it’s supplementation causes reduction in proliferation because of its anti inflammatory and possible role in regulating cell functions such as differentiation, proliferation, and apoptosis in target tissues, including vascular endothelial tissue. Larger randomized trials are needed to establish whether vitamin D supplementation in people with T2DM will have beneficial effects on the development and progression of these microvascular complications.

CONCLUSIONS

This study suggests that mean serum concentration of 25(OH) 2D3 was significantly lower in diabetics than non diabetic. Patients with diabetic retinopathy especially PDR had lower 25(OH) 2D3 level than with less severe diabetic retinopathy. Therefore detection and correction of coexistent Vitamin D deficiency may be an important step in decreasing the onset of diabetes and it plays an important role in prevention and management of life threatening and sight threatening microvascular complications.

REFERENCES


in children and adolescents with type 1 diabetes,”

