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Full Length Research Paper

Does Vitamin D Level Effect the Response to Intravitreal Ranibizumab Therapy for Diabetic Macular Edema ?

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Vitamin D deficiency is considered as an independent risk factor for retinopathy in patients with type 2 diabetes mellitus (DM 2). We investigated the relationship between 25-hydroxy vitamin D (25-OHD) levels and response to intravitreal ranibizumab injection indicated for diabetic macular edema. The records of 34 naive patients with non-proliferative diabetic retinopathy and diabetic edema who underwent three intravitreal injections of ranibizumab (1 mg each injection) administered on day 0, month 1 and month 2 were analyzed. Anthropometric data, serum HbA1c, 25 hydroxy vitamin D (25-OHD), parathyroid hormone, calcium, phosphorus, C reactive protein (CRP) and (total and high density) lipoprotein cholesterol levels were collected. All subjects underwent standardized ophthalmic evaluation at baseline and at month 1, 2 and 3 including visual acuity (VA) measurement and imaging with ocular coherence tomography (OCT) evaluating changes in central retinal thickness (CRT). Stereocolour fundus photographs and fluorescein angiography was performed to evaluate macular edema and exclude patients with proliferative retinopathy. Twenty-nine study participants had a deficient or insufficient level of serum 25-OHD concentration (less than 50 nmol/l). There was no statistically significant relationship between serum 25-OHD, parathyroid hormone, calcium levels and (anatomical or functional) success of the treatment. Serum phosphorus levels positively correlated with anatomical success ($p<0.05$). High levels of HbA1c and body mass index (BMI) negatively correlated with anatomical (CRT) and functional (VA) success rates consecutively ($p<0.05$). The ranibizumab schedule resulted in a continuous improvement in mean BCVA and CRT ($p<0.05$). The levels of 25-OHD does not seem to affect the success rates of ranibizimab administered intravitreally for the treatment of DME. However most of the participants (85.2%) had deficient of insufficient levels of 25-OHD. Thus randomized controlled studies are required to demonstrate that 25-OHD supplementation in diabetics with macular edema will lead to improved outcomes of intravitreal ranibizumab therapy.

Keywords: Diabetic macular edema, vitamin D, ranibizumab, intravitreal injection, parathyroid hormone, calcium, phosphorus

INTRODUCTION

Diabetes affects more than 300 million individuals in the world with significant morbidity and mortality worldwide (Sherwin and Jastrebo, 2012). In the United States, it has

been estimated that the incidence is about 1 million new cases per year (Pittas et al., 2007).

Diabetic retinopathy (DR) is the leading cause of vision

loss in working-age adults in developed countries (Cheung et al., 2010; Fowler, 2008; International Diabetes Federation). Within 20 years after disease onset, nearly all adults with type 1 diabetes and more than 60% of adults with type 2 diabetes develop the complication.

The most common cause of moderate vision loss in patients with DR is diabetic macular edema (DME), a manifestation of DR that can develop at any time during the progression of the disease from the early non-proliferative stage to the advanced proliferative stage (Nguyen et al., 2009; Emerson and Lauer, 2007). Recently intravitreal anti-VEGF agents, with low frequency of ocular and systemic adverse effects, are often preferred for DME treatment. Several VEGF antagonists have been, or are being investigated as intravitreal treatments for DME. In Europe the only anti-VEGF agent currently approved for the treatment of DME is ranibizumab (Lucentis); a recombinant, humanized, monoclonal antibody fragment that binds to and inhibits multiple active forms of VEGF-A (Vinores, 2006).

In parallel to the increase in the prevalence of diabetes mellitus, there has been a resurgence of vitamin D deficiency worldwide (Lips, 2007; Gannage-Yared et al., 2000). Vitamin D has traditionally been associated with calcemic activities, namely, calcium and phosphorus homeostasis and bone. However, recent evidence from various lines of research suggested nontraditional roles of vitamin D in human health including cancer, autoimmune, infectious, respiratory, and cardiovascular diseases (Mohr, 2009; Lappe et al., 2007; Staud, 2005; Nagpal et al., 2005; Janssens et al., 2009; Gibney et al., 2008; Karatekin et al., 2007; Kendrick et al., 2009; Cantorna, 2008; Adorini and Penna, 2008). In addition to the tissues involved in calcium homeostasis, more than 30 target tissues contain calcitriol or vitamin D receptors (Bouillon et al., 1995; Samuel and Sitrin, 2008).

It has various pleiotropic effects like suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors, reduction of albuminuria, immunomodulatory effects, and anti-inflammatory effects (Chabas et al., 2008; Feldman et al., 1997; Garcion et al., 1998; Neveu et al., 1994; American Diabetes Association, 2010; Zhang et al., 2009; Vidotti et al., 2004; Zhang et al., 2007; Taverna et al., 2002). Thus vitamin D is implicated in many ways in the pathogenesis of retinopathy.

A recent animal study reported the possible inhibitory effect of vitamin D on retinal endothelial cell proliferation; a major cause of more severe retinopathy (Albert et al., 2007). There is increasing evidence that vitamin D deficiency may play a role in pathogenesis of DR. In adults with type 2 diabetes, lower 25-hydroxyvitamin D (25-OHD) levels have been associated with proliferative DR (Aksoy et al., 2000; Suzuki et al., 2006).

In this study we assessed the relationship between serum 25-OHD levels and anatomical, functional

response to intravitreal ranibizumab indicated for diabetic macular edema in non-proliferative diabetic retinopathy patients.

PATIENTS AND METHODS

Study population

Medical reports of 34 patients (19 female, 15 male) who underwent monthly intravitreal injections of ranibizumab (Lucentis, Novartis) for the treatment of diabetic macular edema between March 2013- December 2013 were reviewed retrospectively.

Inclusion criteria included patients with type 2 diabetes, glycosylated haemoglobin (HbA 1c) of %6 or higher, non-proliferative diabetic retinopathy (NPDR) and DME involving the center of macula at least in one eye, central retinal thickness (CRT) ≥ 250 μm , Snellen best corrected visual acuity (BCVA) 0.2-1.3 logMAR preoperatively, three consecutive monthly injections of ranibizumab and minimum follow up time of 3 months after the first injection. Only one eye (with the lower BCVA) of each patient was enrolled to the study.

We excluded any individuals who had received prior interventional treatment for diabetic retinopathy [eg, intravitreal injection except ranibizumab, laser (panretinal, grid or focal), cryocoagulation, vitrectomy] . Further exclusion criteria included: neovascularization of the optic disc; neovascularization elsewhere; cataract or other opacities precluding retinal examination and high-quality photography; other retinal diseases; ischemic maculopathy; glaucoma; prevalent 25-OHD supplementation intake; pregnancy; breastfeeding; history of any intraocular surgery during the last 6 months; participation in another clinical trial; any malignant or other life-threatening diseases.

Anthropometric data [including weight (kg), height (m) and body mass index (BMI) (kg/m²)], HbA1c, 25-OHD, parathyroid hormone, calcium, phosphorus, C reactive protein (CRP), total cholesterol, HDL, LDL, triglycerides levels were collected. All patients received intravitreal injection of 1 mg ranibizumab (Lucentis, Novartis) under sterile conditions in the operating theatre.

Using the operation microscope, the injection was transconjunctivally carried out under topical anaesthesia. All subjects underwent standardized ophthalmic evaluation at baseline and at month 1,2 and 3 consisting of visual acuity (VA) measurement, intraocular pressure assessment using applanation tonometer and imaging with ocular coherence tomography evaluating changes in CRT.

Anatomical success is defined as CRT decrease of 100 μm or more at 3th month compared to baseline values. Functional success is defined as gain at least 2 Snellen lines at 3th month.

Table 1. Socio-biographical and clinical variables of the participants

n= 34	
Age (years)	59.21 ± 8.33*
Body weight (kg)	80.68±13.18
BMI (kg/m ²)	30.14±4.84
Duration of DM (years)	10.88±8.49
Calcium (mg/dl)	9.63±0.56
Phosphorus (mg/dl)	3.53±0.50
25-OHD (ng/ml)	14.15±11.55
Parathyroid hormone (pg/ml)	69.77±56.89
HbA1c (%)	8.73±1.88
Fasting glucose (mg/dl)	158.71±50.80
Triglyceride (mg/dl)	193.65±94.22
Total cholesterol (mg/dl)	206.59±39.40
HDL (mg/dl)	44.15±10.29
CRP (mg/l)	8.70±29.44

*Mean ± SD (all such values), BMI: body mass index, HbA1c: glycosylated hemoglobin, 25-OHD: 25-hydroxy vitamin D, HDL: High density lipoprotein, CRP: C reactive protein

Design

Single center, retrospective, case controlled study.

Ethical considerations

The study was approved by the Ethics Committee of the Şişli Hamidiye Etfal Training and Research Hospital and was conducted according to the guidelines laid down in the Declaration of Helsinki.

Statistical analyses

Statistical analyses were performed using SPSS version 19. Values are presented as mean ± SD for continuous variables or numbers (%) for categorical variables. P value < 0.05 was considered significant.

The Spearman's correlation test was used to examine relationships between two parameters such as BCVA, CRT, HbA1c, BMI, diabetes duration and 25-OHD levels. The following correlation coefficient ranges were adopted: $r = 0$ (the variables are not correlated), $0 < r < 0.3$ (small correlation), $0.3 < r < 0.5$ (medium correlation), $0.5 < r < 0.7$ (strong correlation), $0.7 < r < 0.99$ (very strong correlation), and $r = 1$ (complete correlation). Repeated-measures ANOVA with post-hoc Bonferroni tests were used to compare the variables at baseline, month 1, 2, and 3.

RESULTS

A total of 34 participants met the study's inclusion criteria. Their mean age was 59.21 ± 8.33 years (47-70) and 19 of them were women. Table 1 summarizes the comparisons of levels of socio-biographical and clinical variables of the participants. The ranibizumab schedule resulted in a continuous improvement in mean BCVA. The mean BCVA was measured as 0.58± 0.37 at baseline; 0.35 ±0.34 at month 1; 0.28±0.28 at month 2; 0.22± 0.27 at month 3. At month 3, mean BCVA had improved statistically significantly from baseline with monthly administered ranibizumab ($p < 0.05$) (Figure 1).

Of 34 patients 25 (73%) had a normal level of parathyroid hormone (PTH) (15-65 pg/mL); while 9 (27%) had a level over 66 pg/mL. None of these patients were aware of any kind of a parathyroid gland or pituitary disease.

Twelve (35.3%) of individuals were at risk of deficiency; 17 (50%) of them were at risk of insufficiency for vitamin D. We used a guideline setting the following serum 25-OHD thresholds: <30 nmol/l is deficient; 30-50 nmol/l insufficient; >50 nmol/l is sufficient for almost the whole population (IOM, 2011).

The reduction in both foveal, parafoveal and perifoveal CRT from baseline to month 3 was statistically significant ($p < 0.05$) (Figure 2). The baseline and 3th month mean CRT values were 389,62 ±98,75; 288,15 ±78,81 for fovea, 389,09 ±66,97; 328,62 ±51,16 for parafovea and 345,94 ±49,38; 306,35 ±39,10 for perifovea respectively.

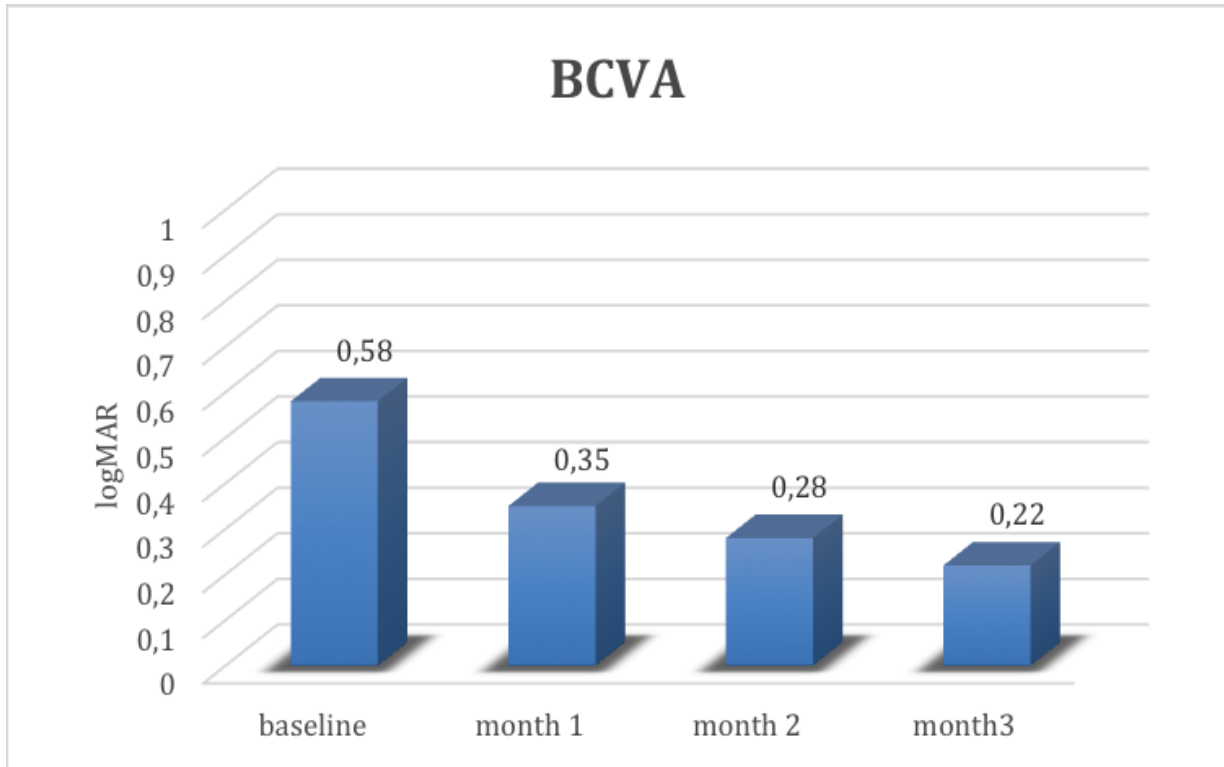


Figure 1. The ranibizumab schedule resulted in a continuous improvement in mean best corrected visual acuity (BCVA). Results for BCVA at baseline (BCVA-1), month 1 (BCVA-2), month 2 (BCVA-3) and month 3 (BCVA-4) are 0.58 ± 0.37 ; 0.35 ± 0.34 ; 0.28 ± 0.28 and 0.22 ± 0.27 respectively.

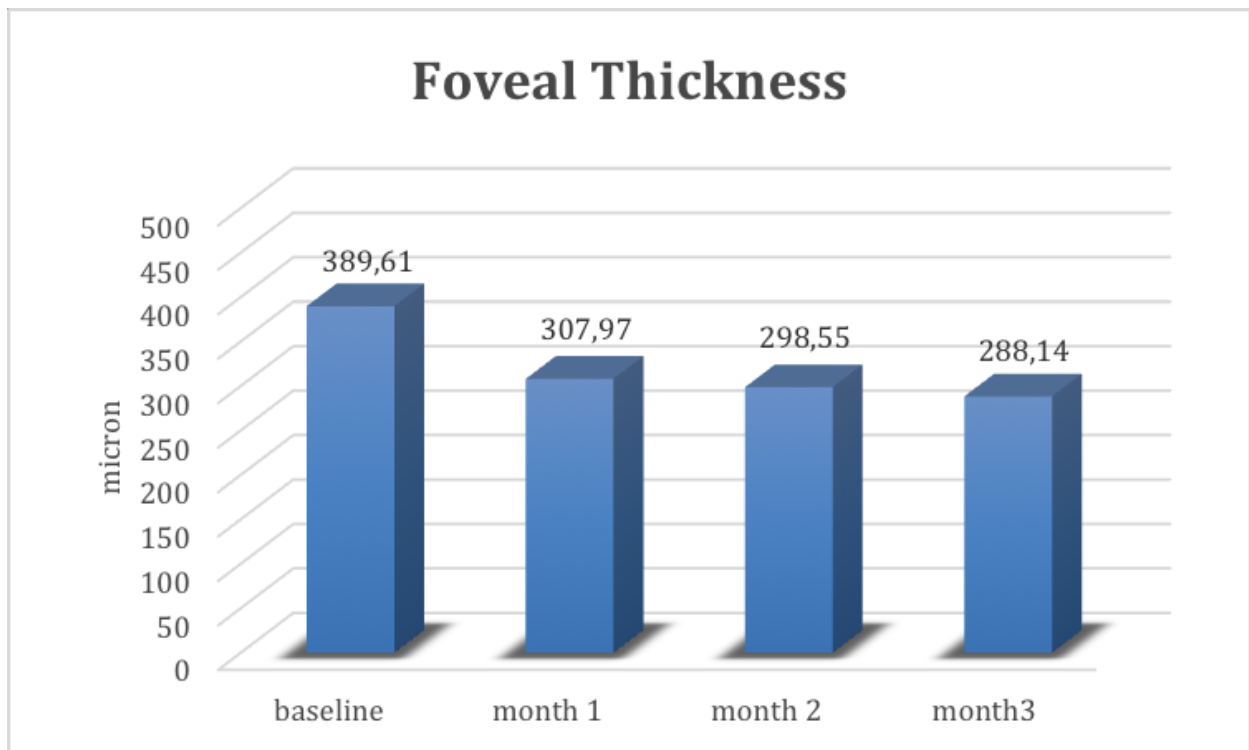


Figure 2.a. The reduction in mean foveal retinal thickness in 3 months. Baseline (MM5_1), month 1 (MM5_2), month 2 (MM5_3) and month 3 (MM5_4) mean foveal thickness values are 389.62 ± 98.75 , 307.97 ± 79.52 , 298.56 ± 76.17 , 288.15 ± 78.81 respectively.

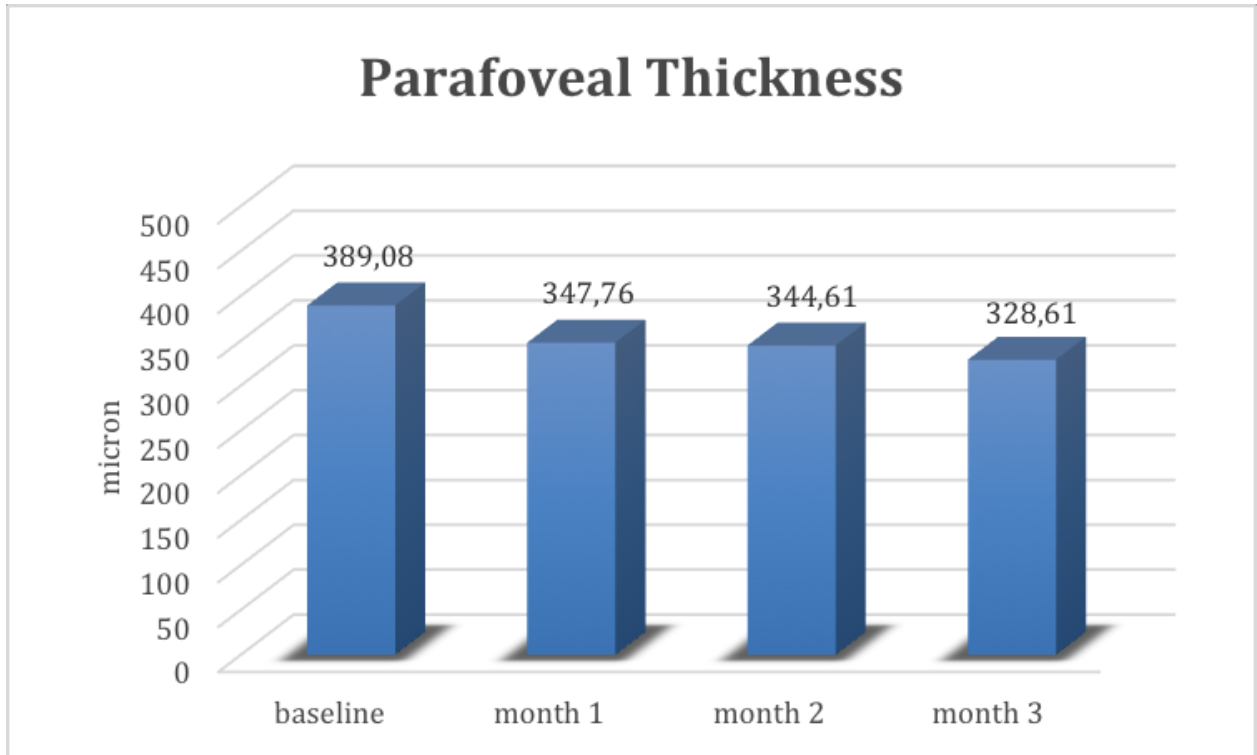


Figure 2.b. The reduction in mean parafoveal retinal thickness in 3 months. Baseline (MM5_1), month 1 (MM5_2), month 2 (MM5_3) and month 3 (MM5_4) mean parafoveal thickness values are 389.09 ± 66.97 , 347.76 ± 53.35 , 344.62 ± 54.04 and 328.62 ± 51.16 respectively

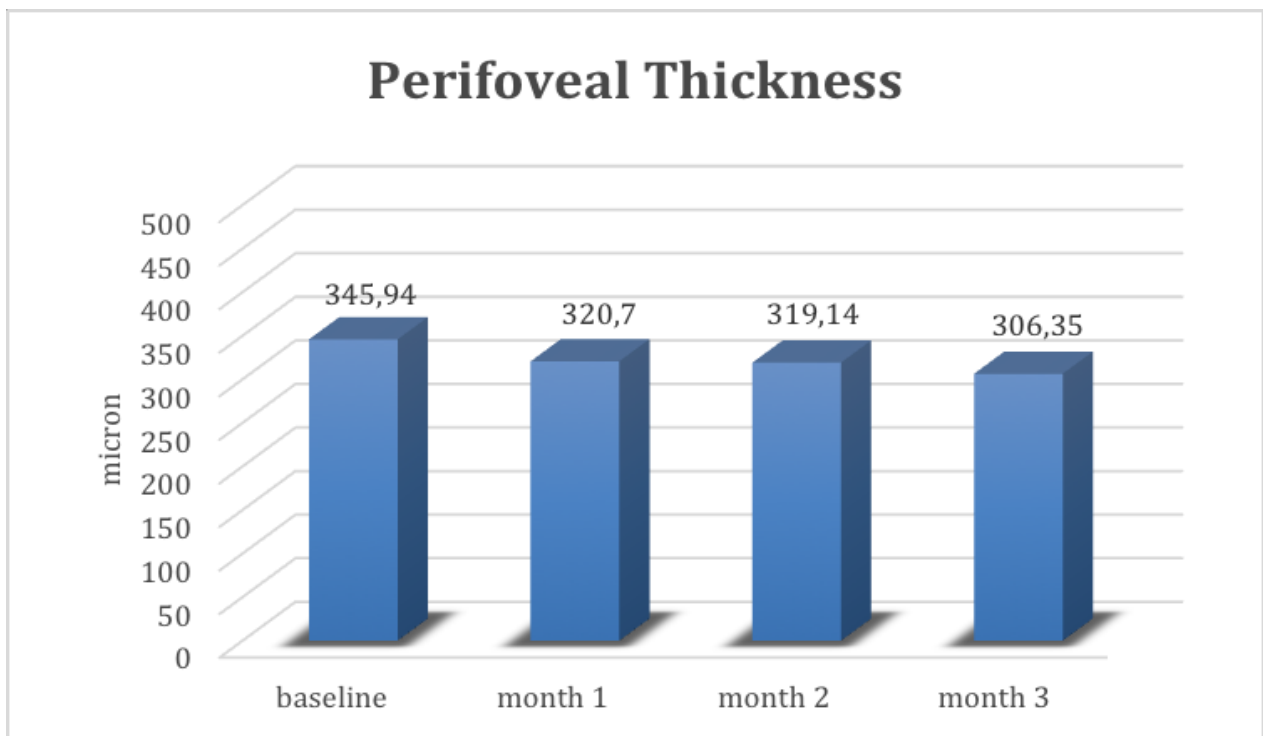


Figure 2.c. The reduction in mean perifoveal retinal thickness in 3 months. Baseline (MM5_1), month 1 (MM5_2), month 2 (MM5_3) and month 3 (MM5_4) mean perifoveal thickness values are 345.94 ± 49.38 , 320.71 ± 34.27 , 319.15 ± 44.90 and 306.35 ± 39.10 respectively

Self-reported height and weight were used to calculate BMI (kg/m²). The mean BMI was 30,14±4,84 kg/m². According to BMI classifications of World Health organization (WHO), of the patients 52.9% were obese (BMI: 30-30.9), 26.5% were overweight (BMI: 25-29.9) and 20.6% were classified into the normal BMI range (18.5-24.9). High levels of body mass index (BMI) inversely associated with functional success rates (p<0.05).

Statistically significant inverse association was assessed between anatomical success rates and HbA1c levels. The higher levels of HbA1c is correlated with lower decrease of mean foveal CRT from baseline to month 3 (p: 0.016; rs:0.411). There was no statistically significant relationship between serum 25-OHD, parathyroid hormone, calcium levels and (anatomical or functional) success of the treatment.

Serum phosphorus levels positively correlated with anatomical success (p<0.05). The higher levels of serum phosphorus related with a more obvious decrease in mean foveal, parafoveal and perifoveal CRT at month 3, 2 and 1 compared to baseline.

DISCUSSION

Lately several studies reported association between vitamin D and progression rate of diabetic retinopathy (Longo-Mbenza et al., 2014; Patrick et al., 2012; Kaur et al., 2011). Vitamin D has important actions on glucose metabolism. These include improved insulin exocytosis, direct stimulation of insulin receptor, improved uptake of glucose by peripheral tissues, improving insulin resistance (Kadowaki and Norman, 1984; Need et al., 2005; Mitri et al., 2011).

Additionally it has various pleiotropic effects such as suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors like nerve growth factor, Glial cell line-derived neurotrophic factor, neurotrophin, suppression of RAAS, reduction of albuminuria, immunomodulatory, antiinflammatory and anti-angiogenic effects were shown (Chabas et al., 2008; Feldman et al., 1997; Garcion et al., 1998; Neveu et al., 1994).

The most common cause of moderate vision loss in patients with diabetic retinopathy is DME. Briefly DME involves retinal swelling or thickening in the macular area due to high levels of vitreous inflammatory factors as vascular endothelial growth factor (VEGF), interleukin (IL)-6 and pigment epithelium-derived factor (PEDF) (Nguyen et al., 2009; Emerson and Lauer, 2007). So different types of corticosteroids are commonly being used for resolution of DME associated with their anti

inflammatory and anti-angiogenic effects (Diabetic Retinopathy Clinical Research Network, 2008).

In a study reported by Funatsu H et al, vitreous fluid samples were obtained during vitreoretinal surgery, and the levels of VEGF, intercellular adhesion molecule (ICAM)-1, IL-6, monocyte chemotactic protein (MCP)-1, and PEDF were measured in 53 patients with DME, 15 patients with nondiabetic ocular disease, and 8 diabetic patients without retinopathy (Funatsu et al., 2009). Levels of VEGF, ICAM-1, IL-6, and MCP-1 were significantly higher in patients with DME than in nondiabetic patients (P<0.05, all respectively) or diabetic patients without retinopathy (P<0.05, all respectively).

In a mouse model of ischemic retinopathy, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] inhibited retinal neovascularization (Albert et al., 2007), while in cell culture it inhibited endothelial cell proliferation (Mantell et al., 2000). Thus vitamin D is implicated in many ways in the pathogenesis of diabetic retinopathy.

On the other hand vitamin D has role in regulating calcium, phosphorus, and bone metabolism (Llach and Velasquez, 2001). It enhances intestinal calcium absorption in the small intestine. Vitamin D deficiency plays a major role in the development of secondary hyperparathyroidism as 1,25 OH₂ D₃ deficiency promotes parathyroid gland hyperplasia and increased PTH synthesis through loss of the ability to up regulate vitamin D receptor expression within parathyroid cells. The end result is elevated serum PTH and abnormal calcium (Ca) and phosphorus (P) balance.

We aimed to explore a hypothesized association between vitamin D inadequacy and success rates of intravitreally administered ranibizumab for the treatment of DME. According to the complicated relationship between Ca, P, PTH and vitamin D we assessed whole of them in our study.

To the best of our knowledge this is the first study in the literature, investigating the relationship between the success rates of intravitreal anti-VEGF injection for the treatment of DME and serum 25-OHD levels.

Although we did not assess any statistically significant relationship between serum 25-OHD and (anatomical or functional) success of the treatment; serum phosphorus levels positively correlated with anatomical success. The importance of this finding needs to be explored, since there was no correlation with either serum 25-OHD or serum Ca levels.

In this study the importance of good glucose control and normal body mass index is emphasized once again. Studies with wider number of participants are needed to investigate the association between levels of vitamin D and intravitreal ranibizumab therapy success rates, including supplementation of vitamin D.

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