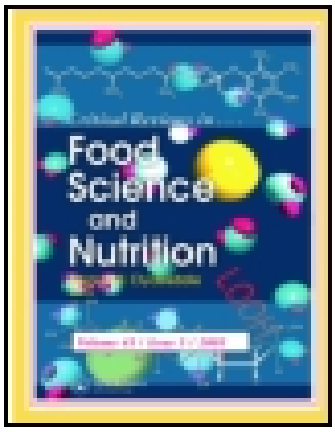

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Modulatory Effects of 1,25-dihydroxyvitamin D3 on Eye Disorders: A Critical Review

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Modulatory effects of 1,25-dihydroxyvitamin D3 on eye disorders: A critical review

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ABSTRACT

Many studies have shown that the presence of 1,25-dihydroxyvitamin D3 in the eye is able to modulate inflammatory responses. In fact, it has been demonstrated that topical administration of vitamin D3 inhibits Langerhans cells migration from the central cornea, corneal neovascularization, and production of cytokines (*i.e.* interleukin-1-6-8) in experimental animals.

Moreover, both *in vitro* and *in vivo* studies have demonstrated that vitamin D is a potent inhibitor of retinal neovascularization. It has been shown that calcitriol, the biologically active form of vitamin D, inhibits angiogenesis both in cultured endothelial cells and in retinas from guinea pigs with retinoblastoma or oxygen-induced ischemic retinopathy. In addition, it seems that this compound is able to prevent the progression from early to neovascular age-related macular degeneration (AMD) and, at the same time, to down-regulate the characteristic inflammatory cascade at the retinal pigment epithelium-choroid interface due to its anti-inflammatory and immunomodulatory capabilities.

Furthermore, 1,25-dihydroxyvitamin D3 and its analogue, 2-methylene-19-nor-1,25-dihydroxyvitamin D3, are able to modulate intraocular pressure (IOP) through gene expression. Several studies have suggested a role in glaucoma and diabetic retinopathy therapies for vitamin D3.

In conclusion, this review summarizes our current knowledge on the potential use of vitamin D3 in the protection and treatment of ocular diseases in ophthalmology.

Keywords: Anti-angiogenesis, cytokines, diabetic retinopathy, eye diseases, glaucoma, 1,25-dihydroxyvitamin D3.

INTRODUCTION

Vitamin D was discovered in 1919, when Huldschinsky noticed that sun exposure was a remedy for rickets. Likewise the most well-known function of vitamin D is its role in calcium and phosphorus homeostasis which contributes to bone and muscle health (Cherniack et al., 2008). After this, several scientific papers suggested that, besides the effects on the skeleton, Vitamin D is also able to regulate proliferation and cell differentiation, apoptosis, angiogenesis, immunity (Holick, 2007), and gene regulation (Pittas et al., 2005). It could therefore be involved in cancer pathogenesis (Tuohimaa et al., 2007), multiple sclerosis (MS) (MacLean, and Freedman, 2009) and cardiovascular diseases (Kendrick et al., 2009). Moreover, vitamin D is required to promote normal brain development, and to maintain pregnancy by immunological changes in the mother to prevent miscarriage. In fact, vitamin D insufficiency has been associated with perinatal growth restriction and altered adult mental health (Morse, 2012; Dror, 2011). There is also evidence from observational studies suggesting that adequate vitamin D during pregnancy (800 U/die) and lactation may prevent the development of immunological diseases such as type 1 diabetes, allergic, respiratory diseases, wheezing and asthma both in the first three years and later in life (Hollis and Wagner, 2004; Camargo, 2007; Dror, 2011).

Various factors have been implicated in the development and/or protection of ocular diseases, including genetic factors and environmental factors as well as dietary components and their interactions. Among the dietary components that have recently attracted attention is vitamin D. In 2012 Lin et al. observed that in the aqueous humor, vitreous and tears from guinea pigs treated with a diet enriched with vitamin D3, the increase of the intraocular concentration of (OH)-vitamin D3 was statistically significant (Lin et al., 2012). They also determined that cultured

corneal epithelial cells were able to synthesize vitamin D3 metabolites in the presence of 7-dehydrocholesterol following ultraviolet-B (UV-B) exposure (two doses of 10 and 20 mJ/cm^2/day for 3 days), this was due to the corneal enzyme alpha hydroxylase, as shown by further PCR assays (Lin et al., 2012; Yin et al., 2011).

Based on the complexity of the anatomical structures of the eye and the specificity required for the treatment of each disease, it would be useful to summarize our current knowledge on vitamin D effects as well as remarking on its potential use for the health of the eyes.

Pharmacodynamics of vitamin D3

Vitamin D is a group of fat-soluble prohormones including vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Cholecalciferol is produced in the skin through the action of UV irradiation (wavelength between 290 and 315 nm) on its precursor 7-dehydrocholesterol and to a limited extent comes from diet. Fortified milk and milk products are among the few foods considered to be good sources of vitamin D (Calvo et al., 2004, Ho et al., 2012). Ergocalciferol (D2) derives from UV irradiation of ergosterol, a membrane sterol produced by some kinds of yeasts and plants (Holick, 2007; Lin et al., 2012). In the liver, cholecalciferol (vitamin D3) is converted to calcidiol, which is also known as calcifediol (INN), 25-hydroxycholecalciferol, or 25-hydroxyvitamin D3 ð abbreviated to 25(OH)D3. Ergocalciferol (vitamin D2) is converted in the liver to 25-hydroxyergocalciferol, also known as 25-hydroxyvitamin D2 ð abbreviated to 25(OH)D2. These are the two specific vitamin D metabolites that are measured in serum to determine a person's vitamin D status. Part of calcidiol is converted by the kidneys to calcitriol (the biologically active form of vitamin D) and, as a hormone in the blood, binds a specific receptor called vitamin D receptor (VDR) on target organs. VDR, in turn, is able to bind retinoid

X receptor (RXR); then the resulting heterodimer is a transcription factor that migrates into the nucleus and, *via* response elements of Vitamin D can activate, directly or indirectly, more than a thousand genes (Holick, 2007).

RXR and VDR translocate into the nucleus by distinct pathways. VDR selectively is associated with importinŬand RXR with importinɓ, but the RXR heterodimerization partner dominates the activity of the heterodimers (Rubina, et al., 2005). (Fig. 1).

Effects of vitamin D3 on immune cells and cytokines

Various authors have investigated the role of vitamin D in the function of some immune cells such as lymphocytes, macrophages, monocytes, as well as microglial and dendritic cells. *In vivo* and *in vitro* animal studies demonstrated that the exposure of vitamin D3 to lymphocytes resulted in decreased B and T cell proliferation, immunoglobulin production, and apoptosis (Chen et al., 2007), with mechanisms that are still unknown. Also the expression of VDR in antigen presenting cells (APC), proliferation and activated immune cells in the central nervous system (CNS), was inhibited by the presence of 1,25(OH)2 vitamin D (Smolders et al., 2008).

Furthermore, the pro- and anti-inflammatory cytokines were down- or up-regulated by immune cells and APC (Maynard and Weaver, 2008). In fact, the production of pro-inflammatory cytokines, including interleukin-2 (IL-2), IL-12, interferon-ɔ (IFN-ɔ), tumor necrosis factor-Ŭ (TNF-Ŭ) can be decreased or suppressed by the presence of vitamin D3; while the production of anti-inflammatory cytokines, e.g., IL-4, IL-10, and tumor growth factor-ɓ (TGF-ɓ), can be enhanced (Penna and Adorini, 2000).

Moreover, vitamin D may influence the release of other cytokines such as osteopontin. This is a pro-inflammatory cytokine that in turn may influence the other cytokines and exerts both

inhibitory and stimulatory effects such as increased macrophage IL-12 production, IFN-ɔ and TNF expression, and decreased regulatory T-cell and IL-10 production (Stromnes and Goverman, 2007).

VITAMIN D3 AND OCULAR DISEASES

Ocular inflammation

Kernacki et al. examined the effects of topical vitamin D3 on the inflammatory response to corneal infection by Pseudomonas aeruginosa in the C57BL/6J strain of mice (Kernacki and Berk, 1994) (Fig. 2, panel A). The main markers of inflammation, ocular IL-1Ŭ, IL-6, and TNF-Ŭ, were detected over an 11 day time period, confirming that 10^{-6} mol/l calcitriol is able to reduce inflammation (Kernacki and Berk, 1994).

In agreement with other studies, it has been demonstrated that vitamin D3 is able to regulate inflammatory marker expression both at the transcriptional and post-transcriptional level in infected cells, while in healthy ones it increases their production (Cole et al., 2001). It was shown, furthermore, that serum levels of IL-8 and IL-6 were statistically reduced due to the vitamin D3-induced down regulation of IL-1ƀ (Cole et al., 2001). In addition, it has been proposed that 10 nM calcitriol is able to increase phagocytic activity by promoting the influx of calcium (Picotto et al., 2012).

In 2009 Tang et al. demonstrated that calcitriol is able to prevent, and to partially reverse, experimental autoimmune uveitis (Tang et al., 2009). This is accompanied by a reduced production of IL-17. Suppression of the Th17 response by calcitriol appears to involve two independent mechanisms, as follows: direct inhibition of IL-17 production by $CD4^+$ T cells and

indirect inhibition of IL-17 lineage commitment by down-regulation of the ability of dendritic cells to support priming of T cells toward the Th17 effector pathway (Tang et al., 2009).

Lee et al. demonstrated that vitamin D3, administration for only 6 weeks in aged mice, significantly reduced retinal inflammation and levels of amyloid beta (Aб) accumulation (Lee et al., 2012). Mice also had significant reductions in retinal macrophage numbers and marked shifts in their morphology. These changes were reflected in a significant improvement in visual function, revealing that vitamin D3 is a route to slowing the pace of age-related visual decline (Lee et al., 2012).

Ocular angiogenesis

Angiogenic ocular conditions represent the leading cause of vision loss in a large number of conditions, for example, age-related macular degeneration (AMD), diabetic retinopathy, retinopathy of prematurity, retinal vascular occlusion, and neovascular glaucoma.

Recent papers have emphasized the calcitriol anti-angiogenic properties, both *in vitro* and *in vivo*. In 2000 Suzuki et al. demonstrated that the topical administration of vitamin D3 on the cornea, at concentrations of 10^{-8} M/ 10^{-9} M, inhibited Langerhans cell migration and corneal neovascularization in mice, whereas moderate inhibition of corneal vascularization was observed in the 10^{-7}M vitamin D3 group but not in the other groups (Suzuki et al., 2000), suggesting that calcitriol may, however, act on corneal epithelial cells and inhibit the production of cytokines such as IL-1, granulocyte-macrophage colony-stimulating factor, and TNF-Ŭknown to induce Langerhans cells migration (Suzuki et al., 2000; Suzuki et al., 2000).

Likewise Albert et al. showed that vitamin D3 is able to significantly reduce retinal neovascularization in mice with oxygen-induced ischemic retinopathy (Albert et al., 2007). In

particular, it has been found that vitamin D3 was able to inhibit morphogenesis in capillary endothelial cells in a dose-dependent way, without affecting proliferation and migration. In fact, in guinea pigs it has been shown that 5 micrograms/Kg calcitriol inhibited retinal neovascularization by more than 90%. In addition, researchers found that doses of calcitriol more than or equal to 50 microM significantly decreased endothelial cell viability in retinal capillaries (Albert et al., 2007; Merke et al., 1989; Hisa et al., 1996; Wang et al., 1997; Lansik et al., 1998), and this effect was not dependent on VDR activation. It is not yet clear how calcitriol affects angiogenesis, but like previous findings on guinea pigs with retinoblastoma, it seems to be able to down-regulate Bcl-2 (that inhibits angiogenesis) both in neoplastic and endothelial cells from retinal capillaries (Shokravi et al., 1995).

It has been also demonstrated that vitamin D3 up-regulates p21 and p53 expression, as well as other cell cycle regulatory proteins, and it can inhibit retinoblastoma growth and endothelial cell apoptosis. This is due to higher p53 expression that, in turn, increases thrombospondin 1 expression as well as many other angiogenesis endogenous inhibitors both in tumoral and endothelial cells (Lee et al., 2003; Bonnefoy et al., 2008; Teodoro et al., 2006).

In 2011 Millen et al. proposed the same mechanism for AMD in postmenopausal women. In this observational study participants aged between 55 and 70 years were enrolled and it was demonstrated that vitamin D from combined food and supplements was able to reduce disease occurrence or prevent progression to advanced stages (Millen et al., 2011) (Fig. 2, panel B). The same study also showed the inverse association between early AMD and 25(OH) vitamin D in women younger than 75 years: the odds of early AMD decreased after 25(OH) vitamin D concentration rose higher than 75 nmol/l, when compared with concentrations lower than

38nmol/l. In addition, a 59% reduced odds of early combined AMD was observed in women younger than 75 years treated with 18 g/day vitamin D from food and supplements, whereas there was no protective effect reported after several hours spent in direct sunlight on early AMD (Millen et al., 2011; Shokravi et al., 1995; Klein et al., 2002; Wang et al., 2007; Jager et al., 2008).

These results might be due to vitamin D anti-inflammatory properties. It is well known, in fact, that VDR is present in activated T and B lymphocytes (Klein et al., 2002; Wang et al., 2007; Jager et al., 2008; Holick, 2004).

In a systematic review based on the relationship between UV irradiance and vitamin D production, Morrison et al. employed a candidate gene approach for evaluating common variations in key vitamin D pathway genes and studied the contribution of vitamin D levels on advanced AMD risk in a family-based cohort of 481 sibling pairs (Morrison et al., 2011). After controlling for established AMD risk factors, including polymorphisms of the genes encoding complement factor H (CFH) and age-related maculopathy susceptibility 2/HtrA serine peptidase (*ARMS2/HTRA1*), as well as smoking history, the authors found that UV irradiance was protective for the development of neovascular AMD ($p = 0.001$). Although serum vitamin D levels were higher in unaffected individuals than in their affected siblings, this finding did not reach statistical significance (Morrison et al., 2011). In light of these findings, it is possible that the required level of 25-hydroxyvitamin D may depend on the specific genotype (Morrison et al, 2011).

Glaucoma

Vitamin D3 has been widely studied in glaucoma and a lower expression of proteins and molecules has been observed in glaucomatous patients, such as (Faralli et al., 2009; Tian et al., 2009; Pescosolido et al., 2012):

- some cytoskeletal proteins, alpha and gamma actin;
- cell adhesion molecules, CEACAM and CD44;
- extracellular matrix (ECM) proteins as tissue specific inhibitors of metalloproteinase type 3 and fibronectin-1.

In addition, after vitamin D3 treatment, metalloproteases type 3,11,13,14 encoding genes are up-regulated. Moreover, calcitriol is able to remodel ECM, as well as cause cell adhesion loss and actin filament rupture that, in turn, causes relaxation of the trabecular meshwork. These events are considered the leading cause of a lower aqueous humour outflow and intraocular pressure (IOP) reduction (Faralli et al., 2009; Tian et al., 2009; Pescosolido et al., 2012).

Vitamin D3 also seems to down-regulate aquaporin 1 (AQP1) expression in the eye at sites of aqueous fluid production and outflow, leading to an IOP regulation by facilitating aqueous fluid secretion across the ciliary epithelium (Liu et al., 2010).

Recent studies in hypertensive rats suggested a strong positive correlation between blood pressure and IOP. ACE expression is markedly decreased by vitamin D. ACE is known to be a key part of the renin angiotensin system that stimulates blood pressure by the conversion of angiotensin I (AngI) to angiotensin II (AngII) (Vaajanen and Vapaatalo, 2011). In recent studies, orally administered angiotensin II type 1 receptor blockers and angiotensin-converting enzyme inhibitors lowered IOP, in fact, many recognized renin-angiotensin system components have also

been identified in the human eye (Vaajanen et al., 2008; Vaajanen et al., 2008; Wallow et al., 1993).

Kutuzova et al. showed that both vitamin D3 and its analogue, 2-methylene-19-nor-(20S) -1 Ŭ, 25 -dihydroxyvitamin D3 (2MD), significantly lower IOP in nonhuman primates following topical application (5µg) (Kutuzova et al., 2012). The effect of vitamin D3 can last for more than 12 h, although 2MD proved to be less effective than vitamin D3 in lowering IOP (Kutuzova et al., 2012). Interestingly, these authors observed two types of IOP responses to vitamin D3 treatment: a strong bilateral IOP lowering response when applied unilaterally without nasolacrimal duct occlusion or a unilateral IOP lowering response when applied unilaterally with nasolacrimal duct occlusion. The mechanism by which 1Ŭ,25-(OH)2D3 reduces IOP is not clear at this time. Vitamin D3 did not decrease aqueous humor formation or increase AH uveoscleral outflow (Kutuzova et al., 2012).

Diabetes mellitus and eye

It is well known that retinopathy and nephropathy are common late type 1 diabetes mellitus complications. The current finding suggests that vitamin D deficiency may have a permissive role in earlier stages of retinopathy. In 2009 Buĺan et al. found that VDR gene BsmI genotypes showed a significant association with cumulative prevalence of diabetic retinopathy (Buĺan et al., 2009). VDR is present in the human retina, and polymorphisms of VDR are related to retinopathy risk in type 1 diabetes (Buĺan et al., 2009).

In addition Kaur et al. speculated that the angiogenic effect of vitamin D deficiency contributes to retinal vascular damage, whereas renal vessels and peripheral nerves may be less susceptible (Kaur et al., 2011). This novel association is supported by studies on adults with type 2 diabetes

and more advanced retinopathy. Vitamin D3 levels were inversely correlated with a higher grade of retinopathy, and lower calcitriol levels were associated with proliferative retinopathy. Consequently, biological models supported a causal role for vitamin D deficiency in proliferative retinopathy, which was characterized by angiogenesis and neovascularization (Kaur et al., 2011). Vice versa, higher serum vitamin D3 was associated with reduced angiogenesis in both a transgenic retinoblastoma model and ischemic retinopathy in mice. This may be explained by an interaction between vitamin D and vascular endothelial growth factor (VEGF). VEGF is a 40 kDa dimeric glycoprotein, that prometes angiogenesis, and can influence cell proliferation, cell migration, proteolysis, cell survival and vessel permeability in a wide variety of biological contexts. In fact, the addition of vitamin D3 reduced VEGF-induced proliferation in aortic endothelial cell cultures and increased the expression of platelet-derived growth factor. (Merke et al., 1989; Shokravi et al., 1995; Wang, 2007; Hadi et al, 2007; Pen et al., 2008).

Optic nerve in multiple sclerosis

Several clinical manifestations of MS imply the involvement of diverse immunological and chronic inflammatory processes in its pathogenesis (Trapp et al., 1999). Areas of demyelination in the CNS, axonal damage, perivascular inflammation, destruction of oligodendroglia, and disintegrated blood brain barrier are the hallmarks of MS disease (Ho et al., 2012). Although the etiology of MS is not known, evidence suggests that its development is associated with immunological, environmental, genetic, and nutritional factors (Trapp et al., 1999). At present, the clinical work-up and follow-up of acute or subacute optic neuritis (ON) patients is characterized by a specific pattern of the optic nerve with visual evoked potentials and visual field damage due to the death of retinal ganglion cells or to inflammation and demyelination of

the optic nerve as in MS (Nebbioso et al., 2013). There are many *in vitro* and *in vivo* studies supporting the immuno-modulative effects of vitamin D in the treatment of ON (Ho et al., 2012). Some of the major functions of vitamin D are: immunoregulation (Gregori et al., 2001), induction of VDR transcription, nerve growth factor (NGF), and the expression of osteopontin, that exert both inhibitory and stimulatory effects on other cytokines. (Chabas et al., 2001; Stromnes and Goverman, 2007). These effects suggest a biological need for vitamin D in the CNS (Garcion et al., 2002; Neveu et al., 1994). Indeed, it appears that vitamin D exerts protective effects on neurons, oligodendrocytes, and astrocytes, although the exact mechanisms are not fully elucidated (Garcion et al., 2002). The therapeutic effects of vitamin D on MS, based on large scale, randomized control trials, are still lacking. A study conducted in the U.S. showed that vitamin D supplementation proved to be beneficial in MS subjects (Ascherio et al., 2014). Several clinical studies showed that vitamin D decreased the number of MS lesions present in magnetic resonance imaging and serum anti-inflammatory cytokine-TGF-ß1 levels can significantly increase after vitamin D supplementation (Kimball et al., 2007, Ascherio et al., 2014).

Moreover, synergistic effects have been observed by combining 1,25(OH)2 vitamin D and immunosuppressive agents such as cyclosporine, sirolimus, or minerals such as calcium in treating MS (Stromnes and Goverman, 2007; Chabas et al., 2001). The possible side effect caused by vitamin D supplementation is a concern for the treatment of MS. High doses of 1,25(OH)2 vitamin D may increase plasma calcium levels and possibly lead to tissue calcifications which could lead to renal or heart failure (VanAmerongen et al., 2004).

CONCLUSIONS

Generally, Vitamin D is believed to exert its protective effects by modulating the immune system, inhibiting inflammation and inhibiting angiogenesis. Vitamin D may modify the regulatory activity of immune-cells and cytokines, e.g., down-regulate T helper cells, T cytotoxic cells and natural killer cells. Animal and *in vitro* studies have demonstrated that vitamin D inhibits angiogenesis by reducing the expression of VEGF, reducing the proliferation of endothelial cells, and increasing the expression of platelet-derived growth factor. In addition, it has been demonstrated that vitamin D plays a role in choroidal neovascularization, the leading cause for AMD. Nevertheless, it is probable that the required level of 25-hydroxyvitamin D may depend on the specific genotype. Moreover, some authors showed that topical application of vitamin D can reduce IOP in glaucomatous primates. An enhancement of fluid outflow has been observed by decreasing the outflow resistance due to disruption of the cellular adhesions and reductions in contractility molecules which, in the anterior segment of the eye, can result in relaxation of the trabecular meshwork. Likewise the beneficial effect of vitamin D on several functions of the immune cells, especially Th1 and regulatory T cells, and the role of vitamin D in influencing the oxidative stress associated with ON in MS needs further elucidation. Therefore, results of epidemiological studies have provided most of the evidence suggesting the importance of vitamin D for newborn infant and adult health; consequently pregnant woman would certainly benefit from vitamin D3 supplementation.

In conclusion, well-designed randomized clinical trials to test the effectiveness of vitamin D and its analogues in eye diseases are still needed. On the other hand vitamin D supplementation may

provide a new therapeutic approach for the treatment of several ocular diseases, but further studies, especially on humans, are needed to assess a safe but effective concentration.

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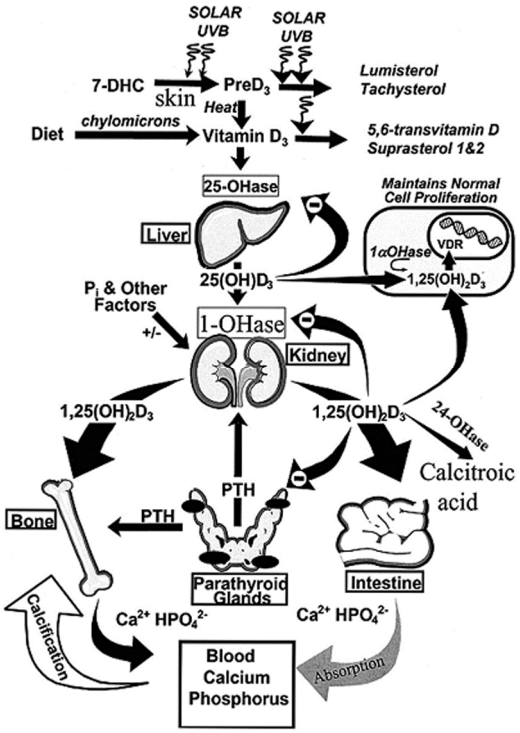

Figure 1. Metabolism of vitamin D3.

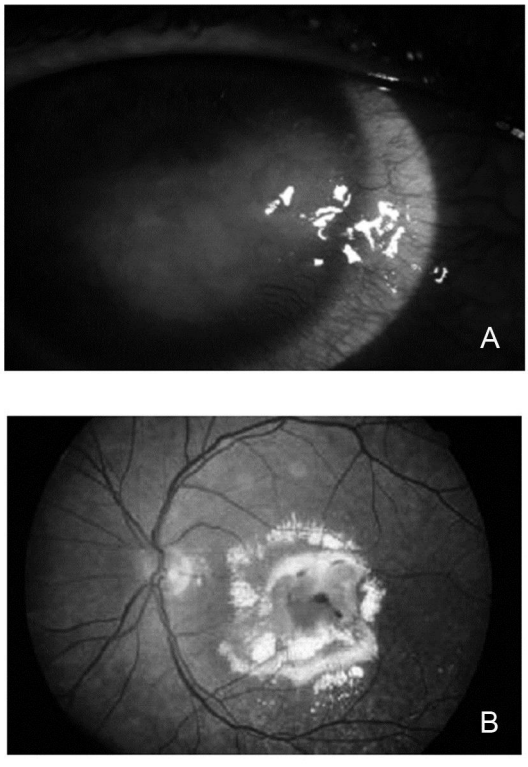

Figure 2. Panel A. Superficial corneal and stromal neovascularization caused by pseudomonas aeruginosa. **Panel B.** Advanced exudative macular degeneration with subretinal neovascularization and hemorrhage.