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Original Research Article

Lipocalin2 and Other Inflammatory and Angiogenic Variables in the Vitreous of Diabetic Macular Edema and Proliferative Retinopathy

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Abstract:

Study participants had either diabetic macular edema (DME) or proliferative diabetic retinopathy (PDR), and researchers looked for angiogenic and inflammatory factors in their vitreous. The study included 30 diabetics; 22 of them had PDR and 8 had DME. The procedure for collecting samples did not include diluting anything. "Thirteen individuals without vitreomacular interface pathology (n = 3), including four with full-thickness macular hole (FTMH), four with vitreomacular traction syndrome (VMT), and five with idiopathic epiretinal membrane (iERM), were also included in the sample pool. Cytometric flow analysis was used to measure the concentrations of several cytokines and growth factors, including IL1b, IL6, IL8, IL27, TNFα, ICAM-1, VCAM, MCP-1, VEGFA, and LCN2. Patients with PDR had elevated median concentrations of the following biomarkers compared to controls: LCN2, IL6, IL8, IL1b, IL27, ICAM-1, VCAM-1, MCP-1, TNFa, and VEGFA. On a related note, the median levels of IL6, IL8, IL27, ICAM-1, VCAM-1, TNFa, and VEGFA were all greater in DME patients compared to controls. The median LCN2 concentration in the diabetes patients was 6,822 pg/ml (IR = 3,645 pg/mL), which is quite a difference compared to the control group's 3,697 pg/ml (IR = 3,456 pg/mL). Further, the median LCN2 levels were 5,896 pg/ml in the PDR group and 7,445 pg/ml in the DME group, with IR = 7,9601 and 3,719, respectively (p = 0.03)." For both PDR and DME, our findings point to the involvement of many inflammatory and angiogenic mechanisms in their pathogenesis. Since elevated vitreous LCN2 levels may correlate with the onset of PDR in individuals with DME, this protein may be an appropriate target for therapy. To provide further evidence, additional long-term study is necessary.

Keywords: diabetic macular edema, proliferative diabetic retinopathy, idiopathic epiretinal membrane, vitreomacular traction syndrome, full-thickness macular hole.

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Introduction

A metabolic condition known as diabetes mellitus (DM) affects 422 million individuals globally. It can lead to two major eye problems: diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR). While 28 million people suffer from DME, 17 million have PDR, and both numbers are projected to rise in the coming years [1,2]. Inflammation and structural changes in blood vessels are the root causes of diabetic retinopathy (DR) [5]. Two issues that might arise from DR are DME and PDR. Unfortunately, the response rate of 30% when treating DME with anti-vascular endothelial growth factor (anti-VEFG) medication is far from optimal [7]. In actual practice, steroids like dexamethasone implants help treat the inflammatory elements of DME [8,9]. Recent studies [10–13] have demonstrated encouraging outcomes for PDR patients with anti-VEGF medications. Neutrophil gelatinase-associated lipocalin (NGAL), or lipocalin-2 (LCN2), is a glycoprotein that has a number of uses, including metabolism and inflammation [14,15]. LCN2 is a biomarker for multiple sclerosis, cardiovascular disease, lupus nephritis, acute renal damage, and other conditions. It has been shown that people with type 2 diabetes had higher blood levels of LCN2 [16]. Additionally, serum LCN2 levels are greater in DR patients [15]. Studies have been done on the role of LCN2 in diabetic neuropathy [17]. Proliferative vitreoretinopathy (PVR) grade and vitreous LCN2 were found to be strongly correlated earlier [18]. Additionally, increased vitreous

NGAL in cases of ocular sarcoidosis and a correlation with vitreous LCN2 in PDR patients have been reported in recent investigations [19, 20]. Nevertheless, no research has looked at the role of LCN2 in DME. The purpose of our study was to ascertain if LCN2 interacts with other inflammatory and angiogenic factors, as well as DME or PDR.

Materials & methods:

The study was conducted between October 2020 to September 2022 in the Regional Institute of Ophthalmology Department, S.C.B. Medical College, Cuttack, Odisha, India.

The study was approved by the Institutional Ethics committee. At the Ophthalmology out-patient Department, all participants were screened and evaluated, and patients gave their informed permission both before and after the recruitment process. The Declaration of Helsinki's guiding principles were followed in the conduct of this investigation.

The study included thirteen healthy controls and twenty diabetes patients. Proliferative diabetic retinopathy (PDR) affected thirteen people with diabetes, whereas diabetic macular edema (DME) affected seven. As a control group, we included five patients with idiopathic epiretinal membrane (iERM), four with full-thickness macular holes (FTMH), and four with vitreomacular traction syndrome (VMT). Each patient underwent a comprehensive eye exam that included imaging techniques such as fluorescein angiography (FA) and optical coherence tomography (OCT).

Every DME patient had previously tried anti-VEGF medication ineffectively using either ranibizumab or aflibercept. Their OCT-CST (central subfield thickness) was 250 μm throughout a 24-week duration, with at least four intravitreal anti-VEGF injections. However, after moving from ranibizumab to aflibercept, their condition did not become any better. In individuals with DME, OCT did not show any notable vitreoretinal interface abnormalities (like ERM). Neither diabetic patient's fundoscopy revealed any tractional components.

In conjunction with fundoscopy, ultrasonography (US) was utilized to rule out tractional retinal detachment in patients involving vitreous hemorrhage (VH). Panretinal photocoagulation (PRP) was the only therapy administered to patients with PDR. Based on the chosen treatment modality (intravitreal injections or PRP), PDR and DME were identified. Proliferative illness was present in patients with PDR but not in those with DME. Individuals who had undergone phacoemulsification surgery in the past, had a history of ocular trauma, had systemic or ocular inflammation, or had cancer were not accepted.

"Every conventional 25G pars plana vitrectomy with the Alcon Constellation system was performed by the same vitreoretinal surgeon. After obtaining each sample, the infusion cannula was withdrawn and the 0.5 cc of core vitreous was frozen at -80 °C. Through the use of flow cytometry, the levels of LCN2, NGAL, IL1b, IL6, IL8, IL27, TNFα, ICAM-1, VCAM, MCP-1, and VEGFA were assessed." Based on many investigations [26, 28-30], these chemicals are believed to play a role in the pathophysiology of PDR. Linking LCN2 levels to the development of PDR or DME suggests a linkage with inflammatory or angiogenic pathways. In order to improve oxygenation and remove angiogenic and inflammatory chemicals from the retinal region, vitrectomy was performed on patients with diabetic macular edema [31]. In order to ensure complete vitreous cortex excision [31] and prevent the formation of ERM after surgery [32], vivid blue dye has been utilized to peel the internal limiting membrane (ILM). Without any complications following surgery, all treatments were successfully completed.

Statistical analysis:

In contrast to continuous variables that are provided with averages, standard deviations, medians, interquartile ranges, minimum and maximum values, and frequency representations, categorical variables are expressed as percentages based on their frequency. The non-normal distribution of continuous variables necessitated the employment of nonparametric methods. a Mann-Whitney test: The U test was utilized in order to compare the continuous variables of the two groups. An independent-samples t-test was used to compare the DR group to the control group in terms of age, and a chi-square test was used to compare the two groups in terms of gender. By employing the two-tailed test, it was determined that a p-value that was lower than 0.05 was found to demonstrate statistical significance.

Results:

Both the diabetic patients and the control group were of the same age and gender. "The average age of the diabetic patients was 68.7 years (SD = 13.0, min = 34, max = 88), whereas the average age of the control group was 69.6 years (SD = 10.5, min = 52, max = 85) (p = 0.11). For instance, the PDR group had an average age of 66.2 years (SD = 14.9, minimum value = 34, maximum value = 87) whereas the DME group had an average age of 71.8 years (SD = 9.8, minimum value = 58, maximum value = 88). In the control group, men accounted for 31% (n = 4), but in the diabetic patient group, they made up 56% (n = 12) (x2 = 1.9, p = 0.38). Hemoglobin A1c averaged 7.6% for DME (mean deviation = 2.4%, median = 6.9%, minimum = 6.2%, maximum = 13%) and 9.6% for PDR (mean

deviation = 2.8%, median = 11%, minimum value = 8%, $p < 0.05$).

Patients with PDR had elevated median levels of the following biomarkers as compared to the control group: LCN2, IL6, IL8, IL1b, IL27, ICAM, VCAM-1, MCP-1, TNFa, and VEGFA.

Additionally, median concentrations of IL6, IL8, IL27, ICAM, VCAM-1, TNFa, and VEGFA were greater in the DME group than in the control group." The control group had an IR of 3,697 pg/ml for LCN2, whereas the diabetic patients had an IR of 3.645 pg/ml, with a median value of 6,822 pg/ml. A median LCN2 level of 7,645 pg/ml (IR = 7,961) and 5,896 pg/ml (IR = 3,719) were found in the DME group and PDR group, respectively. Every single piece of data was normal.

The median levels of LCN2 and IL8 were greater in the PDR group compared to the DME group ($p < 0.05$), whereas the converse was also true ($p < 0.05$) for the PDR group. In terms of LCN2, the median difference between DME and controls (4,059) was nearly double that of PDR and controls (3,200). The median IL8 level difference between the PDR and control groups was 605.6, about three times more than the median IL8 level difference between the DME and control groups (318.8).

Discussion:

In this investigation, it was shown that the vitreous fluid of patients with diabetes had noticeably greater amounts of LCN2. This was a previously unreported finding: the PDR group's median LCN2 concentration was higher than the control groups.

When it comes to the development of DR, DME, and PDR, there are a multitude of angiogenic and inflammatory variables that contribute [33]. A mechanism known as NF-κB is accountable for the regulation of the levels of IL1b, IL6, IL8, TNFa, I-CAM, and MCP-1. This pathway is responsible for controlling these levels. According to study [34], there is evidence that intravitreal LCN2 has the capability to regulate ocular inflammation by inhibiting the NF-κB pathway in rat models. This mechanism is believed to be responsible for this regulation. Based on previous research, it has been established that LCN2 has anti-inflammatory properties on macrophages and the NF-κ pathway [135].

DME is caused by BRB collapse, which is connected to the vascular changes that are associated with DR [36]. It is possible that dysfunctional vascular endothelial cells and junctional proteins are the cause of BRB breakdown when [37]. Researchers have investigated the effect that LCN2 has on the vascular endothelial cells that line the blood vessels in the brain [38]. Endothelial junctional proteins (ZO-1 and VE-cadherin) and the blood-brain barrier (BBB) may be protected against ischemic brain stroke by an endogenous "help me signal" that originates from LCN2 [38]. In contrast, LCN2 is known to stimulate angiogenesis [39, 40]. It is necessary to do further research in order to learn how LCN2 contributes to DME and PDR; the function that it plays in modulating both pro- and anti-inflammatory responses is not yet fully understood [41]. [42-46] Research has shown that MMP-9 plays a significant part in the development and progression of DR into PDR.

Furthermore, there is a connection between MMP-9 and the establishment of DME as well as permanent structural damage to DME [47]. Since LCN2 has an effect on the activity of MMP-9, more research is required [48–51]. Patients with DME and PDR had levels of IL27 that were significantly higher than those discovered in the control group. High levels of IL27 have been found in the aqueous humor of diabetic retinopathy patients in earlier research [29], and IL27 has a known anti-inflammatory function in ocular inflammation [52]. Strong angiogenic factor VEGFA acts as a chemo attractant for granulocytes and macrophages, encouraging vasodilation [53]. A research by Zhang et al. [54] found that in diabetic retinopathy patients' macrophages, IL27 suppresses the production of VEGFA. There is currently little information available to support LCN2's role in diabetes mellitus. However, this glycoprotein could be useful in clinical practice if it functions as a predictive marker in diabetics and a possible target for treatment. These factors, together with the discovery that DME patients had higher LCN2 levels, might strengthen the validity of our study. The authors conclude that our work adds something significant to a field that needs more research because it is understudied. It is crucial to recognize that some restrictions must be taken into account while analyzing the results of this investigation. First, one restriction is the limited sample size of patients that were recruited. Nevertheless, considering that patients with DME frequently have concomitant vitreoretinal disease or a medical cause for vitrectomy, the study results are quite important to clinical practice, even with this restriction. We are unable to draw firm conclusions on the relationship between the differences in LCN2 concentrations and the various treatment modalities (anti-VEGF in DME or PRP in PDR) due to the limited sample size. It is essential to bear in mind that LCN2 has the potential to be an effective target in the treatment of diseases that do not react favourably to anti-VEGF medicine or other treatments. Additionally, when taking into consideration the presence of antibody-based anti-VEGF drugs such as ranibizumab or aflibercept, it is essential to exercise caution when interpreting

the results of VEGF quantification using antibody-based assays [55, 56].

Conclusion:

In conclusion, it has been discovered that vitreous LCN2 levels are higher in PDR and DME patients who are resistant to anti-VEGF medication. These results strengthen the relationship between LCN2 and PDR and DME development, supporting previous studies. Further long-term, comprehensive research is required to evaluate LCN2's potential as a treatment and biomarker.

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