Assessment of inflammatory component of diabetic macular edema pathogenesis

Noha Rabie Bayomy[1], MD and Sameh Mohamed Elgouhary[2], MD

[1]Department of Medical biochemistry, Menoufia University, Egypt.
[2]Department of Ophthalmology, Menoufia University, Egypt.
sameh_elgouhary@yahoo.com

Abstract: Purpose: To evaluate the inflammatory component of diabetic macular edema (DME) pathogenesis by: 1) measuring the interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) levels in the serum as inflammatory mediators in diabetic patients with and without macular edema and 2) measuring the central macular thickness (CMT) in patients with DME before and 3 months after topical bromfenac 0.09% eye drops. **Methods:** This is a prospective observational study recruiting 54 patients suffering from diabetes, classifiedinto two groups. Thirty three patients with DME in group 1, while group 2 included 21 patientswithout DME. We measured serum concentration of TNF-alpha and IL-6 in these 2 groups then patients of group 1(33 patients) were treated with topical bromfenac 0.09% eye drops twice daily for one month. Optical coherence tomography (OCT) was used for measuring the thickness of central macular edema before and 3 months post topical bromfenac. **Results:** There was significant increase in mean serum levels of TNF-alpha and IL-6 in patients with DME (26.8±4.2 and 4.9±0.8 respectively) than in patients without DME (17.4±2.3 and 1.8±0.4 respectively) ($p< 0.05$). Mean CMT was significantly reduced from 423.61±117.37microns at baseline to 347.82± 139.58 microns 3 months after topical bromfenac. **Conclusions:** Inflammation may play an important role in DME pathogenesis and this may be proved by increased serum levels of TNF-alpha and IL-6 (as potent inflammatory mediators) in patients with than without DME and by reduction of CMT in patients with DME after topical bromfenac 0.09% eye drops (asnon-steroidal anti-inflammatory drug).
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1. Introduction:

One of the main causes of visual losses in diabetic patients is the macular edema **[1]**. The initial pathological change in DME is interruption of inner blood barrier of the retina with escaping from abnormal blood vessels in the retina into the macula **[2]**.

Several studies postulated that chronic low grade inflammation andalteration in serum or vitreous levels of many inflammatory cytokines has been contributed to the pathogenesis of diabetic retinopathy and DME **[3-5]**.

Tumor necrosis factor –alpha and interleukine-6 are potent proinflammatory and sometimes angiogenic mediators involved in pathogenesis of diabetic retinopathy and DME **[6].**

There are a number of non-steroidal anti-inflammatory drugs (NSAID) which generally used as a proved therapy for DME as bromfenac, nepafenac, ketorlac and diclofenac **[7-10]**.

The goal from the present investigation is to assess the inflammatory component of DME pathogenesis by: 1) measuring the concentration of both interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in the serum of diabetic patients matched with control, as inflammatory mediators in diabetic patients with and without macular edema and 2) measuring the central macular thickness (CMT) in patients with DME before and 3 months after topical bromfenac 0.09% eye drops.

2. Patients and method:

This is a prospective observational study recruiting 54 diabetic patients (33 patients with DME and 21 patients without DME) attending at the outpatient clinic of Ophthalmology Department of Menoufia University Hospital.

All included patients underwent best corrected visual acuity (VA) assessmentusing Log Mar units, anterior segment examination using slit lamp, intraocular pressure using Goldmannapplanation tonometer, posterior segment examination using slit lamp biomicroscopy with +90 and +78 diopters volk lens. Fundus fluorescein angiography (FFA) and Optical coherence tomography (OCT) were done at base line and 3 months after topical bromfenac.

Exclusion criteria were: 1) macular edema due to causes other than DM as branch vein occlusion, retinal telangiectasia, retinal macro aneurysm, 2) macular edema may be due to causes other inflammation as vitreomacular traction (proved by OCT) and ischemia (proved by enlarged foveal avascular zone (FAZ) in

FFA),3) previous treatment in the form of focal or grid macular laser photocoagulation or intravitreal injection of steroids or anti- vascular endothelial growth factor (VEGF) agents, 4) history of cataract extraction within 6 months (to exclude Irvin- Gass syndrome), and 5) any pathology that may interfere with measurement of CMT by OCT as dense cataract, corneal opacity, and high myopia.

Included patients were divided into 2 groups. Group 1 included 33 patients with DME and group 2 included 21 patients without DME. We measure serum concentration of TNF-alpha and IL-6 in these 2 groups then patients of group 1 (33 patients) were treated with topical bromfenac 0.09%eye drops twice daily for one month. Central macular thickness is measured by OCT before and 3 months after topical bromfenac.

All included patients received complete information about the study and signed a written informed consent. This study was approvedthe clinical research committee of the Menoufia University Hospital and it followed the tenets of the Declaration of Helsinki.

Sample Collection and Assay:

Five mL venous blood was collected under complete aseptic precaution into planetubes, left to clot, then centrifuged for 10 minutes at 4000 r.p.m and the sera were stored at -20 degree centigrade until analysis. Serum TNF-alpha was determined by Enzyme Linked Immunosorbent Assay (ELISA) using Human TNF – alpha ELISA Kit **[11]**. Serum IL-6was determined also by ELISAusing Human IL-6 ELISA Kit **[12] (Eustace et al; 1993).** Both cytokines are determined by technique called Quantitative Sandwich Immunoassay. The micro titer plate has been pre-coated with a monoclonal antibody specific to TNF-alpha or IL-6. Standards or samples are then added to the appropriate micro titer plate wells with biotin –conjugate polyclonal antibody preparation specific for TNF-alpha or IL-6 and incubated. If cytokine present, it will bind and become immobilized by the antibody pre-coated on the wells and then it be sandwiched by biotin conjugate.

Statistical Analysis:

All data were expressed as mean ± standard deviations. Analyses were achieved using SPSS version 16 (SPSS Inc., Chicago, IL, USA). P value of less than 0.05 was considered to be statistically signi-ficant.

3. Results:

Patients' data:

Group one comprised 15 females and 18 males, whereas in group 2, 10 females and 11 males were participating in the study, their ages were averaged 52.5±3.2and 55.5±4.3 in groups 1 and 2 respectively. A non-significant variations concerning the gender or ages was recorded between the two groups in the present study.

Serum TNF-alpha and IL-6:

The results revealed that serum levels of TNF-alpha and IL-6 was elevatedsignificantly in patients with DME (26.8±4.2 and 4.9±0.8 respectively) than in patients without DME (17.4±2.3 and 1.8±0.4respectively) ($p<0.05$).

Central macular thickness:

Mean CMT was significantly reduced from 423.61±117.37 microns at baseline to 347.82± 139.58 microns 3 months after topical bromfenac.

4. Discussion

The exact pathogenesis of DME still unclear. It may be inflammatory supported by the role of topical or intravitreal anti-inflammatory agents or due to vitreomacular traction supported by the role of vitrectomy or ischemic or multifactorial. Which component is more potent? a question still needs an answer.

Hyperglycemia induces the inflammation cascade through many mechanisms as oxidative stress, irregularity in the synthesis of nitric oxide, with tendency for development of advanced glycation end products (AGEP). This cascade ends in the production of a number ofproinflammatory cytokines likeinterleukin-6 (IL-6) and TNF-α, interleukin-1 β (IL-1β), and chemokineslike monocyte chemoattractant protein-1(MCP-1), interleukin-8 (IL-8), stromal cell derived factor-1 (SDF-1) and interferon-γ-inducible protein of 10 kDa (IP-10). Moreover, another key inflammatory proteins such as cyclo-oxygenase-2 (COX-2), matrix metalloproteinase-9 (MMP-9/gelatinase B) and inducible nitric oxide synthase (iNOS). All these mediators lead to vasodilatation and increased permeability of retinal vessels with further development and progression of DME **[13, 14]**.

Tumor necrosis factor alpha is a powerfulproinflammatory multifunctional cytokines released from different cell types and macrophages. TNF-alpha is composed of 157 amino acids, the molecular weight is 17 KDa and induces its biological effects via two receptors TNFR2 and TNFR1. TNF-alpha acting a significant role in theinflammatory processes, cell migration, apoptosis, differentiation and proliferation of cells **[15]**. With respect to diabetic patients, there is evidence ofincreased concentrations of TNF-α in the vitreous fluid **[16]**, in addition, there are a high relationshipamong plasma levels of TNF-α and harshness of diabetic retinopathy and DME has been reported **[17]**. Clauss et al. reported that TNF-α is needed for VEGF-induced endothelial hyperpermeability **[18]**.

Interleukin-6 is a pleiotropic cytokine and may also have essential controlling functions of T cell and immune system. Interleukin-6 may be produced by many cells and with a molecular weight of 26 KDa and is composed of 184 amino acid. Itinduces their function through specific receptors in the cell surface, these receptors are formed of 2 molecular subunits **[19]**. There is evidence of increased levels of interleukin-6 in vitreous fluid of individuals suffering from diabetic macular edema and proliferative diabetic retinopathy (PDR) **[20].**

Bromfenac is a NSAID produces its anti-inflammatory action through inhibition of COX enzymes with reduction of circulating prostaglandins and inflammatory mediators that are responsible for vasodilatation and disruption of inner blood-retinal barrier **[7]**.

The current study was designed for assessment of the inflammatory component of DME pathogenesis by: 1) measuring the serum levels of TNF-alpha and IL-6 as proinflammatory mediators in diabetic patients with and without macular edema and 2) measuring the CMT in patients with DME before and 3 months after topical bromfenac 0.09% eye drops.

This study showed that the concentrations of IL-6 and TNF-alpha in patients serum was significantlyelevated in individuals with DME than in patients without DME.

Shimizu et al. demonstrated that there are a significant relationship (odds ratios = 3.68, 1.70, respectively) between the concentration of IL-6 in the serum and cases of the posterior vitreous detachment (PVD) and the harshness of macular edema **[21]**.

In this study, mean CMT was significantly reduced from 423.61±117.37 microns at baseline to 347.82± 139.58 microns 3 months after topical bromfenac 0.09% eye drops. **Pinna et al**. demonstrated that CMT was significantly reduced from 465.41±118.47 microns at baseline to 388.88± 152.63 microns after topical bromfenac 0.09% twice daily for 30 days **[7]**.

Limitations of this study were that TNF-alpha and IL-6 were measured in the serum only not in the aqueous or the vitreous but taking aqueous or vitreous samples for measurement of these inflammatory markers before and after treatment with bromfenac may carry the risk of endophthalmitis, vitreous hemorrhage or retinal detachment so we measured the levels of theseinflammatory mediators in the serum only and we replaced the aqueous and vitreous measurements of these mediators by less invasive procedure which is measurement of CMT by OCT after topical bromfenac as an NSAID which my give a clue for inflammatory component in DME pathogenesis.

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Short running title: Assessment of inflammatory component of DME

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