Aqueous Humor and Serum Concentrations of Soluble MICA and MICB in Glaucoma Patients

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ABSTRACT

Background: Immune reactions have been reported to be involved in the destruction of retinal ganglion cells (RGCs) in glaucoma. Objective: To investigate the role of major histocompatibility complex class I-related chain A and B (MICA and MICB) molecules in the pathogenesis of glaucoma. Methods: Aqueous humor and serum samples from 15 glaucoma patients and 45 patients with cataract, undergoing ocular surgery, were obtained. The concentrations of MICA and MICB molecules in all samples were measured using ELISA. Results: Both MICA and MICB concentrations were higher in the aqueous humor of patients with glaucoma compared to those with cataract (p=0.013 and p=0.004, respectively); however, in the serum samples, no significant differences were observed. Conclusions: Increased intraocular pressure may be associated with increased expression of the MICA and MICB molecules, which could initiate the destruction of RGCs and consequent development of glaucoma.

Keywords: Aqueous Humor, Glaucoma, MICA, MICB

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INTRODUCTION

Glaucoma is a multifactorial, progressive neurodegenerative disorder. It represents a group of ocular disorders that are responsible for the death of retinal ganglion cells (RGCs) and loss of their axons as well as optic nerve atrophy (1). It has been predicted that by the year 2020, about 80 million people will have been affected by this disease (2). Primary open angle glaucoma (POAG) is the most common form of glaucoma that associates with increased intraocular pressure (IOP); however, there are about 30% of patients with normal IOPs (10 mmHg and even lesser than that); the so-called normal tension glaucoma (3). Moreover, there are some patients with elevated IOP (20-30 mmHg) but normal glial cells and optic nerves, so-called the ocular hypertension. Interestingly, only 10% of patients with ocular hypertension are susceptible to future glaucoma event, indicating that elevated IOP is not the sole cause of glaucoma (loss of RGCs) (4). In this regard, studies have suggested that several pathological conditions other than increased IOP, including oxidative stress, ischemia, and age, may play a role in glaucoma pathogenesis (5). Indeed, it has been suggested that multiple stimuli and complex interactions can cause the selective degeneration of RGCs through an apoptotic pathway, which finally leads to the destruction of the optic nerve in glaucoma (6). Nevertheless, the precise mechanism of destruction of the RGCs has not yet been identified.

One hypothesis is that the immune system recognizes damaged cells, which are under different stress conditions including increased IOP, infection, hypoxia, heat, oxidative stress, ischemia, and malignancy. The presence of abnormal immune responses in patients with glaucoma supports the possible role of autoimmune reactions (7-9). Some of these abnormal reactions include, the presence of antibodies against ocular antigens, such as heat shock proteins (HSPs) (10,11), rhodopsin (12), γ-enolase (13), and glutathione-S-transferase (14), as well as inflammatory cytokines like tumor necrosis factor (TNF)-α (15), which are possible neurodegenerative factors in glaucoma.

Major histocompatibility complex (MHC) class I-related chain A and B (MICA and MICB) molecules are encoded by the MIC gene family located within the MHC class I region of the human chromosome 6. MIC proteins are mostly expressed on epithelial surfaces and are up-regulated in cells which are under stress. Thus, these cells might be recognized and destroyed by intra-epithelial lymphocytes and CD8+ T cells, as well as Natural Killer (NK) cells, via NKG2D receptors (16). Furthermore, tumor cells have been shown to produce metalloproteases that can degrade MICs into soluble forms, which are then released in the serum. This might lead to evasion of tumor cells from the immune system (17). The role of MICs has also been studied in autoimmune disorders. One study reported an increase in soluble MICB but not MICA concentrations in the sera of patients with multiple sclerosis (MS) related to disease activity (18). They also suggested a possible role of MIC in the modulation of immune response activity during relapses (18).

Based on the possible role of autoimmune reactions in the process of destruction of RGCs and optic nerve in glaucoma pathogenesis (19), and given the involvement of MIC molecules in MS and that both glaucoma and MS are neurodegenerative disorders, it can be hypothesized that the immune system recognizes and damages the RGCs via MICA and MICB molecules.

Soluble MICA and MICB concentrations in the aqueous humor can be considered as a marker of their surface expression, since it has been shown that metalloproteases can
cause release of soluble forms of MICA and MICB in gastrointestinal malignancies (17). In order to reveal the role of MIC molecules in the pathogenesis of glaucoma, this study measured the concentration of these molecules in the aqueous humor and serum samples from patients with glaucoma compared to those affected by cataract, as the control group.

MATERIALS AND METHODS

Patients. In this case-control study, patients suspected of having glaucoma or cataract and referred to the ophthalmology clinic of Bou Ali Sina Hospital between January 2010 and December 2011, were examined for enrollment into this study.

To diagnose glaucoma, IOP was measured with Goldman napplation tonometry. Changes in the head of the optic nerve were also evaluated. Visual field was checked using Humphrey 24–2 visual field analyzer.

The four types of glaucoma, including primary open-angle glaucoma (POAG), pseudo-exfoliative glaucoma, closed-angle glaucoma (CAG), and juvenile glaucoma were diagnosed by an ophthalmologist according to the following criteria: increased IOP (more than 21 mmHg) at least in two turns of measurement in different sessions during business hours, opening of the angle of the anterior chamber \( \geq \) than grade 3 on gonioscopy, changes of the head of the optic nerve, and loss of visual field (glaucomatous visual field change) were considered to be OAG. Presence of pseudo-exfoliation material on the lens or on the papillary border associated with increased IOP, glaucomatous optic nerve head, and changes in the visual field was regarded as pseudo-exfoliative glaucoma.

For the diagnosis of the CAG, in addition to increased IOP (more than 21 mmHg), at least in two turns of measurement in sessions during business hours, changes of the head of the optic nerve, loss of visual field, and appositional closure of the irido-corneal angle less than grade 2 on gonioscopy were considered. Finally, glaucoma onset after the age of 3 years and before early adulthood was considered as juvenile glaucoma.

Patients with any type of glaucoma who needed surgery were selected as the case group. Exclusion criteria included: myopia more than -10 diopters, previous history of eye surgery, ocular hypertension, pigmentary glaucoma, lens-induced glaucoma, neovascular glaucoma, 2° OAG, 2° CAG, proliferative diabetic retinopathy (PDR), ocular diseases other than glaucoma, rheumatologic diseases, and systemic infections.

Patients with the diagnosis of cataract who showed an IOP less than 20 mmHg and a cup/disc area ratio of \((C/D)\) \(\leq 0.3\) without any glaucomatous changes were selected as the control group. This study was approved by the Ethical Committee of the Mazandaran University of Medical Science and written informed consent was taken from each participant.

Sample Collection and Analysis. After a retrobulbar block, 0.1 ml of aqueous humor was aspirated via a paracentesis in the peripheral cornea, using a 27-Gauge needle attached to a tuberculin syringe. A sample of blood serum was also taken from each participant. Samples of serum and aqueous humor were kept at -80°C until use. The concentration of MICA and MICB were measured in all samples using a commercial ELISA assay (R&D Systems, Minneapolis, MN). Statistical analyses were performed using SPSS, v.15 software. The results were analyzed by the Student \( t \)-test.
RESULTS

Fifteen patients with glaucoma, consisting of 8 men and 7 women, with the mean age of 53±1.5 years, were enrolled as the case group and 45 patients with cataract, consisting of 32 men and 13 women, with the mean age of 65±1.5 years, were enrolled as the control group of this study. Characteristics of glaucoma patients are shown in Table 1.

Table 1. Characteristics of the glaucoma patients.

<table>
<thead>
<tr>
<th>Type of glaucoma</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-angle glaucoma</td>
<td>9</td>
</tr>
<tr>
<td>Pseudoexfoliative glaucoma</td>
<td>2</td>
</tr>
<tr>
<td>Closed-angle glaucoma</td>
<td>2</td>
</tr>
<tr>
<td>Juvenile glaucoma</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family history of glaucoma</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Optic nerve head Condition</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cup</td>
<td>6</td>
</tr>
<tr>
<td>C/D*: 0.5-0.9 mm</td>
<td>7</td>
</tr>
<tr>
<td>C/D: 0.3-0.5 mm</td>
<td>2</td>
</tr>
</tbody>
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*C/D: cup/disc area ratio

The mean concentrations of MICA and MICB molecules in the aqueous humor and serum samples of glaucoma (N=15) and cataract patients (N=45) are shown in Table 2 and Figure 1.

![Figure 1](image_url)

**Figure 1.** Mean concentrations of MICA and MICB in aqueous humor (A) and serum (B) samples of glaucoma and cataract patients. In the aqueous humor samples, both MICA and MICB concentrations were significantly higher in glaucoma compared to cataract patients (p=0.013, and p=0.004, respectively). In the serum samples, however, they showed no significant difference.
As Table 2 and Figure 1 show, MICA and MICB concentrations in the aqueous humor samples of glaucoma patients were significantly higher than those of patients with cataract (p=0.013, and p=0.004, respectively); however, there were no significant differences in the concentration of MICA and MICB molecules in the sera of the case and control groups (Figure 1B). The concentration of MICA and MICB molecules were further compared among the four groups of glaucoma patients, but no significant difference was observed. The concentrations of MICA and MICB molecules were further analyzed in the three groups of glaucoma patients according to the severity of Optic Nerve Head (ONH) cupping (total cup, C/D: 0.5-0.9, and C/D: 0.3-0.5) and failed to show a significant difference.

Table 2. MICA and MICB concentrations in aqueous humor and serum samples of the case and the control groups.

<table>
<thead>
<tr>
<th></th>
<th>Aqueous humor</th>
<th></th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glaucoma</td>
<td>Cataract</td>
<td>P Value</td>
</tr>
<tr>
<td>MICA concentration (pg/ml) (mean ± SE)</td>
<td>136.72 ± 42.55</td>
<td>51.17 ± 13.14</td>
<td>0.013</td>
</tr>
<tr>
<td>MICB concentration (pg/ml) (mean ± SE)</td>
<td>257.42 ± 18.97</td>
<td>45.50 ± 14.15</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study measured the concentration of MICA and MICB molecules in the aqueous humor and serum samples of glaucoma patients compared to those of patients with cataract, as a control group, and showed significantly higher concentrations of these molecules in aqueous humor samples of glaucoma patients.

The role of MIC molecules in the pathogenesis of glaucoma has not been previously studied, while it has been studied in MS and both diseases are known as neurodegenerative disorders. Moreover, Niwaet et al. in 2012 has reported that the percentages of CD3-CD56+NKG2D+ cells were higher in the patients with Parkinson's disease, another neurodegenerative disease, than in healthy individuals. Since, NKG2D has been defined as a ligand for the MIC molecules; this might support the involvement of MICs in neurodegenerative diseases (20).

Given the possible role of immune responses in the degeneration of RGCs in glaucoma (8), it seems that the balance between protective immunity and the risk of induction of an autoimmune neurodegenerative process is important in the pathogenesis of glaucoma. Indeed, the immunoregulatory functions of glial cells on one side and the presence of tissue stress on the other side seem to be important factors that determine the balance between the opposing roles of the immune system (5). Furthermore, the presence of abnormal immune responses in glaucoma patients supports its autoimmune
pathogenesis. One of these abnormal responses is increased expression of HSPs, like HSP27 and HSP70, which is associated with various autoimmune diseases; it is also responsible for neuro-degeneration in glaucoma (10,11). Several components of the innate immune system, particularly Toll-like receptors (TLRs) and complement proteins have been shown to be over-expressed in the retina and optic nerve head of human glaucomatous eyes (8,21). These components can initiate an immunostimulatory pathway and consequently activate autoimmune reactions. Regarding the adaptive immunity, abnormal auto-antibodies, including non-organ specific antibodies against DNA, RNA, and nuclear proteins, have been identified in the sera of patients with glaucoma, indicating abnormal B cell responses (19). Concerning T cell responses however, they have been reported to cause neuro-degeneration in animal models of glaucoma, but not in human glaucomatous eye (8).

MICA and MICB are encoded by the MHC region and share a common structure and similar sequence (28-35%) with the HLA class-I gene; however, they are neither polymorphic nor expressed on normal cells. Under pathologic conditions, including infection, hypoxia, oxidative stress, and malignancies, the expression of MICA and MICB molecules is induced on the intestinal epithelial cells and also in privilege sites of the immune system like the central nervous system (22). Consequently, intra-epithelial lymphocytes, CD8+ T cells, and NK cells through their NKG2D receptors, may react with MICA and MICB and cause the apoptosis of cells that express these molecules (19, 22). MICA and MICB molecules may be expressed on the surface of RGCs and optic nerve head over a chronic and cumulative period of stress (e.g. elevated IOP or oxidative stress).

Stress conditions have also an effect on glial cells resulting in a chronic activation response by glial cells (8). Therefore, perivascular microglia or astrocytes might produce cytokines like TNF-α and chemokines (8). Moreover, it has been shown in glaucoma that the expression of matrix metalloproteinases is up-regulated by the optic nerve head astrocytes. This may lead to the degradation of the basement membrane of astrocytic and microglial end feet. These changes collectively result in the dysfunction of the perivascular barrier (8). In such circumstances, migration of CD8+ T cells, γδT cells, and NK cells to the retina would take place, and consequent apoptosis of RGCs expressing MICA and MICB may lead to glaucomatous optic nerve damage.

In summary, our study showed increased concentrations of MICA and MICB molecules in the aqueous humor of glaucoma patients. We can thus assume that changes in RGCs under stress conditions and over-expression of MICA and MICB may result in the destruction of these cells. In future, we suggest induction of increased IOP in experimental models, and then evaluating MICA and MICB expression in the RGCs and optic nerve head by using the immunohistochemistry and quantitative PCR.

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REFERENCES