Evaluation of serum interferons in patients with age-related macular degeneration

Mehrdad Afarid[1], Ali Azimi[1], Mahyar Malekzadeh[2]

[1]Department of Ophthalmology,Poostchi Ophthalmology Research Center, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, [2]School of Medicine, Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Environmental, genetic, and immunological factors may play a role in the pathogenesis of age-related macular degeneration (AMD). In an attempt to better understand the pathogenesis of AMD, in this study, we evaluated the serum interferon (IFN) levels in patients with AMD and compared it with persons without AMD. **Materials and Methods:** In this case–control study, 42 patients with AMD and 42 healthy individuals (without AMD) were enrolled as the case and control groups, respectively. The two groups were matched regarding their age and sex. We classified the case group as dry-type and wet-type AMD. Blood samples were obtained and the serum was collected and frozen at −20°C. Alpha-, beta-, and gamma-IFN levels were measured using the sandwich ELISA method and compared between and within the groups. **Results:** The mean beta IFN levels in both case and control groups were 46.88 ± 27.25 pg/ml and 34.90 ± 18.81 pg/ml (P = 0.021), respectively. Regarding gamma and alpha IFN, the serum levels were not detectable in most of the patients and no significant difference was detected between the case and control groups. **Conclusion:** We found that serum beta IFN levels are higher in patients with AMD. This finding may have diagnostic, therapeutic, and prognostic value in AMD patients and can be a beginning for further evaluation.

Key words: Immune system, interferon, macular degeneration

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INTRODUCTION

Decreased vision is one of the most prevalent disabilities that have a tremendous effect on the quality of life. The causes to this disability vary in different regions of the world. Age-related macular degeneration (AMD) is one of the significant causes of blindness in the Western world.[1,2] The prevalence of AMD steadily increases with age, affecting 2% of the population at age 40 and one in four people by age 80. The disease is believed to be more prevalent among the European population than the African population.[2,3]

Many studies have evaluated the causes and mechanisms of AMD. Two main types of AMD have been described as follows: the dry-type AMD and wet-type AMD. Dry-type AMD is a chronic form of the disease and affects about 85%–90% of patients with AMD. The wet type is a rapidly progressive form of AMD affecting a minority of patients.[4]

It has been shown that AMD is a disease of the retinal pigmented epithelial (RPE) cells.[4,5] A number of investigations have shown that some immunological genes may play a role in the pathogenesis of AMD. The upregulation of complement factor H (CFH) in the RPE cells has also been suggested as a cofactor in AMD pathogenesis.[5,6] Cytokines are the immune system mediators. Some cytokines account for the regulation of the immune vascular responses in AMD.

IFNs are a type of cytokines and some studies have shown that the interaction of IFNs with RPE cells

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Address for correspondence: Dr. Ali Azimi, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz Medical school, Shiraz University of Medical Sciences, Shiraz, Iran. E-mail: ali.azimi1365@gmail.com

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and CFH may play a part in the pathogenesis of AMD.[6] Up to our knowledge, we did not find any study about the evaluation of three types of IFNs (alpha, beta, and gamma) in patient with AMD, and our study is unique on this subject. In this study, we evaluated the serum interferon (IFN) levels in patients with AMD and compared it with healthy patients.

MATERIALS AND METHODS

Design and patient selection

The study design was approved by Shiraz University of Medical Sciences (No: 6189). In this case–control study, patients with AMD (cases) were selected from those referring to the Ophthalmology Clinics affiliated to Shiraz University of Medical Sciences.

A total of 42 patients with AMD were considered as the case group and 42 healthy individuals that were matched in terms of age and sex with the case group were considered as the control group. The participants in the control group were among the individuals that referred to the clinic for routine checkup and did not have any systemic or retinal problems.

We excluded participants with a history of autoimmune disorders, infection, surgery, trauma, and use of immunosuppressive or immunogenic drugs 1 month before their admission. For patients who needed bevacizumab injections, the blood sampling was performed before the injection.[7]

The diagnosis of AMD was based on clinical, optical coherence tomography (OCT), and fluorescence angiography findings, namely, central retinal atrophy, scar, choroid neovascularization, multiple (>2) large drusen (more than 124 μ in diameter), and multiple (>5) intermediate drusen (between 64 and 124 micron in diameter) in at least one eye. The drusen size was measured according to the OCT printout or estimation of examiner.[8]

Blood sampling and variables

A 5cc peripheral venous blood sample was obtained from each participant, between 8:00 and 9:00 AM, in order to take into consideration, a possible circadian rhythm of IFN secretion.

The samples were immediately immersed in melting ice. To minimize the source of the platelets, the clot samples were centrifuged for 30 min and stored at −20°C for further analysis.

After meeting the adequate sample size, the samples were transferred to Shiraz Institute for Cancer Research. At this institute, all samples were stored at −20°C. The serum IFN levels of alpha, beta, and gamma IFNs were measured using the commercial sandwich ELISA kits, according to the manufacturer's instructions.

Measurement kits for alpha (IBL Co.), beta (USCN Co.), and gamma (eBioscience Co.) IFNs were utilized with a sensitivity of 500 – 7.8 pg/ml and >2.8 pg/ml and 100 – 1.6 pg/ml, respectively.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences software version 18 (Chicago: SPSS Inc). For comparison of quantitative data between the two groups, the independent *t*-test was used, and for qualitative data, the Chi-square test was used. We also classified the wet and dry types of AMD into two separate groups and for comparison of IFN levels between the three groups (control, wet-type, and dry-type AMD), the one-way ANOVA test was used, and for beta IFN, Bonferroni *post hoc* test was used.

All the data are displayed as mean ± standard deviations unless stated otherwise. $P < 0.05$ was considered as statistically significant.

RESULTS

From the case group, 20 (47/6%) patients had dry-type AMD and 22 (52.3%) patients had wet-type AMD. The mean age of the control group was 66.42 ± 7.58 years and the mean age of the case group was 69.40 ± 7.53 years ($P = 0.075$).

Alpha interferon

The alpha IFN serum levels in all the participants were less than the kit's sensitivity level (500-7.8 pg/ml). There was no statistically significant difference for the alpha IFN serum levels between the case and control groups ($P = 0.99$) [Table 1].

Beta interferon

The results were different for the beta IFN levels. The mean beta IFN serum levels in the control group were 34.90 ± 18.81 pg/ml; however, in the case group, it was 46.88 ± 27.25 pg/ml ($P = 0.021$). When we separated the dry-type AMD and the wet-type AMD as two groups, we detected that the beta IFN serum levels are also different among those with wet type and control groups those with wet-type AMD had higher levels of beta IFN ($P = 0.04$). No significant difference was detected between dry and control groups ($P > 0.05$) and between dry- and wet-type groups ($P > 0.05$) [Table 1].

Gamma interferon

Regarding gamma IFN, the serum levels were less than the kit sensitivity (100-1.6 pg/ml) in all patients in both

Table 1: Interferon serum levels in the groups

IFN types	Control group (*n*=42)	Case group (*n*=42)			*P* values for pairwise comparisons
		Dry type (*n*=20)	Wet type (*n*=22)	Overall (*n*=42)	
Alpha	<7.8	<7.8	<7.8	<7.8*	0.99
Beta	34.89±18.81	42.97±18.46	50.43±33.36	46.88±27.7	0.021 (Wet-control=0.04, Wet-dry=0.916, Dry-control=0.623)
Gamma	2.45±9.5¥	0.266±0.35	4.8±20.7	2.66±15¶	0.938

*In the case group, the alpha IFN levels of the patients were all under the kit sensitivity cutoff point (<0.99 pg/ml) except for one patient who had an alpha IFN level of 5.7 pg/ml, ¥In the control group, the gamma IFN levels of the patients were all under the kit sensitivity cutoff point (<0.99 pg/ml) except for six patients who had gamma IFN levels of 9.73, 57.87, 4.59, 23.16, 1.95, and 6.69 (mean: 17.33 pg/ml), ¶In the case groups, the gamma IFN levels of the patients were all under the kit sensitivity cutoff point (<0.99 pg/ml) except for three patients who had gamma IFN levels of 2.2, 97.79, and 2.29 (mean: 34.09 pg/ml). IFN=Interferon

groups except for three patients in the case group (2.20 pg/ml, 97.79 pg/ml, and 2.29 pg/ml) and six participants in the control group (9.73 pg/ml, 57.87 pg/ml, 4.59 pg/ml, 23.16 pg/ml, 1.95 pg/ml, and 6.69 pg/ml) [Table 1]. The two groups did not show a significant difference in gamma IFN serum levels ($P = 0.938$) [Table 1].

DISCUSSION

As studies have shown that the immune system may play a role in the pathogenesis of AMD, in here, we aimed to evaluate serum IFN levels (as one of the mediators of the immune system) among patients with AMD. We found that beta IFN levels are significantly higher in those who have the disease; furthermore, among those with AMD, individuals with the wet type had higher levels of beta IFN in compared to those with the dry-type AMD.

IFNs may play a role in the process of vascular endothelial growth factor (VEGF) production. Therefore, this function may have an important effect on the progression of wet-type AMD. On this bases, Sun *et al*., in his study in 2014, showed that some types of IFNs could suppress VEGF production.[9] Nowadays, the VEGF is known as the factor responsible for the progression of choroidal neovascularization (CNV) and anti-VEGF drugs are the mainstays of CNV management, and as a result, anti-VEGFs have recently been utilized in the management of wet-type AMD.[4,5]

The effects of autoimmunity are another hypothesis in the pathogenesis of AMD.[10] In some previous studies, it has been documented that CFH is overexpressed in RPE cells in the AMD process.[6]

Kim *et al*.,[4] in his study in 2010, showed that CFH expression in RPE cells was tremendously upregulated by IFNs. They concluded that an interaction between IFNs and CFH may play a part in the pathogenesis of AMD.

Oxidative stress and immune vascular responses are considered as the initiators of AMD,[4,5] and the role of immune vascular modification is not very clear in the pathogenesis of the disease; however, certain studies have demonstrated that immune suppressants might decrease the progression of active CNV, which is the hallmark of wet-type AMD.[4,5]

IFNs have immune regulatory, antitumoral, and antiviral effects.[7] Some types of IFNs could have specific functions in some diseases. For example, alpha IFNs play a major role in the process of systemic lupus erythematosus.[11] Some types of IFNs have been utilized to treat autoimmune disorders. For instance, beta IFNs, with its immune regulatory functions, are used in the treatment of multiple sclerosis.[12,13]

Systemic IFNs have been used to manage uveitis associated with multiple sclerosis and ocular Behcet's disease, thereby improving the visual acuity and uveitis significantly.[14-19]

In a systematic review, the effect of systemic alpha-2a IFN therapy was studied in patients with CNV secondary to AMD. They documented no remarkable improvement with its use.[20]

According to the results of the previous studies, IFNs have important roles in modulating the immune system, by different pathways. For example, in some situations, they have a rate-limiting role in the activity of innate and adaptive immune responses.[21] IFN-gamma plays a role in the regulation of nearly all inflammatory responses, including the activation and differentiation of T-cells, B-cells, natural killer cells, and macrophages. IFN-gamma secretion is a hallmark of Th1 lymphocytes but can be secreted by other types of cells. Each of these cell types secretes IFN-gamma only when activated, especially in response to interleukin (IL)-2 and IL-12.The production of IFN-gamma is inhibited by IL-4, IL-10, and transforming growth factor-beta.[22]

Mo *et al*. showed that IFN-gamma can overexpress protein 10 (IP10) as a biomarker of AMD.[1] Kauppinen *et al*. demonstrated the key role of chemokines and cytokines in AMD.[23] Intravitreal injection of some types of IFNs was effective in controlling endotoxin-induced uveitis in some studies.[24] All of these support the hypothesis of the important role of IFNs in the pathogenesis of uveitis and AMD.

IFNs may have an effect on other factors in the body and through these effects may have an indirect influence on the development of AMD. Some studies showed changes in the serum level of brain-derived neurotrophic factor (BDNF) in patients with AMD.[25,26] Changes in the serum level of these factors (IFNs or BDNF) may be related or unrelated to each other, and for better evaluation of this hypothesis, further studies are needed.

Some studies showed that IFNs may have a valuable application for virus-host interaction by these methods.[27]

IFN beta inhibits B-cell apoptosis and upregulates the expression of MHCI and macrophage cytotoxicity. This IFN may have an antiproliferative effect on some cells.[28]

Deletion of IFN alpha and beta receptors may trigger vascular leakage and CNV, but systemic IFN beta may attenuate macrophage responses and finally CNV size in laser-treated animal models.[29]

Patient with advanced AMD suffered from low vision. Some case reports are present about the recovery of vision in the blind eye after losing vision in the better eye, but it is rare. In this process, some central or peripheral mechanisms may be involved.[30]

Recently, cell replacement therapy is one of the new strategies for treatment of blindness due to AMD. Moreover, many studies have done on differentiation of stem cells to retinal cells.[31]

The role of immunologic and biochemical factors in the process of cell replacement therapy is under investigation.[31]

We showed that the beta IFN level is different in AMD-affected patients, especially among those with wet type AMD. Our study supported the idea that perhaps IFNs may play a part in the pathogenesis of AMD, and based on our results, beta IFNs are probably the most important IFNs in this process.

We cannot claim that this finding is a causal phenomenon. It may be merely an overproduction in response to the inflammation of the retinal tissue.

Our study has some limitations. We only evaluated serum IFN levels, which are not a definite indicator of the role of IFNs in the pathogenesis of AMD; however, to the best of our knowledge, this study is without precedence in literature. Although our study had a simple design, it could be an indicator for the future studies to further evaluate the role of IFNs in the clinical course and pathogenesis of AMD.

CONCLUSION

We found that serum beta IFN levels are higher in patients with AMD. This finding may have diagnostic, therapeutic, and prognostic value in AMD patients and can be a beginning for further evaluation.

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Conflicts of interest

There are no conflicts of interest.

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