Circulating levels of Interferon-Gamma in patients with neovascular age-related macular degeneration in Yogyakarta

Supanji Supanji, PhD[1], Ayudha Bahana Ilham Perdamaian, MSc[1], Firman Setya Wardhana, MD[1], Muhammad Bayu Sasongko, PhD[1], Mohammad Eko Prayogo, MD[1], Angela Nurini Agni, MD[1], Chio Oka, PhD[2]

[1]Department of Ophthalmology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia, [2]Laboratory of Gene Function in Animals, Nara Institute of Science and Technology, Takayama, Ikoma, Nara,Japan

ABSTRACT

Introduction:Neovascular age-related macular degeneration (nAMD) is a major factor contributing to blindness and impaired visual acuity in elderly people. The pathophysiology of nAMD involves excessive inflammation events in the macula. Thus, it is crucial to study the dynamics of an important pro-inflammatory cytokine, interferon-gamma (IFN-γ).

Materials and Methods: This research is aimed to investigate plasma IFN-Ɣ profiles of patients with nAMD. In this cross-sectional study, blood plasma samples of 16 patients with AMD and 23 age-matched controls were collected. Samples were examined for two inflammatory cytokines (IFN-Ɣ) using a commercially available enzyme-linked immunosorbent assay.Acquired data were log transformed to normalize any outliers before conducting student'st-test using the SPSS software.

Results: IFN-Ɣ levels were higher in the control group, without statistically significant difference between the two groups.

Conclusion: IFN-Ɣ levels were not significantly different between patients with AMD and controls.

KEYWORDS:
Age-related macular degeneration, inflammation, inflammatory cytokines, IFN-Ɣ, ELISA

INTRODUCTION

Neovascular age-related macular degeneration (nAMD) is a major risk factor for impaired central visual acuity and irreversible blindness.[1,2] Genetics plays a key role in the onset of nAMD. Epidemiological study conducted in Indonesia showed a strong association of rs11200638 High temperature requirement factor A1 (HTRA1),[3] rs10490924 HTRA1/Age-related maculopathy susceptibility 2 (ARMS2), del443ins54 ARMS2, and rs10490924 Complement factor H (CFH) polymorphisms with the onset of nAMD.[4] However, other studies have failed to show a correlation of interleukin-1beta (IL-1β)[5] and HTRA1[6] protein levels with nAMD pathogenesis.

The aetiology of nAMD is characterized by retinal swelling, which is caused by abnormal vascularization, leading to leaky blood vessel formation in the macula. Genetic and epidemiological evidence indicate the key role of inflammation in nAMD. An important pro-inflammatory cytokine, interferon-gamma (IFN-γ), is considered a vital factor in nAMD onset. Recent studies indicate an emerging relationship between IFN-γ and mechanisms underlying AMD pathogenesis. Alone or along with other pro-inflammatory factors such as IL-1β and tumour necrosis factor-alpha, IFN-γ appears to trigger inflammatory pathways[7] and its associated biomarkers, including the complement cascade,as well as recruit immune cells such as macrophages, microglia, natural killer, and Tcells.[8–11] Reportedly, in the affected eyes, cytokines were observed in the outer retina and drusen.[12–14] Excessive cytokines could compromise photoreceptors,[14,15] leading to central vision impairment or blindness. The pathways activated by IFN-γ are interconnected in a complicated manner and are not fully understood. Furthermore, clinical data related to IFN-γ and AMD therapy are limited. The possibility of using IFN-γ as target for AMD therapy remains debatable. Accordingly, the present research aimed to investigate IFN-γ levels in patients with nAMD and age-matched controls.

MATERIAL AND METHODS

This cross-sectional case control study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing (FK-KMK) UGM, Universitas Gadjah Mada (approval no.: KE-FK-0215-EC-2021). Following screening, 38 patients with AMD and 16 age-matched control were included. Recruitment was conducted from January until August 2019. All participants understood and signed informed consent form before undergoing ophthalmic testing and blood collection. Only patients without other retinal or systemic diseases were included to rule out the effect of other disease. All patients underwent standard eye examination, including visual acuity assessment, fundus imaging, and optical coherence tomography to diagnose nAMD or verify the control eye group. A structured questionnaire was used to collect baseline data regarding lifestyle, including smoking status (active or

Corresponding Author: Supanji S
Email: supanji@ugm.ac.id

Table I: Characteristics of patients with AMD and age-matched control

Variables	nAMD (n:16; %)	Age-matched control (n:23/%)	*p*-value
Sex			
Male	58	52	0.301
Female	42	46	
Age (years)			
<59	23	8	0.235
60–69	35	52	
70–79	35	30	
>80	5	8	
Smoking status			
Yes	23	30.5%	0,23
No	76	69.5%	
Blood pressure (mmHg)			
Hypertension	35.2%	21.7%	0,4
Normal	64.8%	78.3%	
Cytokines (mean (±SD))			
IFN-γ (pg/mL)	14.88 (± 6.96)	18.70 (± 17.93)	0.087

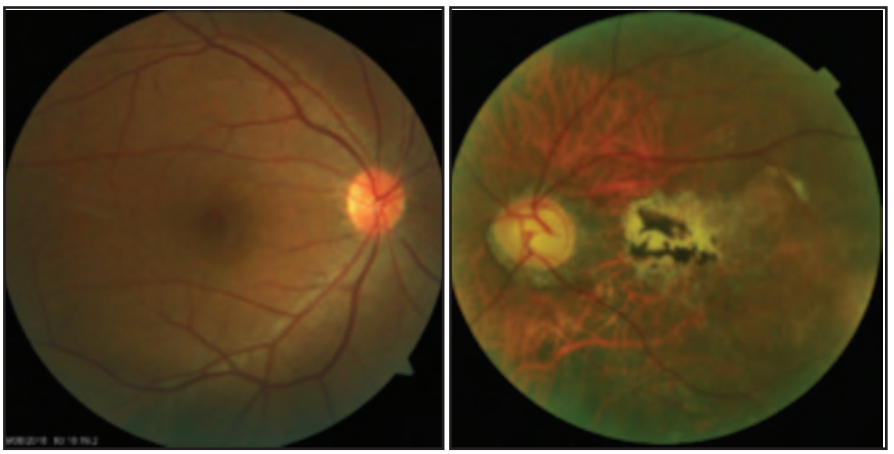

Fig. 1: Funduscopic image of the affected eye (left) and clear normal eye (right). Hemorrhage and neovascularization were observed in the affected eye.

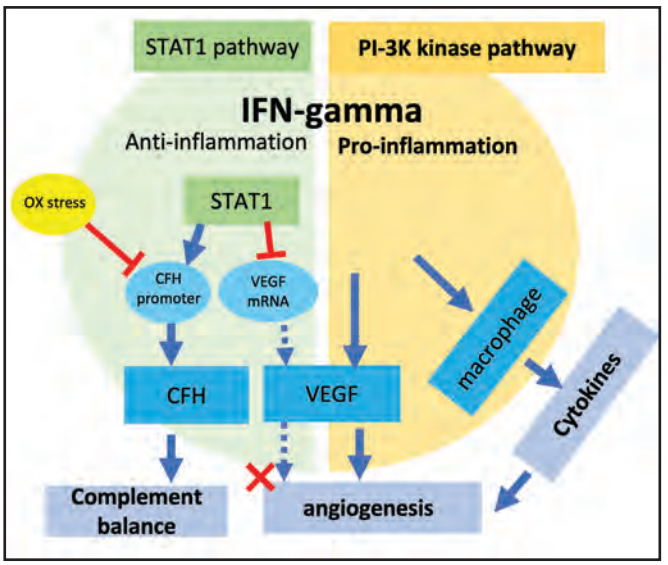

Fig. 2: Pro- and anti-inflammatory roles of IFN-γ in nAMD pathogenesis

passive) and indoor/outdoor working activity of AMD and control groups. The study was conducted at Dr. Sardjito Central General Hospital, Dr. S. Hardjolukito Air Force Main Hospital, and Dr. Yap Eye Hospital, Yogyakarta. ELISA was conducted at the Integrated Research Laboratory, FK-KMK, UGM.

Whole blood was centrifuged (1,000 ×g for 15 min), and the upper layer was collected to retrieve plasma. ELISA was performed in accordance with the manufacturer's protocol (Finetest®, Wuhan Fine Biotech, Wuhan, China). First, 100 µL of the plasma sample and the standard solution each was placed into 96-well plates, followed by incubation for 37°C for 90 min. After discarding the mixture and standard,the empty plate was washed twice, and 100 µLof Biotin was added to each well before incubating the plate at 37°C for 60 min. Next, the mixture was discarded, and the plate was washed three times. Subsequently, streptavidin conjugate buffer was added before incubating at 37°C for 30 min. Finally, the mixture was discarded, and the plate was washed five times. Then, 90 µLof TMB substrate buffer was added before incubating the plate at 37°C for 10–20 min (depending on the change in color) in the dark. Immediately after the color accurately changed according to the standard chart, 50 µLof

stop solution was added. Then,using a microplate reader,the optical density at 450 nm obtained, which was interpolated into nanogram per milliliter using the CurveExpert 1.4 software.

All acquired data were analyzed using the Mann–Whitney Test (SPSS) for unevenly distributed data. The obtained results were expressed as means and standard deviations with 95% confidence interval (CI) to determineany association between IFN- γ level and the incidence of AMD.

RESULTS

All patients with AMD or age-matched controls were diagnosed by an ophthalmologist (Fig. 1). For statistical analysis, the data from 16 patients with AMD and 23 age-matched controls were used.

In the nAMD and control groups, the mean value of circulating IFN-y was 14.88 ± 6.96pg/mLand 18.70 ± 17.93pg/mL, respectively. There was no positive association between cytokine level and AMD (p> 0.05, 95% CI:12.650 – 21.606) (Table I).

Baseline parameterswere similar in the case and control groups. The majority of subjects were non-smokers (76% in nAMD group and 69.5% in control group) and had normal blood pressure (64.8 mmHg in nAMD group and 78.3 mmHg in control group).

DISCUSSION

To our knowledge, this is the first study reporting the association between circulating IFN-γ level and neovascular AMD in an Indonesian population. The results suggest that IFN-γ levels were not associated with AMD. Patients with AMD had lower IFN-γ cytokine level than controls. However, there was no association between IFN-γ cytokine level and nAMD onset (p>0.05, 95% CI).

In exudative nAMD, it is not clear what the role of IFN-gamma is, since IFN-γ can either maintain or inhibit inflammation in different ways (Figure 2). Several reports have demonstrated the role of IFN-γ in initiating immunomodulatory and protective functions. However, IFN-γ was reported as a pro-inflammatory factor in nAMD.[8,16] In addition, IFN-γ inhibits the angiogenic activity of VEGF via the activation of the STAT1 pathway in human endothelial cells1[7], while also down-regulating Vascular endothelial growth factor (VEGF) mRNA in a dose-dependent manner18. This process might be of potential in AMD therapy. Therefore, IFN-γ-associated STAT1 activation may be beneficial.

Interestingly, another study has suggested that IFN-γ can mediate VEGF expression in Retinal pigment epithelium (RPE) cells through the PI-3K/Akt/mTOR/p70 S6 kinase pathway and is independent of STAT1.[19] Another piece of evidence comes from the study demonstrating IFN-γ up-regulating CFH expression in RPE cells.[20] CFH can keep the complement cascade in check and prevent tissue injury from excessive complement activation.[21] CFH is transcriptionally upregulated by STAT1; however, oxidative stress, one of the most important risk factors for AMD, can disrupt this process by acetylating FOXO3, which competes with STAT1 for binding to the CFH promoter.[22,23] Reportedly, STAT1-deficient mice were highly susceptible to autoimmune disorders[24], and considering this response pattern, AMD may be considered an autoimmune disease.[25,26]

One limitation of this study was that only patients with nAMD who were only in the most severe stage of the disease were included. At this stage, IFN-γ may no longer play an important role in inducing or maintaining AMD condition. Further studies should recruit patients in the early and intermediate stages of AMD to compare cytokines levels. Our findings may also be limited due to the lack of samples in the study groups. Future studies with larger number of samples are required to validate and better understand the association between IFN-γconcentration and AMD incidence.

CONCLUSION

Although insignificant, higher concentrations of IFN-γ were observed in the control group than in the AMD group.

ACKNOWLEDGMENT

The authors would like to acknowledge Universitas Gadjah Mada for providing the RTA grant (1185/UN1.P.III/SK/HUKOR/2021).

REFERENCES

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, et al.Global data on visual impairment in the year 2002. Bull World Health Organ 2004; 82(11): 844–51.
2. GBD 2019 Blindness and Vision Impairment Collaborators; Vision Loss Expert Group of the Global Burden of Disease Study. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: The Right to Sight: An analysis for the Global Burden of Disease Study. Lancet Glob Heal 2021;9: e144–60.
3. Supanji S, Perdamaian ABI, Romdhoniyyah DF, Sasongko MB, Agni AN, Wardhana FS,et al.The association of HTRA1 rs11200638 polymorphism with neovascular age-related macular degeneration in indonesia.Ophthalmol Ther 2021.
4. Supanji S, Romdhoniyyah DF, Sasongko MB, Agni AN, Wardhana FS, Widayanti TW,et al.Associations of ARMS2 and CFH gene polymorphisms with neovascular age-related macular degeneration. Clin Ophthalmol2021; 15: 1101–8.
5. Supanji S, Perdamaian ABI, DianratriA, PrayogoME, Sasongko MB, Wardhana FS, et al.The circulating level of IL-1β in patients with age-related macular degeneration (AMD) in yogyakarta: characteristics to disease activity. in Proceedings of the 3rd KOBI Congress, International and National Conferences (KOBICINC 2020).Atlantis Press 2021; 14: 434–6.
6. Supanji S,Perdamaian ABI, DianratriA, SyifarahmahA, WidayantiTW, WardhanaFS.et al. HtrA1 serine protease expression levels on age-related macular degeneration (AMD) patients in Yogyakarta. BIO Web Conf2020; 28: 02004.
7. Jiang K, Cao S, Cui JZ, Matsubara JA. Immuno-modulatory effect of IFN-gamma in amd and its role as a possible target for therapy. J Clin Exp Ophthalmol 2013; Suppl 2, 0071.
8. Nagineni CN, Detrick B, Hooks JJ. Synergistic effects of gamma interferon on inflammatory mediators that induce interleukin-6 gene expression and secretion by human retinal pigment epithelial cells. Clin Diagn Lab Immunol1994; 1: 569–77.

9. Chakrabarty P, Ceballos-Diaz C, Beccard A, Janus C, Dickson D, Golde TE,et al. IFN-γ promotes complement expression and attenuates amyloid plaque deposition in amyloid β precursor protein transgenic mice. J. Immunol 2020; 184: 5333–43.
10. Huang Y, Krein PM, Muruve DA, Winston BW. Complement factor B gene regulation: synergistic effects of TNF-α and IFN-γ in macrophages. J Immunol 2002; 169: 2627–35.
11. Juel HB, Faber C, Udsen MS, Folkersen L, Nissen MH. Chemokine expression in retinal pigment epithelial ARPE-19 cells in response to coculture with activated T cells. Investig Ophthalmol Vis Sci 2012; 53: 8472–80.
12. Dastgheib K,Green WR. Granulomatous reaction to bruch's membrane in age-related macular degeneration. Arch. Ophthalmol1994; 112: 813–8.
13. Penfold PL, Wong JG, Gyory J, Billson FA. Effects of triamcinolone acetonide on microglial morphology and quantitative expression of MHC-II in exudative age-related macular degeneration. in Clin Exp Ophthalmol2001; 29: 188–192.
14. Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration. Prog Retin Eye Res2009; 28: 1–18.
15. Roque RS, Rosales AA, Jingjing L, Agarwal N,Al-Ubaidi MR. Retina-derived microglial cells induce photoreceptor cell death in vitro. Brain Res1999;836: 110–9.
16. AfaridM, Azimi A, Malekzadeh M. Evaluation of serum interferons in patients with age-related macular degeneration. J Res Med Sci2019; 24.
17. Battle TE, Lynch RA, Frank DA. Signal transducer and activator of transcription 1 activation in endothelial cells is a negative regulator of angiogenesis. Cancer Res2006; 66: 3649–57.
18. Kawano Y, Matsui N, Kamihigashi S, Narahara H,Miyakawa I. Effects of interferon-γ on secretion of vascular endothelial growth factor by endometrial stromal cells. Am J Reprod Immunol2000; 43: 47–52.
19. Liu B, Faia L, Hu M, Nussenblatt RB. Pro-angiogenic effect of IFNγ is dependent on the PI3K/mTOR/ translational pathway in human retinal pigmented epithelial cells. Mol Vis2010; 16: 184–193.
20. Fritsche, L. G. Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet2016; 48: 134–143.
21. Patel M,Chan CC. Immunopathological aspects of age-related macular degeneration. Seminars in Immunopathology 2008; 30: 97–110.
22. WuZ, Lauer TW, Sick A, Hackett SF, Campochiaro PA. Oxidative stress modulates complement factor H expression in retinal pigmented epithelial cells by acetylation of FOXO3. J Biol Chem 2007; 282: 22414–25.
23. Beatty S, Koh H, Phil M, Henson D,Boulton M.The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2000; 45: 115–34.
24. Nishibori T, Tanabe Y, Su L, David M. Impaired development of CD4+ CD25+ regulatory T cells in the absence of STAT1: increased susceptibility to autoimmune disease. J Exp Med 2004;199: 25–34.
25. Iannaccone A,Neeli I, Krishnamurthy P, Lenchik NI, Wan H, Gerling IC, et al. Autoimmune biomarkers in age-related macular degeneration: A possible role player in disease development and progression. Adv Exp Med Biol 2012; 723: 11–6.
26. Morohoshi K, Goodwin AM, Ohbayashi M, Ono SJ. Autoimmunity in retinal degeneration: Autoimmune retinopathy and age-related macular degeneration. J Autoimmun2009; 33: 247–54.