The Difference between hs-CRP and Serum IL-6 Levels in Patients with Glaucoma and Non Glaucoma

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Abstract

Background: Glaucoma is a group of eye diseases that have the characteristic of optic nerve damage with a specific pattern and field loss of view with or without increased intraocular pressure (IOP). The objective of the study was to analyze the association between glaucoma incidence with hs-CRP and IL-6 levels.

Method: This study used patients who fulfilled the inclusion criteria. Inclusion criteria were in the form of patients with a primary closed-angle diagnosis, willing to participate in the research. The study was conducted in the period from March to August 2013. The research procedure included patient examination, hsCRP and IL-6 level examination.

Results: Mann-Whitney test result showed difference of hs-CRP with p value of 0.755 and difference of IL-6 level with p = 0.95. Thus, there was no significant difference in the levels of hs-CRP and IL-6 in patients with glaucoma and non-glaucoma.

Conclusion: Hs-CRP and serum IL-6 levels in the glaucoma group did not differ from the non-glaucoma group.

Keywords: *hs-Crp, IL-6, glaucoma, intraocular*

Introduction

Glaucoma is a group of eye diseases that have the characteristic of optic nerve damage with a specific pattern and field loss of view with or without increased intraocular pressure (IOP). WHO States that glaucoma is the second rank as the cause of irreversible blindness in the world. Primary closed-angle glaucoma (PCAG) is the most common form of glaucoma in Asian countries. A typical PCAG in European countries is an acute attack, unlike Asian and African countries that tend to develop gradually as chronically closed angle asymptomatic [1].

Glaucoma disease affects more than 66 million individuals worldwide, causing bilateral blindness at 6.8 million. It is estimated that 3.9 million individuals became blinded by PCAG in 2010. By 2020, this number is assumed to increase to 5.3 million. Eighty-six percent of PCAG patients are in Asia, with an estimated 48.0% in China, 23.9% in India and I4.1% in Southeast Asia.Indonesia has the highest number of blindness in Southeast Asia. From the 1993-1996 health survey, glaucoma caused 13.4% of blindness after cataracts. Mimiwati et al. found chronic PCAG (58.5%) was the most common form followed by subacute (26.8%) and acute group (14.6%) [2].

Glaucoma risk factors such as old age increase the percentage of closed angle and increase the incidence of PCAG. Women are at risk 3 to 4 times compared to men because they are related to the fact that they are gonioscopy which has a shallower eye angle than men. Other risk factors for increased IOP are family history, severe myopia, systemic hypertension, cardiovascular disease, migraine headaches, peripheral vasospasm and previous neurological damage. Acute attacks of closed-angle glaucoma lead to blindness within hours or days, in contrast to primary open-angle glaucoma that is slow

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and gradual loss of vision. In addition, the other most important things are the speed and the accuracy of the management. The accurate diagnosis in the form of intermittent and chronic of PCAG is also important because prophylactic treatment can protect the eyes from acute attacks and prevent damage due to repeated intermittent attacks or chronic closed angle. Loss of vision due to glaucoma is irreversible; however, it can be treated if the diagnosis is established at an early stage. Many individuals with glaucoma become blind because they have not received good help [3].

Retinal ganglion cell (RGC) apoptosis is a key of neurodegenerative glaucoma, but the detail of the underlying process remains unclear. Various mechanisms have been found such as the deprivation of neurotrophic factors, hypoperfusion or ischaemia, glial cell activation, glutamic excitotoxicity and abnormal immune responses which lead to an end result of RGC apoptosis. The in vivo study explains the role of an adaptive immune response in the pathogenesis of glaucoma. Increased IOP and ischemia is a stress that acts as an immunostimulator signal, triggering an activation of an immune response that was initially useful for repairing tissue [4].

IL-6 is a pleitropic cytokine, which both uses a common signal transduction component with other IL-6 family members. A specific IL-6 signal involves the interaction of gp130 with IL-6 alpha receptor present in the membrane (IL-6Rα). Research conducted in vivo and in vitro showed elevated levels of T helper (Th2) lymphocyte cell cytokines which are IL-6 and Th1 cytokines including interferon γ (IFN-γ), IL-1, IL-2, IL-17 and tumor necrosis factor α (TNF-α) associated with retinal damage and RGC. The increase in intraocular IL-6 levels has not been shown to affect serum IL-6 levels [5].

Serum IL-6 examination is influenced by several conditions because these cytokines are produced by various cells after stimulation of infection, trauma, immunological reactions and other systemic inflammation. In addition, IL-6 examination is still not conducted in the clinical laboratory; thus, it needs a practical examination. High-sensitivity C-reactive Protein (hs-CRP) is a solution to this problem, since it is useful in filtering out inflammatory markers such as infection, trauma, immunological reactions and systemic inflammation. Its production in the liver is induced directly by IL-6. Hs-CRP examination has grown rapidly as predicted risk of coronary heart disease, stroke and other peripheral vascular diseases; thus, it needs to be evaluated its potential for the diagnosis of glaucoma [6].

Method

This research is a laboratory analysis research with cross sectional design. The nclusion criteria of respondents arepatients with a primary closed-angle diagnosis and willing to participate in the study. While the exclusion criteria were patients with coronary heart disease, systemic infections, viral conjunctivitis, and diabetes mellitus. The sampling technique used simple random sampling. The research procedure included patient examination, hsCRP examination, and examination of IL-6 levels [7].

Examination of IL-6 levels was conducted by using ELISA method. This examination used the ELISA microreader tool from R&D Systems. The venous blood sample from the cubital vein had previously been disinfected with 70% alcohol, then venous blood was inserted into a 2 ml plain vacutainer tube. Each tube was labeled (patient name, date and time of sampling, and the initials of the subjects taking the sample). Samples were incubated for 30 min and then centrifuged 3000 rpm, 15 min to separate serum. The serum was put into several microcentrifuge tubes [8]. The tube was labeled and immediately shipped to the Bank Jaringan, stored in the -70ºC freezer until further inspection. The retrieval time to sample storage was less than 1 hour [9]. All collected data were arranged in tabular form and processed statistically. The statistical test used to determine the difference of serum hs-CRP and IL-6 levels in glaucoma and non glaucoma patients was Mann-Whitney test with $p < 0.05$ [10].

Results

Examination of hs-CRP levels in study subjects' serum showed that the mean value of hs-CRP in glaucoma patients was not different compared to non-glaucoma control hs-CRP values. The mean values in the control group tend to be larger and the standard deviation was also greater than the mean glaucoma group. The range of variation in hs-CRP values was also significant in the glaucoma group from 0.084 to 5.78 mg/L while in the non-glaucoma control group it was greater than 0.001 to 7.934 mg/L and the median values for each group were 1.362 and 1.710 (Table 1)

IL-6 examination results showed no significant difference in mean values in both groups of study

subjects. The mean value of IL-6 glaucoma group was greater than non glaucoma. The mean rate of IL-6 levels in the glaucoma group was greater than non glaucoma but the mean level of hs-CRP in the glaucoma group was smaller than non glaucoma. This result was not in accordance with physiological conditions, ie when the level of IL-6 increased, it will be followed by increased levels of hs-CRP. Standard deviations in both groups were also relatively large even it was larger in non glaucoma group. The range of IL-6 levels was also high in the glaucoma group from 0.21 to 9.1 pg/ml and the non-glaucoma group from 0.16 to 9.63 pg/ml with median values of 1.910 and 0.775, respectively (Table 1)

Test of normality of difference of hs-CRP level between glaucoma and non glaucoma patient has significance <0.05. Normality test with Kolmogorov-Smirnov on glaucoma group showed p value = 0.004 was in non glaucoma group p = 0.001. The situation showed abnormal data distribution (Table 3). The normality test of difference of IL-6 level between glaucoma and non glaucoma patient had significance <0.05. Test of data normality with Kolmogorov-Smirnov in glaucoma group showed p value = 0.106 was in non glaucoma group p = 0.002. Although there was a value of p >0.05, it remains statistical tests for abnormal data distribution because another p value <0.05 (Table 2).

The normal distribution curve was shown at the level of IL-6 glaucoma which was symmetrically pointed, although the median value slightly shifts toward the left of 1.910 shifts from the mean of 2.319. Non-glaucoma group distribution curve was not normal because the median value of 0.775 shifts away from the mean value of 1828; thus, the curve figure is not symmetrical. The pattern of non-glaucoma group population was more to the right of the median region. The results of normality test of hs-CRP and IL-6 results in glaucoma patients and non glaucoma patients with Kolmogorov-Smirnov showed mostly p <0.05. The value in the normality test of IL-6 data in glaucoma patients with Kolmogorov-Smirnov p >0.05 was p = 0.106 and overall data distribution remained abnormal. Mann-Whitney test result difference of hs-CRP with p value of 0.755 and difference of IL-6 level with p = 0.95. Thus, there was no significant difference in the levels of hs-CRP and IL-6 in glaucoma and non-glaucoma individuals (Table 3).

Discussion

The results of this study have stated that there was no difference in hs-CRP levels in glaucoma and non glaucoma. The results were similar to previous studies; thus, the hs-CRP examination had no clinical significance in the diagnosis of glaucoma.Serum CRP examination is routine in clinical laboratories as a marker of various acute and chronic inflammatory diseases. The production of CRP in hepatic cells are stimulated various proinflammatory cytokines such as IL-1, IL-6, TNF-α, IFN-γ and TGF-β. Small amounts are also produced locally in lymphocytes, and vascular wall muscle tissue in patients with coronary heart disease [11]. CRP production increases in the inflammatory process. The normal range of CRP values in adult individuals ranges from 1.5 mg/L for males and 2.5 for females [12].

IL-6 is a single protein cytokine not only in the production of lymphoid and non-lymphoid cells. IL-6 is also expressed by normal or transformed cells such as macrophages, fibroblasts, synovial cells, endothelial cells, glial cells, keratocytes and tumor cells [13]. IL-6 expression induced by various stimuli includes cytokines such as IL-1, TNF-α, PDGF, bacterial and viral infections and microbial components such as lipopolysaccharide [14]. Its role has been well known in the minimal chronic inflammatory state of obesity, diabetes, cardiovascular disease as well as in infections and sepsis. In healthy individuals independent of inflammatory reactions, the IL-6 concentrations are very low from 0.2 to 7.8 pg/ml and can rise to a high of 1600 pg/ml concentrations in sepsis [15].

IL-6 in the systemic circulation is a pleiotropic cytokine involved in the regulation of the immune system, acute phase reaction and hematopoiesis. IL-6 is associated with an acute phase reaction functionally referred to as hepatocyte stimulating factor (HSF) because it stimulates hepatocytes in producing CRP. The mean rate of IL-6 group of glaucoma was greater than non glaucoma, but the mean hs-CRP level in the glaucoma group was smaller than non glaucoma [16].

This result is not compatible with physiological conditions, ie when the level of IL-6 increases, it will be followed by increased levels of hs-CRP. This suggests that no significant difference in IL-6 levels in the glaucoma and non glaucoma groups needs to be analyzed further [17]. The results of IL-6 studies on serum or systemic circulation do not coincide with IL-6 studies in aqueous humour can be influenced by various factors. Intraocular IL-6 cytokine levels are too minimal to affect levels in the systemic circulation; thus, the threshold

levels of IL-6 glaucoma and non glaucoma are very narrow. The intraocular immune privilege mechanism also limits the local inflammatory process to not spread to the circulation; thus, the levels of IL-6 in the glaucoma group are not different from that of non glaucoma [18].

Conclusion

Based on the results of research, it can be concluded that serum hs-CRP and IL-6 levels in the glaucoma group did not differ from the non glaucoma group. Serum hs-CRP testing can not be utilized in assisting clinical diagnosis of glaucoma, whereas serum IL-6 examination also has not been able to assist in clinical diagnosis of glaucoma.

Ethical Clearance : This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest : The authors swear that there is no conflict of interest related with this paper so far and in the future.

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