Hyperuricemia and Pro Inflammatory Cytokine (IL-1β, IL-6, and TNF-α)

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ABSTRACT

Background. Rugerio et al 2006 reported that there were a positive correlation between the level of hyperuricemia and the level of IL-1β, IL-6, and TNF-α pro inflammatory cytokines value. On the other hand, Choi et al reported a negative correlation between hyperuricemia and the level of pro inflammatory cytokine in the late phase of hyperuricemia.

Methods. Venous blood samples were collected and stored at a temperature of - 80oC from in- and out-patients with hyperuricemia with age of more than 17 years old at Dr. Kariadi Hospital, Semarang. The level of uric acids (mg/dl) were examined with enzymatic colorimetric technique (Roche Diagnostics) whereas the levels of IL-1β, IL-6, and TNF-α pro inflammatory cytokines (pg/ml) were examined with enzyme linked immunosorbent assay (ELISA) technique using ultra sensitive commercial kit (Human ultra sensitive, Biosource International Inc Europe), and ELX 800, 2002 machine. The normality of the data was tested with One-Sample Kolmogorov-Smirnov technique and the correlation was tested with Spearman correlation (data with abnormal distribution) or Pearson correlation (data with normal distribution).

Results. There was a weak positive correlation between the level of hyperuricemia and the level of IL-1 β cytokine in Spearman correlation test with r value = 0.246 and p value > 0.05 in Spearman correlation test. On the other hand, there was a weak negative correlation between the level of hyperuricemia and the level of TNF-α cytokine with r value = - 0.096 and p value > 0.05. There was also weak negative correlation between the level of hyperuricemia and the level of IL-6 cytokine with r value = - 0.072 and p value > 0.05 in Pearson correlation test.

Conclusion. There was a weak positive correlation but not sifnificant between the level of hyperuricemia and the level of IL-1β.

Uric acid is produced from protein (purin) metabolism in the liver by urate oxidase enzyme (uricase) in the liver, and then metabolized (oxidative degradation) into alantoin which is more soluble and easier to excrete through the renal. The function of uric acid (monohydrate uric monosodium) in the body is not clear. It is assumed that uric acid acts as primary antioxidant, binding radical oxygen effectively just like vitamin C does.[1] Hyperuricemia occurs when 1) there is an excess production resulted from high purin diet, alcohol consumption, certain conditions with high level of cells degradation, or enzymatic defect in purin metabolism; 2) there is a disorder in uric acid excretion through the renal. The complications of hyperuricemia include 1) gout, 2) renal stone and renal damage, and 3) an elevated risk factor of ischemic heart disease caused by atherosclerosis and atherothrombosis in blood vessels.[1,2] In animal study, hyperuricemia in joint or structures around will stimulate monocytes/leucocytes (mast cells in early phase, neutrophils in later phase) to produce various chemoattractant protein and Interleukine-1beta (IL-1β), IL-6, and Tumor Necrosis Factor alpha (TNF-α) pro inflammatory cytokines. The role of hyperuricemia in the pathogenesis of atherosclerosis and atherothrombosis in blood vessels is still widely debated. Early phase of vascular inflammation begun by a vascular endothelial injury, and then chemoattractant protein will be produced to attract various inflammatory leucocyte, many adhesion molecules (E selectin, intracellular adhesion molecule-1/ ICAM-1, vascular cell adhesion molecule-1/ VCAM-1), mast cells, and monocytes with all their cytokine products to the injured area.[2,3] In a study of 967 subjects, Rugerio et al 2006 (USA) reported a positive correlation between the level of hyperuricemia (quintile) with the levels of IL-1β, IL-6, and TNF-α cytokines value, causing an increase in the level and activation of the three pro inflammatory cytokines. It is supposed that the cytokines were activated through specific receptors such as the toll like receptor on the leucocyte system aided by a protein called My 88 protein (produced in cytokine stimulated tissues).[5]

METHODS

The subjects were in- and out-patients with hyperuricemia, from both sexes with age of more than 17 years old, at Dr. Kariadi Hospital, Semarang. They agreed to participate in the study and signed the informed consent. The study had the permit from the Ethics Committee of Dr. Kariadi Hospital, Faculty of Medicine, Diponegoro University. Peripheral blood samples were collected from the subjects, serum plasmas were collected and stored at a freezer with temperature of - 80°C. The level of uric acid (mg/dl) was examined by enzymatic colorimetric technique (Roche Diagnostics). Hyperuricemia is

a state when the level of uric acid in the serum exceeds 7 mg/dl in men or 6 mg/dl in women.[2] The levels of IL-1β, IL-6, and TNF-α pro inflammatory cytokines (pg/ml) were examined with enzyme linked immunosorbent assay (ELISA) technique using ultra sensitive commercial kit (Human ultra sensitive, Biosource International Inc Europe). The result of the ELISA reader was an absorbency value (Optical Density/OD) which was converted to pg/ml using an ELX 800,2002 machine. The normality of the data distribution was tested with One-Sample Kolmogorov-Smirnov test. The correlation was tested with Spearman correlation (data with abnormal distribution) or Pearson correlation (data with normal distribution).[6,7]

OBJECTIVE

The objective of the study is to identify the correlation between the level of hyperuricemia value and the levels of IL-1β, IL-6, and TNF-α pro inflammatory cytokines.

RESULTS

There were 44 cases of hyperuricemia (35 men : 9 women) in in- and out-patients at Dr. Kariadi Hospital, Semarang. The mean age was 62.57 ± 9.73 years old, body mass index was 24.81 ± 2.89. The mean level of hyperuricemia was 8.94 ± 1.46 mg/dl whereas the mean level of IL-1β was 36.8 ± 69.3 pg/ml, the mean level of IL-6 was 219.17 ± 130.8 pg/ml, and the mean level of TNF-α was 34.9 ± 24.2 pg/ml (table 1).

Table 1 Raw data of the study, the levels of hyperuricemia, IL-1β, IL-6, and TNF-α

Hyperuricemia (mg/dl) **Mean/SD**	**IL-1β(pg/ml) Mean/SD**	**IL-6 (pg/ml) Mean/SD**	**TNF-α(pg/ml) Mean/SD**
8.94 ± 1.46	36.8±89.3	210.17±130.8	34.9±24.2

One-Sample Kolmogorov-Smirnov test showed that the distribution of the IL-1β (pg/ml), IL-6 (pg/ml) and TNF α (pg/ml) were abnormal, thus the correlation between IL-1β and uric acid were tested with Spearman correlation test.

Spearman* correlation test showed that there was a weak positive correlation but not significant between the level of IL-1β (pg/ml) and the level of uric acid (mg/dl) (r = 0.246 and p = 0.107). The correlation test of the level of TNF-α cytokine value (pg/ml) and the level of uric acid (mg/dl) also showed that there was a negative weak correlation but not significant between them (r = - 0.096 and p = 0.445). Finally the correlation between the level of IL-6 value (pg/dl) and the level of uric acid (mg/dl) also showed that there was a negative weak correlation but not significant between them (r = - 0.072 and p = 0.445) (table 2).

Table 2 The correlation between the level of hyperuricemia and the levels of IL-1β, IL-6, and TNF-α

	Mean / SD	**Hyperuricemia (mg/dl) Mean/SD**	**r value**	**p value**
IL-1β (pg/ml)	36.8±89.3	8.94 ± 1.46 mg/dl	0.24	> 0.05*
IL-6 (pg/ml)	210.17±130.8	8.94 ± 1.46 mg/dl	- 0.096	> 0.05**
TNF-α (pg/ml)	34.9±24.2	8.94 ± 1.46 mg/dl	- 0.072	> 0.05**

DISCUSSION

The correlation between the level of hyperuricemia and the level of IL-1β, TNF-α, and IL-6

In this study there was a weak positive correlation (not significant) between the level of hyperuricemia and the level of IL-1β (figure 1, ROC graph with r = 0.246 and p > 0.05 in Spearman correlation test). Moreover, there was a weak negative correlation (not significant) between the level of hyperuricemia (mg/dl) and the level of TNF-α (pg/ml) (figure 2, ROC graph with r value = -0.096 and p value > 0.05). Finally, there was also a weak negative correlation (not significant) between the level of hyperuricemia (mg/dl) and the level of IL-6 (pg/ml) (figure 3, ROC graph with r value = -0.072 and p value > 0.05). These results were a contrast to the study done by Rugerio et al 2006 (USA). In the study of 967 hyperuricemic patients, Rugerio reported that there were positive correlations between the high level of hyperuricemia (quintile) and the levels of IL-1β, IL-6, and TNF-α cytokines. It meant that an increase of the level of hyperuricemia would be followed by the increase and activation of the three pro inflammatory cytokines.[2] Kim et al (US, 2007) in a five-year cohort study reported that hyperuricemia > 7 mg/dl in a woman was a risk factor of coronary heart disease with an odds ratio of 1.356 in women and 1.121 in men. However, Neogi et al (USA, 2007) in a multicenter analysis reported that hyperuricemia was not related to coronary artery calcification in men and women through helical computed tomography examination technique.[8] Neogi's finding was supported by Choi et al's hypothesis. Choi stated that monocytes and mast cells were responsible for the early phase of acute inflammation by secreting various inflammatory chemicals (chemoattractant, prostaglandin, etc) and also IL-1β, IL-6, and TNF-α inflammatory cytokines. Nevertheless, if the inflammation had continued, the stimulated and differentiated monocytes/macophages would fail to produce and secrete various inflammatory cytokines including IL-1β, IL-6, and TNF-α.[1] This phenomenon was proven with in vitro culture of well differentiated human macrophage for seven days where it would then failed to secrete cytokine.[1] Machophage in chronic phase of gout will produce IL-10 and TGF- β cytokines which are able to force down the inflammatory response or reduce the production of IL-1β, IL-6, and TNF-α.[7]

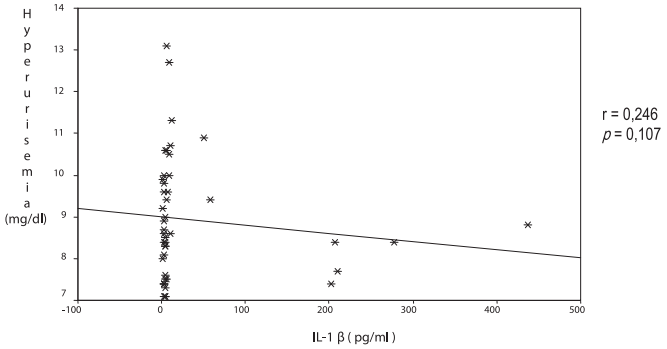

Figure 1 ROC graph of the correlation between the level of hyperuricemia (mg/dl) and the level of IL-1β cytokine (pg/ml) (n=44). There was a weak positive correlation between them with r value = 0.246 and p value >0.05 in Spearman correlation test.

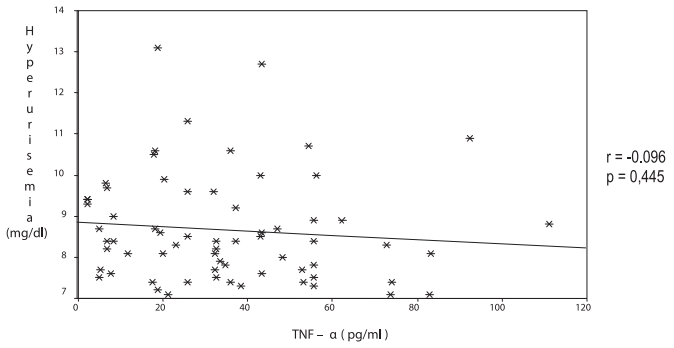

Figure 2 ROC graph of the correlation between the level of hyperuricemia (mg/dl) and the level of TNF-α cytokine (pg/ml) (n=44). It showed a negative correlation between them with r value = -0.096 and p value > 0.05 in Spearman correlation test.

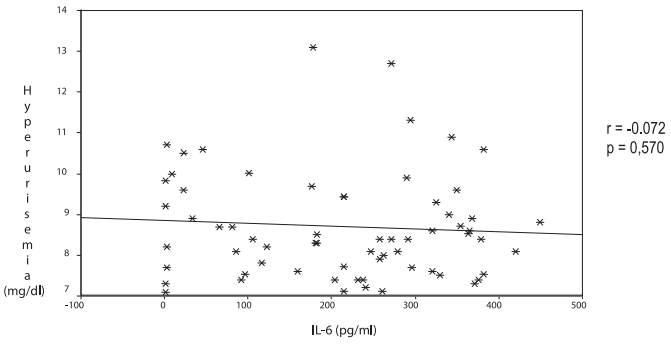

Figure 3 ROC graph of the correlation between the level of hyperuricemia (mg/dl) and the level of IL-6 cytokine (pg/ml) (n=44). It showed a weak negative correlation between them with r value = -0.072 and p value > 0.05 in Spearman correlation test.

CONCLUSION

In this study, there was a weak positivie correlation but not significant between the level of hyperuricemia and the levels of IL-1β.

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