The Effect of Stress on IL-17 Levels in an OVA-immunized Mice Allergic Model

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ABSTRACT

Objective: Evaluate the effect of stress on eosinophil count, levels of cortisol and levels of IL-17 in an OVA-immunized Mice Allergic model. **Method:** Eight teen female BALB/c mice divided into 3 groups. Negative control in group 1, Allergy control in group 2 group received ovalbumin sensitization only and stress treatment in Group 3 received ovalbumin sensitization and stress using water-immersion stress test on day 24th, 26th and 30th. All mice are terminated on 31st day, lungs and blood sample are collected and measured for eosinophil count microscopically, level of cortisol and level of IL-17 using ELISA. **Results:** Mean eosinophil count for group 1 is 0.48 per high power field (hpf), group 2 is 2,13 hpf, group 3 0.8 hpf. Mean cortisol level for group 1 is 9.13 ng/mL, group 2 is 10.85 ng/mL, group 3 25.47 ng/mL. Mean IL-17 level for group 1 66.71 pg/mL, group 2 is 22.36 pg/mL, group 3 is 26.38 pg/mL. **Conclusion:** The number of eosinophils in the allergy control in group 2 (2.13/hpf) was significantly higher than the negative control in group 1 (0.48/hpf) (p = 0.004). Levels of cortisol in the stress treatment in group 3 (25.47 ng/mL) were not significantly different from the allergy control in group 2 (10.85 ng / mL) (p = 0.180). Levels of IL-17 in the stress treatment in group 3 (26.38 pg / mL) did not differ significantly from the allergy control in group 2 (22.36) (p = 0.394).

Keywords: Allergic Rhinitis, Ovalbumin, Stress, Cortisol, IL-17

Prevalence of allergic diseases continue to increased up to 30-40% of the world population, with 10-30% of which suffer from allergic rhinitis with annual cost about 1.6 4.9 billion USD in the United States of America.(1)(2) Stress can worsen allergic symptoms by activation of *Hypothalamic-Pituitary-Adrenal* (HPA) axis and increased the cortisol levels. Cortisol influenced the immune system into a dominant T helper 2 (Th2) cell condition with increased in Th2 cytokines such as Interleukin 4 (IL-4), IL-5 and IL-13 that result in increased eosinophils infiltration and levels of Immunoglobulin E (IgE) thus lowering the success rate of immunotherapy, although the exact mechanism is still unknown.(3)(4)(5)

Recent pblication on allergy has reported that other factors come into play in allergy mechanism that is the activity of *regulatory* T cells [T*reg* CD4 + CD25 +] that produce IL-10, TGF-ß1 (*Transforming Growth Factor Beta-1)* and Th17 cells that produce IL-17 which is a pro-inflammatory mediator that can cause local inflammatory processes.(6) Research on relationship between stress and allergy has been reported that stress can increase levels of IL-4, IL-5, IL-10, IL-13, and worsen allergy symptoms and lower the success rate of allergic rhinitis treatment causing disturbance in daily activity that can lead to frustration, low self-esteem and even depression.(7)(8)(9)(10)(11)(12)(13)

Stress in allergy also affect HPA axis and increase cortisol levels. Cortisol then can reduce the activity of Th1 thus creating a more Th2 dominant condition in immune system that can lead to worse allergic rhinitis manifestation. (14)(15)(16)(17)(18)(19)(20)(21)(22)(23)(24)(25)(26)(27)(28)(29)

Recent research on allergy now focusing in IL-7, a pro-inflammatory cytokine that can reduce neutrophil infiltration and increase eosinophil infiltration that lead to local inflammation in the upper airway.(30)(31)(32)(33)(34) Local inflammation of the upper airway has been related to the increased IL-17 levels in the lungs tissue(35)(36)(37)(38)(39)(40) and also blood serum of allergic patient was related to worsening of symptoms, more drugs usage,

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elevated eosinophil count and worse immunotherapy result.

Animals allergic models used in interventional allergy research can help explain the mechanisms of various process that affected allergy. Stress simulation method such as electrical shock, immobilization, or water stress test can increased levels of the hormone cortisol in the blood[5]. Species that most often used as allergic models BALB/c mice with sensitization using ovalbumin given intraperitoneal and inhalation. Caution should always be used in conclusion before the result can be applied to human.[41][42][43][44]

Hypothesis of this study are i) the number of eosinophils count in allergy control in group 1 is higher than in mice without allergy group, ii) cortisol level in stress treatent in group 3 is higher than in allergy control in group 2, and iii) IL-17 level in stress treatment in group 3 is higher than in allergy control in group 2.

The purpose of this study is to provide evidence of elevated eosinophil count in the allergy control in group 2 compared to the negative control in group 2, and also to provide evidence of elevated cortisol and IL-17 levels in the stress treatment in group 3 compared to allergy control in group 2.

This study is required to established evidence for effective methods of sensitization to create an allergic mice models, evaluate the effectiveness of the water stress test protocol in creating stress in mice and to explore the relationship between stress and IL-17 levels that both seem to influence the worsening of allergic symptoms.

MATERIALS AND METHODS

This is an experimental posttest only control group design with laboratory animals using BALB/c mice. This study is done in Biology Laboratory of Faculty of Science in State University of Semarang and ELISA examination done in GAKI laboratory of Medical Faculty of Diponegoro University Semarang in June 2016 for 30 days. Number of sample are 18 BALB/c mice according WHO criteria in which minimum of 5 mice per group is considered sufficient. Inclusion criteria are female BALB/c, 6-8 weeks of age, weight 22-25 grams, with exclusion criteria of sick mice, and drop out criteria of death mice.

All samples are then divided according to simple random sampling into 3 groups, in which negative control in group 1 in which mice are not sensitized and not given stress treatment. Allergy control in group 2 which mice are sensitized but not given stress treatment. And Stress treatment in group 3 mice are sensitized and given stress treatment.

Sensitization were given by injecting intraperitoneally a mixture of ovalbumin 10μ and 2 mg of *AL(OH)$_3$ in 0,2 mL normal saline* on day 1st , 7th and 14th followed with inhalation of 1% ovalbumin on day $19^{th} - 22^{nd}$ for 30 minutes daily. [7][19][20][45][46] Stress treatment were given using Water-immersion stress test, done by immersing individual mice in a glass jar with the size of 25cm x 12cm x 25 cm filled with 15 cm deep of room temperature water (22±3°C) for 6 – 10 minutes on day 24th, 26th and 30th .[47]

Samples are obtained on day 31st with taking lungs samples to check for the eosinophil count microscopically, blood samples obtained from retrobulbar to evaluate the cortisol levels using Cortisol (mouse/rat) ELISA kit from Biotech and IL-17 levels using Mouse IL-17 Quantikine ELISA kit from R&D Systems in GAKI laboratory of Medical Faculty of Diponegoro University Semarang, Central Java, Indonesia.

Statistical analysis

Data obtained in this study are primary data of eosinophil count, cortisol levels and IL-17 levels. Results are expressed as the mean ± standard deviation (SD). Data were tested for normality with Saphiro-Wilk test and found to have abnormal distribution. Difference between means were analyzed with Kruskal-Wallis and Mann-Whitney test with significance of p≤ 0,05 and 95% confidence interval, and 80% power, using SPSS system.

Ethics

This study used animal as research subject and treated according to animal ethics, in which the animal kept at cage with sufficient airflow, food and drinks during research. Analgesia are used when blood samples are obtained and before termination. Ethical clearance had been obtained from Ethical Review Board of Diponegoro University with document number: 531/EC/FK-RSDK/2016.

RESULTS

Effects of Ovalbumin Sensitization on Eosinophil count

To evaluate the sensitization process, the eosinophil count was performed from the lung tissue of the mice in all group. The statistical analysis done to compare means from allergy control in group 2 and negative control in group 1, as shown in table 1. The eosinophil count in allergy control in group 2 (2.13/hpf) is higher than negative control in group 1, with p=0.004.

Effect of Stress on Cortisol Levels

To evaluate the result of water stress test, cortisol levels was measured from blood serum of the mice in all group using Cortisol (mouse/rat) ELISA kit from Biotech. The statistical analysis done to compare means from stress treatment in group 3 and allergy control in group 2, as shown in table 2. The cortisol levels in stress treatment in group 3 (25.47 ng/mL) is higher than allergy control in group 2, with p=0.180.

Effect of Stress on IL-17 Levels

To evaluate the result of water stress test, cortisol levels was measured from blood serum of the mice in all group using Mouse IL-17 Quantikine ELISA kit from R&D Systems. The statistical analysis done to compare means from stress treatment in group 3 and allergy control in group 2, as shown in table 3. The IL-17 levels in stress treatment in group 3 (26.38 pg/mL) is higher than allergy control in group 2, with p=0.394.

DISCUSSION

Effects of Ovalbumin Sensitization on Eosinophil count

Results from table 1 shows there was a significant higher means of eosinophil count in allergy control in group 2 compared to negative control in group 1. This suggests that the sensitization process in this study managed to increased the means of eosinophils count found in lungs of the allergy control in group 2. This is consistent with several studies of allergy mice models that also use BALB / c mice with intraperitoneal and inhaled ovalbumin sensitivity.(7)(19)(20)(45)(46)

The increased means of eosinophil count in the allergy control in group 2 occurred due to ovalbumin sensitization given from intraperitoneal injection and ovalbumin inhalation, causing eosinophilic inflammation in the peri bronchial region causing the airway hyperresponsivity state.(19)(20) Thus, the sensitization technique done in this study can be used in subsequent research that requires model of allergic mice.

The means of eosinophils count in the Stress treatment in group 3 were higher compared with the negative control in group 1, but lower than the allergy control in group 2. This is because cortisol is a steroid hormone in the glucocorticoids group that can decreased eosinophils count by inhibiting eosinophil survival, increasing eosinophil apoptosis but inhibiting neutrophil apoptosis. Therefore, in a conditions of airway inflammation and increased glucocorticoids in certain levels, will lead to decreased of eosinophils count but at the same time increased the of neutrophil count.(48)

Increased of neutrophils count in the upper respiratory tract has been shown to play a role in worsening asthma symptoms through airway obstruction mechanisms due to epithelial damage and tissue remodeling. The neutrophils count in the airway could still be elevated despite treatment with high doses of corticosteroid, and it has been proven in laboratory setting that corticosteroids can improve neutrophil survival by reducing neutrophil apoptosis.(49)

This is consistent with findings in this study, where eosinophils count in stress treatment in group 3 were lower than the allergy control in group 2, however in this study the neutrophils count is not measured.

Effect of Stress on Cortisol Levels

Results from table 2 shows that the cortisol levels in stress treatment in group 3 is higher than in allergy control in group 2 but the difference is not significant with p=0.180. This suggests that the Water-immersion Stress Test protocol as described by Gupta can increased cortisol levels in stress treatment in group 3[47].(47) However, the difference was not statistically significant could be due to time of stress exposure is not enough. This suggests that the Water-immersion Stress Test protocol if it were going to be used in subsequent research to produce stress should be further investigated, by modification of different time duration of stress exposure.

Interestingly, cortisol level of the allergy control in group 2 were higher than the negative control in group 1. This finding was

contradicting with the results of a Bakkeheim study in which cortisol levels in asthma and allergic rhinitis were lower than those of healthy subject[51].(50)

Effect of Stress on IL-17 Levels

Results from table 3 shows that the IL-17 levels in stress treatment in group 3 is higher than in allergy control in group 2 but the difference is not significant with p=0.394. This suggests a tendency to increase levels of IL-17 in allergic conditions that experience stress. This is in accordance with the results of Miyasaka and Viswanathan research where in patients with asthma who experienced psychological stress there is an increase in IL-17 which results in a decrease in response to glucocorticoid therapy and result in worsening of symptoms as a result of airway inflammation associated with increased neutrophil[52,53].(51)(52) Limitations This study did not measure the number of neutrophils, and other confounding factors that could affect IL-17 levels such as IL-4 and IL-6.

CONCLUSION

The number of eosinophils in the allergy control in group 2 (2.13/hpf) was significantly higher than the negative control in group 1 (0.48/hpf) (p = 0.004), thus the first hypothesis was proven. The cortisol levels in the stress treatment in group 3 (25.47 ng/mL) were not significantly higher than the allergy control in group 2 (10.85 ng/mL) (p=0.180), thus the second hypothesis was not proven.

IL-17 levels in the stress treatment in group 3 (26.38 pg / mL) were not significantly higher than the allergy control in group 2 (22.36) (p = 0.394), thus the third hypothesis were not proven.

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