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The Variation Levels of Serum MC3R in the Group of Active TB Patients, Household Contact and Healthy Person as a Control and Correlation with Their Nutritional Status

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Abstract. Gene melanocortin 3 (MC3R) play a role in various biological systems such as body composition, natriuresis, immune function, and circadian rhythms which associated with pathogenesis of tuberculosis. This study investigated the MC3R serum levels on groups of TB status, household contacts, healthy control, and the nutritional status of different groups. The samples of this study were 53 active TB patients, 49 household contacts, and 30 healthy subjects as controls. We analyzed blood samples using IGRA and ELISA Melanocortin 3 test. The results shown average of MC3R levels on different health status groups are active tuberculosis 1259.55 (10.41 to 11279.04); household contacts 535.15 (51.18 to 3757.27); and control 503.26 (10.91 to 1486.58), significantly different with p = 0.028. The average MC3R levels on different nutritional status groups are BMI less 879.26 (10.91 to 11279.04); normal BMI 861.74 (10.41 to 6421.46); excessive BMI 697.28 (70.85 to 4052.48) There is no significant difference with p = 0.817. High MC3R levels in TB patients associated with its role as the main transducer of the anti-inflammatory effect, whereas high MC3R levels on BMI status is insufficiently linked to the physiological role assigned MC3R encodes MSH receptor which is closely related to Leptin excretion.

1. Preliminary

Tuberculosis is no longer a disease unfamiliar to the world community, especially in Indonesia. TB is a global disease, is found in every country in the world and became one of the 10 causes of death worldwide[1,2,3]. WHO estimates that two billion people or one-third of the world's population infected with the bacteria that cause tuberculosis[1]. TB occurs in every part of the world. According to WHO, 2016, 10.4 million people fell ill with TB and 1.7 million died of TB disease. More than 95% of TB deaths occur in low and middle income countries. The largest number of new TB cases occur in Asia, with 45% of new cases, followed by Africa, with 25% of new cases[2]. TB patients in Indonesia amounted to 842 000 inhabitants in 2017,TB patients consisted of 492,000 men, 349 000 women and 49,000 children. The number of cases of tuberculosis Indonesia is the third biggest in the world after India and China[3].

TB is caused by Mycobacterium tuberculosis that primarily attacks the lungs causing pulmonary disease and is transmitted through exposure to the air[4]. Today, TB transmission so easily occur due to contact with the patient, and the most widely spread are the closest of patients[5]. Not finished until there, the nutritional status of patients with TB then also be important to investigate. Body mass index is low is a known risk factor for TB, TB is often associated with the incidence of malnutrition that bias into factors that worsen the condition of people with TB, it is suspected because of the involvement of genes melanocortin 3 (MC3R) which acts interfere with the metabolism of patients resulting in disruption intake nutrition and the occurrence of anorexia (decreased appetite) in patients[6,7].

2. Research Methods

2.1. Research Design and Respondents

A total of 132 respondents were invited in this study for a blood sample, and divided into three groups: patients with active TB, contact the group at home, and the healthy group. 53 respondents were active TB patients with three categories of body mass index, 49 respondents were household contacts were also selected from three categories of body mass index, and 30 respondents who are healthy individuals were also selected from three different categories of body mass index. TB patients were recruited from the Central Public Health Lung Makassar, South Sulawesi, Indonesia and selected household contacts of TB patient's next of kin. Healthy controls were taken from various places in Makassar, Indonesia with a wide range of ages, genders. All TB patients are diagnosed based on clinical manifestations, thorax, and BTA.

We collected blood samples from each participant as much 6-7cc household contacts and healthy controls for examination in the laboratory IGRA HUM-RC (Hasanuddin University Medical-Research Center) Makassar, Indonesia. Blood samples obtained centrifuged at 4400 rpm for 10 minutes at a temperature of 250C for separating plasma samples. Plasma samples were stored at -200C prior to ELISA.

This study was approved by the Research Ethics Commission of the Medical Faculty of Hasanuddin University in Makassar, South Sulawesi, Indonesia (No. 583 / H4.8.4.5.32 / PP36-KOMETIK / 2018) and all study subjects gave written informed consent.

2.1.1. Statistic Analysis

All the experimental data were analyzed using SPSS 21 software, where $p < 0.05$ was considered statistically significant. Having obtained the results of ELISA analysis will be performed to assess differences MC3R levels in each group. Statistical analysis using ANOVA test to distinguish between the value of the group. Data are presented as mean (min-max), then the Post Hoc test to see significant differences between the two variables.

3. Result

All the experimental data were analyzed using SPSS 21 software, where p <0.05 was considered statistically significant. Having obtained the results of ELISA analysis will be performed to assess differences MC3R levels in each group. Statistical analysis using ANOVA test to distinguish between the value of the group. Data are presented as mean (min-max), then the Post Hoc test to see significant differences between the two variables.

Table 1. Distribution of BMI Status and Status Based on TB.

variables	Mean (Min-Max)	P
Active TB	1259.55 (10.41 to 11279.04)	
Household contacts	535.15 (51.18 to 3757.27)	P = 0.028
Healthy controls	503.26 (10.91 to 1486.58)	
BMI Less	879.26 (10.91 to 11279.04)	
Normal BMI	861.74 (10.41 to 6421.46)	P = 0.817
Excess BMI	697.28 (70.85 to 4052.48)	

The average value of MC3R highest levels are in the group of active TB is 1259.55 and the lowest at 503.26 with a healthy control group p = 0,028 which means there is a significant difference MC3R levels in all three groups. While the average value of the levels of MC3R based nutritional status is different, where the highest values are in the BMI group is less that 879.26 and the lowest at 697.28 which excess BMI group with a value of p = 0.038, which means there are no significant differences in the levels of MC3R in all three groups.

Table 2. Results of Post Hoc Test To Determine The significance of difference between the two variables MC3R levels Based on Their Health Status.

Group	P	Conclusion
Active TB - Household Contacts	0,019	Significantly Different
Active TB - Healthy Controls	0,033	Significantly Different
Contacts at home - Healthy Controls	0,929	Not Significant Contrast

Based on the Post Hoc Test MC3R known differences in levels between the groups and their household contacts of active TB significantly different with p = 0.019 (p <0.05), and a control group of healthy active tb sifnifikan also different with p = 0.033 (p <0.05), while the control group of healthy household contacts and there is no significant difference with p = 0.929 (p> 0.05).

Table 3. Post Hoc Test Results To Know the Difference Significance MC3R levels on two variables Based on Body Mass Index.

Group	P	Conclusion
BMI less - Normal BMI	0,625	Not Significantly different
BMI less - BMI excess	0,625	Not Significantly Different
Normal BMI - BMI excess	0.916	Not significant contrast

Based on Post Hoc LSD test known differences between groups MC3R levels and BMI less normal BMI was not significantly different with p = 0.625 (p> 0.05), less BMI and BMI group excess

sifnifikan also not different with p = 0.625 (p> 0.05), whereas the normal BMI group and excess BMI is not a significant difference with p = 0.916 (p> 0.05).

4. Discussion

4.1 Comparative Levels of MC3R on Health Status of Different Groups

In this group there are significant differences MC3R levels of the three groups with different health status, with p = 0.028. The group of active TB has the highest levels and the healthy control group had the lowest levels.High levels of MC3R in active TB associated with its role in maintaining energy homeostasis, immune and suppresses inflammation[8]. In his role in the regulation of human physiological responses, one of which plays a role in immune activity is α-MSH. α-MSH is one form of MSH polypeptide consisting of 13 amino acids[9]. In the pathophysiology of the disease process, α-MSH suppress inflammation and encourage activation of inflammatory activity and immune regulation through the MC1R, MC3R and MC5R, while in macrophages, α-MSH to mediate macrophage activation alternative which will suppress inflammation through the MC1R and MC3R[10, 11]. Macrophages are the body's defenses, which are part of the innate immune system / innate which gives the body the ability to destroy mikrobakteri attack[12]. In the process of TB pathogenesis, war between Mycobacterium and macrophages will produce two possible if strong macrophage cells, it will destroy Mtb, but if macrophages weak, then Mtb will continue to replicate inside macrophages, the system imuni specific body will continue to work to attack Mtb, macrophages will surround Mtb and forming nucleated cells a lot, this is what will lead to increased levels of melanocortin including MC3R who also plays an important role in encourage anti-inflammatory activity and immune regulation against Mtb[13]. This allegation is certainly supported by the results obtained that MC3R lowest levels are in the healthy control group indicating MC3R stable levels in a healthy condition without inflammation activity.

4.2 Comparative Levels of MC3R on Nutritional Status of Different Groups

In this group there is no significant difference MC3R levels of three groups with different nutritional status, with p = 0.817. Although not significantly different, but the group had a BMI less than the average level of the highest MC3R and excess BMI group had an average of the lowest levels of MC3R. Human body weight is a complex trait that is determined by the interaction between environmental influences and genetic risk factors[14]. Melanocortin 3 is a gene that encodes the H-protein-receptor pairs for melanocyte-stimulating hormone (MSH) and adrenocotropic hormone expressed in tissues other than the adrenal cortex and melanocytes. One of the main regulator of MSH is leptin levels. Leptin is released from adipocytes and acts to modulate appetite and energy expenditure[15]. Leptin levels comparable to the number of adipocytes and remain relatively constant even though there are variations in consumption and daily energy expenditure[16]. MSH increases leptin levels by increasing the expression of pro-opiomelanocortin (POMC), the precursor to MSH[17]. When leptin pass through blood vessels/brain, it binds to the leptin receptor expressed on melanocortin neurons and increased the expression of MSH, binding of MSH to MC3R result in decreased efficiency of appetite[18]. Based on this theory, it is known that high levels of MC3R the low BMI group and low levels of excess BMI MC3R group closely related to the physiological process of neuropeptide above. Other studies that have been done by Butler et al (2000) and Chen et al (2000) after the removal of specific MC3R on rats, the results show an increase in fat mass although not significant in body weight and an increase in food intake measured[19]. As consideration, the present study found many cases low BMI in patients with active TB, and when looking for the link between TB and TB patients with a body mass index less, it can be associated with activation of MSH in maintaining energy homeostasis[8].

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5. Conclution

MC3R high levels in patients with active TB is believed to be closely related to the activity of immunity against Mtb, this also explains the reasons respondents MC3R healthy levels stable, because no significant immune activity occurs. At respondents with a body mass index less, MC3R level was increased compared with two other groups, it is associated with the process of energy homeostasis. The other interesting thing is the majority of TB patients have less body mass index, and researchers believe that the condition is controlled by MSH peptides directly related to MC3R.

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