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Expression Analysis of mRNA Interleukin-8 and (mRNA) Monocytes Chemo Attracting Protein-1 (mRNA MCP-1) in Bruise Age Determination

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Abstract

The number of incidents of injuries in the hospital for a criminal case requires examination report including wound age in accordance with Article 133 Criminal Procedure Code. This study aims to determine the bruise age of the wound through mRNA expression of IL-8 and MCP-1 mRNA in body bruises. Using qPCR techniques: extraction of mRNA of IL-8 and MCP-1 mRNA from blood samples by the method of Boom. Results showed differences in mRNA expression of IL-8 and MCP-1 in healthy bodies (ie 8.70 and 12.74 Log Log). There is an increased expression of mRNA of IL-8 and MCP-1 gradually by the age of the wound 14 hours, 28 hours and 34 hours for mRNA expression of IL-8, while 28 hours and 34 hours for mRNA expression of MCP-1. There are variations in mRNA expression of IL-8 and MCP-1 mRNA in each age group wound. This suggests that there are several other factors that affect mRNA expression of IL-8 and MCP-1 is not yet known, so further research is still needed.

Keywords: mRNA IL-8; mRNA MCP-1; bruises.

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1. Introduction

The increasing cases of injury due to mechanical trauma is one health problem in Indonesia even in the world. Police Wakabareskrim report on the activities of the Ministry of Law and Human Rights in 2012, said that every 91 seconds a crime occurred in Indonesia [1]. This is in line with the number of incidents of injury due to a criminal case is found almost all over the hospital in Indonesia, including in remote health centers. For the sake of justice is very important to explain the life of wounds on the examination report was wounded by a doctor in accordance with Article 133 Book of the Criminal Procedure Law (Criminal Cases) This relates to the series of processes of trauma (patobiology injury).

In Section Forensic Hospital. Bhayangkara Makassar injury cases from 2010 to 2011 were accompanied by requests Visumet Repertum (SPV) by the investigator reaches approximately 60% (Section Forensik-Medikolegal UNHAS). Bruises are the highest first order of all the types of injuries. ie 44% in RS. Bhayangkara Makassar and 43% in RS.Wahidin Makassar. In criminal cases 7 of 12 cases per day (58%) that there are differences in the expected life of bruises that most injuries at age> 24 hours between the results of medical examination with a victim description. namely the age of 1-4 days wound found in brown-skinned patients [1].

The process of changing the color of bruises very many factors that influence it. The body will respond in the form of the inflammatory reaction by releasing several products, among others leukocytes; eg, neutrophils, monocytes, leukocytes as a defense body releases cytokines (IL-1, IL-8, MCP-1, MIP-1α, etc.) as the conducting signal receptor on leukocytes to the site of injury. While cell injury will also release cytokines product to the extent that the healing occurs [2]. The role of monocytes replace neutrophils after 24 hours and ages monocytes are longer than other leukocytes by [2] showed that launched products by leukocytes can be a marker to tell the age of the wound is more than 24 hours after the trauma. While the marker for wound age less than 24 hours performed by Interleukin-8 released by macrophages to give a signal to the neutrophils to the place of injury or injuries [3,4].

To view the production of signal interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) course by analyzing the source of production of the body's cells, especially neutrophils and monocytes through tier level of molecular namely the mRNA of IL-8 mRNA MCP -1. It is known that everything played cell activity information in the cell nucleus (nucleus). Inside the cell nucleus are chromosomes. This DNA is on chromosome. DNA for protein synthesis to send a message with the help of RNA (there are three types of RNA; mRNA, rRNA, tRNA). It is known that interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) is a type of chemokine a family of small proteins expressed by various cell types [5].

DNA sends a message to the synthesis of proteins with the help of mRNA that mRNA expression of interleukin-8 (IL-8) and mRNA Monocyte chemoattractant protein-1 (MCP-1), which brings neutrophils, monocytes and the number of cases of DM in Indonesia known as up-regulation of IL-8 and MCP-1 is important for further research in wound age determination. By knowing the mRNA expression of IL-8 and MCP-1In mRNA wound age is expected to help law enforcement investigate about who, what, where, how, why and when of trauma until the injury occurred.

2. Materials and Methods

Design Prospective observational longitudinal cohort study. Places in Makassar Mappaoudang Police Hospitals. The study population was men and women consist of children and adults, aged 15-45 years with a body weight ideal. Total sample was 25 patients. Samples were divided into 3 major groups: group I (group unharmed, healthy) of 5 patients. Group II (sample number 10) were age bruising <24 hours (8 hours and 14 hours, the same patients) with or without a history of diabetes in the family and group III (sample number 10) were age bruise> 24 hours (28 hours and 34 hours, the same patients) with or without a history of diabetes in the family blood sampling from cubital vein as much as 3 cc.

One drop of blood was added to the tube L6 for examination mRNA levels of IL-8 and MCP-1 mRNA and the rest for examination when blood sugar levels, cholesterol and uric acid to meet the inclusion and exclusion criteria. Up to 25 samples collected and stored in a temperature -200c in Microbiology Laboratory of the University of Hasanuddin. RNA extraction method, Blood was added to 500 mL of lysis buffer L6 on the tube that has a cover [6].

This mixture is then centrifuged at 12,000 rpm for 10 minutes. Sediment samples were concentrated is homogenized for 30 minutes. Before the suspension was added diatoms, L6 buffer mixture which already contains RNA extraction centrifuged 2-3 minutes at a speed of 12,000 rpm, with the aim that RNA results settle to the bottom of the tube. 20 mL added atom suspension into the tube, the suspension of diatom should seal rotated and stirred using a gyratory shaker at 100 rpm for 10 minutes. L6 buffer mixture of diatoms and rotated back to using a centrifuge eppendorfmikrosentrifus at 12:00 rpm speed for 15 seconds. Supernatant from each tube is formed is separated using a suction made of pasteur pipette and connected with a vacuum pump to prevent loss of diatoms in suspension earlier. Approximately 10 mL of the suspension is left [7]. Supernatant was washed two (2) times using 1 ml of buffer washers L2. L2 washing buffer is added 1 ml, rotated and centrifuged at 12,000 rpm for 15 seconds, then the supernatant was discarded. The precipitate is washed again with 1 ml of 70% ethanol for 2 (two) times, and then rotated and centrifuged at 12,000 for 15 seconds. The supernatant was discarded, the precipitate was washed again with 1 ml acetone, rotated and centrifuged at 12,000 rpm for 15 seconds, then re supernatant discarded. Acetone remaining in the sediment (sediment) evaporated by opening the cover tube and heated in an oven at a temperature of 50-550C for approximately 10 minutes. After the sediment dries, TE elution buffer was added about 60 ml then rotated evenly so that the suspension of sediments and soluble. Then the tube was incubated in an oven at 560 for 10 minutes. Then the mixture was centrifuged at a speed of 12,000 rpm for 30 seconds. Supernatant taken as much care as 40-50 mL of the supernatant and put in a new tube. Extraction results stored at a temperature of -800C All PCR repeated 3 times and the data were analyzed by the instrument detection system MX4000 using comparative threshold cycle method (using the comparative threshold cycle method). Standard curve was constructed and is an indication of good amplification efficiency (90-100%).

3. Results and Discussion

Of the 25 patients were divided into 2 age groups 15-30 years and 31-45 years consisted of 6 male patients are

two healthy adult males, one boy and three healthy adult males in the group bruises <24 hours and 19 female patients that two healthy adult women, 7 women in the group of bruises <24 hours, 9 women in the group bruise> 24 hours and one girl in the group bruise> 24 hours.

Results Group I obtained mRNA expression of IL-8 and MCP-1 from the 5 healthy samples showed no difference in expression between healthy samples with each other healthy samples with an average of IL-8 mRNA expression healthy Log 8.70 while the average expression MCP-1 mRNA 12.74 Log. The big difference in mRNA expression of IL-8 and MCP-1 mRNA expression in healthy groups showed that the cells produced MCP-1 is far more than cells that produce IL-8 [8, 9].

In group II, bruises <24 hours as many as 10 samples were obtained difference in the average yield of IL-8 mRNA expression in the age group of 8 hours and the wound 14 hours, as well as the expression of MCP-1 mRNA 8 hours and 14 hours. Increased IL-8 mRNA expression was found in the age of the wound 14 hours, whereas the mRNA expression of MCP-1 at the age of 14 clock wound together with MCP-1 mRNA expression in the healthy group. This can be explained by the theory that in the age of the wound 14 hours, neutrophils are the most powerful cells play a role among other leukocyte cell injury beginning. Age wound 8 hours showed mRNA expression of MCP-1 lower than the value of MCP-1 mRNA expression in the healthy group. This means that at the age of 8 hours of the injury in the event of damage, the mRNA expression of MCP-1 is not active, therefore this cell played roles by macrophage cells that exist in the network. Macrophages play an important role in the wound healing process. In early injury, the macrophages release cytokines that trigger the response of inflammatory cells by activating additional leukocytes. Macrophages also responsible for inducing and clean the cells undergoing apoptosis (i.e. neutrophils), so it is a way to resolisi inflammation [10, 11].

In group III, bruise> 24 hours as many as 10 samples were obtained difference in the average yield of mRNA expression of IL-8 in the age group of the wound 28 hours and 34 hours, as well as the mRNA expression of MCP-1 in the age group wound 28 hours and 34 hours, There is an increased expression of the two markers. Indirectly show neutrophils and monocyte cells play a role at the same time and together in the age of the wound 28 hours and 34 hours for the process of hemostasis, inflammation, proliferation, the process returns to normal (remodeling) [12,13].

4. Conclusion

Obtained differences in mRNA expression of IL-8 and MCP-1 in healthy bodies (ie 8.70 and 12.74 Log). There is an increased expression of mRNA of IL-8 and MCP-1 gradually by the age of the wound 14 hours, 28 hours and 34 hours for IL_8 mRNA expression, whereas 28 hours and 34 hours for mRNA expression of MCP-1. Not a lot of some of the sample variations in mRNA expression of IL-8 and MCP-1 mRNA in each age group wound. This suggests that there are several other factors that affect mRNA expression of IL-8 and MCP-1 is not yet known, so further research is still needed Not a lot of some of the sample variations in mRNA expression of IL-8 and MCP-1 mRNA in each age group wound. This suggests that there are several other factors that affect mRNA expression of IL-8 and MCP-1 is not yet known, so further research is still needed

Conflict of Interest

Author declare no conflict of interest within this study

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