

# Role of Superoxide Dismutase (SOD) Gene Ala16Val in Sepsis

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**Abstract:- Sepsis is a systemic inflammatory response syndrome (SIRS) accompanied by clinical manifestations of infection. Sepsis is a common condition in the intensive care unit that is often associated with mortality and morbidity. Increased reactive oxygen species (ROS) or oxidants will occur in sepsis conditions especially by polymorphonuclear leukocytes (PMNs) in response to bacterial endotoxin.**

**The manganese superoxide dismutase (MnSOD) antioxidant enzyme provides the main defense against ROS in mitochondria. Structural or functional single nucleotide polymorphisms (SNP) in the MnSOD coding gene are relevant for ROS detoxification. In particular, MnSOD Ala16Val SNP shows that it can change the localization of enzymes and mitochondrial transport, and affect the balance of redox status.**

**This study aimed to determine the MnSOD Ala16Val polymorphism genotypic in 60 patients with sepsis compared to 60 healthy subjects. The results showed that the distribution of SOD gene Ala16val genotype, the Val/val genotype had the highest frequency both in the sepsis group and in the healthy control group, but showed no statistically significant differences.**

**Keyword:- Sepsis, MnSOD, Ala16Val.**

## I. INTRODUCTION

Sepsis is a systemic inflammatory response syndrome (SIRS) accompanied by clinical manifestations of infection in the form of temperatures  $>38.3$  C or  $<35.6$  C, heart rate  $>90$  times/minute, frequency of breath  $>20$  times/minute or PaCO<sub>2</sub>  $<32$  torr ( $<4.3$  kPa), leukocyte count  $>12,000$  cells/mm<sup>3</sup> or  $<4000$  cells/mm<sup>3</sup> or found  $>1\%$  of immature cells. Sepsis is a common condition in the intensive care unit that is often associated with mortality and morbidity. (Lever and Mackenzie, 2007). The World Health Organization (WHO) estimated that in 2011, 12 million Indonesian people diagnosed with critical illness of sepsis with mortality at the age of 15 - 60 years which equals to 366/1000 populations with 200 men and 166 women (WHO, 2013).

Increased reactive oxygen species (ROS) or oxidants will occur in sepsis conditions especially by

polymorphonuclear leukocytes (PMN) in response to bacterial endotoxin. ROS is very reactive so it is easy to react with other compounds such as lipids, DNA and proteins. The oxidation of ROS to these compounds causes damage to tissues or organs so that it can cause disease. ROS that continuously increases beyond the ability of cell tolerance causes endogenous antioxidant depletion which is also a risk factor for sepsis mortality (Leonardo et al, 2015). The antioxidant defense system works against free radicals. Every antioxidant reduces the work of free radicals with different mechanisms. One of the antioxidants that experienced depletion in the sepsis condition is superoxide dismutase. Superoxide dismutase is an antioxidant that acts to convert O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub> to prevent organ dysfunction due to cell wall lysis. Organ dysfunction is a risk factor for high morbidity and mortality in critically ill patients and sepsis conditions. (Paludo et al, 2013)

The MnSOD (Manganese superoxide)/SOD gene is located in chromosome 6q25. This is the only antioxidant enzyme known today in mitochondria. Seeing the relevance of MnSOD as the first line of defense against the production of ROS, structural and functional SNP (single nucleotide polymorphism) from coding the MnSOD gene is very important in maintaining the level of ROS in cells. In humans, at least 190 SNPs have been identified as MnSOD. (Bresciani et.al, 2013). Ala16Val is the MnSOD functional gene variant and the most widely studied in exon 2 SOD2 genes. A functional polymorphism in exon 2 of the Ala16Val SOD2 gene has been identified to cause structural changes in the mitochondrial targeting domain, which has implications for reducing the potential for limited antioxidants in post-transcription transportation. (Banerjee M, Vats P., 2013). The replacement from C to T (GCT to GTT), alanine to valine, results in structural changes in the mitochondrial targeting domain from sheet  $\beta$  to  $\alpha$ -helix, which induces a 30-40% increase in MnSOD activity in mitochondria. (Vats P, Sagar N, Singh TP, Banerjee M., 2015).

We hypothesized that the MnSOD gene variant Ala16Val might influence the functional activity of the MnSOD enzyme on mitochondrial function. This can affect the mitochondrial response to sepsis. The aim of this study was to determine genotypes in patients with sepsis compared to healthy subjects and the functions of Ala16Val polymorphisms.

**II. RESEARCH METHODS**

➤ *Types of Research*

This study is a case-control study, an analytic study by looking at the genetic variation - SOD ala16val (C/T) in septic patients compared to healthy people.

➤ *Place and Time of Research*

This research was conducted at the University of North Sumatra Faculty of Medicine Integrated Laboratory and the University of North Sumatra Hospital. This research was conducted after obtaining approval from the University of North Sumatra Medical Research Ethics Commission. The research period started from April 2018 to November 2018.

➤ *Research Population And Samples*

1. The research population was septic patients who had been diagnosed by specialist doctors and healthy people as controls.
2. The research subjects were a portion of the population who met the research criteria selected by consecutive sampling, stated their willingness to participate in the study in writing, and had signed the subject's consent sheet.

➤ *Sample Size*

The minimum number of samples used in the study is 60 samples for each group

➤ *Work Procedures*

The researchers first requested the approval of the Ethical Clearance Committee of the University of North Sumatra Faculty of Medicine.

➤ *Stages of Sample Inspection*

1. DNA isolation was carried out using manual techniques, with commercial kits. The material used was 5x reaction buffer, nuclease free water, enzyme mix, spike in (sp6), micropipette tips (1-10 µL).
2. PCR-RFLP was performed to see the polymorphism. The tools used were micropipette (1-10 µL; 100-1000 µL), vortex, Eppendorf tube, PCR tube, PCR-RFLP. The ingredients used were Primary, master mix, agarose, DNA leader, TAE buffer, micropipettes tips (1-10 µL; 100-1000 µL) and restriction enzymes each Nco1 for SOD gene ala16val (C/T)

➤ *Data Analysis*

The data would be analyzed statistically using the SPSS program. Genotypic distribution and frequency of individual alleles in equilibrium were analyzed using Hardy Weinberg (HWE) analysis. From the results of the analysis, it is found that the population is said to be in equilibrium if the p-value <0.05. Genotypic distribution and frequency of alleles and their role in DM disease with TB will be tested for significance by using chi-square test (x2)

**III. RESULTS**

➤ *Research Result*

A series of studies had been conducted, recorded, the blood samples had been taken and the data had been analyzed to determine SOD gene variants ala16val (C/T) in septic patients compared to healthy people. This research was conducted after the Ethical Clearance was issued by the Ethics Commission of University of North Sumatra Faculty of Medicine.

This study is a case-control study, an analytic study by looking at the genetic variation - SOD ala16val (C/T) in septic patients compared to healthy people. To assess sepsis, the MSOFA score was calculated when the patient entered the Intensive care unit, then a venous blood sample was taken for laboratory examination. The blood was stored in an EDTA tube and taken to a laboratory with a storage temperature of 2 - 8°C. The assessment of the SOD gene variant ala16val (C/T) was carried out at the University of North Sumatra Faculty of Medicine Integrated Laboratory. From the process of 2 times sampling of sepsis patients and control samples (healthy people) the characteristics of the samples of the study can be seen in table 4.1 below:

Variables	Sepsis Patients	Control
Age (years old)	53,14 ± 13,46	45,2 ± 10,9
Male	36 (60%)	24 (40%)
Female	32 (54%)	28 (36%)

Table 5.1 Characteristics of the study samples

Based on table 5.1 above, it is seen that the characteristics of the research subjects in sepsis patients based on sex were 36 males in the highest percentage of 60%.

The process of DNA isolation in samples that produce DNA isolates had been examined with DNA purity that was feasible to be used as research samples. PCR and RFLP results from the SOD gene ala16val (C/T) are shown in figures 1 and 2 below.

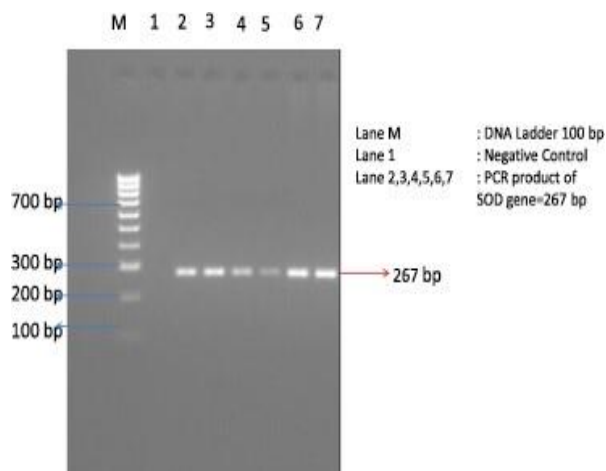


Fig 1:- PCR of the product from the SOD gene ala16val (C/T) at 267bp

Ala16val gene SOD genotype from sepsis patients and healthy controls was assessed by direct counting and analyzed the relationship with alias chi square with the results shown in table 2

Allele	Sepsis	Control	P
Ala/ala	5 (71,4%)	2 (28,6%)	0.312
Ala/val	10 (40%)	15 (60%)	0.304
Val/val	45 (51,1%)	43 (48,9%)	0.875

Table 2:- Comparison of genotype frequencies of the SOD gene Ala16val polymorphism in the sepsis group with the healthy control group

This study is the first study to report the Ala16val genotype in septic patients. In table 2, the distribution of the SOD gene Ala16val genotype shows that the Val/val genotype has the highest frequency both in the sepsis group and in the healthy control group.

The results of this study showed that there were more val/val genotypes in sepsis patients compared to healthy controls but it did not show statistically significant differences. In line with a study reported in a community in Brazil (Montano MA et al 2009), Ala16val is also associated with other metabolic diseases. Val/val genotypes are also associated with obesity, regardless of sex, age, diabetes, dyslipidemia, hypertension, and metabolic syndrome. In another study, we found an association between this SNP MnSOD genotypes and hypercholesterolemia and biomarkers of oxidative stress, with pro-oxidative status associated with val/val genotypes in hypercholesterolemic patients (Duarte MM et al. 2010)

#### IV. CONCLUSIONS

Conclusions of the 120 respondents, there were more male samples than female. The SOD gene Ala16val (C/T) showed the Val/val genotypes as the most variant found in the Sepsis group and control group. There was no association of SOD genotype ala16val in the sepsis group with the control group

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