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The effect of antibiotic therapy on Salivary Catalase kinetic parameters in neonatal at risk of Sepsis



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ABSTRACT

Introduction: This study describes the effect of antibiotic therapy on salivary catalase kinetic parameters (CAT) at neonatal at risk of sepsis.

Methods: Study is conducted from February – June 2015. Salivary samples are obtained from 20 neonates (5 normal-healthy neonates and 15 neonates at risk of sepsis) at Ulin General Hospital, Banjarmasin, Indonesia. The samples were placed in four different groups: P0 as control group (saliva + hydrogen peroxide/H₂O₂), P1 (saliva + 2 mg Ampicillin + H₂O₂), P2 (saliva + 0.2 mg Gentamicin + H₂O₂), P3 (saliva + 2 mg Ampicillin + 0.2 mg Gentamicin + H₂O₂). The solution is incubated at 37°C and 40°C before catalase (CAT) activity is measured. Catalase activity is measured by using a spectrophotometer at 240 nm. Kinetic parameters are measured at different concentrations of H₂O₂ and temperature. Kinetic

parameters are represented by the Michaelis-Menten constant (*K_m*) and the maximum reaction speed (*V_{max}*) obtained through the Lineweaver-Burk curve plot.

Results: The *K_m* of Catalase on the saliva of neonates at risk of sepsis treated with antibiotics (4.37, 1.84, 0.12, and 0.23, 3.74, 1.5, for P1, P2, P3 respectively) was lower than the control group (17.61 and 12.54), both at 37°C and 40°C. Similarly, *V_{max}* of the neonates at risk of sepsis treated with antibiotics (0.46, 0.34, 0.04 and 0.07, 0.20, 0.24) was lower than the control group (1.47 and 0.53) at 37°C and 40°C.

Conclusion: The study shows that the Catalase activity at the saliva of newborns at risk of sepsis treated with antibiotic was lower than the control group.

Keywords: Ampicillin, Gentamicin, Catalase, kinetic parameters, neonatal sepsis, saliva

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INTRODUCTION

Neonatal sepsis remains the highest cause of neonatal morbidity and mortality despite much progress in the management of neonatology.^{1,2} In developing countries, the number of neonatal deaths due to sepsis is 34 per 1000 live births, mainly occur in the first week of life.³ In Indonesia, the infant mortality rate is 19 per 1000 live births, or around 236 babies die every day. Neonatal sepsis contributes to 20.6% deaths at the first 28 days and about 12% deaths at the first six days.⁴

Neonatal sepsis is a clinical syndrome of a systemic disease that is accompanied by bacteremia and occurs in the first month of life. This condition could be defined clinically and/or microbiologically by positive blood or cerebrospinal fluid cultures.⁵ In neonatal sepsis, the activation of leukocytes and inflammatory cells results in massive production of reactive oxygen species (ROS) in the body.⁶ Initially, ROS are generated by enzymes as a defense mechanism against infective agents.⁷ Hydrogen peroxide (H₂O₂) is part of the enzymatic, mechanical

defense that can be found in blood and saliva.⁸ It is not specific and can harm the patient because it is also toxic to human cells.⁹ The production of ROS in neonatal sepsis has been documented in various studies, including our previous studies that have shown an increase in ROS such as peroxide in neonatal sepsis.¹⁰ The increased ROS level is one of the predisposing factors for disease severity.¹¹

An excessive increase in ROS will activate endogenous enzymatic antioxidants such as Catalase (CAT) to prevent the accumulation of ROS in the body.¹² Human Catalase belongs to the monofunctional catalase group that contains heme, which is present in almost all aerobic organisms. Catalase is mainly intracellular (in cells) with the highest concentration found in red blood cells, liver, kidneys, and saliva.^{13,14} Catalase is a tetrameric protein with a molecular weight of 244 kDa and symmetry of 222, consisting of four identical subunits weighing 59.7 kDa. Each subunit contains 527 amino acid residues, a heme group called iron (III) protoporphyrin IX, and a tightly bound

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NADPH molecule. Catalase is responsible for the decomposition of H_2O_2 into water and oxygen. Under prolonged exposure to H_2O_2 , Catalase binds to NADPH, oxidizes NADPH to $NADP^+$ and its activity falls to one-third of its initial activity.¹³

Ampicillin, Gentamicin or a combination of both are the first-line antibiotics that have been widely used in the management of neonatal sepsis.^{15,16} In its development, it is believed that antibiotic therapy plays a certain role in the production of ROS and the disruption of enzymatic antioxidants activity such as catalase (CAT).¹⁷⁻¹⁹ There have been several studies on the effects of antibiotic therapy on catalase activity. The study of Dwyer et al. proves that bactericidal antibiotics dynamically alter cellular respiration and induce lethal levels of H_2O_2 . On the other hand, antioxidants significantly reduce the process of killing cells by antibiotics.¹⁷ However, how antibiotics affect the endogenous enzymatic defense system or vice versa remains unknown to date. This research will measure the saliva catalase kinetic parameters in neonates at risk of sepsis and the effect of antibiotics on the CAT kinetic parameters in vitro.

METHODS

The study was conducted in February-June 2015 at the neonatal intensive care department of the Ulin General Hospital Banjarmasin. Biochemical analysis was carried out in the biochemistry laboratory of the Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia. The samples include 20 neonates, consisting of 5 healthy infants (without risk factors for sepsis) as a control group and 15 infants at risk of sepsis. Infants with sepsis risk were defined as newborns with at least one major risk factor and or two minor risk factors. Major risk factors were: premature rupture of membranes >24 hours, intrapartum fever with temperature >38°C, chorioamnionitis, fetal heart rate >160 times/minute and persistent, smelly green amniotic fluid. Minor risk factors were: premature rupture of membranes >12 hours, intrapartum fever >37.5°C, low APGAR score (<first 5 minutes, <fifth 7 minutes), very low birth weight (<1500 grams), gestational age <37 weeks, multiple pregnancies, itchy vaginal discharge with a foul smell, mothers with urinary tract infections or suspected untreated urinary tract infections.⁸

About three ml saliva specimens were taken from the oropharynx of each neonates using mucus extractor/suction according to the standard neonatal resuscitation procedures. The parents were informed about the study and has given written consent prior to the procedure. The procedure has been approved by local Ethical commission prior

to the study. The saliva from the control group was mixed with H_2O_2 (Saliva + H_2O_2), Saliva samples from infants at risk of sepsis were divided into three groups, P1 (Saliva + 2 mg ampicillin + H_2O_2), P2 (Saliva + 0.2 mg Gentamicin + H_2O_2), and P3 (Saliva + 2 mg ampicillin + 0.2 mg gentamicin + H_2O_2). All the solutions were incubated at 37°C and 40°C for an hour.

Catalase activity was measured by the Aebi method using a spectrophotometer at a wavelength of 240 nm.²⁰ The activity was defined as the amount of H_2O_2 (in mmol) used per minute in a phosphate buffer solution (50 mM, pH 7). The study was conducted at two different temperatures, 37°C dan 40°C. The kinetic parameters were determined based on five different substrate concentrations of H_2O_2 . The substrate concentrations used were 10, 20, 30, 40 and 50 mM. Kinetic parameters in the form of Michaelis-Menten constant (K_m) and maximum speed (V_{max}) were determined using the Lineweaver-Burk plot of the Michaelis-Menten equation, which is as follows:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

V is the reaction speed, V_{max} is the maximum reaction speed, K_m is the Michaelis-Menten constant which represents the number of substrates needed to reach half the reaction rate, and S is the substrate concentration.²¹

RESULTS

The overall CAT activities in two different temperature were shown in Table 1 and plotted in Figure 1 and 2. The Lineweaver-Burk plot (Figure 1 and 2) shows the reverse graph of the substrate concentration plotted against the inverse of the H_2O_2 reaction rate. Figure 1 and 2 also show the linear regression equation and correlation index (r) for each group. Table 1 shows the K_m and V_{max} values calculated through the linear regression equation obtained from each group. It can be seen that the K_m value of the treatment groups (P1, P2, P3) is lower than the control group. This means that the administration of antibiotics reduces the K_m value of salivary Catalase.

DISCUSSION

The value of K_m is determined by the concentration of the enzyme, and kinetically describes the enzyme affinity for the substrate. The smaller the K_m value, the higher the affinity between the enzyme and

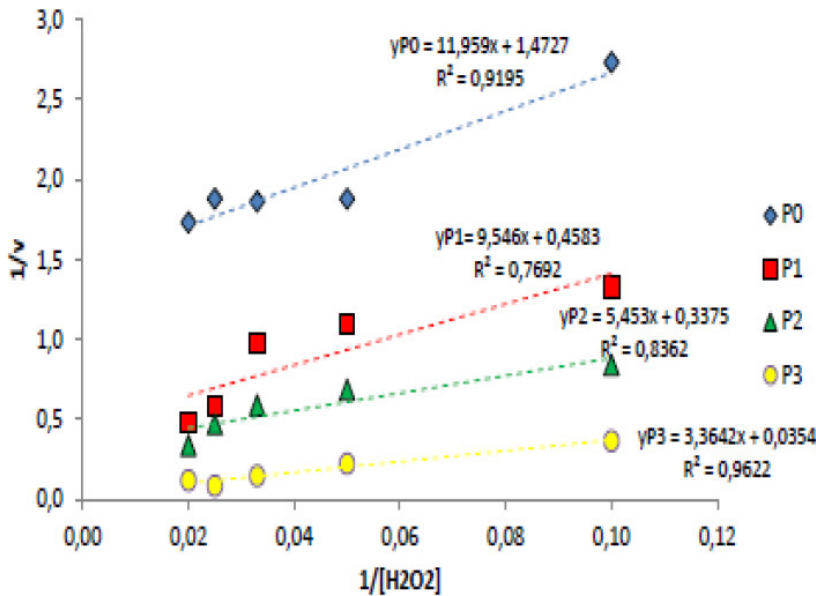


Figure 1. Lineweaver-Burk plot of the four groups at 37°C.

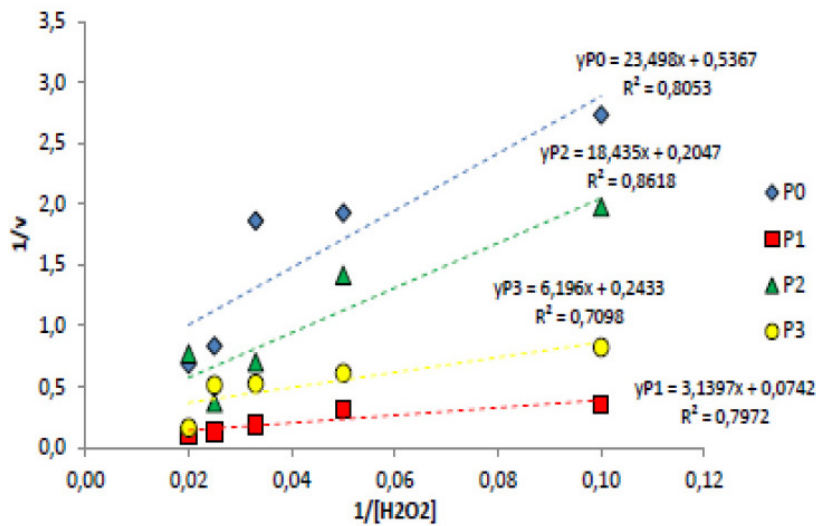


Figure 2. Lineweaver-Burk plot of the four groups at 40°C

Table 1. Effect of treatment on CAT kinetic parameters and correlation coefficient between substrate concentration and CAT activity

Group	Temperature					
	37°C			40°C		
	<i>Km</i>	<i>Vmax</i>	<i>r</i>	<i>Km</i>	<i>Vmax</i>	<i>r</i>
P0	17.61	1.471	0.959	12.545	0.532	0.899
P1	4.369	0.456	0.878	0.232	0.074	0.893
P2	1.840	0.337	0.915	3.742	0.203	0.928
P3	0.119	0.035	0.981	1.504	0.243	0.843

Km: Michaelis-Menten constant; *Vmax*: maximum reaction rate; *r*: correlation coefficient of substrate concentration against Catalase activity.

the substrate. *Km* indirectly shows the catalytic efficiency of the enzyme. The higher the value of *Km*, the better the catalysis power of the enzyme on the substrate.²¹ The result of the study indicates that the administration of antibiotics can affect the activities of enzymatic endogen by increasing the affinity of CAT-H₂O₂ complex in saliva. Nevertheless, it can be concluded that antibiotic therapy, including Ampicillin, Gentamicin, and a combination of both, can increase the affinity of CAT-H₂O₂. The highest affinity was found in the P3 group (a combination of ampicillin and Gentamicin), while the lowest affinity was found in the control group (P0) at 37°C (Table 1).

Ampicillin and Gentamicin have been used as a combination of antibiotics in empirical therapy for neonatal sepsis when bacteriological culture results are yet available. Ampicillin is a penicillin derivative and belongs to the beta-lactam group. It is bactericidal and potent, especially against gram-positive bacteria. Ampicillin is different from penicillin because of its amino group structure. The amino group in ampicillin helps the drug to penetrate the outer membrane of gram-negative bacteria. Ampicillin works as a competitive inhibitor of the transpeptidase enzymes that bacteria need to form cell walls. Ampicillin inhibits the bacterial cell wall synthesis.²² On the other hand, Gentamicin is a bactericidal aminoglycoside antibiotic. Gentamicin inhibits bacterial protein synthesis by binding to the bacterial ribosomal site. Gentamicin is used mainly for gram-negative bacterial infections.²³ The combination of Ampicillin and Gentamicin results on synergistic action against various pathogens that cause sepsis such as *Group B Streptococcus (GBS)*, *Enterococci*, *Listeria monocytogenes*, *Enterobacteriaceae (Enterobacter spp., Proteus spp., Escherichia coli)*. This combination has been used as a first-line treatment for neonatal sepsis in both developing and developed countries.²⁴

The result of this study shows that the administration of antibiotics (ampicillin and Gentamicin) indirectly helped the defense mechanism by increasing the H₂O₂ level in saliva. In vitro studies show that bactericidal antibiotics increase oxidative stress by directly inducing the ROS formation.^{19,25} One of the induction mechanism is believed to be through the Fenton reaction.¹⁷ In this study, Ampicillin and Gentamicin have been shown to increase ROS indirectly through CAT inhibition. The *Km* and *Vmax* value of CAT in this study decreased in the treatment group, especially in the P3 group with a combination of ampicillin and Gentamicin. Furthermore, the *Km* value decreased more than the *Vmax* value in all treatment groups. This indicates that CAT inhibition is more likely to

occur due to increased enzyme-substrate affinity than interference with CAT catalytic ability. This study also investigated the effects of temperature exposure on CAT kinetic parameters. K_m and V_{max} values were lower in all treatment groups at 40°C (Table 1). These results indicate that the increase in temperature also affects the increased affinity of the CAT- H_2O_2 complex of saliva with antibiotics.

CONCLUSION

Based on the findings above, it can be concluded that Ampicillin and/or Gentamicin reduce the value of K_m and V_{max} salivary Catalase in neonatal with a risk of sepsis. It is believed that Ampicillin and Gentamicin play a role in inhibiting Catalase by increasing the affinity of the CAT- H_2O_2 complex.

CONFLICT OF INTEREST

All authors declare there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors have contributed substantially during all phases of the study, involve in drafting and revising the manuscript, giving final approval and have agreeing to be accountable.

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