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**Submission date:** 24-Feb-2021 09:49AM (UTC+0700)
**Submission ID:** 1516678786
**File name:** of_Some_Co-Susbtrate_in_Saliva_of_Newborn_at_Risk_of_Sepsis.pdf (362.31K)
**Word count:** 2860
**Character count:** 14924

# Kinetic Parameter Analysis of Myeloperoxidase in The Presence of Some Co-Susbtrate in Saliva of Newborn at Risk of Sepsis

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## Abstract

**Background:** During infection like sepsis, myeloperoxidase (MPO) enzyme will be generated from the primary granules of neutrophil. MPO will catalyze the oxidation of halide or pseudo halide ($Cl^-$, $I^-$, $SCN^-$, $Br^-$) by using hydrogen peroxide ($H_2O_2$). However, the mechanism by which oxidation of these several co-substrates in neonatal sepsis is unknown. There is common belief that MPO will catalyze the oxidation of $Cl^-$ in sepsis rather than another compound. Here, we have compared the kinetic parameter of MPO in saliva of newborn at risk of sepsis with or without the presence of these several co-substrates.

**Methods:** In this experiment, saliva samples were taken from newborn in health (n=20) and risk of sepsis (n=20) condition. Saliva samples from each condition then homogenized and divided into four groups with; group 1 served as control which contains saliva + $H_2O_2$; group 2 which contains saliva + $H_2O_2$ + $Cl^-$; group 3 which contains saliva + $H_2O_2$ + $I^-$; and group 4 which contains saliva + $H_2O_2$ + $SCN^-$. After 1-hour incubation, the kinetic parameters (Km and Vmax) were analyzed.

**Results:** The result shows that in sepsis condition, compared without the presence of some co-substrates, it seems the addition of some co-substrate in sepsis condition will lead to decreasing of Km value, which means increasing affinity between MPO, $H_2O_2$, and the co-substrate. Between the three co-substrates, it seems in sepsis condition MPO will oxidize $Cl^-$ than another co-substrate.

**Conclusion:** In conclusion, in sepsis condition MPO works by a common mechanism, i.e., oxidizing $Cl^-$ to HOCl than another co-substrate.

**Keywords**: Keywords contain three to eight words/phrases separated with coma.

## Introduction

Neonatal sepsis (NS) is still a problem in the worldwide because it is one of the leading causes of neonatal death. It is estimated that more than 40% death of children under-five years old occur in neonates, and each year there are 3.1 million neonates who die in the world (1). The death of the neonate is mostly caused by NS. NS is responsible for 30-50% of all total neonatal deaths especially in developing countries (2).

NS is a clinical syndrome which is characterized by a hemodynamic, respiratory, and metabolic disturbances processes due to systemic infection that can trigger an abnormal systemic inflammatory response syndrome (SIRS) in infants (3-4). The signs and symptoms of NS are varied and not specific. This causes the diagnosis of NS to be difficult. Until now, only blood culture can be a definitive diagnosis for NS, but as it is known, blood culture takes a long time and is quite expensive. The difficulties of this diagnosis makes some of clinicians used "Suspected sepsis" to diagnoses neonatal sepsis before the blood culture results (5).

The pathomechanism of NS is still unclear, but several studies have suggested that myeloperoxidase (MPO) enzyme was s involved in theese pathomechanism. Our previous result study shows that the level of MPO was significantly higher in saliva of newborn with risk of sepsis compared to healthy newborn (6). The MPO level elevation in NS are thought to be caused by an immune and inflammatory responses during NS. These response was important in these time, because it play an important role in killing invading parasites and pathogens (5).

MPO is a heme-containing enzyme which is produced by neutrophils during phagocytosis. MPO play an important role an unique ability in killing pathogens thorugh the formation of reactive oxidants which acts as an antimicrobial (7-8). Also, because MPO is one of the peroxidases family enzyme, it also can act as a catalyst of hydrogen peroxide ($H_2O_2$) removal depending on the conditions under which it operates. If the MPO plays a role in the defense mechanism against the pathogen, MPO will use $H_2O_2$ and halide (iodide, bromide, chlroide, and thiocyanate; I, Br, Cl, and SCN) to produce pseudohalide such as, hypoiodous acid (HOI), hypobromous acid (HOBr). Hypochlorous acid (HOCl), and hypothiocyanate acid (HOSCN). Conversely, if the MPO plays a role for $H_2O_2$ removal, then the MPO will catalyze the change of $H_2O_2$ into water ($H_2O$) and oxygen ($O_2$) (9-10).

Study of kinetics parameters of an enzyme is important in order to explain the work mechanism of the enzyme. From this point of view, it is important to know the kinetic parameters of the MPO in NS considering that many reactions catalysed by MPO. Due to important role of MPO in NS, the work mechanism in these diseases have attracted much attention. In this regard and for better understanding of the possible mechanisms of MPO with the presence of some co-substrates (I, Cl, and SCN) in NS, changes in the kinetic parameters of MPO upon interaction with those co-substrates was studied.

## Methods

### 1. Participants

This was a prospective study conducted from December 2016 to June 2017. Ethical approval was obtained from the the Ethics Commission of the Faculty of Medicine, Lambung

Mangkurat University, Banjarmasin, South Kalimantan, Indonesia (approval number: 2010019). Written informed consent was obtained from all guardians on behalf of the newborn participants involved in the study. Patients who were participated in this study are newborn with risk of SN (n=15) based on The American Congress of Obstetricians and Gynecologist (ACOG) guidelines criteria for NS. Patients who were participated must at least have 1 major criteria or 2 minor criteria according to these criteria. Healthy newborns (n=15) were included as controls.

**2. Saliva samples collection**

The patient's salivary sample is taken from the mouth of the newborn at the time of clearing the oral cavity by using a sterile polypropylene transfer pipettes. The saliva was taken by giving 0.9% NaCl solution slowly by using the transfer pipettes at the bottom of the patient's tongue and then the mixture of the saliva and NaCl was taken again as much as 5 ml. Samples were transported on ice to the laboratory where they were centrifuged at 176 g for 5 minutes and the supernatants stored until further analysis.

**3. Experimental section**

Samples were divided into 4 groups from each conditions (health and risk of sepsis). Group 1 contained healthy/sepsis newborn saliva + $H_2O_2$ and SN saliva + $H_2O_2$, respectively. Group 2 contained healthy/sepsis newborn saliva + $H_2O_2$ + 0.1 M of NaCl and SN saliva + $H_2O_2$ + 0.1 M of NaCl, respectively. Group 3 contained healthy/sepsis newborn saliva + $H_2O_2$ + 0.1 M of KI and SN saliva + $H_2O_2$ + 0.1 M of KI, respectively. Group 4 contained healthy newborn saliva + $H_2O_2$ + 0.1 M of SCN and SN saliva + $H_2O_2$ + 0.1 M of SCN, respectively.

**4. MPO activity analysis**

MPO activity was measured spectrophotometrically using o-dianisidine (Sigma-Aldrich) and $H_2O_2$. In the presence of $H_2O_2$ as oxidizing agents, MPO catalyzes the oxidation of o-dianisidine yielding a brown coloured product, oxidized o-dianisidine, with a maximum absorbance at 470 nm. One unit (U) of MPO activity was defined degrading 1 $\mu$mol of $H_2O_2$ per minute at 25°C (11).

**5. MPO kinetic parameter analysis**

Kinetic parameters were determined by using five different concentrations of the substrate, $H_2O_2$. The substrate concentrations are 6.25, 12.5, 25, and 50 mM of $H_2O_2$. The kinetic parameters, Vmax, and Km, were determined using the Lineweaver-Burk version of the Michaelis-Menten equation, as follows:

$$\frac{1}{V} = \frac{km}{V\max} \cdot \frac{1}{[S]} + \frac{1}{V\max}$$

V is the reaction velocity. Vmax is the maximum reaction velocity. Km is the Michaelis constant (the substrate concentration at half-maximal reaction velocity) and [S] is the

substrate concentration (5).

**Results and Discussion**

In this present study, the kinetic parameters of MPO with and without the presence of some co-substrate in saliva of newborn at risk of sepsis were investigated. Kinetic parameters of salivary MPO were determined using Lineweaver-Burke plot. From this plot, a Michaelis-Menten equation used to determine the Km and Vmax values for both groups with and without the presence of different co-substrate. The results show in figure 1, 2, and table 1. From the figure 1 and table 1, the Vmax of the salivary MPO in healthy newborns group ranges from 0.035-0.041 $sec^{-1}$, while the Vmax values in sepsis newborn group are within the range of 0.035-0.043 $sec^{-1}$. Also, from the figure 2 and table 1 the Km values in healthy newborn group are within the range of 0.070-0.0.235, while the Km values in sepsis newborn group are within range the range of 0.064-0.338. The highest Vmax values was found in the group 3 of healthy newborn group, while in the sepsis newborn group was found in the group 4. However, the Vmax values were found not much different between each group. The highest Km values was found group 1 of both healthy and sepsis newborn, while the lowest Km values was found in the group 2 of healthy newborn and group 3 of sepsis newborn.

To best of our knowledge, the Vmax value illustrates the ability of an enzyme to catalyze the substrate, and the Km value illustrate enzyme and substrate affinity. From this point of view, the higher value of Vmax reflects a better enzyme's ability to catalyze its substrate. In contrast to the Vmax value, a higher Km value reflects the low affinity of the enzyme against its substrate. This means, the reaction will shift to the left. The shift in the reaction results in a decrease in the product level (13-14).

Results of this present study indicated that the addition of some co-substrate do not affect the catalytic ability of MPO both in normal or sepsis condition. However, different things happen at Km value. According to our results, it seems sepsis condition decrease the affinity between MPO and $H_2O_2$. It can be seen from the higher value of Km in sepsis group compare to normal group without the addition of some co-substrate. Interesting results can be found at the value of Km after some co-substrate additions. In sepsis condition, the additions of some co-substrate seem to increase the affinity between MPO and the substrate and co-substrate. The strongest affinity of MPO was found in chloride addition.

The increasing of affinity of MPO in sepsis condition means the binding between MPO and $H_2O_2$ might be stronger by the presence of those co-substrates. This condition will increase the concentration of hypochlorous acid, hypothiocyanite, and hypoiodous acid as a product. Between these three products, it seems MPO rather to oxidize chloride than the two others. It can be seen from the lowest Km value in chloride group than another group. To best of our knowledge, as far as we are aware, this the first study of MPO kinetic parameter analysis in saliva of newborn at risk of sepsis. However, several previous studies only stated that there was an increasing level or activity of MPO in saliva and plasma of newborn at risk of sepsis (6, 14-16).

It is well known that MPO is a haem enzyme that uses $H_2O_2$ to oxidize some co-substrate including $Cl^-$, $I^-$, $Br^-$, and $SCN^-$. The activity of MPO to oxidize those co-substrate is

relatively non-specific. However, between those co-substrate, $Cl^-$ was accepted as a physiological co-susbtrate because a higher concentration in plasma than another co-substrate. This is in line with our results study. The result of this present study indicated that with the present of $Cl^-$, $I^-$, and $SCN^-$, MPO rather to oxidize $Cl^-$ than $I^-$ and $SCN^-$ (9). The production of HOCl, HOI, and HOSCN by MPO is essential in host defense mechanisms. These productions deploy bactericidal activity on the bacteria ingested in the phagosome (17).

**Conclusion**

From this present study, it can be concluded that the addition of some co-substrate in sepsis condition will lead to decrease the Km value of MPO, but not Vmax. It seems the presence of some co-substrate in sepsis condition will activated MPO to catalyze the oxidation of $Cl^-$, $I^-$, and $SCN^-$, which is important to killing the bacteria during sepsis condition. The result also shows that in sepsis condition, MPO will oxidize $Cl^-$ to form HOCl than another co-substrate.

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**Table 1.** Kinetic parameters (Km and Vmax) and coefficient correlation for salivary MPO in different group in normal and sepsis newborn. Group 1 contain saliva and $H_2O_2$; Group 2 contain saliva, $H_2O_2$, and NaCl; group 3 contain saliva, $H_2O_2$, and KI; and Group 3 contain saliva, $H_2O_2$, and SCN.

| Condition | Group | Parameters | | |
|---|---|---|---|---|
| | | Km | Vmax | r |
| Normal | 1 | 0.235 | 0.035 | 0.981 |
| | 2 | 0.070 | 0.037 | 0.960 |
| | 3 | 0.216 | 0.039 | 0.917 |
| | 4 | 0.080 | 0.041 | 0.984 |
| Sepsis | 1 | 0.338 | 0.035 | 0.951 |
| | 2 | 0.064 | 0.039 | 0.907 |
| | 3 | 0.080 | 0.039 | 0.941 |
| | 4 | 0.148 | 0.043 | 0.981 |

**Figure 1**. The Vmax value comparison of salivary MPO between group in normal and sepsis conditions. Group 1 contain saliva and $H_2O_2$; Group 2 contain saliva, $H_2O_2$, and NaCl; group 3 contain saliva, $H_2O_2$, and KI; and Group 3 contain saliva, $H_2O_2$, and SCN.

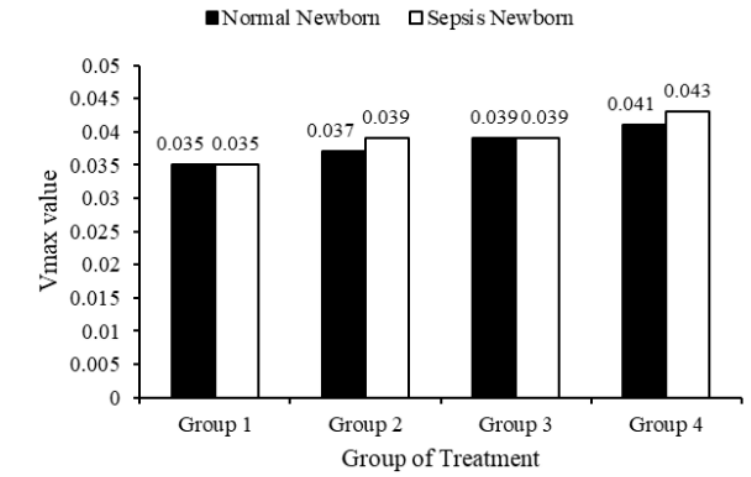
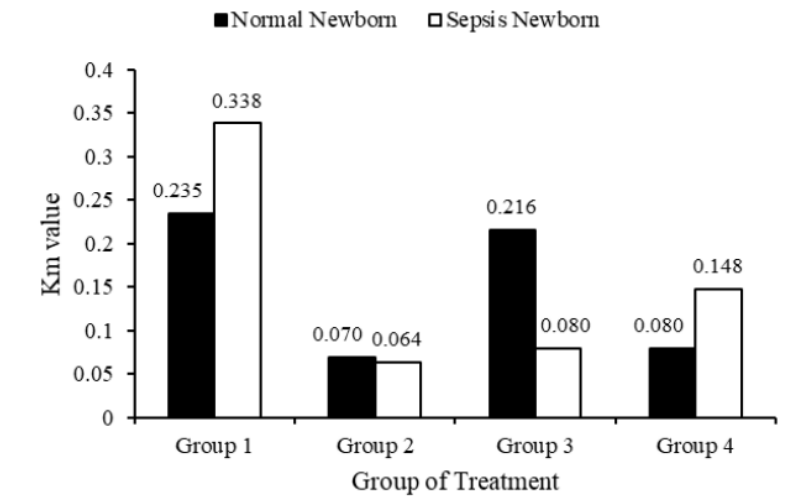


**Figure 2**. The Km value comparison of salivary MPO between group in normal and sepsis conditions. Group 1 contain saliva and $H_2O_2$; Group 2 contain saliva, $H_2O_2$, and NaCl; group 3 contain saliva, $H_2O_2$, and KI; and Group 3 contain saliva, $H_2O_2$, and SCN.



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