

# Salivary Antioxidative Index in Newborns at Risk of Sepsis as Novel Parameter for Early-Onset Neonatal Sepsis

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**Abstract**—Neonatal sepsis is a clinical syndrome in the first months of infant life due to a systemic response caused by the presence of pathogenic microorganisms or their products in the blood. Sepsis promotes the unbalanced production of oxidant and anti-oxidant substances, causing an excess of free oxygen radicals. Early markers of neonatal sepsis have been studied in recent years, and we proposed another parameter to detect early-onset neonatal sepsis with salivary antioxidative index (SAOI), saliva has been shown as blood representatives and to have many benefits. This study was conducted in April - June 2012, saliva specimens were taken from 57 newborns, in which 32 infants were at risk of sepsis and 25 infants were healthy and served as a control group. Data was analyzed by Mann-Whitney test. We concluded that sepsis possibility 3,7 fold when there are low AOI. This parameter may be used as another marker to detect early-onset neonatal sepsis.

**Index Terms**—Early-onset neonatal sepsis, free oxygen radicals, salivary antioxidative index

## I. INTRODUCTION

Early-onset neonatal sepsis (EONS) which manifests in the first 72 hours of life (up to 7 days), and late-onset neonatal sepsis (LONS) which incidence peaks in the 2nd to 3rd week of postnatal life. Differentiating early from late neonatal sepsis is clinically important because in early neonatal sepsis, the infectious organisms are acquired during the delivery, whereas in late sepsis the infecting organisms are mostly acquired after birth, from either hospital or community sources. Activation of natural immune system describe defense mechanism against infectious agents. Immune cells that roled in respond to this activity such as monocyte, macrofage, dendritic cell, and neutrophil [1]-[3].

Neutrophil plays a role as one of the frontline of body defense against infection. These cells use bactericidal pathways that oxygen-dependent or oxygen-independent as a weapon to eliminate infectious agents. In response to

bacterial pathogens entrance, neutrophils inserted into the infected tissue, then activate to form a reactive oxygen compounds. This event called respiratory burst involving the NADPH oxidase activation. At the respiratory burst, there was a rapid uptake of molecular oxygen and transformation into reactive oxygen compounds, which is a representation of the host defense mechanisms in the inflammatory site. It is important that relevant reactive oxygen compounds physiological concentrations able to modulate the redox-sensitive signaling cascade and improve immunological cellular function [4].

Reactive oxygen compounds such as hydrogen peroxide, superoxide anion, hydroxyl radical, etc can trigger oxidative damage to macromolecules, leading to lipid peroxidation, amino acid chains oxidation, cross links protein formation, polypeptide chain oxidation forming protein fragmentation, DNA strands ruptured. Furthermore, through the degranulation process, occured secretion of several chemical compounds, especially superoxide dismutase (SOD) and peroxidase enzyme [4].

Both enzymes were involved due to oxygen consumption by neutrophil which produce anion superoxide than dismutated into hydrogen peroxide. Hydrogen peroxides were able to kill bacterias on sepsis when they are in high concentration while superoxides anions were not able to kill bacterias directly. Hydroxyl radicals were oxygen radicals that most reactive and very cytotoxic. Hydrogen peroxide ( $H_2O_2$ ) and superoxide anion ( $O_2^-$ ) were less reactive and have longer half time than hydroxyl radicals. [3].

Myeloperoxidase is an haeme-containing enzyme which secreted by the phagocytes after an activation from respiratory burst system. Myeloperoxidases are usually used as tissues neutrophil accumulation and neutrophil activity marker on plasma assays. Myeloperoxidase use hydrogen peroxides to oxidize amount of aromatic species (RH) by one electron mechanism to form aromatic radical ( $R^\bullet$ ). This is typical, therefore they are ready to oxidize the strong non radical reactive oxygen species, the HOCl ions. HOCl is reactive oxygen species that produced by neutrophils and very bactericidals [3].

Several methods to diagnose early-onset neonatal sepsis has been reported, such as procalcitonin and c-reactive protein [5]-[7]. We used different approach by using other parameter to detect early onset neonatal sepsis through salivary antioxidative index (SAOI). SAOI is ratio of salivary enzymatic antioxidant activity to salivary hydrogen peroxide level.

## II. MATERIAL AND METHODS

The cross-sectional prospective study was conducted from April to June 2012 in the Division of Neonatology, Department of Child Health, Lambung Mangkurat University Faculty of Medicine/Ulin General Hospital, Banjarmasin. Laboratory tests were conducted at Medical Chemistry/ Biochemistry Department Faculty of Medicine Lambung Mangkurat University, Banjarmasin. Saliva specimens were taken from 57 newborns, of which 32 infants were at risk of sepsis and 25 infants were healthy and served as a control group. All subject's parents provided with a written informed consent. Saliva specimens (3 ml each) were taken via suction from the oropharynx according to standard procedures for neonatal resuscitation.

Subjects in the sepsis risk group were included on the basis of having at least 1 major criteria or 2 minor criteria. Major risk criteria were membranes ruptured for > 24 hours, maternal fever with intrapartum temperature > 38 °C, chorioamnionitis, fetal heart rate persisting at > 160 times/minute or foul-smelling amniotic fluid. Minor risk criteria were membranes ruptured for > 12 hours, maternal fever with intrapartum temperature > 37.5 °C, low Apgar score (<5 at the 1st minute, <7 at the 5th minute), very low birth weight baby (VLBWB) of <1500 grams, gestational age < 37 weeks, multiple pregnancy, foul-smelling vaginal discharge, maternal urinary tract infection (UTI) or suspected untreated maternal UTI [8].

### Data Analysis

- Determination of SOD activity  
The SOD activity in supernatant was measured by the method of Misra and Fridovich [9]. The supernatant (500 µl) was added to 0.800 ml of carbonate buffer (100 mM, pH 10.2) and 100 µl of epinephrine 3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec.
- Determination of Peroxidase activity  
Determination of Peroxidase activity was measured by method of Pruitt et al [10]. The assay was performed by mixing 1.0 ml phosphate buffer (pH 7.0), 1.0 ml guaiacol solution and 1.0 ml of a saliva sample. The reaction was started by adding 1.0 ml of H<sub>2</sub>O<sub>2</sub> stock solution. Absorbance at 470 nm (A) and time (T) data were monitored.
- Determination H<sub>2</sub>O<sub>2</sub> concentration  
Determination of peroxide concentration by the modified FOX2 method [11]. Solutions measured spectrophotometrically at λ = 505 nm
- Determination of AOI

AOI is ratio of enzymatic antioxidant activity to hydrogen peroxide level.

$$\text{SAOI} = \frac{\text{SOD activity} + \text{Peroxidase activity}}{\text{Peroxide level}}$$

### Statistical Analysis

Data are presented as means ± SD. The determinations were performed saliva from 32 newborns with sepsis risk (Case group) and saliva from 25 healthy newborns (Control group) The differences were examined by the Mann-Whitney test and the correlation were examined by odds ratio. For all outcomes, a nominal p-value of p < 0,05 was considered significant.

## III. RESULTS

TABLE I. MANN-WHITNEY SALIVARY COMPARISON RESULTS (MEAN±SD)

Parameter	Case group (n = 32)	Control group (n = 25)	p-value
Peroxide level (mM)	22.5 ± 8.6	14.3 ± 2.3	0.001*
SOD activity (µM min <sup>-1</sup> )	1.1 ± 0.9	6.6 ± 2.1	0.000*
Peroxidase activity (µM min <sup>-1</sup> )	8.4 ± 2.7	9.1 ± 3.5	0.505
Saliva Antioxidative Index (10 <sup>-3</sup> )	3.3 ± 2.7	5.7 ± 2.6	0.000*

\*) = a significant difference/ significantly (p < 0.05)

From Table I. Mean of peroxide level, SOD activity, in the saliva from the Case group higher than the Control group, and low SAOI in Case group (< 3,5x10<sup>-3</sup>) and SAOI in Control group (> 3,5x10<sup>-3</sup>). Odds ratio (3,7) between high and low SAOI showed there are significant correlation between Case group and Control group (p < 0,05). This suggests that occurred oxidative imbalance in the Case group, which is characterized by a lower value of SAOI. There is about 3,7 fold of sepsis possibility when there are low SAOI.

## IV. DISCUSSION

Under normal physiological conditions, a homeostatic balance exists between the formation of reactive oxidizing/oxygen species and their removal by endogenous antioxidant scavenging compounds. Oxidative stress occurs when this balance is disrupted by excessive production of reactive oxygen species, including superoxide, hydrogen peroxide and hydroxyl radicals, and/or by inadequate antioxidative defences, including superoxide dismutase (SOD), catalase, and peroxidase [12].

Superoxide is converted to hydrogen peroxide by the SOD enzyme. SOD is believed to play a major role in the less reactive species H<sub>2</sub>O<sub>2</sub>. In the absence of transition metal ions, H<sub>2</sub>O<sub>2</sub> is fairly stable. It does, however, allow neutrophils to oxidize chloride ions, via myeloperoxidase, into hypochlorous acid, providing additional cytotoxic activity [13], [14]. Excess hydrogen peroxide is normally converted to water by the action of catalase, and other peroxidases. Hydroxyl radicals can be formed by the

reaction of superoxide with hydrogen peroxide in the presence of metal ions (usually iron or copper). Hydroxyl free radicals are much more reactive than superoxide anions. Iron-catalysed hydroxyl generation requires that the iron is in its reduced, ferrous form ( $\text{Fe}^{2+}$ ), whereas most iron existing in cells and plasma is in the oxidized form ( $\text{Fe}^{3+}$ ). As well as its involvement with hydrogen peroxide in hydroxyl radical formation, superoxide can also reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , thereby further promoting hydroxyl production. However, most iron in the plasma exists in a bound form as a protective measure, as it is the free component which is able to participate in biochemical reactions. Biological mechanism has used molecules for iron metabolism (heme proteins), storage (ferritin) and transport (transferrin) that lock the iron in a state where free radical production cannot occur [15].

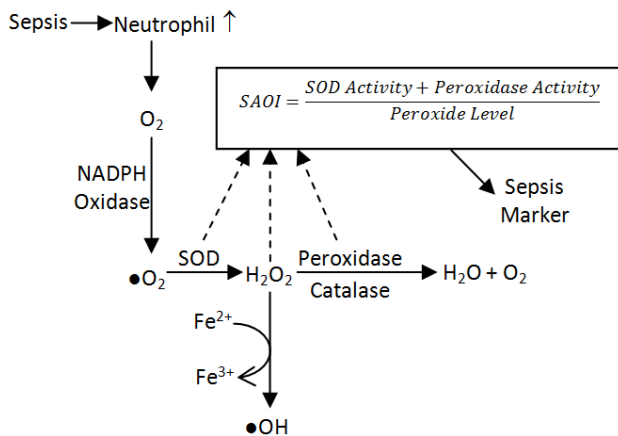


Figure 1. Salivary Antioxidative Index as Neonatal Sepsis Marker

In sepsis, there are several potential sources of reactive oxygen species, including the mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischaemia and reperfusion, the respiratory burst associated with neutrophil activation, and arachidonic acid metabolism. Activated neutrophils produce superoxide as a cytotoxic agent as part of the respiratory burst via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the free radical nitric oxide ( $\text{NO}^{\bullet}$ ), which can react with superoxide to produce peroxynitrite, a powerful oxidant, which may decompose to form the hydroxyl radical. Under ischaemic conditions followed by subsequent reperfusion, the xanthine oxidase enzyme catalyses the formation of uric acid with the coproduction of superoxide. Superoxide release results in the recruitment and activation of neutrophils and their adherence to endothelial cells, which stimulates the formation of xanthine oxidase in the endothelium, with further superoxide production [12], [14].

Saliva has been shown as blood representatives [4] and to have many benefits [16], [17] such as containing antimicrobial compounds and biomarkers of infectious diseases [18], [19], and malignancy [20], [21]. In our study, there were some significant differences between peroxide level, SOD activity, and the SAOI. But unlikely in peroxidase activity which describe there is

domination of SOD activity to act as neutralizer on defense mechanism to oxidative stress induced by early-onset neonatal sepsis. The saliva specimens from the case group had significantly low SAOI compared to that of the control group. Level of SAOI and the risk of sepsis shows correlation significantly. Any decrease of SAOI will contribute presence of sepsis about 3,7 fold. This suggest a marker role of SAOI to detect oxidative stress on early-onset of neonatal sepsis which describe in Fig. 1.

The SAOI method is similar with a study by Suhartono *et al* [22]. They proposed the used peroxidative index to detect improvement of oxidative mechanism during medication of lung tuberculosis. Peroxidative index is a parameter that show the ratio of peroxides and the peroxidases enzymes. The high value of peroxidative index is show that the formation of oxidants are higher than the enzymatic antioxidant. Otherwise, the low value of peroxidative index is show that the enzymatic antioxidant activity is more active therefore the peroxides may be catalyzed to water and oxygen. They concluded that there were significant correlation between peroxidative index and duration of medication.

## V. CONCLUSION

There were significant correlation between salivary antioxidative index to newborn at risk of sepsis. This suggests that occurred oxidative imbalance in the Case group, which is characterized by a lower value of SAOI. There is about 3,7 fold of sepsis possibility when there are low SAOI, therefore this parameter may be used as another marker to detect early-onset neonatal sepsis.

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