

Advance Oxidation Protein Products (AOPPs) of Newborn at Risk of Sepsis as Novel Parameter for Early-Onset Neonatal Sepsis

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Abstract—Neonatal sepsis is a clinical syndrome of bacteremia and divided into two different types, early-onset neonatal sepsis and late-onset neonatal sepsis. Sepsis promotes the unbalanced production of oxidant and anti-oxidant substances, causing an excess of free oxygen compounds. Early markers of neonatal sepsis have been studied in recent years, and this study proposed another parameter to detect early-onset neonatal sepsis with the use of advance oxidation protein products (AOPPs). This study was conducted in April - June 2012, saliva and serum specimens were taken from 81 newborns, in which 39 infants were at risk of sepsis and 42 infants were healthy and served as a control group. Data was analyzed by using Mann-Whitney test and Spearman correlation test. This study suggested that there is significant difference between AOPPs level on the case group compare to the control group. This study also found that there is significant difference between salivary AOPPs level compare to serum AOPPs level. As the conclusion, this study proposed that the AOPPs parameter may be used as another marker to detect early-onset neonatal sepsis.

Index Terms—Early-onset neonatal sepsis, free oxygen compounds, AOPPs.

I. INTRODUCTION

Neonatal sepsis remains a leading cause of neonatal morbidity and mortality despite recent advances in neonatal intensive care. Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Sepsis divided into two different types, early-onset neonatal sepsis and late-onset neonatal sepsis. 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 and 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 describes defense mechanism against infectious agents.

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Immune cells that ruled in respond to this activity such as monocyte, macrofage, dendritic cell, and neutrophil [1]-[3].

Neutrophils 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. As a consequence of respiratory burst, a number of oxygen-containing compounds are produced. In this process NADPH oxidase catalyzes the formation of superoxide anions part of which are converted to hydrogen peroxide and singlet oxygen. Moreover, superoxide anion can react with hydrogen peroxide resulting in the formation of hydroxyl radicals and more singlet oxygen. Altogether, these reactions result in the production of toxic oxygen compounds such as superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radicals. Myeloperoxidase is released into the phagosome as azurophilic granules fuse with the phagosome. This enzyme utilizes hydrogen peroxide and chloride ions to form hypochlorite, which represents a highly toxic substance that kills pathogens [4].

Reactive oxygen species 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, occurred secretion of several chemical compounds, especially superoxide dismutase (SOD) and peroxidase enzyme [4].

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

Protein carbonyl derivates (aldehydes and ketones) are produced on protein side chains when they are oxidized by the reactive oxygen species. Protein carbonyl content is actually the most general indicator and by far the most commonly used marker of protein oxidation, and accumulation of protein carbonyls has been observed in several human diseases including Alzheimer's disease (AD), diabetes, inflammatory bowel disease (IBD), and arthritis [5]. Several methods to diagnose early-onset neonatal sepsis have been reported, such as procalcitonin and c-reactive protein

[6]-[8]. Recently, another type of oxidized protein beside protein carbonyl has been investigated called advance oxidation protein product (AOPPs). AOPPs is dityrosine containing cross linked protein products, a definition that is important as it excludes protein aggregates that are formed by disulphide bonds as a result of oxidative stress. Therefore, AOPPs is a good oxidative stress marker, which originates under oxidative and carbonyl stress and increase global inflammatory activity. Several studies have use AOPPs as a marker for several disease. AOPPs has been used as oxidative stress marker for nasal polyposis and acute coronary syndrome and also as inflammatory marker for liver cirrhosis and chronic renal failure [6]-[9]. The well-established relationship between AOPP response and induced damage makes this simple, fast and inexpensive automated technique applicable in daily routine laboratory practice for assessing and monitoring oxidative stress in critically ill or other patients [10].

The use of AOPPs as marker for early-onset of neonatal sepsis (EONS) were never been investigated, therefore a study to use this method as a marker for EONS should be performed. We used other parameter to detect EONS through salivary and serum AOPPs, therefore AOPPs can be used as new parameter for EONS.

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 and serum specimens were taken from 81 newborns, of which 39 infants were at risk of sepsis and 42 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. While serum specimens were taken from peripheral veins blood.

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 [9].

A. AOPPs Determination

Salivary and serum AOPPs measurement were made by spectrophotometric methods as describe by Witko-Sarsat *et al* [11].

B. Protein Carbonyl Assay

Sample derivatization: Two 1-mg aliquots are needed for each sample to be assayed. Samples are extracted in a final

concentration of 10% (w/v) TCA. The precipitates are treated with 500 µL of 0.2% DNPH or 500 µL of 2 M HCl. Samples are incubated at room temperature for 1 h with vortexing at 5-min intervals. The proteins are then precipitated by adding 55 µL of 100% TCA. The pellets are centrifuged and washed three times with 500 µL of the ethanol:ethyl acetate mixture. The pellet is then dissolved in 600 µL of 6M guanidine hydrochloride. The carbonyl content is determined by reading the absorbance at the optimum wavelength 390 nm [5], [12]-[14].

C. Hydrogen Peroxide Assay

90 ml of sample, 10 ml of methanol and 900 ml of xylenol orange reagent containing ferrous ions were added successively and absorbance was noted at 560 nm [15].

Data are presented as means ± SD. The determinations were performed on saliva and serum from 39 newborns with sepsis risk (Case group) and 42 healthy newborns (Control group). The differences were examined by the Mann-Whitney test and the correlation were examined by Spearman Correlation using SPSS for Windows version 17. For all outcomes, a nominal *p*-value of *p* < 0.05 was considered significant.

III. RESULTS

From Table I. Mean of AOPPs, carbonyls, and hydrogen peroxides level both, from saliva and serum samples shows that the case group is higher than the control group and it is significant (*p*<0.005).

TABLE I: MANN-WHITNEY COMPARISON OF PARAMETERS RESULTS BY RISK FACTOR (MEAN±SD)

Parameter	Case group (n = 39)	Control group (n = 42)	<i>p</i> -value
Salivary			
AOPP (µM/L)	7.363±4.849	3.893±1.924	0.000*
Carbonyl (µM)	6.040±4.451	1.699±1.565	0.000*
H ₂ O ₂ (µM)	79.659±0.237	37.465±0.314	0.000*
Serum			
AOPP (µM/L)	11.569±9.142	6.271±2.267	0.000*
Carbonyl (µM)	8.450±6.871	2.102±1.364	0.000*
H ₂ O ₂ (µM)	79.106±0.825	36.736±0.747	0.000*

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

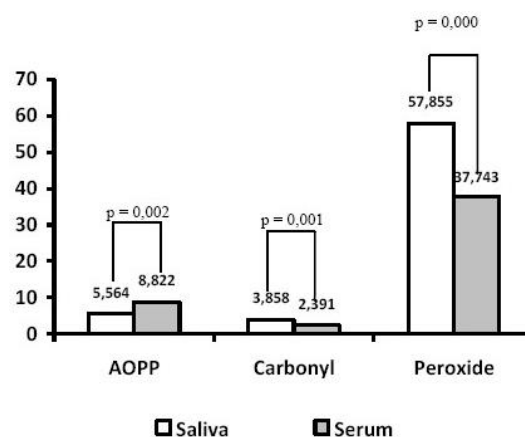


Fig. 1. Salivary and serum parameters comparison by Mann-Whitney test.

From Fig. 1, Mean of salivary and serum AOPPs, carbonyls, and hydrogen peroxides comparison shows there is significant difference between both samples (*p*<0.05).

IV. DISCUSSION

Neonatal sepsis causes significant mortality and morbidity in infants, especially preterm infants. Despite considerable advances of neonatal intensive care medicine, the pathogenesis of neonatal sepsis with its rapid progression from infection to a systemic inflammatory response still remains little understood. Beyond doubt, sepsis is a clinical manifestation of a dysregulated immune response to invasive pathogens. However, numerous clinical trials with immunomodulatory approaches for neonates with suspected or proven infections failed to improve survival [16].

In physiological conditions, there is a balance condition between the formation of reactive oxidizing/oxygen species and their elimination by endogenous antioxidant scavenging compounds. Oxidative stress occurs when this balance is disrupted by inadequate antioxidative defences, including superoxide dismutase (SOD), catalase, and peroxidase and/or by excessive production of reactive oxygen species, including superoxide, hydrogen peroxide and hydroxyl radicals [9].

In vivo measurement of oxidative stress is complicated, and practical and easy methods of measurement are still being investigated. Several methods has been performed to measure oxidative stress, such as measurement of lipid peroxidation and protein carbonyl compound as oxidative products from reactive oxygen species activity. Protein carbonyls believed chemically more stable than oxidative modification products from lipid. Another type of oxidative modification of protein is AOPPs. AOPPs are produced during oxidative stress as a result of myeloperoxidase activity in activated neutrophils acting on hypochloric acid and chloramines; these are a reliable marker to measure the oxidative modification of proteins [5]. 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].

The oxidative modifications of proteins are more stable than lipids, making proteins good oxidative stress markers. Protein oxidation can result from the formation of protein carbonyls, formation of cross linked molecules by sulphhydryl group oxidation, and formation of advanced oxidation protein products (AOPPs). AOPPs can be defined as dityrosine that includes cross linked proteins. This definition is important because it excludes protein aggregates that are formed as a result of disulphide bonding. Therefore, AOPPs may have a function as a marker for oxidative stress [6].

In sepsis, there are several potential sources of oxidative stress, 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 (NO^{\bullet}), which can reacts 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 [17], [18].

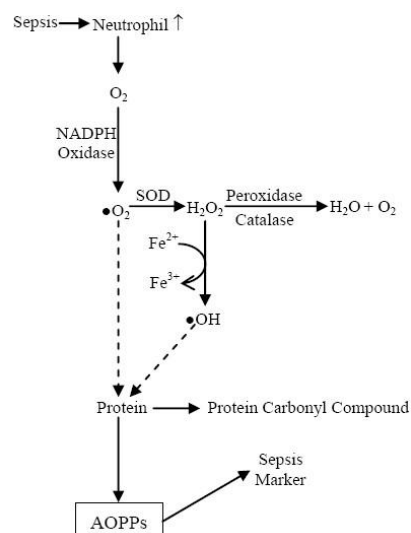


Fig. 2. AOPPs as novel neonatal sepsis marker.

This study suggests that there are some significant differences between AOPPs level, both from salivary and serum, on the case group (sepsis risked) and the control group (normal). It is similar with the protein carbonyl compounds level that shows its level higher in the case groups and it is significant while the production of hydrogen peroxides as the reactive oxygen species is also higher in the case group significantly. This study shows production of AOPPs and carbonyl compounds are significantly increase when there are any risk factor for neonatal sepsis. There is significant difference between salivary AOPPs level to the serum AOPPs level, likely the carbonyl compounds and the hydrogen peroxides. This study indicates, both AOPPs from saliva as well as from serum, significantly produced in neonatal sepsis and although amount of AOPPs that produce in saliva were different compare to serum AOPPs level. This suggested a marker role of AOPPs to detect oxidative stress on early-onset of neonatal sepsis which describe in Fig. 2.

AOPP has been used recently as oxidative stress marker for several disease. The involvement of AOPPs on oxidative stress has been used as marker for chronic renal failure, acute coronary syndrome, liver cirrhosis, and nasal polyposis [7], [8], [11], [17].

Several methods to diagnose early-onset neonatal sepsis have been reported, such as procalcitonin and c-reactive protein [9], [19], [20]. Based on the result of this study we proposed the use of AOPPs as the new marker for early-onset of neonatal sepsis. This study is similar with our previous study which uses salivary antioxidative index (SAOI) as a marker for early-onset of neonatal sepsis. Salivary antioxidative index is ratio of salivary enzymatic antioxidant activity to salivary hydrogen peroxide level, shows that any decrease of SAOI will contribute presence of sepsis about 3.7 fold. This study suggests a marker role of SAOI to detect oxidative stress on early-onset of neonatal sepsis [21].

V. CONCLUSION

As a conclusion, this study shows that there is significant difference of AOPPs level between the case group and the control group and also there is significant difference between salivary AOPPs level to serum AOPPs level of the newborn at risk of sepsis. This result suggests that this parameter may be used as another marker to detect early-onset neonatal sepsis.

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