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# UV-Vis and FTIR Spectroscopy: Can early risk of neonatal sepsis be investigated?

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**Abstract.** Neonatal sepsis (NS) is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. NS is one of the most common causes of neonatal mortality. It is responsible for 30-50% of neonatal deaths. The manifestation is mostly nonspecific. Blood culture as a gold standard for diagnosis still have many limitations, such as invasive, time-consuming, and highly cost. A strategy to reduce mortality of NS is early detections using biomarkers but most of them are PCR- and "-omic" technology-based and using blood as its sample. The alternative method is a spectroscopy technique using saliva as the sample because the procedure is non-invasive and does not require trained personnel and special equipment. This method is based on the absorption of light by a compound in a certain wavelength to detect biochemical changes in the early phases of disease since the biochemical changes will precede the morphological changes of the disease itself. The utilization of this method to analyze biomarkers in NS has been popular recently. Furthermore, additional research might be needed to study this method for early detection in NS.

## 1. Introduction

Spectroscopy is a method to analyze the interactions between molecules with light at certain wavelengths. The light that is passed through can be visible (Vis), ultraviolet (UV) and infrared (IR). UV wavelengths cover a range approximately 185-400 nm, Vis 400 - 700 nm and IR 700 - 15,000 nm [1]. In recent years, spectroscopy method has been used to analyze biological samples such as blood plasma, serum, and tissue. Besides, this method can explain the pathomechanism of disease and its molecular interactions, for example interactions between Pb as a cause of osteoporosis and damage to proteins by metals [2,3,4]. Thus, this method is able to detect biochemical changes in the early phases of a disease, including NS [5].

NS is a clinical syndrome of systemic disease caused by bacteraemia that occurs in the first month of life [6]. The clinical manifestations of NS are non-specifics, so the diagnosis is difficult to establish. Blood culture, the gold standard of diagnosis, has many limitations such as using

blood as a sample, time-consuming, and expensive. Blood sampling is not a simple procedure especially in newborns because it is invasive and traumatic [7].

Based on the above reasons, the non-invasive and non-traumatic samples are indispensable, such as saliva. Several studies have proposed using saliva as a sample for early detection of NS. For example, a study conducted by Yunanto *et. al*. [8] recorded that there were changes in the salivary protein structure of neonate at risk of sepsis compared to healthy infants using FTIR spectroscopy. Suhartono *et. al*. [5] also state that saliva can be used to detect hypoxia in neonate at risk of sepsis using UV-Vis spectroscopy. Furthermore, a question arises whether the spectroscopic method can be used for early detection of NS.

## 2. Neonatal Sepsis

Neonatal sepsis is a clinical syndrome of systemic disease caused by bacteraemia that occurs in the first month of life. This condition can be defined clinically and/or by microbiological examination, in the presence of positive blood cultures and/or cerebrospinal fluid [6]. The pathomechanism of NS is not fully understood. Some literature mention that there is a role of phagocytic cells such as neutrophils in NS. Invasive microorganisms activate the innate immune system as the body's defense mechanism. The immune response to invasive pathogens has an important role in clinical manifestations that arise in NS [9]. Although the inflammatory response is initiated by the presence of invasive pathogens, the inflammatory process that occurs itself is the result of the production of endogenous inflammatory mediators [10].

Previous studies showed that there is an involvement of oxidative stress in the pathomechanism of NS. Activation of neutrophils by invasive pathogens trigger the release of the myeloperoxidase enzyme (MPO) from its azurophilic granules. This activation is known as the respiratory burst. The respiratory burst process that occurs during neutrophil activation not only causes the release of the MPO enzyme from neutrophils but also activates the nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), an enzyme that catalyses the reaction between NAPDH and oxygen to produce superoxide radicals ($\bullet O_2$). Molecules $\bullet O_2$ will undergo further reactions to produce other reactive oxygen species (ROS) such as hydroxyl radicals (•OH) and hydrogen peroxide ($H_2O_2$). ROS production is important as a defense mechanism against pathogenic bacteria that are present at NS. The ROS is used to destroy invasive pathogens through a series of chemical reactions [11,12].

Besides, ROS produced through oxidative stress processes in NS can cause macromolecular damage, lipid peroxidation, oxidation of amino acid chains, the formation of protein cross-bonds, oxidation of polypeptide chains that form protein fragmentation, and DNA damage. Carbonyl protein derivatives (aldehydes and ketones) are produced by protein side chains oxidized by ROS. Carbonyl protein is a biomarker of protein oxidation markers that commonly used recently [11].

Changes in the internal and external environment of cells through the stress oxidation process can also cause cellular injury. The cellular injury that occurs can cause damage in the phospholipid membrane which is a major component of cell walls [13] It causes the release of arachidonic acid that is metabolized by prostaglandin synthase and cyclooxygenase to produce prostaglandins and thromboxane as inflammatory mediators [14]. The release of inflammatory mediators then promotes leukocytes-endothelial cell adhesion, production of nitric oxide, reactive oxygen, and nitrogen species which cause further reactions and induce endothelial dysfunction, vasodilation, and loss of vascular control [15].

Some inflammatory biomarkers that have been studied for early detection of NS are Advanced Oxidation Protein Products (AOPP), myeloperoxidase (MPO) enzymes, and carbonyl proteins [11]. This is based on the pathomechanism of NS through the oxidative stress pathway as mentioned above [11,12].

## 3. Saliva as the Biological Sample in Neonatal Sepsis

Saliva plays an important role in a variety of biological processes that occur in the oral cavity, including as a lubricant, chewing, food ingestion, and cleaning action, as well as protecting against

dental caries. Also, the salivary function is known to diagnose various diseases. The use of saliva as a biological sample for diagnostic convenience has several advantages compared to blood and urine. It is non-invasive, does not require skilled personnel and special equipment. Besides, saliva components do not change at room temperature and can avoid the risk of infectious diseases, such as HIV and hepatitis [16,17].

Saliva can determine the physiological and pathological situations of the human body. Saliva contains water, protein, electrolytes, urine, nitrogenous products, and enzymes. Since two thousand years ago, saliva has been used as a medium to help diagnose disease. Ancient Chinese medicine experts claimed that saliva and blood are "close relatives" in the human body. Both come from the same source that causes salivary components are derived from the blood through active transport or passive diffusion so that the biochemical and immunological levels in saliva can reflect conditions in the blood [18]. It has been proven that saliva has many benefits including containing antimicrobial compounds, monitoring the bone marrow function, and their utilization as disease biomarkers such as malignancy [19].

Until now, there is no standardization for saliva sampling methods. Two types of methods currently used for saliva sampling are aspiration and absorption. An aspiration method is using a pipette or suction to aspirate saliva. Meanwhile, an absorption method is using cotton buds or other absorbent devices such as filter paper or sterile cotton rolls to absorb saliva. Each of the methods has advantages and disadvantages. The aspiration method using suction is considered to be able to injure the neonatal mucous membranes which are very fragile and pollute the sample with blood, especially at strong suction pressure, but the sample obtained can be taken in quite a large volume. On the other hand, the absorption method is said to be safer and not injurious, but the volume is less [20].

Over the past few years, saliva is a biological product that has been widely studied for diagnosing disease. Some studies suggest that saliva can be used to diagnose systemic diseases such as diabetes, respiratory system diseases, chronic heart failure, Cushing's syndrome, and disorders related to stress and depression [21]. A study conducted by Yunanto *et. al.* [11] suggests that saliva may be used as an alternative sample examination of inflammatory biomarkers for early detection of NS. The results of the study showed AOPP levels increased in the blood and saliva of the neonate at risk of sepsis. This shows that saliva can reflect conditions in the blood.

## 4. Nikotinamid Adenin Dinukleotida Fosfat Oksidase (NADPH) Detection Using UV-Vis Spectroscopy

NADPH is an essential electron donor/cofactor produced from $NADP^+$ via the pentose phosphate pathway by glucose-6-phosphate dehydrogenase (G6PD) enzyme. NADPH has an important role in the enzymatic reaction of cell components biosynthetic such as DNA and lipids. Besides, NADPH plays a role in the formation of ROS and as an antioxidant defense mechanism in the oxidative stress process [24]. In NS, neutrophil activation by invasive pathogens triggers the release and activation of the MPO enzyme known as the respiratory burst process. The process will activate NADPH oxidase to catalyse the reaction between NAPDH and oxygen to produce ROS [11,12].

Plenty of work has shown the role of NADPH in sepsis. Exposure to Gram-negative bacterial lipopolysaccharide (LPS) in cardiomyocyte models with sepsis can induce the overexpression of NADPH. In a sepsis condition, an activity of the enzyme NADPH oxidase increases which will tend to consume NADPH as its cofactor, thus a decrease in NADPH in sepsis may be found. However, other studies pointed out that there was an increase in $NADP^+$ levels which might be a compensatory mechanism to increase NADPH after LPS exposure [23,24]. The result of an investigation by Poggi *et. al.* [12] showed that there was an overexpression of NADPH in the model cardiomyocytes with sepsis which caused overexpression of the cyclooxygenase-2 (COX-2) enzyme. The direct activity of NADPH oxidase and the increase in the COX-2 enzyme induced by NADPH also contribute to increasing the production of cytoplasmic $O_2$ cells. Meanwhile, the involvement of Gram-positive bacterial components in inducing overexpression of NADPH in NS is still unknown.

The measurement of NADPH concentration using the UV-Vis spectroscopy method has been done previously. Research by Karakaya *et. al.* [25] showed an increase in NADPH concentration in bacterial cells *Synechocystis sp.* and *E. coli* using the UV-Vis spectroscopy method by looking at its absorbance at a wavelength of 340 nm. However, the NADPH measurement as another inflammatory biomarker in NS, especially using spectroscopic methods has never been done.

## 5. Arachidonic Acid Detection Using FTIR Spectroscopy

Arachidonic acid is one of the essential unsaturated fatty acids formed from linoleic acid which is widely available in the body. Arachidonic acid is generally found in phospholipid membrane cells especially skeletal muscles, brain, liver, spleen, and retina which will be released in response to various stimuli such as physical, chemical, and biological stress [26].

In sepsis, invading pathogens causes biological stress that can trigger cellular injury by activating the innate immune system as a body's defense mechanism. Immune cells that play a role in this response are macrophages. Macrophages will be activated and produce cytokines and pro-inflammatory enzymes, activation of the coagulation system cascade, complement system and production of proteases and oxidant compounds which ultimately cause damage to phospholipid membrane in the cell wall and release of arachidonic acid by enzyme phospholipase $A_2$ [6]. Moreover, ROS produced by oxidative stress in sepsis can cause damage to macromolecules and cell phospholipid membranes [11].

The first step in the arachidonic pathway is the release of arachidonic acid from the phospholipid membrane by the enzyme phospholipase $A_2$. Arachidonic acid is produced then converted to eicosanoids through three pathways: cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P-450 (cyt P450). In the COX pathway, arachidonic acid is converted by COX to prostaglandin $H_2$ ($PGH_2$). $PGH_2$ will be reprocessed by various terminal synthase enzymes into active prostanoids. Prostanoids produce many biological effects and play an important role in the physiology and pathology of the body. The types of prostanoids synthesised through the cyclooxygenase pathway include prostaglandin $D_2$, prostaglandin $E_2$, prostacyclin, and thromboxane $A_2$ as inflammatory mediators. Also, ROS can convert arachidonic acid into isoprostane [26].

Regarding this matter, researchers also have conducted some investigations. Bruegel *et. al.* [27] showed an increase of arachidonic acid concentration in adult patients with sepsis compared with the healthy ones. The increase was statistically significant with a p-value <0.01. Arachidonic acid concentration can be measured using FTIR spectroscopy. Kiefer *et. al.* [28] in their study used FTIR spectroscopy to measure the concentration of arachidonic acid in *Porphyridium purpureum* microalgae by looking at the absorbance at a wavelength of 3200-2700 (2857, 2872, 2928, and 2958) nm.

## 6. Conclusions

Spectroscopy is a potential alternative method for early detection of NS because it has several advantages. This method can detect biochemical changes in the early phases of a disease it is fast and inexpensive and can be used with saliva as the sample (the sampling procedures are non-invasive, do not require skilled personnel and special equipment). Therefore, further research is necessary to study this method for early detection in NS.

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