

## UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

### Abstract

**Background:** This present study was aimed to employ the UV-Vis spectroscopic techniques to detect the changes in blood of Neonatal Sepsis (NS) subject for a deeper understanding in the pathomechanism of NS.

**Methods:** The cross-sectional ~~prospective~~ study was conducted from February to May 2017 in the Neonatology Division, Department of Pediatric, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. Blood specimens were taken from newborns, of which 15 each of newborns at risk of sepsis and without risk of sepsis. Data was analyzed by using Mann-Whitney U test.

**Results:** The result of this present study suggested that there is a significant difference of the average of absorbance ratio parameter using UV-Vis ~~spectroscopic~~ ~~spectrophotometric~~ methods on the case group compare to the control group. Also, there is a significant difference between AOPPs and SCN level in newborn at risk of sepsis.

**Conclusion:** In conclusion, the present study demonstrated there were significant differences between the average of absorbance ratio parameter for protein and oxy hemoglobin region using UV-Vis ~~spectroscopic~~ ~~spectrophotometric~~ methods in healthy subjects and newborn at risk of sepsis.

**Keywords:** Neonatal Sepsis, Oxidative Stress, Spectrophotometric.

**Commented [InaB11]:** Terminology of The Study Design is not appropriate because design Cross Sectional cannot be combined with Prospective Study(longitudinal Study). I think Case Control Method Or Cross Sectional Method more appropriate. You have to mention this in the Methods too.  
**Answer :** I agreed to delete prospective word because this is the cross sectional method research.

**Commented [IH2]:** Delete this word

**Commented [IH3]:** Change the word of spectroscopic with spectrophotometric

**Commented [IH4]:** Delete this word

**Commented [IH5]:** Change the word of spectroscopic with spectrophotometric

## 23 Introduction

24 Neonatal Sepsis (NS) is a clinical syndrome that is characterized by systemic signs  
25 and symptoms of infection in neonates which is an important cause of mortality in newborns  
26 and life threatening disorder in infants (1-2). Incidence rate of neonatal sepsis in developed  
27 countries ranged between 3-5 per 1,000 live births. WHO (2007) reported the Case Fatality  
28 Rate (CFR) in the case of neonatal sepsis in the world is still high by 40% (3). In 2015, 4.5  
29 million children died in first year of life (4). Forty five percents occurred in first month of life  
30 (newborn babies). The causes of death in infants were various. Neonatal sepsis contributed  
31 for seven percent of the total cause of infant mortality (5,6). The current gold standard for  
32 diagnosis of sepsis is blood culture, suffers from low sensitivity and a reporting delay of  
33 approximately 48–72 hours (7).

34 The pathomechanism of NS is remain unclear. Some previous studies suggested that  
35 the pathomechanism of NS was through oxidative stress mechanism (2,8-9). Infectious agents  
36 in NS caused the activation neutrophils which promote a further reaction to activate NADPH  
37 oxidase. These activation will cause the formation reactive oxygen species (ROS). Also, the  
38 activation of neutrophil induced the releasing of Myeloperoxidase (MPO). MPO use  $H_2O_2$  to  
39 catalyses the oxidation of halide (Cl<sup>-</sup>, Br<sup>-</sup>) and pseudohalide (thiocyanate ion, SCN<sup>-</sup>) to form  
40 hypothiocyanous acid (HOSCN). The ROS, ~~halide and pseudohalide oxidation~~ oxidized  
41 halide and pseudohalide is known to play an important role in killing invading parasites and  
42 pathogens. Furthermore, the ROS, halide and pseudohalide oxidation products could  
43 oxidized protein and resulted in the formation of oxidized protein known as Advance  
44 Oxidation Protein Products (AOPPs) (2,10).

45 UV-Visible spectrophotometric ~~spectroscopic~~ methods was one known method that  
46 have been used extensively for several qualitative analysis of medical biological samples

Commented [A6]: I agreed

Commented [A7]: I agreed to change these words to "oxidized halide and pseudohalide"

Commented [InaBJ8]: Spectroscopic or spectrophotometric? The title uses "spectrophotometry" term. The author should understand clearly the difference between the two.  
Answer : I agreed to used spectrophotometric word for this term

47 such as, blood plasma, sera, or tissues (11). Spectroscopy is a technique that measures the  
 48 interaction of molecules with electromagnetic radiation. ~~Electromagnetic radiation in this  
 49 method is obtained from light that will trigger the excitation of electrons in a molecule.~~

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50 Furthermore, when the light was absorbed, a spectrum is obtained as a function of its  
 51 frequency of wavelength. It depend on chemical nature on the molecular environment of its  
 52 chromophores. Absorption spectroscopy is therefore an excellent technique for following  
 53 ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and  
 54 nucleic acids (12).

55 Generally, the blood experiences significant changes in compound and biochemical  
 56 properties in all diseases. The use of an examination method such as UV-Visible (UV-Vis)  
 57 ~~spectroscopy~~ ~~spectrophotometric~~ to determine changes in blood is an inevitability. This is  
 58 due to ~~reduces~~ reduction in time, resources, and cost (11). The advantage in the ever  
 59 improving sensitivity of contemporary spectrometers with sophisticated computational  
 60 techniques proved that UV-Visible (UV-Vis) ~~spectroscopy~~ ~~spectrophotometric~~ could be  
 61 exploited to explore the various biochemical alterations on the molecular and structural  
 62 differences of the biofluids of the human body (13).

Commented [IH10]: Delete this word

Commented [IH11]: Change the word of spectroscopic with spectrophotometric

Commented [A12]: I agreed to delete this word and change it to "reduction"

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Commented [IH14]: Change the word of spectroscopic with spectrophotometric

63 In recent years, ~~spectroscopy~~ ~~spectrophotometric~~ was used for the diagnosis for  
 64 several diseases. Kanagathara et al and Ibrahim et al has been employed UV-Vis  
 65 ~~spectroscopic~~ ~~spectrophotometric~~ methods to study the spectral differences in the serum of  
 66 normal blood samples (11,14). Gunasekaran et al has been demonstrated using UV-Vis  
 67 ~~spectroscopic~~ ~~spectrophotometric~~ method to differentiate the healthy sera from the jaundice,  
 68 leukemia, anemia, cirrhosis liver, thalassemia and diabetes (13,15-16). Still, there is no study  
 69 in the literature examining the characteristics of blood in NS using UV-Vis ~~spectroscopic~~  
 70 ~~spectrophotometric~~ methods. Therefore, the present experimental study aimed to employ the

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Commented [IH20]: Change the word of spectroscopic with spectrophotometric

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71 UV-Vis ~~spectroscopic~~ spectrophotometric techniques to detect the changes in blood of NS  
72 subject and also for a deeper understanding the pathomechanism of NS.

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Commented [IH24]: Change the word of spectroscopic with spectrophotometric

73

74

## 75 Methods

### 76 1. Subjects

77 The cross-sectional study was conducted on infants with and without risk of sepsis  
78 after informed consent was obtained from them. It was approved by the Ethics Commission  
79 of the Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South  
80 Kalimantan, Indonesia. The protocol of this study was approved by the Ethics Committee of  
81 Faculty of Medicine, Lambung Mangkurat University (No. 331/KEPK-FK  
82 UNLAM/EC/IV/2017) according to the Declaration of Helsinki. The participants gave us  
83 their written informed consent before the study. The study was conducted from February to  
84 May 2017 in the Neonatology Division, Department of Pediatric, Ulin General  
85 Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South  
86 Kalimantan, Indonesia. All laboratory tests were conducted at Department of Medical  
87 Chemistry/ Biochemistry, Faculty of Medicine Lambung Mangkurat University, Banjarbaru,  
88 South Kalimantan, Indonesia.

Commented [IH25]: I change the with these, and added the word of cross-sectional for answer the comment og InaBJ1

89 A total of 30 infants subjects were recruited for this study. Subjects were divided into  
90 2 groups: group I were infants at risk of sepsis and served as a case group, while group II  
91 were infants without risk of sepsis and served as a control group. Subjects in the sepsis risk  
92 group were included on the basis of having at least 1 major criteria or 2 minor criteria for  
93 sepsis according to The American Congress of Obstetricians and Gynecologist (ACOG)  
94 guidelines (17-18). Major risk criteria were premature ruptured of membranes (PROM) for >

Commented [InaBJ26]: Inclusion and exclusion criteria for each group?  
Answer : I agreed to clarify the inclusion and exclusion criteria. You can view in the several sentences in the end of this paragraph.

24 hours, maternal fever with intrapartum temperature > 38°C, chorioamnionitis, fetal heart rate persisting at > 160 times/min or bad smelling of amniotic fluid. Minor risk criteria were PROM for > 12 hours, maternal fever with intrapartum temperature > 37.5°C, low Apgar score (<5 at the 1st min, <7 at the 5th min), very low birth weight baby (VLBWB) of <1500 gr, gestational age < 37 weeks, multiple pregnancy, bad smelling of vaginal discharge, maternal urinary tract infection (UTI) or suspected untreated maternal UTI. Age and gender matched healthy newborns were chosen as controls. Newborns with birth asphyxia, low birth weight, preterm and congenital malformations were excluded from this study.

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## 2. Samples collection

Sampling was carried out by accidental sampling method, with criteria specified in subjects section as mentioned earlier. The sampling took place from February-May 2017. In that time, 30 samples were obtained, with 15 samples of newborn at risk of sepsis and 15 normal samples. Samples of blood were taken from umbilical cord (5 ml each) and collected using vacutainers containing EDTA. The samples were centrifuged for 15 min at 2000 rpm and stored at -20C until further analysis. Each sample then washed with cold saline phosphate buffer with pH 7.4 for the spectroscopic analysis, and the estimation of SCN and AOPPs levels.

Commented [InaBJ27]: The way of collection of sample is not clearly stated; how is sample size as 15 for cases and 15 for control was collected. What is the basic assumption for calculation of sampling minimal? What is the sampling procedure to get 15 for cases and 15 for control?  
Answer : I agreed to clarify the way of collection of sample. I add a clearer explanation.

Commented [IH28]: This several sentences is the way of sample collection.

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Commented [InaBJ30]: Which part of the blood were used for analysis? Plasma? Is there any aliquoting procedure after centrifugation? Please give more detail procedure.  
Answer : I agreed to clarify the way of collection of sample. I add a clearer explanation. We used blood for analysis without centrifugation and aliquoting procedure.

Commented [IH31]: This sentences was made to answer the InaBJ30 comment.

## 3. Spectroscopic analysis

Each blood sample was diluted with normal saline at concentration of 5 µL/mL. The spectra were scanned in the region between 200-700 nm. Amide chain of proteins present in the blood absorbs strongly at 210 nm. Absorption of tyrosine and tryptophan is at around 280

Commented [InaBJ32]: What about interference in spectroscopic analysis, such as: hemolysis, icterus? If there is any, please mention in the methods section. Also, please mention the instrument used for spectroscopic analysis.  
Answer : I agreed. I add the instrument used for spectroscopic analysis in this section. I add kind of proteins we mean too in this section.

119 nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-Oxy  
120 hemoglobin (13,15). Each blood sample was diluted with normal saline at concentration of 5  
121  $\mu\text{L}/\text{mL}$ . The spectra were scanned in the region between 200-700 nm using T80+ UV/VIS  
122 spectrometer at Medical Chemistry/Biochemistry Laboratory, Faculty of Medicine, Lambung  
123 Mangkurat University, Banjarbaru, South Kalimantan, Indonesia. Amide chain of proteins  
124 present in the blood absorbs strongly at 210 nm. Absorption of tyrosine and tryptophan is at  
125 around 280 nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-  
126 Oxy-hemoglobin (13,15). According to Gunasekaran et al. (15) from these spectral  
127 differences, three absorbance ratio could be measured. They were  $A_{278}/A_{210}$  in the protein  
128 region and  $A_{543}/A_{417}$ ,  $A_{578}/A_{417}$  in the oxy-hemoglobin region.

#### 131 4. SCN and AOPPs level analysis

132 SCN concentration was measured spectrophotometrically as described by Aune and  
133 Thomas. AOPPs measurement were made by spectrophotometric methods as describe by  
134 Witko-Sarsat et al., with slight modification (10).

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Commented [A33]: I add the procedure to measuring SCN and AOPP in this section.

#### 136 4.5. Statistical Analysis

137 The comparison of absorbance between healthy blood and risk of sepsis blood were  
138 examined by Mann Whitney U test with  $p < 0.05$ . Data analysis using SPSS for Windows  
139 version 16.

#### 141 Results and Discussion

142 Spectroscopic method technique has become a promising tool for a better

143 understanding in pharmaceutical, biological material, and pathomechanism of a disease. It was  
 144 first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral  
 145 differences of several diseases like diabetes, leukemia, and thyroid. In this present study we  
 146 try to employed the spectral differences between healthy ~~serum~~ blood and ~~serum-blood~~ of  
 147 newborn at risk of sepsis. The result is presented in Table 1.

148 Table 1 gives the statistical results of the analysis of UV-Visible spectral data in  
 149 protein and oxy-~~haemoglobin~~. The data clearly shows the discrimination of the newborn at  
 150 risk of sepsis blood from that of the healthy one. The result data indicated that the absorbance  
 151 ratio of oxy-~~haemoglobin~~ is lower in newborn at risk of sepsis. The decreasing of this ratio  
 152 may be due to the changes in oxy-~~haemoglobin~~.

153 It is well known that sepsis will activate the natural immune system which are mostly  
 154 roled by neutrophils. In this condition, neutrophil will inserted into infected tissue to induced  
 155 a process known as respiratory burst. This process use an oxygen to form ROS which is  
 156 useful to attack the bacterial pathogens (2). The result clearly indicated that there is a  
 157 structural chages in oxy-~~haemoglobin~~ This is thought to be due to oxygen release from oxy  
 158 ~~haemoglobin~~ used to produce ROS such as, radical superoxide ( $\bullet\text{O}_2$ ), hydrogen peroxide  
 159 ( $\text{H}_2\text{O}_2$ ), and radical hydroxyl. Also, the release of oxygen from oxy hemoglobin will lead to  
 160 hypoxia. Hypoxia it self will increased the ROS level. The basic mechanism how the hypoxia  
 161 increased the ROS level is mitochondria dysfunction which is activate ~~some enzymes~~ some  
 162 of the enzymes that play a role in the formation of ROS-like NADPH oxidase and superoxide  
 163 dismutase (SOD) (19).

164 The result data from table 1 also shows ~~that the absorbance ratio of protein~~ is also  
 165 lower in newborn at risk sepsis. This result indicated that the protein levels in blood of  
 166 newborn at risk of sepsis was decreased. This may be due to protein damage due to oxidative

**Commented [InaBJ34]:** Serum or plasma? In the methods, the author mentioned that blood were collected using EDTA vacutainer tubes.  
 Answer : I change the " serum" word with "blood". It is not serum or plasma but we use blood for the analysis. I already mentioned in the method section.

**Commented [IH35]:** Add "." in this part

**Commented [IH36]:** Delete the "a" letter on haemoglobin word. I rather to use hemoglobin than haemoglobin.

**Commented [IH37]:** Delete the "a" letter on haemoglobin word. I rather to use hemoglobin than haemoglobin.

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**Commented [IH39]:** Add "." in this part

**Commented [IH40]:** Delete the "a" letter on haemoglobin word. I rather to use hemoglobin than haemoglobin.

**Commented [IH41]:** Delete the "a" letter on haemoglobin word. I rather to use hemoglobin than haemoglobin.

**Commented [InaBJ42]:** Please be consistent in pronouncing haemoglobin or hemoglobin?  
 Answer : I rather to use hemoglobin than haemoglobin. I already change all haemoglobin to hemoglobin.

**Commented [IH43]:** Delete this two word.

**Commented [InaBJ44]:** Ratio of which proteins? Please mention clearly in methods section.  
 Answer : I explain clearer the proteins that we mean in methods section

167 stress conditions. To investigate how the protein damage during sepsis condition, in this  
168 present study we also measured the level of SCN and AOPPs. The results shows are shown in  
169 Table 2.

170 The result from Table 2 shows that the level of SCN is higher in the case group  
171 compare to cotrol group. It is in line with our previous reports which investigated the  
172 comparison of SCN levels in saliva of newborn at risk of sepsis with and without antibiotic  
173 therapy. In that study, the level of SCN seems more higher at newborn with risk of sepsis  
174 without antibiotic theraphy (10). The increasing of SCN level may be due to the activation of  
175 MPO which is used H<sub>2</sub>O<sub>2</sub> and SCN as a co-substrate to form hypothiocyanate acid (HOSCN)  
176 whic is also useful to attack bacterial pathogens (20).

177 Both ROS and HOSCN that produced in sepsis condition are powerful oxidants which  
178 promote a further raction result in host tissue damage (21). One of the targets that can be  
179 damaged by the molecule is protein. It is in line with the result from the table 2. The result  
180 from table 2 indicated that the level of AOPPs is increase. AOPPs in known as a novel  
181 marker in several condition which is related to oxidative stress (10). AOPPs is known as a  
182 cross-linking protein products which is formed by a modification of amino acid caused by  
183 oxidation by an ROS (22-23).

184

## 185 Conclusion

186 In conclusion, the present study demonstrated there were significant differences  
187 between the average of absorbance ratio parameter for protein and oxy hemoglobin region  
188 using UV-Vis spectroscopic methods in healthy subjects and newborn at risk of sepsis. It can  
189 be concluded that there were changes in blood of newborn at risk of sepsis and it may explain  
190 the pathomechanism of neonatal sepsis for our better understanding. This result study also

**Commented [InaBJ45]:** Please mention the procedure for measuring SCN and AOPPs in methods section.  
Answer : I agreed to add the procedure to measuring SCN and AOPP in methods section

**Commented [IH46]:** I agree to delete this word

**Commented [IH47]:** I agree with this change



191 suggests that UV-visible spectroscopic may be useful as a tool ~~to~~ for early diagnosis of NS.

192 Further research is needed to explore spectrophotometric analysis as a diagnosis method of

193 NS, the spectroscopic analysis as a tool for diagnosis of NS.

194

**Commented [InaBJ48]:** Repetition of the previous sentences. Please rephrase or find another statement.  
Answer : I change the previous sentences and change it with other statement.

195 **References**

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257

258 **Tables**

259 **Table 1.** UV-Visible spectral analysis of absorbance ratio parameters of healthy and newborn at risk of sepsis

260

Region	Intensity ratio parameter	Average ± SD
Protein	A <sub>278</sub> /A <sub>210</sub>	
	Normal	13.95 ± 5.714
	Sepsis	1.963 ± 0.776*
Oxy hemoglobin	A <sub>543</sub> /A <sub>417</sub>	
	Normal	1.725 ± 0.758
	Sepsis	0.852 ± 0.252*
	A <sub>578</sub> /A <sub>417</sub>	
	Normal	1.543 ± 0.876
	Sepsis	1.258 ± 0.126*

261

\* Values are significantly different from (P < 0.05), according to Mann-Whitney U tests.

262

263 **Table 2.** SCN and AOPPs level comparison between case and control group

264

Parameters	Control group	Case group
SCN (mM)	0.232 ± 0.053	0.667 ± 0.053
AOPPs (µM)	0.724 ± 0.968	3.867 ± 1.340

265

\* Values are significantly different from (P < 0.05), according to Mann-Whitney U tests.

266

## UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

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Ratih Kumala Sari<sup>2</sup>, Niarsari Anugrahi Putri<sup>2</sup>, Iskandar Thalib<sup>2,4</sup>

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Banjarmasin 70232, South Kalimantan, Indonesia

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### Abstract

**Background:** This ~~present~~ study was aimed to employ the ~~UV-Visible~~ (UV-Vis) spectroscopic techniques to detect the changes in blood of Neonatal Sepsis (NS) subject for a deeper understanding in the pathomechanism of NS.

**Methods:** The cross-sectional study was conducted from February to May 2017 in the Neonatology Division, Department of Pediatric, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. Blood specimens were taken from newborns, of which 15 each of newborns at risk of sepsis and without risk of sepsis. Data was analyzed by using Mann-Whitney U test.

**Commented [IH1]:** Agree with the deletion of this word

**Commented [InaBJ2]:** Abbreviation for?

**Commented [IH3R2]:** This is the abbreviation for UV-Visible. My correction is highlight with green mark

**Commented [InaBJ4]:** Spectrophotometry

**Commented [IH5R4]:** I think it should be spectrophotometry not spectroscopic.

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27 **Results:** The result of this present study suggested that there is a significant difference of the  
28 average of absorbance ratio parameter using UV-Vis spectrophotometric methods on the  
29 case group compare to the control group. Also, there is a significant difference between

30 Advanced Oxidation Protein Products (AOPPs) and thiocyanate (SCN) level in newborn at  
31 risk of sepsis.

32 **Conclusion:** In conclusion, the present study demonstrated there were significant differences  
33 between the average of absorbance ratio parameter for protein and oxy hemoglobin region  
34 using UV-Vis spectrophotometric Vis spectrophotometric methods in healthy subjects and  
35 newborn at risk of sepsis.

36  
37 **Keywords:** Neonatal Sepsis, Oxidative Stress, Spectrophotometric.  
38

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Commented [InaBJ7]: Abbreviation for?

Commented [IH8R7]: AOPPs for Advanced Oxidation Protein Products and SCN for thiocyanate

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39 **Introduction**

40 Neonatal Sepsis (NS) is a clinical syndrome that is characterized by systemic signs  
 41 and symptoms of infection in neonates which is an important cause of mortality in newborns  
 42 and life threatening disorder in infants (1-2). Incidence rate of neonatal sepsis in developed  
 43 countries ranged between 3-5 per 1,000 live births. WHO (2007) reported the Case Fatality  
 44 Rate (CFR) in the case of neonatal sepsis in the world is still high by 40% (3). In 2015, 4.5  
 45 million children died in first year of life (4). Forty five percents occurred in first month of life  
 46 (newborn babies). The causes of death in infants were various. Neonatal sepsis  
 47 contributed for seven percent of the total cause of infant mortality (5,6). The current gold  
 48 standard for diagnosis of sepsis is blood culture, suffers from low sensitivity and a reporting  
 49 delay of approximately 48–72 hours (7).

50 The pathomechanism of NS is remain unclear. Some previous studies suggested that  
 51 the pathomechanism of NS was through oxidative stress mechanism (2,8-9). Infectious agents  
 52 in NS caused the activation neutrophils which promote a further reaction to activate  
 53 Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase. These activation will  
 54 cause the formation reactive oxygen species (ROS). Also, the activation of neutrophil  
 55 induced the releasing of Myeloperoxidase (MPO). MPO use  $H_2O_2$  to catalyses the oxidation  
 56 of halide ( $Cl^-$ ,  $Br^-$ ) and pseudohalide (thiocyanate ion,  $SCN^-$ ) to form hypothiocyanous acid  
 57 (HOSCN). The ROS, oxidized halide and pseudohalide is known to play an important role in  
 58 killing invading parasites and pathogens. Furthermore, the ROS, halide and pseudohalide  
 59 oxidation products could oxidized protein and resulted in the formation of oxidized protein  
 60 known as Advance Oxidation Protein Products (AOPPs) (2,10).

61 UV-Visible spectrophotometric methods was one known method that have been used  
 62 extensively for several qualitative analysis of medical biological samples such as, blood

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**Commented [InaBJ11]:** Abbreviation for?

**Commented [IH12R11]:** NADPH is abbreviation for Nicotinamide Adenine Dinucleotide Phosphate

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63 plasma, sera, or tissues (11). ~~Spectroscopy~~ Spectroscopy is a technique that measures  
 64 the interaction of molecules with electromagnetic radiation. Furthermore, when the light was  
 65 absorbed, a spectrum is obtained as a function of its frequency of wavelength. It depend on  
 66 chemical nature on the molecular environment of its chromophores. ~~Absorption~~  
 67 ~~spectroscopy~~ This method is therefore an excellent technique for following ligand-binding  
 68 reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids (12).

69 Generally, the blood experiences significant changes in compound and biochemical  
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 72 in time, resources, and cost (11). The advantage in the ever improving sensitivity of  
 73 contemporary spectrometers with sophisticated computational techniques proved that ~~UV-~~  
 74 ~~Visible (UV-Vis)~~ spectrophotometric could be exploited to explore the various biochemical  
 75 alterations on the molecular and structural differences of the biofluids of the human body  
 76 (13).

77 In recent years, spectrophotometric was used for the diagnosis for several diseases.  
 78 Kanagathara et al and Ibrahim et al has been employed UV-Vis spectrophotometric methods  
 79 to study the spectral differences in the serum of normal blood samples (11,14). Gunasekaran  
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 81 healthy sera from the jaundice, leukemia, anemia, cirrhosis liver, thalassemia and diabetes  
 82 (13,15-16). Still, there is no study in the literature examining the characteristics of blood in  
 83 NS using UV-Vis spectrophotometric methods. Therefore, the present experimental study  
 84 aimed to employ the UV-Vis spectrophotometric techniques to detect the changes in blood of  
 85 NS subject and also for a deeper understanding the pathomechanism of NS.

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**Commented [InaBJ15]:** Once being shortened in the beginning, no need to write the full term

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87

88 **Methods**89 **1. Subjects**

90 These cross-sectional study was conducted on infants with and without risk of sepsis  
91 after informed consent was obtained from them. It was approved by the Ethics Commission  
92 of the Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South  
93 Kalimantan, Indonesia. The protocol of this study was approved by the Ethics Committee of  
94 Faculty of Medicine, Lambung Mangkurat University (No. 331/KEPK-FK  
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96 their written informed consent before the study. The study was conducted from February to  
97 May 2017 in the Neonatology Division, Department of Pediatric, Ulin General  
98 Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South  
99 Kalimantan, Indonesia. All laboratory tests were conducted at Department of Medical  
100 Chemistry/ Biochemistry, Faculty of Medicine Lambung Mangkurat University, Banjarbaru,  
101 South Kalimantan, Indonesia.

102 A total of 30 infants subjects were recruited for this study. Subjects were divided into  
103 2 groups: group I were infants at risk of sepsis and served as a case group, while group II  
104 were infants without risk of sepsis and served as a control group. Subjects in the sepsis risk  
105 group were included on the basis of having at least 1 major criteria or 2 minor criteria for  
106 sepsis according to The American Congress of Obstetricians and Gynecologist (ACOG)  
107 guidelines (17-18). Major risk criteria were premature ruptured of membranes (PROM) for >  
108 24 hours, maternal fever with intrapartum temperature > 38°C, chorioamnionitis, fetal heart  
109 rate persisting at > 160 times/min or bad smelling of amniotic fluid. Minor risk criteria were  
110 **Premature Rupture of Membrane**, **PROM** for > 12 hours, maternal fever with intrapartum

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Premature Rupture of Membrane

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111 temperature > 37.5°C, low Apgar score (<5 at the 1st min-, <7 at the 5th min), very low birth  
 112 weight baby (VLBWB) of <1500 gr, gestational age- < 37 weeks, multiple pregnancy, bad  
 113 smelling of vaginal discharge, maternal urinary tract infection (UTI) or suspected untreated  
 114 maternal UTI. Age and gender matched healthy newborns were chosen as controls. Newborns  
 115 with birth asphyxia, low birth weight, preterm and congenital malformations were excluded  
 116 from this study.

117

## 118 2. Samples collection

119 Sampling was carried out by accidental sampling method, with criteria specified in  
 120 subjects section as mentioned earlier. The sampling took place from February-May 2017. In  
 121 that time, 30 samples were obtained, with 15 samples of newborn at risk of sepsis and 15  
 122 normal samples Samples of blood were taken from umbilical cord (5 ml each) and collected  
 123 using vacutainers containing EDTA. Each sample then washed with cold saline phosphate  
 124 buffer with pH 7.4 for the spectroscopic analysis, and the estimation of SCN and AOPPs  
 125 levels.

126

127

## 128 3. Spectrophotometryseopie analysis

129 Each blood sample was diluted with normal saline at concentration of 5 µL/mL. The  
 130 spectra were scanned in the region between 200-700 nm using T80+ UV/VIS spectrometer at  
 131 Medical Chemistry/Biochemsity Laboratory, Faculty of Medicine, Lambung Mangkurat  
 132 University, Banjarbaru, South Kalimantan, Indonesia. Amide chain of proteins present in the  
 133 blood absorbs strongly at 210 nm. Absorption of tyrosine and tryptophan is at around 280  
 134 nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-Oxy-

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Commented [IH20R19]: SCN for thiocyanate and AOPPs for Advance Oxidation Protein Products. I thin already mentioned in introduction section. You can see in page 3 line 55 and 59.

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135 hemoglobin (13,15). According to Gunasekaran et al. (15) from these spectral differences,  
136 three absorbance ratio could be measured. They were  $A_{278}/A_{210}$  in the protein region and  
137  $A_{543}/A_{417}$ ,  $A_{578}/A_{417}$  in the oxy-hemoglobin region.

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138

#### 139 4. SCN and AOPPs level analysis

140 SCN concentration was measured spectrophotometrically as described by Aune and Thomas.  
141 AOPPs measurement were made by spectrophotometric methods as describe by Witko-Sarsat  
142 et al., with slight modification (10).

143

#### 144 5. Staistical Analysis

145 The comparison of absorbance between healthy blood and risk of sepsis blood were  
146 examined by Mann Whitney U test with  $p < 0.05$ . Data analysis using SPSS for Windows  
147 version 16 (SPSS Inc., Chicago, Illinois, USA).

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#### 149 Results and Discussion

150 Spectrophotometry seopie method techniqe has become a promising tool for a better  
151 understanding in phmarceutical, biological material, and pathomechanism of a disease. It was  
152 first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral  
153 differencies of several diseases like diabetes, leukemia, and thyroid. In this present study we  
154 try to employed the spectral differencies between healthy blood and blood of newborn at risk  
155 of sepsis. The result is presented in Table 1.

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156 Table 1 gives the statistical results of the analysis of UV-Visible spectral data in  
157 protein and oxy-hemoglobin. The data clearly shows the discrimination of the newborn at risk  
158 of sepsis blood from that of the healthy one. The result data indicated that the absorbance

159 ratio—of oxy-hemoglobin is lower in newborn at risk of sepsis. The decreasing of this ratio  
160 may be due to the changes in oxy-hemoglobin.

161 It is well known that sepsis will activate the natural immune system which are mostly  
162 roled by neutrophils. In this condition, neutrophil will ~~inserted~~have ~~inserted~~ into infected  
163 tissue to ~~induced~~induce a process known as respiratory burst. This process ~~use~~uses an oxygen  
164 to form ROS which is useful to attack the bacterial pathogens (2). The result clearly indicated  
165 that there is a structural chages in oxy-hemoglobin This is thought to be due to oxygen  
166 release from oxy hemoglobin used to produce ROS such as, radical superoxide ( $\bullet\text{O}_2$ ),  
167 hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and radical hydroxyl. Also, the release of oxygen from oxy  
168 hemoglobin will lead to hypoxia. Hypoxia itself will increased the ROS level. The basic  
169 mechanism how the hypoxia increased the ROS level is mitochondria dysfunction which is  
170 activate some of the enzymes that play a role in the formation of ROS-like NADPH oxidase  
171 and superoxide dismutase (SOD) (19).

172 The result data from table 1 also shows that the absorbance ratio of protein is also  
173 lower in newborn at risk sepsis. This result indicated that the protein levels in blood of  
174 newborn at risk of sepsis was decreased. This may be due to protein damage due to oxidative  
175 stress conditions. To investigate how the protein damage during sepsis condition, in this  
176 present study we also measured the level of SCN and AOPPs. The results ~~shows~~are shown in  
177 Table 2.

178 The result from Table 2 shows that the level of SCN is higher in the case group  
179 compare to cotrol group. It is in line with our previous reports which investigated the  
180 comparison of SCN levels in saliva of newborn at risk of sepsis with and without antibiotic  
181 therapy. In that study, the level of SCN seems more higher at newborn with risk of sepsis  
182 without antibiotic therapy (10). The increasing of SCN level may be due to the activation of

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183 MPO which is used H<sub>2</sub>O<sub>2</sub> and SCN as a co-substrate to form hypothiocyanate acid (HOSCN)  
184 whic is also useful to attack bacterial pathogens (20).

185 Both ROS and HOSCN that produced in sepsis condition are powerful oxidants which  
186 promote a further raction result in host tissue damage (21). One of the targets that can be  
187 damaged by the molecule is protein. It is in line with the result from the table 2. The result  
188 from table 2 indicated that the level of AOPPs is increase. AOPPs in known as a novel  
189 marker in several condition which is related to oxidative stress (10). AOPPs is known as a  
190 cross-linking protein products which is formed by a modification of amino acid caused by  
191 oxidation by an ROS (22-23).

192

### 193 Conclusion

194 In conclusion, the present study demonstrated there were significant differences  
195 between the average of absorbance ratio parameter for protein and oxy hemoglobin region  
196 using UV-Vis ~~spectrophotometricseopie~~ methods in healthy subjects and newborn at risk of  
197 sepsis. It can be concluded that there were changes in blood of newborn at risk of sepsis and  
198 it may explain the pathomechanism of neonatal sepsis for our better understanding. This  
199 result study also suggests that UV-visible ~~spectrophotometricseopie~~ may be useful as a tool  
200 ~~te~~ for early diagnosis of NS. Further research is needed to explore spectrophotometric  
201 analysis as a diagnosis method of NS.

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265

266 **Tables**

267 **Table 1.** UV-Visible spectral analysis of absorbance ratio parameters of healthy and newborn at risk of sepsis

268

Region	Intensity ratio parameter	Average ± SD	P value
Protein	A <sub>278</sub> /A <sub>210</sub>	13.95 ± 5.714	
	Sepsis	1.963 ± 0.776*	0.003
Oxy hemoglobin	A <sub>543</sub> /A <sub>417</sub>	1.725 ± 0.758	
	Sepsis	0.852 ± 0.252*	0.003
	A <sub>578</sub> /A <sub>417</sub>	1.543 ± 0.876	
	Sepsis	1.258 ± 0.126*	0.003

269 \* Values are significantly different from (P < 0.05) **1.963 ± 0.776\***, according to Mann-Whitney U tests.

271 **Table 2.** SCN and AOPPs level comparison between case and control group

272

Parameters	Control group	Case group	P value
SCN (mM)	0.232 ± 0.053	0.667 ± 0.053	0.003
AOPPs (µM)	0.724 ± 0.968	3.867 ± 1.340	0.003

273 \* Values are significantly different from (P < 0.05) **3.867 ± 1.340**, according to Mann-Whitney U tests.

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## UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

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Ratih Kumala Sari<sup>2</sup>, Niarsari Anugrahing Putri<sup>2</sup>, Iskandar Thalib<sup>2,4</sup>

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### Abstract

**Background:** This present study was aimed to employ the UV-Vis spectroscopic techniques to detect the changes in blood of Neonatal Sepsis (NS) subject for a deeper understanding in the pathomechanism of NS.

**Methods:** The cross-sectional prospective study was conducted from February to May 2017 in the Neonatology Division, Department of Pediatric, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. Blood specimens were taken from newborns, of which 15 each of newborns at risk of sepsis and without risk of sepsis. Data was analyzed by using Mann-Whitney U test.

**Results:** The result of this present study suggested that there is a significant difference of the average of absorbance ratio parameter using UV-Vis spectroscopic methods on the case group compare to the control group. Also, there is a significant difference between AOPPs and SCN level in newborn at risk of sepsis.

**Conclusion:** In conclusion, the present study demonstrated there were significant differences between the average of absorbance ratio parameter for protein and oxy hemoglobin region using UV-Vis spectroscopic methods in healthy subjects and newborn at risk of sepsis.

**Keywords:** Neonatal Sepsis, Oxidative Stress, Spectroscopy.

## Introduction

Neonatal Sepsis (NS) is a clinical syndrome that is characterized by systemic signs and symptoms of infection in neonates which is an important cause of mortality in newborns and life threatening disorder in infants (1-2). Incidence rate of neonatal sepsis in developed countries ranged between 3-5 per 1,000 live births. WHO (2007) reported the Case Fatality Rate (CFR) in the case of neonatal sepsis in the world is still high by 40% (3). In 2015, 4.5 million children died in first year of life (4). Forty five percents occurred in first month of life (newborn babies). The causes of death in infants were various. Neonatal sepsis contributed for seven percent of the total cause of infant mortality (5,6). The current gold standard for diagnosis of sepsis is blood culture, suffers from low sensitivity and a reporting delay of approximately 48–72 hours (7).

The pathomechanism of NS is remain unclear. Some previous studies suggested that the pathomechanism of NS was through oxidative stress mechanism (2,8-9). Infectious agents in NS caused the activation neutrophils which promote a further reaction to activate NADPH oxidase. These activation will cause the formation reactive oxygen species (ROS). Also, the activation of neutrophil induced the releasing of Myeloperoxidase (MPO). MPO use  $H_2O_2$  to catalyses the oxidation of halide ( $Cl^-$ ,  $Br^-$ ) and pseudohalide (thiocyanate ion,  $SCN^-$ ) to form HO $SCN^-$ . The ROS, halide and pseudohalide oxidation is known to play an important role in killing invading parasites and pathogens. Furthermore, the ROS, halide and pseudohalide oxidation products could oxidized protein and resulted in the formation of oxidized protein known as Advance Oxidation Protein Products (AOPPs) (2,10).

UV-Visible spectroscopic methods was one known method that have been used extensively for several qualitative analysis of medical biological samples such as, blood plasma, sera, or tissues (11). Spectroscopy is a technique that measures the interaction of

molecules with electromagnetic radiation. Electromagnetic radiation in this method is obtained from light that will trigger the excitation of electrons in a molecule. Furthermore, when the light was absorbed, a spectrum is obtained as a function of its frequency of wavelength. It depend on chemical nature on the molecular environment of its chromophores. Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids (12).

Generally, the blood experiences significant changes in compound and biochemical properties in all diseases. The use of an examination method such as UV-Visible (UV-Vis) spectroscopy to determine changes in blood is an inevitability. This is due to reduces in time, resources, and cost (11). The advantage in the ever improving sensitivity of contemporary spectrometers with sophisticated computational techniques proved that UV-Visible (UV-Vis) spectroscopy could be exploited to explore the various biochemical alterations on the molecular and structural differences of the biofluids of the human body (13).

In recent years, spectroscopy was used for the diagnosis for several diseases. Kanagathara et al and Ibrahim et al has been employed UV-Vis spectroscopic methods to study the spectral differences in the serum of normal blood samples (11,14). Gunasekaran et al has been demonstrated using UV-Vis spectroscopic method to differentiate the healthy sera from the jaundice, leukemia, anemia, cirrhosis liver, thalassemia and diabetes (13,15-16). Still, there is no study in the literature examining the characteristics of blood in NS using UV-Vis spectroscopic methods. Therefore, the present experimental study aimed to employ the UV-Vis spectroscopic techniques to detect the changes in blood of NS subject and also for a deeper understanding the pathomechanism of NS.

## Methods

### 1. Subjects

The study was conducted on infants with and without risk of sepsis after informed consent was obtained from them. It was approved by the Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. The protocol of this study was approved by the Ethics Committee of Faculty of Medicine, Lambung Mangkurat University (No. 331/KEPK-FK UNLAM/EC/IV/2017) according to the Declaration of Helsinki. The participants gave us their written informed consent before the study. The study was conducted from February to May 2017 in the Neonatology Division, Department of Pediatric, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. All laboratory tests were conducted at Department of Medical Chemistry/ Biochemistry, Faculty of Medicine Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia.

A total of 30 infants subjects were recruited for this study. Subjects were divided into 2 groups: group I were infants at risk of sepsis and served as a case group, while group II were infants without risk of sepsis and served as a control group. Subjects in the sepsis risk group were included on the basis of having at least 1 major criteria or 2 minor criteria for sepsis according to The American Congress of Obstetricians and Gynecologist (ACOG) guidelines (17-18).

### 2. Samples collection

Samples of blood were taken from umbilical cord (5 ml each) and collected using vacutainers containing EDTA. The samples were centrifuged for 15 min at 2000 rpm and stored at -20C until further analysis.

### 3. Spectroscopic analysis

Each blood sample was diluted with normal saline at concentration of 5  $\mu\text{L}/\text{mL}$ . The spectra were scanned in the region between 200-700 nm. Amide chain of proteins present in the blood absorbs strongly at 210 nm. Absorption of tyrosine and tryptophan is at around 280 nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-Oxy hemoglobin (13,15).

### 4. Statistical Analysis

The comparison of absorbance between healthy blood and risk of sepsis blood were examined by Mann Whitney U test with  $p < 0.05$ . Data analysis using SPSS for Windows version 16.

## Results and Discussion

Spectroscopic method technique has become a promising tool for a better understanding in pharmaceutical, biological material, and pathomechanism of a disease. It was first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral differences of several diseases like diabetes, leukemia, and thyroid. In this present study we try to employed the spectral differences between healthy serum and serum of newborn at risk of sepsis. The result is presented in table 1.

Table 1 gives the statistical results of the analysis of UV-Visible spectral data in protein and oxy haemoglobin. The data clearly shows the discrimination of the newborn at risk of sepsis blood from that of the healthy one. The result data indicated that the absorbance ratio of oxy-haemoglobin is lower in newborn at risk of sepsis. The decreasing of this ratio



may be due to the changes in oxy hemoglobin.

It is well known that sepsis will activate the natural immune system which are mostly roled by neutrophils. In this condition, neutrophil will inserted into infected tissue to induced a process known as respiratory burst. This process use an oxygen to form ROS which is useful to attack the bacterial pathogens (2). The result clearly indicated that there is a structural chages in oxy haemoglobin This is thought to be due to oxygen release from oxy hemoglobin used to produce ROS such as, radical superoxide ( $\bullet\text{O}_2$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and radical hydroxyl. Also, the release of oxygen from oxy hemoglobin will lead to hypoxia. Hypoxia it self will increased the ROS level. The basic mechanism how the hypoxia increased the ROS level is mitochondria dysfunction which is activate some enzymes some of the enzymes that play a role in the formation of ROS-like NADPH oxidase and superoxide dismutase (SOD) (19).

The result data from table 1 also shows that the absorbance ratio of protein is also lower in newborn at risk sepsis. This result indicated that the protein levels in blood of newborn at risk of sepsis was decreased. This may be due to protein damage due to oxidative stress conditions. To investigate how the protein damage during sepsis condition, in this present study we also measured the level of SCN and AOPPs. The results shows in table 2.

The result from table 2 shows that the level of SCN is higher in the case group compare to cotrol group. It is in line with our previous reports which investigated the comparison of SCN levels in saliva of newborn at risk of sepsis with and without antibiotic therapy. In that study, the level of SCN seems more higher at newborn with risk of sepsis without antibiotic theraphy (10). The increasing of SCN level may be due to the activation of MPO which is used  $\text{H}_2\text{O}_2$  and SCN as a co-substrate to form hypothiocyanate acid (HOSCN) whic is also useful to attack bacterial pathogens (20).

Both ROS and HOSCN that produced in sepsis condition are powerful oxidants which promote a further reaction result in host tissue damage (21). One of the targets that can be damaged by the molecule is protein. It is in line with the result from the table 2. The result from table 2 indicated that the level of AOPPs is increase. AOPPs in known as a novel marker in several condition which is related to oxidative stress (10). AOPPs is known as a cross-linking protein products which is formed by a modification of amino acid caused by oxidation by an ROS (22-23).

### **Conclusion**

In conclusion, the present study demonstrated there were significant differences between the average of absorbance ratio parameter for protein and oxy hemoglobin region using UV-Vis spectroscopic methods in healthy subjects and newborn at risk of sepsis. It can be concluded that there were changes in blood of newborn at risk of sepsis and it may explain the pathomechanism of neonatal sepsis for our better understanding. This result study also suggests that UV-visible spectroscopic may be useful as a tool to early diagnosis of NS. Further research is needed to explore the spectroscopic analysis as a tool for diagnosis of NS.

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**Tables**

**Table 1.** UV-Visible spectral analysis of absorbance ratio parameters of healthy and newborn at risk of sepsis

Region	Intensity ratio parameter	Average ± SD
Protein	$A_{278}/A_{210}$	
	Normal	13.95 ± 5.714
	Sepsis	1.963 ± 0.776*
Oxy hemoglobin	$A_{543}/A_{417}$	
	Normal	1.725 ± 0.758
	Sepsis	0.852 ± 0.252*
	$A_{578}/A_{417}$	
	Normal	1.543 ± 0.876
	Sepsis	1.258 ± 0.126*

\* Values are significantly different from ( $P < 0.05$ ), according to Mann-Whitney U tests.

**Table 2.** SCN and AOPPs level comparison between case and control group

Parameters	Control group	Case group
SCN (mM)	0.232 ± 0.053	0.667 ± 0.053
AOPPs (μM)	0.724 ± 0.968	3.867 ± 1.340

\* Values are significantly different from ( $P < 0.05$ ), according to Mann-Whitney U tests.



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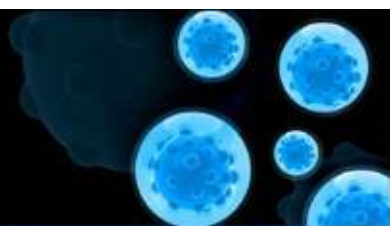
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Section: Research Article

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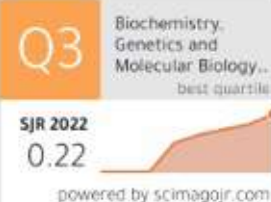
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