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 Nenonatal 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 averange 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.

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Introduction

Neonatal Sepsis (NS) is a clinincal syndrome that is charazterized 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 hypothiocyanous acid (HOSCN). The ROS, ~~halide and pseudohalide oxidation~~ oxidized halide and pseudohalide is known to play an important role in killing invading parasites and pathogens. Furthermore, the ROS, halide and pseudohalide oxidation products could oxidizied protein and resulted in the formation of oxidized protein known as Advance Oxidation Protein Products (AOPPs) (2,10).

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

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Answer : I agreed to used spectrophotometric word for this term

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~~ spectrophotometric to determine changes in blood is an inevitability. This is due to ~~reduces~~ reduction 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~~ spectrophotometric 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~~ spectrophotometric was used for the diagnosis for several diseases. Kanagathara et al and Ibrahim et al has been employed UV-Vis ~~spectroscopic~~ spectrophotometric 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~~ spectrophotometric 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~~ spectrophotometric methods. Therefore, the present experimental study aimed to employ the

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

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Methods

1. Subjects

These cross-sectional 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.

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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). Major risk criteria were premature ruptured of membranes (PROM) for >

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24 hours, maternal fever with intrapartum temperature > 38ºC, 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 > 37.5ºC, 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 spectoscopic analysis, and the estimation of SCN and AOPPs levels.

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Answer : I agreed to clarify the way of collection of sample. I add a clearer explanation.

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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.

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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~~

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~~nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-Oxy hemoglobin (13,15).~~ 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 using T80+ UV/VIS spectrometer at Medical Chemistry/Biochemsitry Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia. 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). According to Gunasekaran et al. (15) from these spectral differences, three absorbance ratio could be measured. They were A_{278}/A_{210} in the protein region and A_{543}/A_{417}, A_{578}/A_{417} in the oxy-hemoglobin region.

4. SCN and AOPPs level analysis

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

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~~4.~~5. 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 techinique has become a promising tool for a better

understanding in phmarceutical, biological material, and pathomechanism of a disease. It was first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral differencies of several diseases like diabetes, leukemia, and thyroid. In this present study we try to employed the spectral differencies between healthy ~~serum~~ blood and ~~serum~~ blood 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-~~h~~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-h~~a~~emoglobin 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-h~~a~~emoglobin This is thought to be due to oxygen release from oxy h~~a~~emoglobin used to produce ROS such as, radical superoxide (•O2), hydrogen peroxide (H_2O_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

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Answer : I rather to use hemoglobin than haemoglobin. I already change all haemoglobin to hemoglobin.

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Answer : I explain clearer the proteins that we mean in methods section

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~~ are shown 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 H_2O_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 raction 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

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suggests that UV-visible spectroscopic may be useful as a tool ~~to~~ for early diagnosis of NS. Further research is needed to explore spectrophotometric analysis as a diagnosis method of NS. ~~the spectroscopic analysis as a tool for diagnosis of NS.~~

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Answer : I change the previous sentences and change it with other statement.

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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.

UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

Eko Suhartono[1,*], Ari Yunanto[2], Edi Hartoyo[2], Nia Kania[3], Adelia Anggraini Utama[2], Ratih Kumala Sari[2], Niarsari Anugrahing Putri[2], Iskandar Thalib[2,4]

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Abstract

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Results: The result of this ~~present~~ study suggested that there is a significant difference of the averange of absorbance ratio parameter using UV-Vis spectrophotometric methods on the case group compare to the control group. Also, there is a significant difference between Advanced Oxidation Protein Products (AOPPs) and thiocyanate (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 spectrophotometric~~Vis spectrophotometric methods in healthy subjects and newborn at risk of sepsis.

Keywords: Neonatal Sepsis, Oxidative Stress, Spectrophotometric.

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Introduction

Neonatal Sepsis (NS) is a clinincal syndrome that is charazterized 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 NS 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).

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

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plasma, sera, or tissues (11). Spectrophotometry ~~Spectroscopy~~ is a technique that measures the interaction of molecules with electromagnetic radiation. 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~~This method is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids (12).

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If they used spectrophotometry, then just elaborate more about this method.
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Methods

1. Subjects

These cross-sectional 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). Major risk criteria were premature ruptured of membranes (PROM) for > 24 hours, maternal fever with intrapartum temperature > 38ºC, chorioamnionitis, fetal heart rate persisting at > 160 times/min or bad smelling of amniotic fluid. Minor risk criteria were Premature Rupture of Membrane [PROM] for > 12 hours, maternal fever with intrapartum

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temperature > 37.5ºC, 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.

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. Each sample then washed with cold saline phosphate buffer with pH 7.4 for the spectoscopic analysis, and the estimation of SCN and AOPPs levels.

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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.

3. Spectrophotometryscopic analysis

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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 using T80+ UV/VIS spectrometer at Medical Chemistry/Biochemsitry Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia. 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). ~~According to Gunasekaran et al.~~ (15) from these spectral differences, three absorbance ratio could be measured. They were A_{278}/A_{210} in the protein region and A_{543}/A_{417}, A_{578}/A_{417} in the oxy-hemoglobin region.

4. SCN and AOPPs level analysis

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

5. Staistical 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 (SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

Spectrophotometry ~~scopic~~ method techinique has become a promising tool for a better understanding in phmarceutical, biological material, and pathomechanism of a disease. It was first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral differencies of several diseases like diabetes, leukemia, and thyroid. In this present study we try to employed the spectral differencies between healthy blood and blood 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-hemoglobin. 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

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ratio —of oxy-hemoglobin 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~~have inserted into infected tissue to ~~induced~~induce a process known as respiratory burst. This process ~~use~~uses 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-hemoglobin This is thought to be due to oxygen release from oxy hemoglobin used to produce ROS such as, radical superoxide (•O2), hydrogen peroxide (H_2O_2), and radical hydroxyl. Also, the release of oxygen from oxy hemoglobin will lead to hypoxia. Hypoxia itself will increased the ROS level. The basic mechanism how the hypoxia increased the ROS level is mitochondria dysfunction which is activate 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~~ are shown 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

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MPO which is used H_2O_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 raction 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 spectrophotometric~~scopic~~ 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 spectrophotometric~~scopic~~ may be useful as a tool ~~to~~ for early diagnosis of NS. Further research is needed to explore spectrophotometric analysis as a diagnosis method 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	P value
Protein	A_{278}/A_{210}		
	Normal	13.95 ± 5.714	
	Sepsis	1.963 ± 0.776*	0.000
Oxy hemoglobin	A_{543}/A_{417}		
	Normal	1.725 ± 0.758	
	Sepsis	0.852 ± 0.252*	0.000
	A_{578}/A_{417}		
	Normal	1.543 ± 0.876	
	Sepsis	1.258 ± 0.126*	0.000

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

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

Parameters	Control group	Case group	P value
SCN (mM)	0.232 ± 0.053	0.667 ± 0.053*	0.001
AOPPs (µM)	0.724 ± 0.968	3.867 ± 1.340*	0.000

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

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UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

Eko Suhartono[1,*], Ari Yunanto[2], Edi Hartoyo[2], Nia Kania[3], Adelia Anggraini Utama[2], Ratih Kumala Sari[2], Niarsari Anugrahing Putri[2], Iskandar Thalib[2,4]

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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 Nenonatal 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 averange 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 clinincal syndrome that is charazterized 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 HOSCN. 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 oxidizied 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.

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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.

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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 techinique has become a promising tool for a better understanding in phmarceutical, biological material, and pathomechanism of a disease. It was first described by Gunasekaran et al. (13,15-16) study which is investigated the spectral differencies of several diseases like diabetes, leukemia, and thyroid. In this present study we try to employed the spectral differencies 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.

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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.

The Indonesian Biomedical Journal

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Document Number : 003/F16/IBJ/I/2018
Subject : Proof reading

Jakarta, January 7, 2018

Dear Dr. Eko Suhartono,

Thank you for your contribution to The Indonesian Biomedical Journal. In this opportunity, we would to inform you that your manuscript is entering publishing process. We need your kind assistance to check an attached proof of your manuscript and please respond before January 12, 2018. Please do any necessary corrections.

When you completed all your corrections/comments, please scan your commented copy and send it along with your filled "Proof Reading Approval by Author" to Secretariat@InaBJ.org.

Thank you for your kind attention and cooperation. Looking forward for your future manuscript submission.

Sincerely,

Nurrani Mustika Dewi
Secretarial Officer

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Dear Managing Editor of The Indonesian Biomedical Journal,

Hereby I confirm that I have already checked the "proof reading" of my manuscript entitled:

UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis

I am agreed that the manuscript : (please tick one)

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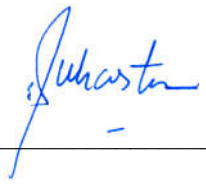

Eko Suhartono

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#360 Editing

SUMMARY | REVIEW | EDITING

Submission

Authors	Eko Suhartono, Ari Yunanto, Edi Hartoyo, Nia Kania, Adelia Anggraini Utama, Ratih Kumala Sari, Niarsari Anugrahing Putri, Iskandar Thalib
Title	UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis
Section	Research Article
Editor	Marita Kaniawati

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The Indonesian Biomedical Journal

#360 Review

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Submission

Authors	Eko Suhartono, Ari Yunanto, Edi Hartoyo, Nia Kania, Adelia Anggraini Utama, Ratih Kumala Sari, Niarsari Anugrahing Putri, Iskandar Thalib
Title	UV-Visible Spectrophotometric as a Prospective Tool in Neonatal Sepsis
Section	Research Article
Editor	Marita Kaniawati

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#360 Summary

SUMMARY | REVIEW | EDITING

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Authors	Eko Suhartono, Ari Yunanto, Edi Hartoyo, Nia Kania, Adelia Anggraini Utama, Ratih Kumala Sari, Niarsari Anugrahing Putri, Iskandar Thalib
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