ORIGINAL ARTICLE

Serum Melatonin in Early Onset Neonatal Sepsis

[1]Mohammed EL Barbary, [1]Mostafa G. Mostafa, [2]Walaa A.Y. Kabiel, [1]Hebatallah A. Shaaban*
[1]Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]Department of Clinical pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Key words:
Neonatal sepsis, melatonin, oxidative stress

Corresponding Author:
Hebatallah A. Shaaban, Department of Pediatrics, Faculty of Medicine, Ain Shams University, Abbassia Square, P.O. Box 11381, Cairo, Egypt.
Tel: +201227169267
Heba_Ali@med.asu.edu.eg

ABSTRACT

Background: *Neonatal sepsis is the result of inflammatory and oxidative stress events leading to cellular oxidative damage. Endogenous melatonin is an antioxidant and free radical scavenger. The objective of this study is to evaluate serum melatonin level as a marker of early onset neonatal sepsis (EOS) in preterm infants.* ***Methodology:*** *Serum melatonin was measured in 20 preterm infants with EOS, and 20 healthy matched preterm controls on day1, then serum melatonin level was estimated in all patients 72 hours after the start of the empirical antibiotics.* ***Results:*** *There was a statistically significant positive correlation between serum melatonin and TLC, CRP, and I/T ratio (r= 0.7458, p=0.001) (r= 0.76, p = 0.01) (r= 0.758, p= 0.001) respectively after empirical antibiotic. Also, there was significant positive correlation between CRP percentage of change and serum melatonin percentage of change after antibiotics (r= 0.752, P<0.001). However, there was no statistically significant difference between patients' initial serum melatonin level and control. Also, there was no statistically significant correlation between initial patients' serum melatonin and initial hemoglobin, TLC, platelet count, I/T ratio, or CRP.* ***Conclusion:*** *Endogenous melatonin could not be used as a marker for EOS; however, it might be helpful in the follow up of preterm patients with EOS.*

INTRODUCTION

Neonatal sepsis a systemic infection in infants ≤28 days of life is one of the leading causes of morbidity and mortality in the neonatal population and is responsible for 30-50% of the total neonatal deaths each year in developing countries. One study in a Nigerian tertiary hospital revealed that incidence of early onset neonatal sepsis (EOS) was 63.5% with 24.1% of them had culture-proven sepsis. In preterm, EOS is occurring in the first 72 hours of life and is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery [1, 2, 3].

Neonatal sepsis is the result of infection and systemic inflammatory syndrome stimulated by pathogens, followed by robust immune system response that activates a redox cascade in the intracellular compartment, leading to mitochondrial, cell and organ dysfunction [4].

Rapid diagnosis of EOS is challenging and complicated because signs and symptoms are subtle in different organs, including apnea, hypothermia/fever, poor perfusion, lethargy/irritability, hypotonia/seizures, and feeding intolerance [5]. Moreover, proven sepsis diagnosis depends on blood culture, which is time-consuming, and with low positivity rate, in addition, it is affected by inadequate blood volume inoculated, intrapartum antibiotic use, low-grade bacteremia and laboratory capabilities[6]. Until recently, no single marker has advantage over others, and laboratory tests are limited. Complete blood count indices are insufficiently sensitive to exclude EOS. C-reactive protein (CRP) the most extensively investigated acute-phase reactants, its reliability within the early phase of sepsis is neither able to diagnose nor rule out infection [6,7].

Many studies either on septic neonates or on animals demonstrated that pro-oxidant pathways paralleled by an increased activity of antioxidant defensive systems; however, redox imbalance supports oxidative stress, proved by increased markers of oxidative damage in sepsis group compared to controls[4].

Melatonin showed different anti-inflammatory and antioxidant effects, through direct scavenging behavior against reactive oxygen species (ROS) and by indirect mechanisms through excitation of antioxidant agents. High melatonin levels found in critically ill children compared to normal age-matched group may be a response to oppose the elevated oxidative stress associated with serious diseases [8].

An ideal diagnostic laboratory study for EOS needs to be rapid, sensitive, and specific, we hypothesized that endogenous serum melatonin level in preterm infants with EOS would be high in comparison with healthy preterm, and it can be utilized as a marker for sepsis in neonates, additionally, we aimed to evaluate serum melatonin level before and after treatment with empirical antibiotic in EOS.

METHODOLOGY

Study population:

This prospective observational cohort study was carried out at Neonatal Intensive Care Unit (NICU), Ain Shams University Hospitals. An informed written consent was taken from the parents or legal guardians. The protocol was approved by the Research Ethical Committee of Ain Shams University hospitals; ID: FMASU MS 118/2019 and are in accordance with the Helsinki Declaration of 1975.

This study included any preterm neonate ≤ 36 weeks gestational age (GA), having early onset neonatal sepsis depending on signs and laboratory parameters including Rodwell's hematological score ≥3[9]. Preterm with hypoxia, infants with high oxygen needs either on invasive or non-invasive mechanical ventilation, maternal causes of increased free radicals in preterm (maternal pre-eclampsia, diabetes) were excluded from our study.

Clinical examination and routine neonatal care:

Perinatal history and clinical examination were recorded for all babies. GA was calculated by maternal last menstrual period and approved by the Ballard score [10]. Birth anthropometric measures: birth weight, birth length, and occipitofrontal circumference were recorded. All neonates were managed according to our NICU protocol.

Twenty consecutively inborn preterm infants with EOS and 20 matched healthy preterm controls were enrolled, in the clinical examination.

Laboratory analysis:

For complete blood count (CBC) analysis, venous blood samples were obtained on potassium-ethylene diamine tetra acetic acid (K2-EDTA) in sterile vacutainers, which were placed in a cold ice box at 4–8 °C. The samples were transported and analyzed on the same day. CBC was performed using the automated hematology analyzer; Sysmex XT-1800i (Sysmex, Kobe, Japan) based on electric impedance or lasers (fluorescence flow cytometry).

For analysis of CRP and serum melatonin; venous blood samples were obtained on gel tubes without addition of anticoagulant and centrifuged for 15 min for separation of serum. For serum melatonin, samples were stored at −70 °C till assay. Repeated freeze-thaw cycles were avoided. Serum melatonin assay was repeated 72 hours after antibiotic initiation.

CRP levels were measured using AVITEX CRP; a rapid latex agglutination test kit (CRP Omega Diagnostics Ltd. Omega House, Scotland, United Kingdom). The AVITEX CRP latex particles are coated with antibodies to human CRP. When the suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 min. The detection limit for CRP is 6 mg/L.

Blood cultures were performed on day 1 using BD BACTEC PEDS PLUS/F culture vials (BENEX Limited, Shannon, County Clare, Ireland). 3-5 mL of venous blood were withdrawn from the neonates under complete aseptic conditions and immediately withdrawn into the culture bottles, then transported immediately to the laboratory, and processed using the BD BACTEC FX40 systems (Becton–Dickinson Microbiology Systems, Sparks, MD), and blood cultures were incubated for a 5 to 7 days (unless they flag positive). Blood cultures which flagged positive (based on CO2 production) were cultured on standard media using routine microbiological techniques.

- Serum melatonin analysis by Enzyme Linked Immune Sorbent Assay (ELISA):
- Serum melatonin assay was analyzed using sandwich kit, supplied by Bioassay Technology Laboratory (Shanghai Korain Biotech Co., Ltd.).

Principle of the test:

The plate used has been pre-coated with human MT antibody. MT in the sample was added and binds to antibodies coated on the wells, then biotinylated human MT Antibody was added and binds to MT in the sample. Streptavidin-HRP was added and binds to the Biotinylated MT antibody. After incubation unbound Streptavidin- HRP was washed away during a washing step. Substrate solution was then added, and color develops in proportion to the amount of human MT. The reaction was terminated by addition of acidic step solution and absorbance was measured at 450 nm.

Calculation of Result:

A standard curve was constructed by plotting the OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and the best fit curve was drawn through the points on the graph.

Sample size:

Using PASS program setting alpha error at 0.05 power at 90%, results from previous study El-Mashad et al[11], showed Melatonin concentration was high among sepsis patients compared to control group (27.2± 3.3 vs. 11.4±3.2 pg/mL) so assuming Melatonin after treatment was 23.2±5.1 pg/mL, based on this we needed sample of 20 sepsis cases before and after treatment with antibiotics.

RESULTS

Table 1 Comparison between control and patients' groups as regards sex, GA, and mode of delivery:

		Control group No. = 20	Patients group No. = 20	Test value	P-value	Sig.
Sex	Female	9 (45.0%)	8 (40.0%)	0.102*	0.749	NS
GA (weeks)	Mean ± SD	34.40 ± 1.23	33.75 ± 1.02	1.818•	0.077	NS
	Range	32 – 36	32 – 35			
Mode of delivery	NVD	11 (55.0%)	6 (30.0%)	2.558*	0.110	NS

GA gestational age, NVD normal vaginal delivery
P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test; •: Independent t-test

There was no statistically significant difference between patients and controls regarding sex, GA (weeks) and mode of delivery as shown in table (1).

Table 2 Clinical presentations of sepsis in patients:

Clinical presentations	Patients group No. = 20
Apnea	8 (40.0%)
Pallor	3 (15.0%)
Temperature instability	
Hypothermia	3 (15.0%)
Hyperthermia	6 (30.0%)
Circulatory dysfunction	
Hypotension	7 (35.0%)
Prolonged CRT	10 (50.0%)
GIT dysfunction	
Feeding intolerance	6 (30.0%)
Jaundice	8 (40.0%)
Neurological dysfunction	
Irritability	4 (20.0%)
Hypotonia	10 (50.0%)
Lethargy	10 (50.0%)
Poor reflexes	10 (50.0%)
Hemorrhagic diathesis	
Petechiae	8 (40.0%)
Bleeding from puncture sites	7 (35.0%)

CRT: capillary refilling time, GIT: gastrointestinal tract

Table (2) shows the clinical presentation of sepsis reported among our patients, with prolonged capillary refill time, hypotonia, lethargy and poor reflexes were the most common presentations 50 % each. The length of NICU stay for patients in (days) was median (IQR) 15.5 (10.5 – 19) with range 6 – 28.

As regards perinatal history of septic infants; maternal fever was reported in 11 mothers (55%), UTI in 9 (45%), and PROM in 6 (30%). Patients median APGAR score at 1 min was 4(3.5-5) and at 5min it was 8 (7.5-9), their mean birth weight was 1.88 ± 0.42Kg.

Table 3 Comparison of investigations done for the patients before and after antibiotics administration

		Before	After	Test value	P-value	Sig.
Hemoglobin (gm/dL)	Mean ± SD Range	15.82 ± 1.75 12.2 – 18.1	13.38 ± 2.73 5.1 – 16.5	4.844•	<0.001	HS
White blood cells (10^9 /µL)	Mean ± SD Range	20.41 ± 4.86 11.7 – 29.3	18.22 ± 6.49 7.8 – 30.2	4.080•	0.001	HS
Platelets (10^9 /µL)	Median (IQR) Range	333 (222 – 457) 10 – 712	320 (212 – 399) 84 – 612	-2.391≠	0.017	S
I/T Ratio	Mean ± SD Range	0.24 ± 0.05 0.19 – 0.35	0.22 ± 0.06 0.15 – 0.32	2.760•	0.012	S
CRP	Negative Positive	0 (0.0%) 20 (100.0%)	1 (5.0%) 19 (95.0%)	1.026*	0.311	NS
CRP	Median (IQR) Range	24 (12 – 48.5) 6 – 96	12 (6 – 56.5) 4 – 114	-0.284≠	0.776	NS
Serum Melatonin (pg/mL)	Median (IQR) Range	127 (112 – 135) 83 – 312	125 (100 – 136.5) 60 – 240	-1.251≠	0.211	NS

I/T Ratio: immature neutrophils against total neutrophils CRP: C-reactive protein

Table (3) shows the comparison of investigations of the patients at time of EOS diagnosis and after antibiotics administration.

Ninety five percent of the patients had negative blood cultures. The only positive blood culture was for *Staph aureus* microorganism, it was reported for the only mortality case with the longest hospital stay 28 days. Interestingly, this case showed the highest initial TLC 29.3 10^9 /µL, moreover, the 3rd day labs showed the highest TLC, lowest platelet count, highest I/T ratio, and the highest serum melatonin, 30.2 10^9 /µL, 84 10^9 /µL, 0.32 and 240 pg/mL respectively.

Although the patients' initial serum melatonin was higher than that of the control, yet it did not reach statistically significant difference (127 (112 – 135) vs 102.5 (77.5 − 150)) (pg/mL) (p= 0.297).

There was no statistically significant correlation between initial patients' serum melatonin and initial hemoglobin, total leucocytic count, platelet count, I/T ratio, or CRP, also there was no statistically significant correlation between initial patients' serum melatonin and length of stay, feeding intolerance or fate.

As regards correlation between serum melatonin and CBC parameters after antibiotic therapy we demonstrated that there was no statistically significant correlation between serum melatonin and hemoglobin or platelet counts, however, there was statistically significant positive correlation between serum melatonin and TLC, CRP, and I/T ratio (r= 0.7458, p=0.001) (r= 0.76, p = 0.01) (r= 0.758, p= 0.001) respectively. In addition, there was significant positive correlation between percentage of change of CRP and percentage of change of serum melatonin after antibiotics treatment (r= 0.752, P<0.001).

DISCUSSION

Sepsis is one of the main causes of morbimortality in neonates. Rapid detection of sepsis in newborn represents a diagnostic problem as symptoms are often nonspecific, and frequently used tests lack enough sensitivity and specificity which is required to provide timely treatment to improve the outcome. Additionally, empirical broad spectrum antibiotics treatment leads to increase antimicrobial resistance [12].

The inflammatory and oxidative stress events involved in the pathogenesis of sepsis in newborn ultimately results in cell oxidative injury and mitochondrial impairment in a self-sustaining and self-promoting pathological process termed the '*sepsis redox cycle*', regardless the incriminated pathogens themselves[4]. In neonatal sepsis, both oxidative stress related pathways and antioxidant defenses would seem to be induced [8].

Melatonin, an indolamine endogenously produced by pineal body from the neurotransmitter serotonin, is a robust broad-spectrum antioxidant and readily beneficial scavenger of ROS beyond its chronobiologic functions [13]. We hypothesized that serum melatonin level in preterm infants with EOS would be higher in comparison with healthy preterm.

Ninety-five percent of our patients revealed negative blood cultures and the only positive one was Staph aureus. El Shimy et al [14], declared that blood culture was positive in 45.8% with Staph aureus documented in 10.4% followed by Coagulase negative staph and Citrobacter 8.3% each. Jatsho et al [15], in Bhutan in south Asia only 10.30% showed positive blood culture with Coagulase-negative staph followed by Klebsiella Pneumoniae. In Ghana, Aku et al[16], revealed 17.3%

culture proven sepsis with Staph epidermidis followed by Staph aureus were the most to be cultured.

In the developed countries the most frequent pathogen of EOS is group *B Streptococcus* (GBS) 50 %, followed by *Escherichia coli* 25 %. The remaining cases are caused by *Staph aureus*, Coagulase negative Staph (CoNS), *Listeria monocytogenes* and other gram-negative bacteria while, Klebsiella followed by *Staph aureus* are the commonest pathogens in developing countries [17].

In our study, the patients' initial serum melatonin was higher than that of the control, yet it did not show statistically significant difference (p= 0.297).

Also, we did not find statistically significant correlation between initial patients' serum melatonin and initial hemoglobin, TLC, platelet count, I/T ratio, or CRP. Additionally, our results did not show statistically significant correlation between initial patients' serum melatonin and length of hospital stay, feeding intolerance or fate. However, we found significant positive correlation between serum melatonin and TLC, CRP, and I/T ratio (r= 0.7458, p=0.001) (r= 0.76, p = 0.01) (r= 0.758, p= 0.001) respectively after antibiotic therapy. Also, we found significant positive correlation between CRP percentage of change and serum melatonin percentage of change after antibiotics treatment (r= 0.752, P<0.001).

Bagci et al [18], did not find difference between septic and non-septic children as regards nocturnal serum melatonin, however, nocturnal melatonin was elevated more in children with septic shock and liver dysfunction, moreover, in non-survivors septic patients or those with liver dysfunction, urinary metabolite of melatonin appeared lower than those without.

Lorente et al [19], concluded that non-survivor septic adult patients showed higher serum melatonin than in survivors. However, Pavlyshyn et al [20], could not find associations of urinary melatonin and the incidence of EOS in preterm newborns.

While El-Mashad et al[11], showed that serum melatonin was higher in full term neonates with late onset sepsis compared with age matched control. Additionally, serum melatonin positively correlated with CRP and I/T ratio (r=0.952, p=0.001) and (r= 0.326, p= 0.015), respectively, but not TLC. Also, the sensitivity and specificity of melatonin as a marker of sepsis, was 93.5% and 85.6%, respectively, and both were increased by combining serum melatonin with CRP.

This variable serum melatonin or its urinary metabolite in septic patients can not be explained by only one factor. In fact, it appears to be multifactorial and complex process, which modifies either manufacturing melatonin in pineal gland or its hepatic metabolism and excretion [18].

Circulating melatonin is mostly hydroxylated by hepatic cytochrome P450 (CYP) mono-oxygenases, then conjugated with sulfate and finally pass in urine. Many studies have found that CYP isoforms are diminished by mediators incriminated in inflammation, and the reduced hepatic perfusion in septic shock [18]. Also being metabolized by cytochrome P450 system, interactions between melatonin and other medicines, like caffeine might have effect [21].

Furthermore, circadian rhythm of melatonin secretion from pineal body might be affected by several factors common in pediatric intensive care unit (PICU), some studies demonstrated reduced melatonin or dyssynchronization of its rhythm in sedated or mechanically ventilated prepubertal children in PICU. Moreover, opioids encourage melatonin release, while benzodiazepines diminish its secretion. Similarly, stress-related endogenous catecholamines, inotropic supports, steroids, phototherapy, and lack of normal lighting/ dark cycle affect the pineal gland reaction in critical illness. Therefore, it is not surprising to find wide variable results in septic patients under different clinical conditions in ICU [18, 22].

Additionally, GA and postnatal age might affect serum melatonin, for instance in the present study we included ≤ 36 weeks GA with EOS that is in the 1st 72 hours after birth, conversely, El-Mashad et al [11], included neonates ≥ 37 weeks GA and more than 3 days of life.

Many studies showed serum melatonin was higher in older GE [23, 24, 20]. This is in consistence with Kennaway et al [25], who reported diminished urinary melatonin metabolite in the first 90 days in preterm compared to term babies. Bojkowski et al[26], postulated that prematurity might affect the brain maturational process thus influencing circadian rhythms through the first weeks after birth.

These discrepancies between studies may be linked to the difference in perinatal variables, inclusion criteria, ICU environment, developmental care, the comorbidities in neonates and different medications that can interact with endogenous melatonin secretion and metabolism.

Small sample size, and the short period of follow-up, are of the major limitations of our study. Therefore, additional research with a larger sample size and a more length follow-up period with more serial testing for serum melatonin is recommended.

CONCLUSION

Endogenous melatonin might be beneficial in the follow up of preterm neonates with EOS but not as a marker for diagnosis.

Funding Source: None

Disclosure statement: The authors declare that they have no competing interests.

Abbreviations:
CRP: C reactive protein
EOS: Early onset neonatal sepsis
I/T: Immature to Total neutrophil ratio
ROS: Reactive oxygen species
TLC: Total leucocytic count

REFERENCES

1. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21-47.
2. Arowosegbe AO, Ojo DA, Dedeke IO, Shittu OB, Akingbade OA. Neonatal sepsis in a Nigerian Tertiary Hospital: Clinical features, clinical outcome, aetiology and antibiotic susceptibility pattern, Southern African. Journal of Infectious Diseases. 2017; 32:4, 127-131.
3. Getabelew A, Aman M, Fantaye E, Yeheyis T. Prevalence of Neonatal Sepsis and Associated Factors among Neonates in Neonatal Intensive Care Unit at Selected Governmental Hospitals in Shashemene Town, Oromia Regional State, Ethiopia, 2017. Int J Pediatr. 2018; 2018: 7801272.
4. Poggi C, Dani C. Sepsis and Oxidative Stress in the Newborn: From Pathogenesis to Novel Therapeutic Targets. Oxid Med Cell Longev. 2018 Aug 2;2018:9390140.
5. Shah BA, Padbury JF. Neonatal sepsis: an old problem with new insights. Virulence. 2014;5(1):170-178.
6. Iroh Tam PY, Bendel C. Diagnostics for neonatal sepsis: current approaches and future directions. Pediatr Res. 2017; 82, 574–583.
7. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics. 1998 Oct;102(4): E41.
8. D'Angelo G, Chimenz R, Reiter RJ, Gitto E. Use of Melatonin in Oxidative Stress Related Neonatal Diseases. Antioxidants (Basel). 2020 Jun 2;9(6):477.
9. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988; 112:761–767
10. Ballard, J. L. et al. New ballard score, expanded to include extremely premature infants. J. Pediatr. 1991;119, 417–423.
11. El-Mashad AR, Elmahdy H, El-Dib M, Elbatch M, Aly H. Can melatonin be used as a marker for neonatal sepsis? J Matern Fetal Neonatal Med. 2016;29(17):2870-3.
12. Eichberger J, Resch E, Resch B. Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. Front Pediatr. 2022; 8; 10:840288.
13. Marseglia L, Gitto E, Laschi E, Giordano M, Romeo C, Cannavò L, et al. Antioxidant Effect of Melatonin in Preterm Newborns. Oxid Med Cell Longev. 2021; 19;2021:6308255.
14. El Shimy MS, El-Raggal NM, El-Farrash RA, Shaaban HA, Mohamed HE, Barakat NM, et al. Cerebral blood flow and serum neuron-specific enolase in early-onset neonatal sepsis. Pediatr Res. 2018;84(2):261-266.
15. Jatsho J, Nishizawa Y, Pelzom D, Sharma R. Clinical and Bacteriological Profile of Neonatal Sepsis: A Prospective Hospital-Based Study. Int J Pediatr. 2020; 26;2020:1835945.
16. Aku FY, Akweongo P, Nyarko KM, Mensah LG, Amegan-Aho K, Kumi L, et al. Factors associated with culture proven neonatal sepsis in the Ho municipality 2016. Pan Afr Med J. 2020; 14;36:281.
17. Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr. 2015;61(1):1-13.
18. Bagci S, Horoz ÖÖ, Yildizdas D, Reinsberg J, Bartmann P, Müller A. Melatonin status in pediatric intensive care patients with sepsis. Pediatr Crit Care Med. 2012;13(2): e120-3.
19. Lorente L, Martín MM, Abreu-González P, Pérez-Cejas A, López RO, Ferreres J, et al. Serum melatonin levels during the first seven days of severe sepsis diagnosis are associated with sepsis severity and mortality. Enferm Infecc Microbiol Clin (Engl Ed). 2018;36(9):544-549. English, Spanish.
20. Pavlyshyn H, Sarapuk I, Kozak K. Peculiarities of melatonin levels in preterm infants. Wien Klin Wochenschr. 2022; 24.
21. Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan DX, et al. Melatonin and Pregnancy in the Human. Reprod. Toxicol. 2008; 25:291–303.
22. Foster JR. Melatonin in Critically Ill Children. J Pediatr Intensive Care. 2016 Dec;5(4):172-181.
23. Muñoz-Hoyos A, Bonillo-Perales A, Avila-Villegas R, González-Ripoll M, Uberos J, Florido-Navío J, et al. Melatonin Levels during the First Week of Life and Their Relation with the Antioxidant Response in the Perinatal Period. Neonatology. 2007; 92:209–216.
24. Biran V, Decobert F, Bednarek N, Boizeau P, Benoist JF, Claustrat B, et al. Melatonin Levels in Preterm and Term Infants and Their Mothers. Int J Mol Sci. 2019; 27;20(9):2077.

25. Kennaway DJ, Stamp GE, Goble FC. Development of Melatonin Production in Infants and the Impact of Prematurity. J. Clin. Endocrinol. Metab. 1992; 75:367–369.

26. Bojkowski CJ, Arendt J, Shih MC, Markey SP. Melatonin Secretion in Humans Assessed by Measuring Its Metabolite, 6-Sulfatoxymelatonin. Clin. Chem. 1987; 33:1343–1348.