Research Article

Evaluation of Serum Melatonin Level In Late Onset Neonatal Sepsis

Mohammed F. Afifi MD*, Abd El-Hakim A. Abd El-Hakim MD*, Bosinah A. Kamel MD, Mohammed M. moustafa MSc*.**

* Department of Pediatric, faculty of medicine, Minia University.

** Department of Biochemistry, faculty of medicine, Minia University.

Abstract

Background: Melatonin, an indolamine endogenously produced by pineal body, has important role as an anti-oxidant, anti-inflammatory and anti-apoptotic. Whether melatonin concentration changes in neonatal sepsis and whether it can be used as a marker of sepsis is unknown. **Objective(s):** The objective of this study is to evaluate serum melatonin as a marker for late onset neonatal sepsis and compare it to standard markers. **Study design:** This prospective study included 80 neonates; 50 diagnosed with late neonatal sepsis (LOS neonates group) and 30 apparently healthy, age and sex-matched neonates (control group). Markers of sepsis and serum melatonin were compared between both groups. **Results:** The LOS neonates group had significantly increased immature to total neutrophils ratio (I/T ratio), and C-reactive protein (CRP), and decreased platelet count in comparison with control group. Serum melatonin was significantly increased in LOS neonates group when compared to control group ($p = 0.001$). In addition, Serum melatonin had a weak insignificant positive correlation with CRP ($r = 0.02$, $p = 0.91$) and weak insignificant negative correlation with I/T ratio ($r = -0.15$, $p = 0.28$). When serum melatonin was combined with CRP to detect neonatal sepsis, sensitivity and specificity were increased to 92.2 and 93.9%, respectively. **Conclusions:** Endogenous serum melatonin is increased in late neonatal sepsis and can potentially be used as a marker for sepsis especially when combined with CRP.

Keywords: Anti-oxidant; infants; late-onset sepsis; marker

Introduction

Neonatal sepsis remains a major problem associated with high morbidity and mortality in the neonatal period. Sepsis in infants hospitalized in intensive care unit (NICU) is a common problem. Bacteremia has been found to occur in 32.3% of infections with mortality rates ranging from 15% to 50% (Gonzalez et al., 2004).

Neonatal sepsis can be categorized as early (EOS) and late onset (LOS) depending upon whether the onset of symptoms is before 72 h of life (early onset) or later (late onset). Late onset sepsis is caused by the organisms present in the external environment of the home or the hospital (Sankar et al., 2008).

Oxidative stress has been implicated in the pathogenesis of multiple highly prevalent diseases. Improvement in the understanding of sepsis and septic shock pathophysiology has contributed to establish a relationship between these settings and the occurrence of oxidative stress. However, the consensus guidelines for the management of patients with these conditions do not include the enhancement of antioxidant potential (Han et al., 2003).

Melatonin is the main pineal gland product and it functions as "time-giver" in the regulation of circadian rhythms, among others. But the actions of melatonin are not only restricted to the neuro-endocrine physiology (Inwald et al., 2009).

Melatonin has been also associated with the cellular antioxidant defense. It can develop its action at two levels: as a direct antioxidant, due its ability to act as a free radical scavenger, and as an indirect antioxidant, since it is able to induce the

expression and/or the activity of the main antioxidant enzymes (Ma et al., 2005).

The presence of specific melatonin-binding sites in the lymphoid cells provides evidence for a direct effect of melatonin on the regulation of the immune system. Melatonin's immuno-enhancing effect depends not only upon its ability to enhance the production of cytokines, but also upon its antiapoptotic and antioxidant actions. (Nunnari et al., 2003)

Therefore, this study was proposed to evaluate the level of serum melatonin in full term neonates suffering from late onset neonatal sepsis

Methods

This prospective study was conducted on 80 neonates who were admitted in the neonatal intensive care unit and/or followed up in Neonatal Care Clinic - Minia University Children Hospital from January 2017 to May 2017.

Neonates were divided into 2 groups:
Group I (LOS neonates group): It included 50 full term neonates with late onset neonatal sepsis diagnosed by clinical examination and investigation.
Group II (control group): It included 30 apparently healthy age and sex-matched full term neonates with no clinical signs or laboratory evidence of sepsis.

The inclusion criteria:

Postnatal Age: More than 6 days age.
Body Weight: 2.5 – 4 kg.
Gestational Age: 37 th – 42 th week

The Exclusion criteria:

Intra-uterine growth retardation
Preterm infants
Perinatal asphyxia
Infant of diabetic mother. (I.D.M).
Any Major or lethal congenital anomalies

All subjects included in this study were subjected to the followings:

I- Complete maternal and perinatal history taking.
II- Thorough clinical examination.
III- Routine laboratory investigations (Complete Blood Count, Quantitive C-reactive protein, Blood culture in LOS neonates group)
IV- Serum Melatonin level by ELISA kit using competitive-ELISA.

Statistical analysis

All statistical analyses were performed using SPSS 21 (Statistical Package for Social Science). Results were expressed as means ± SD for parametric data and by No.(%) for non-parametric data.
Comparisons between the groups were conducted by Student's t test for parametric data and by Chi-square test or Mann Whitney test for Non-parametric data. Correlation between serum melatonin and other markers for late onset neonatal sepsis were carried by Pearson's correlation coefficients. P-value was considered significant if less than 0.05.

Results

There was no significant difference between LOS neonates group and control group regarding Hb level (P = 0.10) and WBCs count (P = 0.08) and significant difference regarding PLTs count (P = 0.001) and I/T ratio (P = 0.001) (Table 1).

Serum melatonin level was significantly higher in LOS neonates group than in Control group (P. value 0.00) (Table 2).
Serum melatonin level was showing insignificant weak positive correlation with CRP Level, Hb level & PLTs count and insignificant weak negative correlation with WBCs count and I/T ratio (Table 3).

In this study, combination of serum melatonin level and CRP level had the highest sensitivity and specificity for diagnosis of LOS in comparison with serum melatonin level and CRP level alone (Table 4, Figure 1 and 2).

Table (1): Comparison between LOS neonates group & control group regarding CBC

Variable	Group (I) LOS neonates (n=50)	Group (II) Control (n=30)	P. value (Sig.)
Hemoglobin level (g/dl)	11.75 ± 2.57 (9-17.3)	12.94 ± 2.18 (10.2-17.8)	0.10
White blood cells (c/mm^3)	10.29 ± 3.74 (4.4-15.4)	8.89 ± 2.86 (4.8-10.8)	0.08
Platelets (c/mm^3)	166.7 ± 68.5 (37-317)	248.1 ± 83.8 (122-410)	**0.001***
I/T ratio	0.42 ± 0.11 (0.10-0.61)	0.16 ± 0.02 (0.10-0.19)	**0.001***

*** Significant (p. ≤0.01).**

Table (2): Comparison between LOS neonates group and Control group regarding serum melatonin level

Variable	Group (I) LOS neonates (n=50)	Group (II) Control (n=30)	P. value (Sig.)
Melatonin (pg/ml) Cutoff point 8.35 pg/mL)	27.52 ± 10.1 (8.1-46.2)	13.36 ± 6.26 (6.71-38.4)	**0.001***

*** Significant (p. ≤0.01).**

Table (3): Correlation of Serum Melatonin level with laboratory data

variable	Melatonin	
	r	P. value (Sig.)
CRP	0.02	0.91
Hemoglobin (g/dl)	0.05	0.73
White blood cells (c/mm^3)	-0.01	0.97
Platelets (c/mm^3)	0.11	0.45
I/T ratio	-0.15	0.28

*** Significant (p. ≤0.01).**

Table (4): Cutoff point, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Sensitivity and Specificity of serum CRP and Melatonin level in LOS

Variable	Cutoff point	PPV	NPV	Sensitivity	Specificity
CRP	20.5	92.0	90.0	90.1	91.8
Melatonin	8.35	94.0	92.0	92.2	93.9
CRP and Melatonin	-	91.2	90.8	94.1	95.5

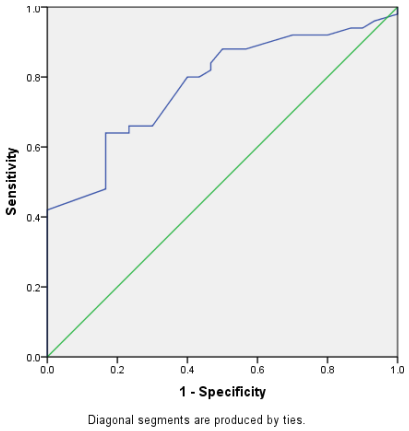

Figure (1): Roc curve of serum Melatonin level

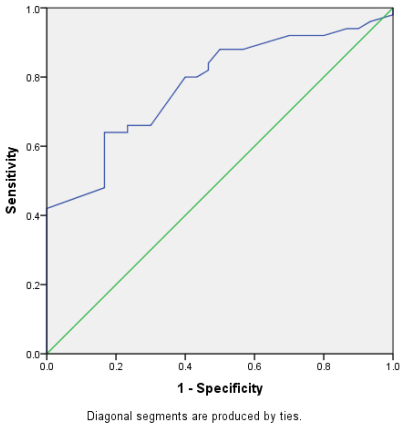

Figure (2): Roc curve of serum CRP level

Discussion

Neonatal sepsis remains a major problem associated with high morbidity and mortality in the neonatal period. Sepsis in infants hospitalized in intensive care unit (NICU) is a common problem. Bacteremia has been found to occur in 32.3% of infections with mortality rates ranging from 15% to 50% (Gonzalez et al., 2004).

For many years, researches have been ongoing to find predictors for neonatal sepsis that identify effectively patients who are at risk of infection (Ahrens et al., 2004).

Sepsis is known to induce a variety of metabolic and endocrinal changes that include increase of serum melatonin level (Haque, 2005).

Melatonin appears to have several immune-modulating effects. Via melatonin receptors, it is capable of stimulating cytokine production by T-helper 1 lymphocytes, including interleukin-2 (IL-2) and gamma-interferon. Melatonin may also potentiate the immunostimulatory properties of IL-2 by increasing T lymphocytes, natural killer cells, and eosinophils. Depressed circadian biosynthesis of melatonin has also been linked to reversible immunosuppression (Maestroni, 1995).

As regard complete blood count, this study demonstrates that white blood cell count has no significant difference between LOS neonates and control groups, which may be related to small sample size. Result was on contrary with Hornik et al., 2012 who worked on 204 septic newborn and found that high and low white blood cell counts were associated with neonatal sepsis. In contrast to this result Morag et al., 2008 who studied 345 septic newborn and found that there was leuocytosis in septic newborn and Lee et al., 2012 and Bhandari et al., 2008 who studied on 24 septic newborns and found that there is higher white blood cell counts in neonatal sepsis.

In the current study, PLT count is significantly lower in patient group than in control group, this result was in agreement with Charoo et al., 2009 and Torkman et al., 2009 who noticed that sepsis is associated with thrombocytopenia, Makhoul et al., (2006) who found that thrombocytopenia is a significant predictors of sepsis and Lee et al., (2012) who found that platelet counts were significantly lower in patients with LOS than control group.

In the present study, I/T ratio was significantly higher (above 0.2) in LOS neonate group than in control group, this result is in agreement with Makhoul et al., (2006) who found that I/T >0.2, CRP >6.0 mg/dl at onset of sepsis are significant predictors of proven neonatal sepsis and also in agreement with Bhandari et al., (2008) who found that there was a higher white blood cell counts, absolute neutrophil counts, absolute band counts, and immature/total neutrophil ratios but lower platelet counts in neonatal sepsis group and Khair et al., (2012) who found that immature to total

neutrophil ratio (>0.2), immature to ratio (>0.3) have optimal sensitivities and negative predictive values and De Assis Meireles et al., (2011) who found that the total number of neutrophils and the number of immature neutrophils are a good parameters to differentiate between the confirmed and unconfirmed sepsis.

In this study we found CRP level was significantly higher in LOS neonates group than in control group, that came in agreement with Khair et al., (2012), Makhoul et al., (2006), Lee et al., (2012) and Vasiljevic et al., (2008) who did his study on 130 neonates and defined a relevant CRP response to sepsis as a concentration of > 10 mg/dl for full-term.

Up to our knowledge, few studies were done on the level of serum melatonin in late onset neonatal sepsis.

In the current study, we found that serum melatonin level was significantly higher in LOS neonates group than in control group. This result was in agreement with Bojkowski et al., (1987) who found very significantly high melatonin concentrations in septic neonates. On the contrary, the results of this study disagreed with Olofsson, (2004) in which melatonin levels in blood and urine were studied over 3 consecutive days in 8 critically ill infants during deep sedation and mechanical ventilation at the ICU. The circadian rhythm of melatonin release was abolished in all. Also Mundigler et al., (2002) who have measured melatonin levels in critically ill infants to find out a possible correlation between melatonin and intensity of septic shock, the study carried out in intensive care unit (NICU) patients, 17 septic NICU patients, 7 NICU non septic patients and 21 controls were examined results was significantly lower in septic patients. Also, In the study of Perras et al., (2006) which measured serum melatonin concentrations in 302 septic patients consecutively admitted to the NICU, overall analysis for the whole group of patients revealed no or very weak correlation between serum melatonin levels and illness severity.

Regarding sensitivity and specificity of CRP in the current study we found that CRP had 90.1 % sensitivity and 91.8 % specificity this come in agreement with Choo et al., (2012) who that CRP had 91% sensitivity and 83% specificity, while Scharma et al., (2008) found that CRP had 92% sensitivity and 99% specificity and according to Khair et al., (2012) CRP had 74% sensitivity and 73% specificity but Zaki Mel et al., (2009) noticed that CRP had 86% sensitivity and 97% specificity.

In this study, the cut off value of serum melatonin level was 8.35pg/ml with sensitivity 92.2%, specificity 93.3% while melatonin together with CRP had the highest sensitivity 94.1% and specificity 95.5% in diagnosis of neonatal sepsis. Which indicate that combination of melatonin and CRP had the highest sensitivity and specificity for diagnosis of neonatal sepsis in comparison with serum melatonin level and CRP level alone.

Conclusion

Serum Melatonin level is high in late onset neonatal sepsis.

Melatonin together with CRP had the highest sensitivity and specificity for diagnosis of neonatal sepsis in comparison with serum melatonin level and CRP level alone.

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