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The Cortisol Serum Measurement as a Marker of Stress in Neonates

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Abstract

The first few days of life are crucial; the neonates are particularly vulnerable to stress and infection during this period. Stress can cause changes in metabolic and endocrine, including cortisol level. The disease causes an indirect impact on cortisol level through cytokines and enzymes. Elevation of cortisol level can disturb hemostasis and cause long terms complications. This research aimed to compare the difference in cortisol level between healthy and neonatal with high suspicion of sepsis. The study was an observational study using the cross-sectional method, conducted in Ananda mother and child hospital Makassar, from February until April 2018. Sixty neonates who fulfill the criteria were included in the study. They were divided into control group (CG) and patient group (PG). The blood samples in CG were taken a right after birth before the routine procedure was performed. Meanwhile, blood samples in PG were made at the time of diagnosis. The level of serum cortisol was measured Duplo with ELISA.

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The average serum cortisol level in CG and PG ranged between 9,98 - 120 ng/mL (average of 55,64 ng/mL) and 125,56 - 273,06 ng/mL (182,09 ng/mL) respectively. The correlation of cortisol level between PG and CG was statistically significant with p-value 0.000 ($p < 0.05$), 95% CI -143,59475 – (-109, 31159). In this study, the gender, gestational age, birth weight, length of stay, and Apgar score 5 minutes were not correlated with cortisol level in PG and CG. The only mode of delivery correlated with serum cortisol level with p-value 0.000 (95% CI 34,20958 – 101,67470). The serum cortisol level is an accurate method to indicate neonatal stress, and it is significantly higher in ill neonates compare to the healthy ones. It can be used as a prognostic marker, and continuous monitor of its dynamic changes may help us understand better about the implication of stress in early life.

Keywords: Cortisol; newborn; neonatal stress; sepsis; stress marker.

1. Introduction

The neonates are very vulnerable to any kind of stress, particularly premature or neonates with low birth weight. Aside from the immaturity of the organs, infection or other critical diseases may worsen the condition. Sepsis, respiratory distress, neonatal asphyxia, prematurity are the common causes of morbidity in neonates, particularly in developing countries. The stress causes metabolic and endocrine changes, not to mention that the neonates with critical illness will require specific management, causing them to be separated from the mothers and exposed to many painful medical procedures [1]. Any real or perceived threat to the organism will cause a reaction, which leads to a disorder in the homeostasis, causing a cascade of a complex chain reaction. It causes a disturbance in the dynamic equilibrium between the organism and its environment. This is what stress in human can be defined. If the impact of acute stress can be this big, we can imagine how chronic stress in human can cause a more significant effect on our body [2–4]. The stress causes direct effects on serum cortisol through the activation of hypothalamic-pituitary-adrenal (HPA) axis. It also stimulates activation of sympathetic pathway, causing tachycardia, hypertension, tachypnea, and renin-angiotensin release. It also altered the immunity system through the works of inflammatory mediators. Chronic stress can change the neuroplasticity of the spinal cord and central nervous system, as well change the central processing, causing anxiety and fear. It can lead to many psychological disorders like depression, aggressive behavior, sleep deprivation, anorexia, and some study correlate it with autism spectrum disorders [5]. Neonates also vulnerable to infection that can happen in prenatal, antenatal, or postnatal periods. Initial signs of sepsis in neonates usually not specific, which lead to screening and identification of risks problem became crucial. Sepsis can be divided into early onset sepsis (EOS), late onset sepsis (LOS), and nosocomial sepsis. According to Indonesian Pediatric Society consensus, neonates can be diagnosed as having highly suspicion of sepsis if they fulfill the criteria in category A and B. Clinical manifestations in A group include: respiratory distress, seizure, unconsciousness, body temperature instability, delivery in the less hygienic environment, and the overall condition deteriorate rapidly. Clinical signs in B category consist of: tremor, lethargy, reduced activity, irritable, vomiting, abdominal distension, signs of infection after four days, amniotic meconium fluid, poor feeding in the previously well-fed baby. Highly suspicion of sepsis can be diagnosed if the mother has a risk factor of infection, premature rupture of membrane (PROM), and the neonates show 2 or more clinical symptoms in A category, or it has 3 or more clinical signs in B category [6]. Infection impacts on endocrine changes are mainly regarded as a non-specific response of the

body, mostly mediated through cytokines and enzyme [7–9]. Many studies have been conducted to review the correlation of critical illness with cortisol level. The increase of serum cortisol in neonates with infection were not only caused by the activation of HPA axis but also because of the reduction of cortisol removal. The increase in serum cortisol usually correlates to the severity of illness [10]. The mother's serum cortisol level during delivery also plays an important role in it, and it also needs consideration [11–13]. Seriously ill neonates are often forced to live and develop in a stressful environment. They are forced to be separate from the mothers and underwent many painful medical procedures. They also exposed to many other stressful stimuli, such as noise and lighting intensity in NICU. This event notably longer in premature neonates compared to full-term neonates. This all will cause jeopardy in the endocrine system, particularly cortisol serum [3,14,16]. Some study also showed a significant low cortisol level and relative adrenal insufficiency in critically ill preterm and full-term neonates. This data suggest that low level of cortisol may increase mortality, morbidity, and caused detrimental effects on systemic inflammatory processes [17]. The difference in cortisol level between healthy newborns and sick newborns varied significantly. The current study aimed to compare the difference in cortisol level between healthy neonates and those with high suspicion of sepsis.

2. Materials and methods

2.1. The Study Design

The study was conducted in Ananda mother and child hospital Makassar, from February until April 2018. The study was an observational study using the cross-sectional method. Sixty neonates, divided into control and patient groups, were studied for serum cortisol at the time of admission and diagnosis. Thirty neonates in the control group (CG) were healthy neonates, had normal weight birth, full term, delivered through the regular and cesarean section, without congenital disabilities. They do not need any specific treatment and were roomed in with their mothers soon after birth. Thirty neonates in the patient group (PG) were diagnosed with high suspicion of sepsis based on mother's risk factors, and clinical sign in neonates, which fulfill criteria in A and B category. They showed clinical features and laboratory criteria for the diagnosis of neonatal infection. All the neonates received antibiotics according to the standard medical procedure. None of them received corticosteroids prenatally or during therapy. Those who received prenatal corticosteroids, born with congenital disabilities or brain problems were excluded from the study.

2.2. Blood samples collection

Blood samples in CG were collected through the umbilical cord before clamping, before the injection of vitamin K or Hepatitis B vaccine. The neonates in the PG have also received through umbilical cord at the time of diagnosis. Samples were collected in tubes, allowed to clot for 2 hours at room temperature, centrifuged for 20 minutes at approximately 1000xg. The level of serum cortisol was measured Duplo with ELISA using LSBio kit for human cortisol.

2.3. Data Analysis

Statistical analysis was performed using SPSS 23 (statistical package for the social science), to compare the

average cortisol serum values between control and patient group. The Wilcoxon signed-rank test and Pearson correlation test were used, and the level of significance was set at 0.05.

2.4. Approval of Research by Ethics Board

Parents of the neonates gave informed consent for all aspects of the protocol. This study was performed following approval from The Ethics research Committee of Medical Faculty at the University of Hasanuddin on February 8, 2018.

3. Results

The PG and CG both consisted of 30 neonates. The characteristic difference between PG and CG can be seen in table 1.

Table 1: The characteristic differences between control and patient group

Variables	Control group (n=30)	Patient group (n=30)
Gender		
- Male	14 (46,7%)	17 (56,7%)
- Female	16 (53,3%)	13 (43,3%)
Mode of delivery		
- Spontaneously	6 (20%)	18 (60%)
- Cesarean Section	24 (80%)	12 (40%)
Gestational age (weeks)		
- < 37 weeks	0	3 (10%)
- 37 – 42 weeks	30 (100%)	27 (90%)
- Mean	38,4667	37,9667
- Median	35,5	38
- Modus	39	37
Birth weight (gram)		
- 1500 – 2500 gram	0	2 (6,67%)
- ≥ 2500 gram	30 (100%)	28 (93,34%)
- Mean	3165	3031
- Median	3125	3125
- Modus	2700	3300
Apgar score 5 minutes		
- Mean	9 (0-10)	9 (0-10)
- Median	10	10
- Modus	10	10
Cortisol serum level (ng/mL)		
- Mean	55,6403	182,0934
- Median	53,7820	176,7191
- Modus	24	125,56
Length of stay (days)		
- Mean	3 days	5,5 days
- Median	3 days	3 days
- Modus	3 days	3 days

Serum Cortisol Level (SCL)

For the CG, the SCL ranged between 9,98 ng/mL and 120 ng/mL, with an average of 55,64 ng/mL. For the PG, SCL ranged between 125,56 ng/mL and 273,06 ng/mL, with an average of 182,09 ng/mL.

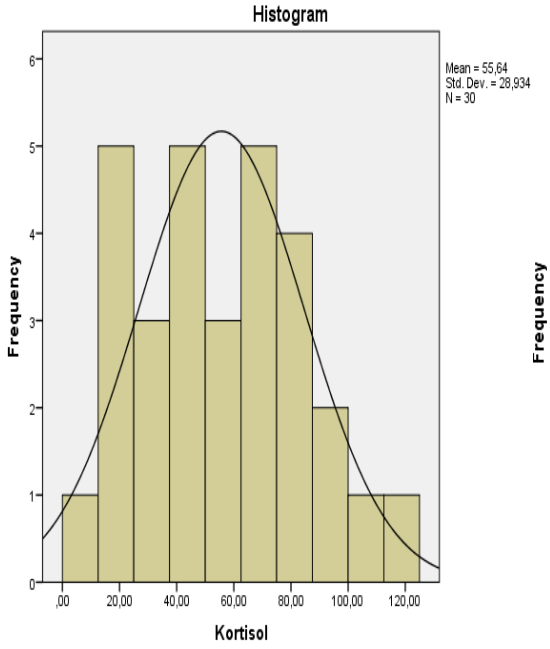

Figure 1: SLC in control group

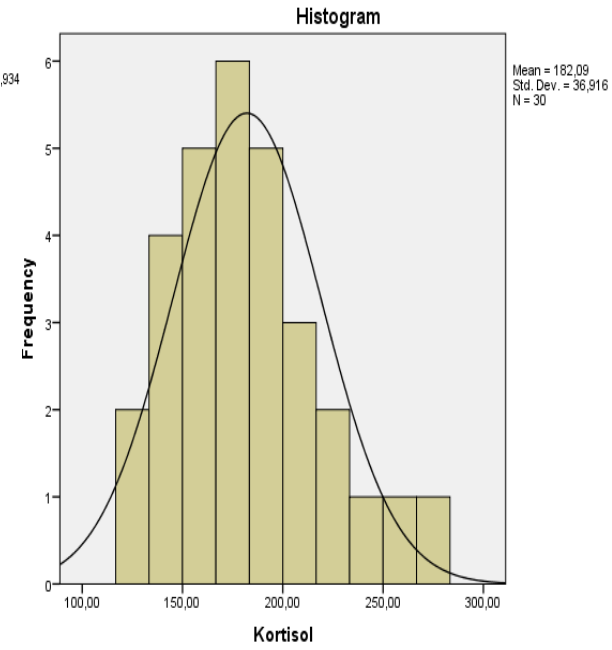

Figure 2: SLC in patient group

The correlation of SCL between PG and CG was analyzed using independent sample t-test. The result showed statistical significance p-value 0.000 ($p < 0.05$), with 95% CI -143,59475 – (-109, 31159).

The next step is to see the correlation between gender, mode of delivery, gestational age, birth weight, Apgar score 5 minutes, and length of stay with serum cortisol level in PG and CG. It was analyzed using Pearson correlation, and the result can be seen in table 2

Table 2: The correlation between gender, mode of delivery, gestational age, birth weight, Apgar score 5 minutes, and length of stay with serum cortisol level in PG and CG

Variables	Serum Cortisol Level
Gender	
- Sig. (2-tailed)	0,255
- 95% CI	-15,74462 – 58,25172
Mode of delivery	
- Sig. (2-tailed)	0,000
- 95% CI	34,20958 – 101,67470
Gestational age	0,085
Birth weight	0,126
Apgar score 5 minute	0,046
Length of stay	0,748

According to table 2, only mode of delivery is statistically significant, p-value 0.000 ($p < 0.05$) with 95% CI 34,20958 – 101,67470. Other variables were not statistically significant in this study.

4. Discussion

In our study, the cortisol serum level was different significantly between healthy neonates compared to neonates with high suspicion of sepsis. The difference was threefold compared to healthy neonates. This indicates that neonate with medical problems, like infection, was already in a stressful condition due to the illness. They also have to be separated from the mother right after birth, which even a stressful event. In neonatal intensive care unit (NICU) or high care unit (HCU), the neonates will have to be subjected to many painful medical procedures. The later will undoubtedly cause a surge in the cortisol serum level. This finding correlates with Boonen study in critically ill patients submitted in ICU. Boonen study showed that in a critically ill patient, there was 83% increase in cortisol production. This reflects the ongoing stimulus to cortisol secretion during the patient's stay in ICU. Inflammation also plays a role in the elevation of cortisol serum level, through the work of cytokines. It appears that the cytokines level positively correlates with the cortisol serum level [10]. This result is different from Khashana study which found a lower level of cortisol despite high cortisol precursors in neonates with infection [18].

This finding showed that neonates with high suspicion of sepsis already under significant stress even before treatment. The severely ill neonates will be subjected to more stressful events during their hospital stay, not only physical anxiety, but also psychological stress. This goes way higher in premature neonates. The first hours of premature neonates life are dominated with the effort to adjust to the extrauterine environment. The physical immaturity leads to complications like respiratory distress syndrome, congenital heart disease, body temperature instability, metabolic disorders, and vulnerability to infections. They will stay in the hospital for a longer duration of time, as well a higher number of invasive and traumatic procedures. All of these will cause a surge in serum cortisol level. Indeed, high cortisol level can increase intracranial pressure and could lead to intraventricular hemorrhage in premature neonates [2,19–22].

Based on this findings, we can deduct that serum cortisol level in ill neonates can be used as a prognostic marker to monitor the level of stress during hospitalization. A study showed a decrease in cortisol level in neonates with asphyxia during hypothermic therapy and correlated positively with IL-6 reduction [17]. The use of non-pharmacological treatment to reduce stress in ill neonates will play a significant role in reducing the side effect of chronic stress in these patients, including parents involvement in treatment.

Our study has some limitations. First, it only measured the serum cortisol serum at the beginning of hospitalization in both groups. The dynamic changes of the cortisol serum level were not followed and compared to other factors like the number of painful stimuli during admission or the length of stay. Second, the samples consist mainly of full-term neonates. According to the previous study, the premature neonates showed higher cortisol serum level and subjected to more extended hospital care. It implicates that the premature

neonates will show a higher serum cortisol level. Studies with larger samples and more patients group are needed to investigate further. Third, there was no investigation to obtain data about the breakdown of cortisol in critically ill neonates. That way, the diagnosis of adrenal failure cannot be proposed in this setting. It is only logical that the reduction of cortisol breakdown also contributed to the cortisol serum level elevation.

5. Conclusion

The serum cortisol level is different significantly in ill neonates compared with healthy neonates. Although the isolation of stressor has not been possible in our subject, we can measure stress in the neonates and can be used further as a prognostic marker of treatment. A continuous monitor of the dynamic changes of serum cortisol level in ill neonates may help us also understand the implication of stress in early life.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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