Relative Adrenal Insufficiency in Full-Term and Preterm Neonates with Neonatal Sepsis

Abstract

Background: Neonatal sepsis is the third most common cause of death in newborns and a significant issue for public health, particularly in developing nations. This study aimed to assess the hypothalamic-pituitary-adrenal axis (HPA) response in full-term and preterm newborns diagnosed with neonatal sepsis in the neonatal intensive care unit (NICU).

Methods: This cross-section observational research was done on neonates who were categorized into two main groups Group I: sepsis neonates who were further subdivided into two groups according to the gestational age into 30 full-term neonates with neonatal sepsis and 30 preterm neonates with neonatal sepsis. Group II: Included 30 healthy control neonates.

Results: HSS score and C reactive protein (CRP) level were correlated with serum cortisol level at (9 am: r=-0.273, P<0.035 and r=-0.447, P<0.001), (9 pm: r=-0.447, P<0.001and r=-0.477, P<0.001) ACTH at (9 am: r=-0.314, P<0.015 and r=-0.377, P<0.003) and at (9 pm: r=-0.362, P<0.005 and r=-0.448, P<0.001) and cortisol level post ACTH stimulation (r=-0.345, 0.007 and r=-0.497, P<0.001) respectively. Regardinginotropes, sepsis type and oxygen support there was significant difference between both groups.

Conclusions:75% of the patients we evaluated with neonatal sepsis had relative adrenal insufficiency (RAI).ACTH and Cortisol values were correlated to CRP and HSS score as an inflammatory marker. This reflects suppressedHPAstatus in this critical clinical condition.

Keywords: ACTH, Adrenal Insufficiency,Full-Term Neonates, Preterm Neonates, Neonatal Sepsis

Introduction:

Neonatal sepsis ranks as the third most common cause of infant death and is a significant public health concern, particularly in developing nations. Despite the progress made in medical advancements, there are still several difficulties in diagnosing and treating newborn illnesses. The diagnosis of neonatal sepsis is challenging due to the frequent occurrence of noninfectious diseases that mimic sepsis, particularly in premature newborns, and the lack of ideal diagnostic techniques[1].

Due to the high-risk nature of neonatal sepsis, particularly in premature newborns, healthcare professionals are obligated to deliver antibiotics to infants who display risk factors and/or show indicators of suspected sepsis in an empirical manner. Regrettably, the use of broad-spectrum antibiotics and extended courses of empirical antibiotics is linked to negative consequences and higher rates of antimicrobial resistance. Due to the significant occurrence and death rate of sepsis in premature newborns, as well as its lasting effects on their growth and development, it is crucial to prioritize measures aimed at reducing infection rates in this vulnerable group within neonatal care[2].

The presence of acute and chronic stressful events in life initiates a meticulously coordinated physiological response with the goal of maintaining homeostasis[3-7].The activated stress response system induces alterations in cardiovascular function, intermediary metabolism, and immune-mediated inflammation. The main components of the stress response are situated in the hypothalamus and brain stem, whilst the other components consist of the HPA axis, as well as the systemic and adrenomedullary sympathetic system[3-7].

The production of cortisol is controlled by the hypothalamus via the release of corticotropin-releasing hormone (CRH), which then stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). The production of cortisol or the administration of glucocorticoids from an external source has a suppressive effect on the synthesis and release

of CRH and ACTH, hence ensuring a highly controlled system. While ACTH controls the production of dehydroepiandrosterone (DHEA) and its sulfated derivative, DHEA-S, via the zona reticularis, these hormones do not directly affect the negative feedback loop that influences ACTH secretion. The physiological effects of DHEA and DHEA-S are significant, but their role in severe disorders is not fully understood[8].

The exact cause of RAI in septic patients is uncertain. However, it is possible that reduced amounts of substances needed for steroid production (such as cholesterol), widespread inflammation in the body, and impaired blood flow to the adrenal glands due to circulatory dysfunction may play a role in the development of RAI[9].

Remarkably, in very unwell newborns experiencing sudden sepsis, both widespread inflammation and impaired blood circulation are the primary causes of organ failures[10, 11], The characteristic of early worsening of newborns' clinical state is a syndrome that is closely linked to poor short-term survival of these individuals. Nevertheless, the research on RAI in the context of septic shock is lacking[12].

Hence, we conducted this research to assess the HPA axis reaction in neonates diagnosed with neonatal sepsis, both full term and preterm, who are receiving treatment in the NICU.

Methods:

This cross-section observational study was carried out on 60full term and preterm neonates, same ageand sex, diagnosed with neonatal sepsis according to clinical suspicion[13] and 30 healthy control neonates.

The research was conducted under the consent of the Ethical Committee of Tanta University Hospitals in Tanta, Egypt. Relatives of the patients provided an informed written permission.Exclusion criteria were neonates with suspected adrenal insufficiency due to: [ambiguous genitalia, cardiac causes, adrenal haemorrhage, brain trauma, congenital

anomalies, chromosomal anomalies, metabolic disorders, maternal corticosteroid therapy during pregnancy] and neonates with postnatal steroid treatment.

All neonates included in the study were categorized into two main groups *Group I:* patients were categorized into two groups according to the gestational age into 30 full-term neonates with neonatal sepsis and 30 preterm neonates with neonatal sepsis [14]. Group II (control): Included 30 healthy control neonates (age and sex matched).

The World Health Organization (WHO) categorized patients according to their gestational age based on definitions with replacing label "term" with the designations[15, 16] into[The different stages of gestation are categorized as follows: early term (between 37 0/7 weeks and 38 6/7 weeks), full term (between 39 0/7 weeks and 40 6/7 weeks), late term (between 41 0/7 weeks and 41 6/7 weeks), and post term (42 0/7 weeks and beyond)].The diagnosis was confirmed by Rodwell's scoring system (HSS score):[Total leukocyte count (TLC): ≤5000/µl scores 1, ≥25000 at birth scores 1, ≥30000 at 12-24 hours scores 1, ≥21000 on day 2 onwards scores 1, total Neutrophil Count: 1800-5400 scores 0, No mature PMN seen scores 2, Increased/Decreased scores , immature Neutrophil count:600 scores 0,>600 (Increased) scores, immature: Total (I: T) Neutrophil Ratio:0.120 scores 0,>0.120 (Increased) scores 1, immature: Mature(I:M) Neutrophil Ratio: <0.3 scores 0, ≥0.3 (Increased) scores l, degenerative changes in neutrophils: Toxic granules/Cytoplasmic vacuolations scores 1 and platelet Count: ≤150000/µl scores 1].Total score ≤2 Sepsis is unlikely; 3 or 4 Sepsis is possible and ≥5 Sepsis or infection is very likely[17].

All patients were subjected to: history taking (peri-natal and natal history of labor and delivery), clinical examination (APGAR scoring in the first 5 minutes of birth, Downes' scoring), routine laboratory investigations, blood culture, urine analysis, prothrombin time (PT), partial thromboplastin time (PTT), international normalised ratio (INR) and blood gases

(ABG/CBG]and routine Imaging workup[Chest x-ray, pelviabdominal x-ray, echocardiography, transcranial Ultrasound and pelviabdominal Ultrasound].

Adrenal functions assessment:Two samples are taken at 9:00 am and 9:00 pm for serum cortisol level assessment, 1 ml volume for each using Gel barrier tube (SST or Tiger top tube) then centrifuged for 10 minutes and The BioactivadiagnosticaGmBH Cortisol test kit is used for quantitative measurement of cortisol level [18]. Also, ACTH level at 9:00 am and 9:00 pm was assessed by taking two samples at 9:00 am and 9:00 pm, 1 ml volume for each using EDTA tube (Lavander top tube) covered with ice then centrifuged in cooled centrifuge for 15 minutes at 1000xg and Develop ACTH ELISA kit is used for quantitative measurement of ACTH levels[19].

ACTH stimulation test or Short Synacthen Test (SST) was performed by taking 1 ml of 250 µg/mL tetracosactrin and diluted under sterile conditions with 49 mL of normal saline to make a concentration of 5µg/mL. Mix well. Then 1 ml of 5 µg/ml solution and 4 mL normal saline to make a 1 µg/mL solution. And it's given at a dose 1 ml of 1µg/ml solution (any age, any size) IM or IV[20]. Interpretation based on measuring cortisol at baseline, at 30 and 60 minutes after HPA axis stimulation[21, 22].

Absolute adrenal insufficiency was defined as serum cortisol level <6 ug/dl (60 ng/ml)[23].

RAI is defined as serum cortisol level 7-15 ug/dl (70-150 ng/ml), and or delta cortisol less than 9 ug/dl (90 ng/ml). Cortisol plasma level ≥16 ug/dl (≥160 ng/ml) was considered normal[22].

Statistical analysis

The statistical analysis was performed using SPSS v27 (IBM©, Chicago, IL, USA). The normality of the data distribution was evaluated by doing the Shapiro-Wilks test and examining histograms. The quantitative parametric data were presented as the mean and standard deviation (SD) and were examined using an analysis of variance (ANOVA) test with

a post hoc test (Tukey). The quantitative non-parametric data were presented using the median and interquartile range (IQR). The data were analysed using the Kruskal-Walli's test, followed by the Mann Whitney-test, to compare each group. The qualitative variables were represented as frequency and percentage (%) and were assessed using the Chi-square test. A two-tailed P value less than 0.05 was deemed to be statistically significant.

Results:

There demographic and baseline characteristics were showed in table 1. Regarding APGAR score, and sex no significant difference between the full-term group and control group in APGAR at 1min and 5 min, while preterm with sepsis had significantly lower APGAR score at 5 min compared to control (P<0.03). Comparing the antenatal risks, both full term and preterm neonatal groups had more frequent antenatal risks (chorioamnionitis- fetal distress-UTI-IDM) compared with the control group (P<0.001). PROM and MAS were more prevalent in preterm neonates with sepsis compared with control group (P<0.001). Only pre-eclampsia was more prevalent in control in comparison to both group of neonates with sepsis.The mean value of heart rate, and axillary temperature were significantly higher in full term and preterm neonates with sepsis compared with control (P<0.001), but no significant difference between the full term and preterm neonatal groups. Estimated MBP was significantly lower in both neonatal groups with sepsis compared to control group (P<0.001) for each.

Only the control group had 53% with normal respiratory rate, but none in neonatal groups with sepsis. 70% of full-term group and 83% of preterm group were intubated and RD II was prevalent in 23% of full- term neonates and 13% in preterm neonates and 20% in control group, RD III was only existing in full term neonates with sepsis.Table 1

Table 1:Demographic, clinicaldata, hemodynamics,CBCand HSS score in study group

		Full term neonates	Preterm neonates	Control	P-value
GA (Weeks)		38.03±0.8	34.07±1.1	35.3±2.9	**p <0.001*, p1<0.001*, p2<0.001* P3 0.025***
Postnatal age (Days)		8.9±3.9	8.8±3.8	9.2±4.382	**0.927**
Weight (kg)		3.3±0.3	2.2±0.4	3.1±0.5	**p<0.001*, P1<0.001*, P2=0.534, P3<0.001**
Sex	**Male**	16(53.3%)	13(43.3%)	14(46.7%)	**0.732**
	Female	14(46.7%)	17(56.7%)	16(53.3%)	
Antenatal risk	**No risk**	0(0.00%)	0(0.00%)	21(70.0%)	**<0.001***
	Chorioaminitis	8(26.7%)	6(20.0%)	0(0.00%)	
	Fetal distress	6(20.00%)	0(0.00%)	0(0.00%)	
	UTI	10(33.3%)	7(23.3%)	0(0.00%)	
	IDM	6(20.0%)	3(10.0%)	2(6.7%)	
	PROM	0(0.0%)	11(36.7%)	0(0.00%)	
	MAS	0(0.0%)	3(10.00%)	0(0.0%)	
	Pre- eclampsia	0(0.0%)	0(0.0%)	7(23.33%)	
Hemodynamics					
HR		149.8±11.0	149.2±11.1	137±9.4	**p<0.001*, P1=0.974, P2<0.001*, P3<0.001***
SBP		59.7±4.2	54.2±4.409	63.7±6.66	**P<0.001*, P1<0.001*, P2=0.011*, P3<0.001***
DBP		34.6±3.5	33.86±3.401	42.033±4.853	**P<0.001*, P1=0.717, P2<0.001*, P3<0.001***
MBP		42.3±3.011	40.800±4.444	48.967±5.129	**P<0.001*, P1=0.337, P2<0.001*, P3<0.001***
Temp		38.0±0.590	37.330±1.075	36.907±0.297	**P=0.001*, P1<0.001*, P2<0.001*, P=0.068**
RR	**Not Distressed**	0(0.00%)	0(0.00%)	17.7	**<0.001***
	Intubated	21(70.00%)	25(83.33%)	0(0.00%	
	RD I	1(3.33%)	1(3.33%)	7(23.33%)	
	RD II	7(23.33%)	4(13.33%)	6(20.00%)	
	RD III	1(3.33%)	0(0.00%)	1(3.33%)	

Data are presented as mean ± SD or frequency (%).P1: significance between full term neonates and preterm neonates, p2: significance between full term neonates and control, p3: significance between preterm neonates and control. *Significant p value <0.05, ANOVA: analysis of variance, GA: Gestational age, CS: Cesarean Section, UTI: urinary tract infection, IDM: Immune-mediated diabetes mellitus, PROM: Premature rupture of membranes, MAS: meconium aspiration syndrome, HR: heart rate,SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: mean blood pressure, RR: Respiratory rate, RD:Respiratory distress.

Regarding oxygen support, inotropes and sepsis type, there was significant difference between both group(P<0.001).Regarding the causative micro-organisms in neonatal groups with sepsis, Klebsiella followed by E coli and pseudomonas were the most prevalent micro-organisms in the neonatal groups with sepsis.Table 2

Table 2:Oxygen support, inotropes, sepsis type andblood culture in neonates with sepsis in study group

	Full term neonates	Preterm neonates	P value
Oxygen support			
SIMV	11(36.67%)	8(26.67%)	<0.001*
HFNC	8(26.67%)	4(13.33%)	
Room Air	1(3.33%)	1(3.33%)	
HFOV	1(3.33%)	3(10.00%)	
PCV	9(30.00%)	14(46.67%)	
Nasal O2	0(0.00%)	0(0.00%)	
Inotropes			
Dopamine and Dobu	9(30.00%)	11(36.67%)	<0.001*
Dopamine	18(60.00%)	15(50.00%)	
Sepsis type			
EOS	10(33.3%)	13(43.33%)	<0.001*
LOS	20(66.67%)	17(56.67%)	
Blood culture			
Klebsiella	18(60.00%)	14(46.67%)	0.411
E-Coli	6(20.00%)	4(13.33%)	
Pseudomonas	4(13.33%)	6(20.00%)	
MRSA	1(3.33%)	5(16.67%)	

Data are presented as frequency (%). *Significant p value <0.05, SIMV: Synchronized intermittent mechanical ventilation, HFOV:High frequency oscillation ventilation, PCV: Pressure controlled ventilation, LOS: late onset sepsis, EOS:early-onset sepsis, MRSA: Methicillin resistant staph aureus.

CRP was markedly elevated in both neonatal groups with sepsis compared to control group (P<0.001, 0.001) respectively, but no significant difference between the full term and preterm groups. Furthermore, blood urea was significantly elevated only in full term group compared with the control group, serum creatinine was higher in preterm group with sepsis compared with control(P<0.01) while PTT, INR,ANC, I:M ratio, I:T ratios and HSS was significantly higher in both neonatal groups with sepsis in comparison to control group. The platelets count, ACTH at 9 am and at 9 pmwere significantly lower in both full term and preterm neonatal groups compared with the control group, but no significant difference between the full term and preterm group. After ACTH stimulation test, there was no significant difference

in both groups compared to the baseline cortisol level. RAI was diagnosed in 47 from 60 neonates with sepsis (78.3%), 22/30 (73.3%) in full term neonates and 25/30 (83.3%) in preterm neonates with sepsis, while the remaining 13 (21.7%) showed absolute adrenal insufficiency. Echo and transcranial ultrasound showed highly significant difference between both groups (P<0.001).

Table 3

Table 3: Routine laboratory findings, CBC and HSS score and serum cortisol and ACTH level in study group

		Full term neonates	Preterm neonates	Control	P value
Routine laboratory findings					
CRP		147.100±56.917	151.200±81.636	4.767±1.478	P<0.001*, P1=0.959, P2<0.001*, P3<0.001*
Urea		47.267±13.334	42.133±13.161	38.767±10.258	P=0.031*, P1=0.246, P2=0.024*, P=0.543
Creat		0.556±0.225	0.650±0.228	0.505±0.120	P=0.018*, P1=0.162, P2=0.570 P3=0.015*
ALT		45.533±40.617	39.800±17.511	38.700±10.841	0.560
AST		67.200±156.421	41.100±10.746	39.200±10.772	0.414
Total Bilirubin		6.127±3.792	5.443±3.761	5.320±3.533	0.662
Direct bilirubin		0.473±0.160	0.456±0.248	0.467±0.207	0.950
S. albumin		3.353±0.429	3.240±0.385	3.543±0.302	P=0.009*, P1=0.475, P2=0.129 P3=0.007*
PT		15.810±7.494	15.137±1.863	13.087±0.707	P=0.054*, P1=0.830, P2=0.054*, P3=0.185
PTT		35.527±6.118	40.154±12.392	30.467±2.726	P<0.001*, P1=0.076, P2=0.047*, P3=<0.001*
INR		1.283±0.594	1.282±0.222	1.015±0.016	P1=0.007*, P1=1.000,P2=0.016*,P3=0.016
CBC and HSS score					
Hb		12.017±1.791	12.003±1.481	12.143±1.185	
Platelet		64.3±28.6	81.2±58.77	302.8±78.36	P1=0.510, P2=<0.001*, P3<0.001*
TLC		15.850±8.025	15.149±8.948	7.385±2.566	P1=0.922, P2=<0.001*, P3<0.001*
ANC		8411.3±4946.0	7786.±5411.0	4413.0±1996.0	P1=0.846, P2=0.002*, P3=0.010*
I:M ratio		0.428±0.128	0.471±0.139	0.074±0.035	P1=0.296, P2=<0.001*, *P3<0.001
I:T ratio		0.285±0.069	0.320±0.077	0.066±0.022	P1=0.070, P2=<0.001*, P3<0.001*
HSS score		5.000±0.830	4.733±0.828	1.067±0.640	P1=0.377, P2=<0.001*, P3<0.001*
Serum cortisol and acth levels					
Serum cortisol (ng/ml)	**9Am**	106.1±38.1	102.71±35.4	314.6±37.065	P<0.001*, P1=0.931, *P2=<0.001*, P3<0.001

	9 Pm	111.7±23.6	119.6±26.2	317.0±24.036	P<0.001*, P1=0.433, P2=<0.001*, P3<0.001*
(ACTH (pg/ml)	**9Am**	106.0±47.9	135.8±33.9	202.2±87.209	P<0.001*, P1=0.144, P2=<0.001*, P3<0.001*
	9 Pm	106.1±52.4	132.4±37.2	202.8±72.973	P<0.001*, P1=0.170
Post-ACTH Stimulation (ng/ml)		102.65±33.24	109.69±34.14	-±-	P=0.422,
Ultrasound findings					
Echo	**Normal**	6(20.00%)	3(10.00%)	18(60.00%)	**<0.001***
	Septal defect	13(43.33%)	9(30.00%)	7(23.33%)	
	PDA	5(16.67%)	8(26.67%)	1(3.33%)	
	PFO	1(3.33%)	7(23.33%)	4(13.33%)	
	PPHN+PDA	5(16.67%)	3(10.00%)	0(0.00%)	
Transcranial US	**Normal**	4(13.33%)	6(20.00%)	24(80.0%)	**<0.001***
	IVH grade I	19(63.33%)	16(53.33%)	6(20.00%)	**<0.001***
	IVH grade II	7(23.33%)	8(26.67%)	0(0.0%)	

Data are presented as mean ± SD.* Significant p value <0.05, P1: significance between full term neonates and preterm neonates, p2: significance between full term neonates and control, p3: significance between preterm neonates and control, CBC: complete blood count, CRP: C -reactive protein, ALT: alanine transaminase, AST: aspartate aminotransferase, Hb: hemoglobin, TLC:Total Leukocyte Count, ANC: absolute neutrophil count PT: prothrombin time, PTT: Partial thromboplastin time, INR: international normalized ratio, HSS:Hematological Scoring System, ACTH: tropic hormone produced by the anterior pituitary, Echo: echocardiogram, PDA: Patent ductus arteriosus, PFO: patent foramen ovale, PPHN: Persistent pulmonary hypertension of the newborn.

Serum cortisol Serum Cortisol and ACTH levels in relation to sex, mode of delivery, anesthesia and sepsis type revealed that no significant difference (P value <0.05). Table 4

Table 4: Serum Cortisol and ACTH levels in relation to sex, mode of delivery, anesthesia and sepsis type

		Sex		**P value**
		Male	**Female**	
S. cortisol	**9Am**	99.004±38.706	109.494±34.304	0.270
	9 Pm	112.018±25.350	119.162±24.691	0.273
ACTH	**9Am**	111.996±44.603	129.282±42.162	0.128
	9 Pm	110.611±49.783	127.389±43.521	0.169
Post-ACTH stimulation		100.388±34.260	111.583±32.569	0.200
Mode of delivery				
		CS	**NVD**	
S. cortisol	**9Am**	104.953±36.642	98.604±39.416	0.714
	9 Pm	116.956±24.601	102.000±28.895	0.204
ACTH	**9Am**	123.224±2.939	95.663±51.153	0.181
	9 Pm	121.615±45.263	93.587±63.647	0.205
Post-ACTH stimulation		107.512±33.225	91.433±37.986	0.309
Anesthesia				

		SA	GA	
S. cortisol	**9Am**	107.234±36.543	96.777±37.403	0.387
	9 Pm	117.520±26.493	114.933±16.898	0.751
ACTH	**9Am**	126.783±42.666	110.472±43.286	0.248
	9 Pm	124.337±45.746	111.861±43.980	0.404
Post-ACTH stimulation		109.012±34.612	102.139±28.367	0.531
Sepsis type				
		EOS	**LOS**	
S. cortisol	**9Am**	93.914±38.874	110.957±33.967	0.079
	9 Pm	111.950±25.686	118.046±24.724	0.364
ACTH	**9Am**	113.135±48.063	125.771±40.969	0.282
	9 Pm	107.756±49.041	126.442±44.898	0.136
Post-ACTH stimulation		99.121±34.388	110.555±32.786	0.202

Data are presented as mean ± SD.* Significant p value <0.05, ACTH: tropic hormone produced by the anterior pituitary, CS: Cesarean Section, NVD: Natural vaginal delivery, SA: spinal anesthesia, GA: general anesthesia, EOS: Early-Onset Sepsis, LOS: Late-Onset Sepsis.

There was no significant difference between serum cortisol (9 am& 9 pm) and ACTH levels (at 9 am & 9 pm), and sex, modes of delivery, maternal anesthesia, oxygen support, inotropes, grades of respiratory distress, blood culture, transcranial ultrasound and sepsis ztype. Table 5

Table 5: Serum Cortisol and ACTH levels in relation to oxygen support, blood culture, respiratory distress, inotropic drugs and transcranial ultrasound

		Oxygen support					P value
		SIMV	**HFNC**	**Room Air**	**HFOV**	**PCV**	
S. cortisol	**9 Am**	107.422±33.170	107.889±35.059	72.614±42.842	111.152±57.008	101.734±37.722	0.747
	9 Pm	118.677±20.481	118.010±22.112	96.459±42.334	125.393±30.536	112.047±28.503	0.634
ACTH	**9 Am**	123.944±40.052	117.116±42.242	83.074±64.480	129.279±62.279	122.263±45.568	0.778
	9 Pm	122.383±45.094	118.721±49.554	73.313±76.216	144.479±63.921	116.621±43.505	0.535
Post-ACTH stimulation		108.872±29.302	110.817±30.855	91.410±43.054	112.488±43.434	101.704±37.944	0.872
Blood culture							
		No Growth	**Klebsiella**	**E-Coli**	**Pseudomonas**	**MRSA**	
S. cortisol	**9Am**	111.238±28.234	107.187±39.524	102.548±34.137	98.587±33.642	100.266±40.463	0.965
	9 Pm	121.605±33.401	116.712±27.342	110.661±24.055	113.725±22.350	120.117±22.542	0.941
ACTH	**9Am**	129.888±65.501	124.217±44.740	111.973±44.792	114.518±41.851	126.000±47.905	0.920
	9 Pm	117.217±56.317	122.550±49.826	105.129±47.590	116.560±44.343	130.644±42.472	0.846
Post-ACTH		110.006±44.71	108.301±	100.241±26	103.844±30.1	107.308±31.94	0.974

stimulation		0	37.840	.469	54	5	
Respiratory distress							
		Intubated	**RD I**		**RD I**	**RD III**	
S. cortisol	**9Am**	104.903± 36.928	117.804±21.066		97.601±39.396	130.684±0.000	0.768
	9 Pm	115.946±25.416	127.620±1.734		112.114±27.428	120.547±0.000	0.875
ACTH	**9Am**	123.567±43.809	130.624±2.766		108.095±49.819	121.251±0.000	0.761
	9 Pm	121.424±45.503	125.893±1.856		108.874±59.801	121.880±0.000	0.884
Post-ACTH stimulation		105.602±34.490	122.106±0.356		105.468±35.359	108.259±0.000	0.929
Inotropic Drugs							
		None	**Dopamine& Dobu**		**Dopamine**		
S. cortisol	**9Am**	102.31±41.32	110.37±39.32		101.267±34.550		0.678
	9 Pm	116.4±34.3	117.7±28.0		114.34±21.60		0.894
ACTH	**9Am**	122.2±61.6	123.2±47.6		119.26±38.48		0.949
	9 Pm	111.2±64.6	126.9±49.0		116.33±42.46		0.655
Post-ACTH stimulation		110.0±35.0	107.2±39.9		104.67±29.91		0.916
Transcranial Ultrasound							
		Normal	**IVH grade I**		**IVH grade II**		0.299
S. cortisol	**9Am**	116.168±31.629	105.800±35.519		93.383±41.170		0.044*
	9 Pm	126.573±18.602	118.108±21.194		102.870±32.520		0.225
ACTH	**9Am**	134.461±44.554	123.764±39.530		105.287±51.223		0.100
	9 Pm	132.466±50.143	124.943±38.687		97.273±57.812		0.027*
Post-ACTH stimulation		123.375±29.914	108.845±29.696		88.467±38.377		0.299

Data are presented as mean ± SD.* Significant p value <0.05, ACTH: tropic hormone produced by the anterior pituitary, HFNC: High flow nasal cannula, HFOV: High frequency oscillation ventilation, SIMV: Synchronized intermittent mechanical ventilation, PCV: Pressure controlled ventilation, MRSA: Methicillin resistant staph aureus, RD: Respiratory distress, IVH: Intraventricular hemorrhage.

HSS score and CRP level were correlated with serum cortisol level at 9 am (r=-0.273, P<0.035 andr=-0.447, P<0.001), at 9 pm (r=-0.447, P<0.001and r=-0.477, P<0.001) ACTH at 9 am (r=-0.314, P<0.015 and r=-0.377, P<0.003) and at 9 pm (r= -0.362, P<0.005 and r=-0.448, P<0.001) and cortisol level post ACTH stimulation (r=-0.345, 0.007 and r=-0.497, P<0.001) respectively. **Table 6**

Table 6:Correlation coefficient between serum cortisol and ACTH levels and clinical and laboratory variables

	S. cortisol				ACTH				Post-ACTH stimulation	
	9Am		9Pm		9Am		9Pm			
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
GA (Weeks)	0.100	0.447	-0.074	0.573	-0.251	0.053*	-0.203	0.121	-0.033	0.802
Postnatal age (Days)	0.197	0.131	0.172	0.189	0.166	0.205	0.228	0.080	0.189	0.149
Weight (kg)	0.083	0.530	-0.069	0.601	-0.240	0.064	-0.171	0.191	-0.042	0.748
HR	-0.177	0.177	-0.191	0.145	-0.175	0.182	-0.205	0.116	-0.227	0.081
SBP	0.049	0.708	-0.068	0.608	-0.146	0.267	-0.073	0.579	0.011	0.931
DBP	0.094	0.475	0.100	0.447	0.096	0.463	0.141	0.284	0.149	0.254
MBP	0.029	0.828	-0.003	0.985	-0.046	0.726	-0.005	0.969	0.054	0.681
Temp	0.123	0.351	0.076	0.561	-0.043	0.743	0.021	0.876	0.092	0.484
Hb	0.050	0.707	0.003	0.983	0.023	0.863	-0.013	0.923	-0.031	0.813
Platelet	0.060	0.647	0.132	0.314	0.084	0.523	0.078	0.556	0.090	0.494
TLC	0.032	0.810	0.015	0.910	0.024	0.858	-0.027	0.840	0.054	0.683
ANC	0.035	0.793	-0.004	0.977	0.002	0.988	-0.018	0.888	0.042	0.752
I:M ratio	0.032	0.809	0.046	0.730	0.114	0.386	0.085	0.520	0.079	0.550
I:T ratio	0.022	0.867	0.051	0.697	0.131	0.317	0.089	0.498	0.085	0.520
HSS score	-0.273	0.035*	-0.329	0.010*	-0.314	0.015*	-0.362	0.005*	-0.345	0.007*
CRP	-0.447	<0.001*	-0.477	<0.001*	-0.377	0.003*	-0.448	<0.001*	-0.497	<0.001*
Urea	-0.128	0.328	-0.142	0.278	-0.190	0.147	-0.181	0.168	-0.127	0.332
Creat	-0.331	0.010*	-0.247	0.058	-0.219	0.092	-0.195	0.136	-0.294	0.022*
ALT	-0.175	0.181	-0.083	0.530	-0.180	0.170	-0.119	0.363	-0.090	0.494
AST	-0.199	0.127	-0.118	0.370	-0.219	0.093	-0.137	0.298	-0.148	0.259
Total Bilirubin	-0.238	0.067	-0.194	0.137	-0.207	0.113	-0.283	0.028*	-0.217	0.096
Direct bilirubin	-0.078	0.551	0.012	0.926	-0.060	0.646	-0.053	0.685	-0.049	0.712
S.	-	0.382	-	0.323	-	0.729	-0.107	0.416	-0.029	0.827

albumin	0.115		0.130		0.046					
PT	-0.167	0.203	-0.073	0.578	-0.181	0.166	-0.100	0.447	-0.134	0.309
PTT	0.146	0.266	0.187	0.153	0.190	0.146	0.181	0.167	0.136	0.298
INR	-0.218	0.094	-0.102	0.439	-0.203	0.119	-0.134	0.308	-0.170	0.194

r: Pearson*Significant p value <0.05, ACTH: tropic hormone produced by the anterior pituitary, GA: Gestational age, Apgar: appearance, pulse, grimace, activity, and respiration, HR: heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: mean blood pressure, RR: Respiratory rate, Hb: hemoglobin, TLC: Total Leukocyte Count, ANC: absolute neutrophil count, PT: prothrombin time ,PTT: Partial thromboplastin time , INR: international normalised ratio.

Discussion

The main finding in the present study showed that the most of preterm neonates were moderate to late preterm between 32- 36 weeks of gestation, they showed significant decline in plasma cortisol level and failure to augment its release after ACTH stimulation test described as RAI when exposed to the stress of sepsis.RAI occurs when the patient's cortisol reaction is insufficient for their amount of stress, even if their cortisol level seems to be within the normal range. This occurs as a result of malfunctioning at any stage of the HPA axis or as a consequence of resistance to glucocorticoids[24].

Cortisol level was studied in plasma of full term and preterm neonates in response to sepsis. RAI was diagnosed in 47 from 60 neonates with sepsis (78.3%), 22/30 (73.3%) in full term neonates and 25/30 (83.3%) in preterm neonates with sepsis, while the remaining 13 (21.7%) showed absolute adrenal insufficiency.

In this study, cortisol level in neonates with sepsis was markedly reduced (106±38 ng/ml) [10.6±3.8 ug/dl) in full term neonates and 102± 35ng/ml [10.2±3.5 ug/dl) in preterm neonates with sepsis compared with 314±37 ng/ml [31.4±3.7 ug/dl] in the control group.Naveen Kumar et al.[23]A study with 35 newborns revealed that 8 of them had cortisol levels below 6 ug/dl, 9 (25.71%) had serum cortisol levels ranging from 7 to 16 ug/dl, 7 had cortisol levels between 17 and 23 ug/dl, and 11 had elevated serum cortisol levels. According to Asare et al. [35], RAI is defined as a cortisol level below 25 ug/dl that is randomly measured. Random

cortisol testing is a valuable diagnostic tool for determining the presence of adrenal insufficiency. On the contrary, Fernandez et al.[25] A research conducted on 32 newborns revealed that 18 (56%) of them had cortisol levels below 15 mcg/dl. A total of 21 newborns received hydrocortisone treatment, out of whom 13 had cortisol levels below 15 mcg/dl.

In the present study, there was no relation between the level of serum cortisol and the weight of the neonate. A comparable observation was detected in the study done by Baker et al. [26]concluded that serum cortisol concentrations showe The current investigation found no correlation between blood cortisol levels and neonatal weight. In research conducted by Baker et al. [26] a similar finding was observed, which revealed that there was no association between blood cortisol concentrations and the weight of the newborns. d no correlation with the weight of the neonates.

The present investigation revealed no association between blood cortisol levels and the gestational age (GA) of the neonates, which aligns with the findings of the study done by Baker et al. [26]. In contrast, Scott et al. [27] discovered a negative correlation between gestational ageand cortisollevels, indicating that the youngest newborns had the greatest cortisol levels. In this study, ACTH stimulation test failed to improve or increase serum cortisol level in neonates with sepsis both in preterm and full-term groups. This might support the use of hydrocortisone as one of management strategy in neonatal sepsis. In research conducted by NaveenKumar et al. [23], it was shown that administering an early stress dosage of hydrocortisone at the beginning of newborn septic shock resulted in significant clinical improvement and increased survival rates. In research done by Kumar et al. [23], it was shown that hydrocortisone medication effectively improved the condition of vasopressor resistance hypotension, resulting in a more favorable clinical outcome. Tantivit et al. [28]demonstrated that they were able to enhance blood pressure and stable full-term infants who were experiencing persistent low blood pressure that was unresponsive to

treatment. Administering a stress dosage of hydrocortisone may effectively treat adrenal insufficiency and perhaps alleviate hemodynamic impairment, therefore altering the progression of the condition[29].

The present study showed that low serum cortisol level had strong inverse correlation with HSS score of neonatal sepsis and CRP as a marker of inflammatory response to sepsis.Sam et al. [30]Indicated that 25% of newborns experiencing septic shock had a complete lack of adrenal function when the shock began, and around 30% of newborns with septic shock may have a partial lack of adrenal function.

Researchers have examined the molecular processes behind septic shock in order to identify possible interventions[31]. Corticosteroids have the potential to serve as prognostic indicators due to their interactions with immunological responses[32]. Adrenal insufficiency was not detected in non-septic severely unwell newborns during the investigation on neonatal shock. Hence, the underlying mechanisms of adrenocortical dysfunction in septic shock are expected to be distinct from those seen in non-septic causes of shock[33].

In addition, there was no significant difference in serum cortisol and ACTH am and pm levels comparing preterm and full terms neonates with sepsis and also in the control neonates without sepsis.In agreement with our result,Beishuizenet al.[34] stated that diurnal rhythms were still absent in first 2 months, and the typical circadian rhythms of the plasma cortisol levels were present in infants aged 3 months. In agreement with that study two other studies[35, 36] confirmed that the normal circadian rhythm of cortisol secretion, (characterized by achievement of the highest concentration in the morning and subsequently decreased nadir in the evening), is lost during critical illness. Schumer[37] demonstrated that treatment with dexamethasone or methylprednisolone improved patients' survival in severe sepsis.

As a result, large well-designed randomized controlled studies were performed to find out whether the corticosteroid treatment has any benefit in the treatment of severe sepsis or septic

shock[38]. However, later in several meta-analyses, it was concluded that high-dose corticosteroids should not be used in the treatment of severe sepsis or septic shock.

Thus, adrenal insufficiency especially the RAI as our studied population is an important diagnosis to consider in any sick newborn infant that require early laboratory investigation and prompt treatment.

The limitations of this study was small sample size, despite the large number of neonates admitted to NICU during study period, there were meticulous selection of the studied population to avoid many confounders that might adversely affect the study results like respiratory distress, gastrointestinal symptoms, arrhythmias or tachyarrhythmias and bradyarrhythmia and so on, Second, our study was cross sectional without short term or long term follow up to examine the impact of adrenal insufficiency on the neonatal outcome, Third, some newborns were receiving hydrocortisone during septic shock and were not excluded. This altered the serum cortisol level measured. However, serial measurement at 9 am and 9 pm and following ACTH stimulation test could reflect well the HPA axis and detected relative or absolute AI.

Conclusions:

Neonatal cortisol and ACTH plasma level of single NICU series of preterm infants, between 32 and 36 weeks of GA and full-term neonates diagnosed with sepsis. RAI was detected in 75% of our studied population with neonatal sepsis. Even though low cortisol levels have not been frequently linked to clinical characteristics of the studied population, cortisol and ACTH values were correlated to HSS score and CRP as an inflammatory marker. This reflects suppressed HPA axis status in this critical clinical condition. Assessment of HPA axis in neonates with sepsis is compulsory in such critical status as it might change the management strategy.

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