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Salivary Cortisol Level as a Marker of Adrenal Function in Children with Systemic Inflammatory Response Syndrome in Egypt

Amany El-Kelany[1], Maha Enany[2], Adel Elbaih[3]

[1]Department of Pediatrics
[2]Department of Clinical Pathology
[3]Department of Emergency Medicine,
Suez Canal University,
Ismailia, Egypt

Corresponding Author:
Dr. Adel Hamed Elbaih
Department of Emergency Medicine,
Suez Canal University,
Ismailia, Egypt
Mobile No: 00201154599748
e-mail: *elbaihzico@yahoo.com*

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Abstract

Objective: Pediatric systemic inflammatory response syndrome (SIRS) criteria is widely used to identify patients who need specialized, advanced therapies. Cortisol plays an important anti-inflammatory in SIRS. Adrenal status and use of corticosteroid in children with SIRS still controversial. The aim of study is to assess salivary in children with SIRS and its relation to outcome in PICU of Suez Canal University hospital.

Methods: Prospective case control study was carried out. We analyzed 45 patients with SIRS and45 healthy control children for morning salivary and total serum cortisol level

Results: Children with SIRS have significantly higher morning salivary and total serum cortisol level than control group (p=.00). Mortality was associated with higher levels of both salivary and total cortisol.

Conclusion: Salivary cortisol can be used to estimate cortisol levels in pediatric with SIRS and can foretell outcome.

Key words: *Systemic Inflammatory Response Syndrome, Children, Salivary, Cortisol*

Introduction

SIRS is nonspecific disorder and is described as the body's immune response to inflammation due to diversity of infectious and non-infectious causes. In fact, it has pro- and anti-inflammatory components. SIRS early identification is critical in confirming good results [1]. Non-infectious SIRS is more common nearly twice as often as infectious SIRS [2].

Non-infectious SIRS etiology include trauma, burns, pancreatitis, ischemia, and hemorrhage, adrenal insufficiency, anaphylaxis, surgical complications and Drug overdose [3] SIRS in the occurrence of or as a consequence of suspected or proven infection is considered sepsis [4].

The prevalence of SIRS is very prohibitive, affecting more than 50% of all ICU patients [1] and 21.7% of pediatric presenting to the emergency [5]

and as high as 72% among hospitalized children with abnormal temperature [6].

Cortisol plays a key role in the compensatory anti-inflammatory responsesyndrome (CARS) and the occurrence of systemic inflammatory response syndrome (SIRS) [7- 8].

Salivary cortisol is a cost effective and non -invasive accurate measure of bioavailable cortisol and can be used in place of plasma cortisol level in critically ill children [9-12]

Salivary cortisol also offer a practical approach to evaluating pituitary-adrenal function continuously during and after short-term corticosteroid therapy [13].

Assessment of unstimulated early morning cortisol has been proposed as a first line parameter to assess adrenal function in suspected secondary adrenal insufficiency [14].

Salivary cortisol represents the biologically active and free fraction of cortisol. Free cortisol (FC) rather than protein-bound cortisol is responsible for the protean actions of this hormone [15-16].

Serum free and salivary cortisol values correlate in critically ill children [9].

The aims of this study were to assess the cortisol level in children with SIRS admitted to PICU of Suez Canal University hospital and its relation to outcome. Secondary aims were to correlate serum total and salivary cortisol in children with SIRS, healthy controls, and to create a reference value for salivary cortisol in children in Egypt.

Materials and Methods

A prospective cross sectional case control study was carried out from at Pediatric Intensive Care Unit of Suez Canal University Hospital.

A total of 90 children aged 2-10 years old, 45 patients with SIRS and 45 healthy normal children matched for age and sex as a control group.

Diagnosis of SIRS was based on Criteria of SIRS definition and diagnosis approved by International pediatric sepsis consensus conference 2005 to diagnose children with SIRS, they should meet two criteria out of four major criteria but at least one of them is abnormal temperature or leukocyte count: (a) core body temperature > 38.5°C or < 36°C or (b) increased or depressed leukocyte count for age not secondary to chemotherapy induced leukopenia (Age 2 to 6 years WBCs Count >15.500 or <6000 ,if Age 6 to 12 years WBCs Count >13.500 or 4.500 or > 10% bands. The other two criteria are c) Tachycardia, defined as a mean heart rate >2 SD above normal for age without external stimulus (if Age 2 to 6 years heart rate >140 per minute Age 6 to 12 years heart rate >130 per minute) D) Tachypnea with mean respiratory rate >2 SD above normal for age (if age 2 to 6 years respiratory rate >22 per minute, and if Age is 6 to 12 years respiratory rate >18 per minute) or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia [4].

Children were receiving systemic steroid or drugs that affect steroid secretion as phenytoin, phenobarbitone, or rifampicin within the previous month were excluded. We also exclude patients with known hypothalamic- pituitary dysfunction, chronic illness or children with increased BMI >85th centile for Egyptian growth curves.

After parental informed consent consistent with the ethical principles of the International Conference of Harmonisation guidelines, Good Clinical Practice (ICH-GCP) was obtained for each child, full history and complete physical examination was done. Saliva and Blood samples were collected simultaneously, between 8:00 am and 10:00 am in the first 24 hour of admission.

Salivary samples were taken at least one hour after nothing per mouth and no visible blood in oral cavity.

The salivary samples were collected using special saliva sampling devices (Salivette, Sarstedt Inc., Rommelsdorf, Germany) the cotton swap introduced in the mouth till completely soaked with saliva but not more than 20 minutes, sample then transferred in the sampling plastic tube. The tubes were centrifuged for 5 to 10 minutes and stored at -20°C until analyzed. Salivary cortisol concentrations were determined using a competitive enzyme immunoassay by DRG Salivary Cortisol ELISA KIT (DRG Instruments GmbH, Germany) after at least one freezing, thawing, and centrifugation cycle (centrifugation to separate the mucins).

All patients were monitored and got standard treatment. No changes in the standard treatment protocol were made based on the cortisol assay results.

Adults expected normal values by same kits are 0.12 – 1.47 µg/dL .

Blood samples were centrifuged immediately and the sera of the samples were stored at temp less than 20 °C. Serum Cortisol levels were estimated using the IMMULITE technique. The serum total cortisol was measured using ELISA. RIA method (Immunotech; Prag, Czech Republic)

Statistical Analysis

Statistical analysis was performed using SPSS software version 21. Continuous variables were expressed as mean and standard deviation and were compared by t-test or Mann-Whitney test. Categorical variables are stated as frequencies and percents and studied using chi-square . Pearson's correlation was used to estimates the correlation between quantitative variables. P value less than 0.05 was considered statistically significant.

Results

Demographic data are summarized in table 1and showed that there was no statistical difference between both groups regarding the mean age and sex.

Among patients diagnosed with SIRS, infectious SIRS (sepsis) were more common (70%) and non-infectious SIRS (30%).

Infectious SIRS started as pneumonias 45%, bacteremia15%, central nervous system infections 15% wound and skin infection 10% gastrointestinal infection 10%. Infectious SIRS was culture positive only in 11/29 and caused by gram negative organism in 6 patients, gram positive organism in 4 and two as a mixed infection.

Noninfectious SIRS caused by seizures (9/16), Hypoxic–ischemic encephalopathy (2/16), postoperative (2/16) and other diagnoses (3/16).

Table 1. Demographic data.

	Patient	Control	P value
Age in month			
Mean St. deviation	54.9 27.78	56.6 25.69	0.76
Age group:			
2-6 years	33	35	0.6
≥6-10 years	12	10	
Sex			
Male/ female	27/18	23/22	0.39

Table 2. Characteristics of cases with SIRS.

Cause of SIRS	No.	Frequency
Infectious :	29	64%
Site of infection:		
Pneumonias	19	42%
Skin and soft tissue	1	2.2%
CNS	5	11%
Blood	2	4.4%
Gastrointestinal	2	4.4%
Skin and soft tissue	1	2.2%
Culture-proven infection	9	20%
Gram –ve infections	5	11%
Gram +ve infections	3	6.6%
More than one organism	1	2.2%
Non infectious	16	35.5%
Cause of non infectious SIRS		
Seizures	9	20%
Hypoxia–ischemia–reperfusion,	2	4.4%
Postoperative	2	4.4%
Other diagnoses	3	6.6%
SIRS criteria		
Temp>38	33	66.6%
Temp >36	2	4.4%
Leukocytosis	26	57.7%
Leucopenia	2	4.4%
Tachycardia	8	17.7%
Bradycardia	1	2.2%
Tachypnea	23	51.1%
Need for Mechanical Ventilation	27	60%
Mortality		
Total mortality	27/45	60%
Non infectious	10/16	62.5%
Infectious	17/29	58.6%

Concerning SIRS criteria, fever was the commonest criteria (33/45), hypothermia in (2/45) patients, leukocytosis in (26/45), leucopenia only in (2/45) tachypnea in (23/45), Mechanical ventilation was needed for (26/45), tachycardia

Table 3. Basal serum cortisol and Salivary cortisol level levels in cases and controls.

	SIRS group	Control	P value
Basal cortisol level (µg/dl)			
Mean ± st. deviation (Min – max)	49.61±13.58 (20.6-76.9)	13.28±2.12 (9.45-18.3)	
Basal cortisol survive	35.97 ±8.6	-	0.00
Basal cortisol died	55.84 ±10.12	-	
Salivary cortisol level (µg/dl)			
Mean ± st. deviation (Min – max)	2.57±.82 (1.65-4.4)	0.64±.13 (0.34-0.98)	
Mean ± st. deviation in survived	3.06 ± .56	-	0.00
Mean ± st. deviation in died	2.34 ±0.42	-	

in (8/45), bradycardia only in (2/45)

Salivary cortisol level was significantly higher in patient than control (2.79±.59 µg/dl versus 0.64±.13 µg/dl, p=0.00)

Basal total cortisol level was significantly higher in patient than control (47.95±13.54 µg/dl versus13.28± 2.12 µg/dl. p=0.00)

Overall mortality was 60%. (10 noninfectious and 17 infectious SIRS)

The results of the mean basal and salivary cortisol levels were higher in died (54.7±11.15µg/dl &3.05±0.53µg/dl) than survived (36.78±9.04µg/d &2.37±0.41µg/dl.) p = 0.00.

Logistic regression analysis was performed to determine the odds ratio between salivary cortisol levels, basal serum cortisol and mortality. The patients who died had higher serum salivary cortisol levels (with OR 3.2;95% CI 1.23- 12.4; p=.047) and had higher basal serum cortisol (with OR 1.2; 95% CI 1.03- 2.4; p=0.015)

Lower total serum cortisol level also correlated with higher probability of survival.

Pearson Correlation between basal total and salivary cortisol in patients was 0.77 (r^2 0.59 95% CI, 0.65-0.87) p value= 0.00. The coefficient of determination suggests that 59% of variation in salivary cortisol is due to variation of serum cortisol.

Discussion

The prevalence of SIRS criteria in our study was nearby to prevalence in study by Jana Pavare 2009. They found diagnosis of SIRS in hospitalized children was depend on a combination of abnormal temperature with elevated respiratory rate >2 SD above normal for age in 76% of cases, a combination of abnormal temperature with abnormal leukocyte count in 50%, and abnormal temperature with tachycardia >2 SD above normal for age in 15% [6].

Carvalho et al. [17] in a study conducted in a pediatric intensive care unit found that increased heart rate >2 SD above normal value for age occurred in 85% of SIRS patients, abnormal leukocyte count in 77%, and increased respiratory frequency >2 SD above normal value of age in 47.5%.

Infection was the most common (53%) associated etiology, followed by trauma (10%) [5]. We found no gender difference among SIRS patients, in agreement with [6, 17].

Normal children's salivary cortisol was reported also in some studies (usually a control group). The expected range of values were.01mcg/dl to less than 1.0mcg/dl [10,18,19,20,21].

For healthy younger children aged 45 days to 36 months Silva et al., 2007 found the Mean (±standard error) morning cortisol levels were 557.86±37.72 nmol/L, with a range of 76.88 to 1,620.08 nmol/L (percentiles 2.5 to 97.5) [22].

We studied the cortisol level in pediatric patients with SIRS. We detected higher basal total and salivary cortisol in patients than the matched control group.

Elevated baseline cortisol level found in children with SIRS found in our study was in agreement with results found in adults with SIRS [23].

The baseline salivary cortisol among children with fluid unresponsive septic shock was higher (19.8, 7.2-42.4 nmol/L) than healthy children (2.6, 1.3-7.6 nmol/L) (P=0.001) [24].

Our study showed that both basal and salivary cortisol was higher in died than survived. Similar results were observed in children with septic shock [25, 26].

Same results by Stepani B et al., 2008 [27] they reported that non-survivors critically ill patients had significantly higher total cortisol concentrations (980 ± 458 nmol/L) than survivors (704 ± 383 nmol/L, P = 0.002).

Conclusion

Salivary cortisol measurement is easy non- invasive and feasible test to assess adrenal function in pediatrics. We have demonstrated that salivary cortisol can be used to assess adrenal status in children with SIRS with very good correlation to serum total cortisol. High salivary cortisol levels can predict mortality children with SIRS.

Competing Interest: None

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