RESEARCH BRIEF

Salivary Cortisol Estimation to Assess Adrenal Status in Children with Fluid Unresponsive Septic Shock

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We evaluated the adrenal status by estimating baseline and ACTH stimulated salivary cortisol in 51 children with fluid unresponsive septic shock at 30 and 60 minutes, and basal salivary cortisol (9-11 am) in 79 healthy children. The baseline salivary cortisol (median,IQR) among patients (19.8, 7.2-42.4 nmol/L) was higher than healthy children (2.6, 1.3-7.6 nmol/L) (*P*=0.001). Non-survivors and those with catecholamine refractory shock had higher baseline cortisol level, though difference was statistically insignificant. Absolute adrenal insufficiency (baseline salivary cortisol <1.3nmol/L) was diagnosed in 8 (15.7%) patients. Relative adrenal insufficiency (rise in cortisol level above baseline value after stimulation <25nmol/L) was observed in 68.6% of all patients; 71.9% among non-survivors, and in 71.4% patients with catecholamine refractory shock. Salivary cortisol estimation appears to be feasible in children with septic shock. Relative adrenal insufficiency is common in these children.

Key words: *Salivary cortisol; Fluid unresponsive septic shock; Adrenal insufficiency.*

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Septic shock remains a major cause of mortality among children in intensive care unit. Activation of hypothalamic-pituitary-adrenal (HPA) axis in response to stress is crucial for adaptation and maintenance of homeostasis. Studies have found a high incidence (40-65%) of adrenal dysfunction in septic shock patients [1-3]. Assessment of adrenal status in septic shock by measuring serum cortisol has prognostic and therapeutic value [4, 5].

Salivary cortisol estimation is non-invasive, easy to perform, represents the biologically active, free fractions of cortisol, and hence offers advantage over serum cortisol [6,7]. Studies on salivary cortisol in children with septic shock remain scanty. We conducted this study to assess the baseline and corticotropin stimulated salivary cortisol in children with fluid unresponsive septic shock, and its association with mortality and refractoriness of shock.

METHODS

This observational study was done at a tertiary care hospital from August 2010 to July 2011. The study was approved by institutional ethics committee and consent was obtained. We enrolled consecutive children aged 1 to 12 yr with septic shock who were unresponsive to a fluid challenge of 60 mL/kg of isotonic fluids (normal saline in aliquots of 20 mL/kg) administered in the first hour [8,9]. The fluid unresponsiveness was defined as persistence of signs of hypotension or hypo-perfusion (blood pressure <5th percentile or systolic BP <2 SD below normal for age or need for vasopressors to maintain BP; or two of the followings: capillary refill time >5 sec, core to peripheral temperature gap >3°C, urine output <0.5 mL/kg/hr, or unexplained metabolic acidosis -base deficit >5 mEq/L) despite the fluid challenge. Patients already receiving steroids or who had received steroid (2 weeks or more) in the past 6 months, patients with primary adrenal insufficiency, chronic organ dysfunction/chronic illness, known hypothalamic/ pituitary dysfunction, and those receiving phenytoin, phenobarbitone or rifampicin were excluded. Study entry was the time when all criteria for fluid unresponsive septic shock were met. Baseline parameters including age, gender, nutritional status (Z score), days of prior hospitalization and admission diagnosis were recorded. Pediatric Risk of Mortality (PRISM III) score [10], use of vasopressors and final outcome were recorded. All patients were monitored and received standard treatment. Catecholamine refractory shock was diagnosed if shock persisted despite the use of epinephrine or norepinephrine. We also enrolled 79 healthy children attending outpatient department between 9-11 am for vaccination or for minor ailments, for baseline salivary cortisol estimation, and saliva was

obtained before any painful procedure.

Saliva sample (2 mL) was collected either by spitting method into a plastic screw cap tube or from oral cavity using sterile cotton swab and then transferring it into the tube. The samples were centrifuged and stored frozen at -20°C until analysis (within 1-2 months). Three saliva samples from each patient were obtained, one at baseline (soon after a diagnosis of fluid unresponsive septic shock) and others after low dose ACTH (1μg intravenous Synacthen injection) stimulation at 30 and 60 min. We collected salivary samples irrespective of any specific time of the day as most critically ill patients lose the diurnal variation in their cortisol levels [11]. The mouth was cleaned at least half hour before post-stimulation sampling. No saliva sample was contaminated with blood. Salivary cortisol was estimated using the Cortisol ELISA (IBL International Germany), minimal analytical sensitivity was 0.138 nmol/L and functional sensitivity 0.828 nmol/L. Results of salivary cortisol assay were not available for deciding about use of steroids in any patient. Basal salivary cortisol values to define absolute adrenal insufficiency and hypercortisolism, were based on our own controls and another study [12]. Relative adrenal insufficiency was diagnosed if the peak increment in salivary cortisol after ACTH stimulation was <25 nmol/L (greater of the value at 30 or 60 min after stimulation minus baseline value), based on previous studies reporting total serum cortisol response to corticotrophin stimulation in septic shock [4, 13, 14]. Statistical analysis was performed using SPSS software version 16. Continuous variables were compared by *t*-test or Mann-Whitney test and categorical variables using chi-square or Fisher's exact test.

RESULTS

The median (IQR) basal salivary cortisol level in healthy control group (n=79) was 2.6 (1.3-7.6) nmol/L. There were no significant variation in basal salivary cortisol values among healthy controls in reference to different age groups and sex. The median (IQR) basal salivary cortisol level (nmol/L) was significantly higher (P<0.001) in patients with septic shock [19.8 (7.2-42.4)] compared to healthy children [2.6 (1.3-7.6)], however, patients in septic shock group were more often undernourished (weight for age <2 SD score: 82.5% *vs* 46.9%). The mean age and gender distribution were similar in both the groups. Of the 51 patients with fluid refractory shock, eight had positive blood culture results.

The baseline parameters and salivary cortisol were compared between survivors and non-survivors **(*Table* I)**. Absolute adrenal insufficiency was diagnosed in 8 (15.7%), and relative adrenal insufficiency in 35 (68.6%) patients. Post-stimulation salivary cortisol was higher at 30 min compared to that at 60 min, however, the difference was insignificant (P=0.715). Amongst patients who had absolute adrenal insufficiency (n=8), seven (87.5%) also demonstrated relative adrenal insufficiency (6 died); and six (75%) had catecholamine refractory shock (all died). ***Web Table* I** shows various parameters in relation to relative adrenal insufficiency.

The difference in 30 min post-stimulation and baseline salivary cortisol (nmol/L) was significantly higher in survivors (mean±SD 39.9±70.5) compared to non-survivors (29.1±90.1), (P=0.657) and those with fluid refractory shock (mean±sd=42.7±76.1) in comparison to catecholamine refractory shock (28.7±86.4), (P=0.582).

DISCUSSION

We studied the adrenal status in children with fluid refractory septic shock using salivary cortisol measurements. We observed absolute adrenal insufficiency in 15.7% and relative insufficiency in 68.6% of these children. A good correlation between salivary cortisol and free serum cortisol has been reported among adult patients with septic shock, in morning samples [13]. Furthermore, a greater relative increase in salivary cortisol than total serum cortisol after stimulation has been observed [5]. This would be expected when the binding capacity of available cortisol-binding globulin (CBG) is exceeded above saturation point, and it could be responsible for the high variability of post-stimulatory values of total serum cortisol and also poor correlation with salivary concentrations at individual times [5]. Thus, in situations of having low CBG concentration, with its maximum saturation, and lower albumin level one would expect more rise in free cortisol level than total serum cortisol level after stimulation. Therefore, salivary cortisol can be used as surrogate of serum free cortisol.

No consensus exists as to what constitutes the lower limit of salivary cortisol in critically ill children. Safarzadeh, *et al*. [12] established a normal range for basal salivary cortisol concentration (1.69-12.81 nmol/L) from healthy children aged 6-14 years. For defining absolute adrenal insufficiency, we used a cut-off of 1.3 nmol/L salivary cortisol value representing 25th centile; and a cut-off of 60 nmol/L for hypercortisolism corresponding to 97th centile of our control group. A study evaluating adrenal status in children with septic shock using low dose stimulation similar to ours, had taken an increment in total serum cortisol <9 µg/dL to define relative adrenal insufficiency [14]. In another study, a post-stimulation increment of <9 µg/dL in total

TABLE I SALIVARY CORTISOL IN CHILDREN WITH FLUID UNRESPONSIVE SEPTIC SHOCK

Parameters	All patients (*n*=51)	Died (*n*=32)	Survived (*n*=19)	*P* value
Age (yr) (mean±sd)	5.3 +3.4	5.2± 3.5	5.3±3.3	0.92
1-5 yr	32 (62.7)	21 (65.6)	11 (57.9)	0.58
>5 yr	19 (37.3)	11 (34.4)	8 (42.1)	
Male: Female	36 : 15	20 : 12	16 : 3	0.10
Weight for age: -2 to -3 SD score	12 (23.5)	8 (25)	4 (21.1)	0.87
Weight for age: > -3 SD score	25 (49.0)	16 (50)	9 (47.4)	
Prior hospitalization: ≤ 3 days	3 (5.9)	3 (9.4)	0	0.31
>3 days	23 (45.1)	15 (46.9)	8 (42.1)	
Catecholamine resistant shock	35 (68.6)	31 (96.9)	4 (21.1)	<0.001
PRISM score ≥8	43 (84.3)	31 (96.9)	12 (63.2)	0.005
#Basal Salivary cortisol, median(IQR)	19.8 (7.2-42.4)	25.2 (5.0-60.2)	17.2 (7.4-34.0)	0.86
Low <1.3nmol/L	8 (15.7)	7 (21.9)	1 (5.3)	
Normal 1.3-60 nmol/L	32 (62.7)	17 (53.1)	15 (78.9)	0.15
High >60 nmol/L	11 (21.6)	8 (25.0)	3 (15.8)	
#Post-ACTH salivary cortisol* median(IQR)				
At 30 min	33.7 (7.0-73.8)	30.5 (4.8-66.6)	39.0 (14.5-99.8)	0.31
At 60 min	24.4 (7.7-68.2)	22.3 (5.6-74.2)	24.5 (13.1-67.5)	0.67
*Rise in salivary cortisol Median (IQR),	7.4 (-0.1- 45.2)	3.5 (-3.2 - 42.5)	17.9 (0.6-47.6)	0.33
Rise <25 nmol/L	35 (68.6)	23 (71.9)	12 (63.2)	0.52
Rise ≥25 nmol/L	16 (31.4)	9 (28.1)	7 (36.8)	

*Figure in parentheses indicates percentage unless specified; SD= standard deviation, IQR= interquartile range, *Highest post-ACTH salivary; #salivary cortisol values in nmol/L.*

serum cortisol was associated with poor prognosis [4]. Converting this value for cortisol in nmol/L (multiplying by 27.6), and taking unbound cortisol represented in saliva as 10% of the total serum cortisol, an post-stimulation salivary cortisol increment of <24.84 nmol/L can be derived and would represent relative adrenal insufficiency [4,13,14].

The baseline salivary cortisol was higher in patients than control. This may be because of an initial adequate response to the stress induced by septic shock. Patients in non-survivor group had higher baseline value, and subsequent increment after stimulation was less compared to those who survived. Similarly, patients with catecholamine refractory shock had higher baseline value, and subsequent increment after stimulation was smaller compared to those who had only fluid refractory shock, though statistically insignificant. These smaller post-stimulation increments may be due to the fact that the HPA axis is already maximally stimulated, but it may be also a result of interference with the capacity of the adrenal cortex to produce glucocorticoids, and a lack of cortisol reserve [15]. We diagnosed primary adrenal insufficiency in 15.7% and relative adrenal insufficiency in 68.6% of children with fluid unresponsive septic shock, higher than reported by others [1, 14]. This may be because our children were sicker, all fluid unresponsive septic shock cases. Sarthi, *et al*. [14] reported relative adrenal insufficiency in 30% children with septic shock and primary insufficiency in none and there was no association with mortality but with catecholamine refractory shock. Though they used low-dose stimulation test similar to ours, they estimated serum cortisol. Although inconclusive due to small sample size, our data gives insight about incidence of absolute and relative adrenal insufficiency among fluid unresponsive septic shock children, and its association with catecholamine refractory shock and survival, which may have therapeutic implication.

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Contributors: SNS conceived and supervised the study, analyzed data and finalized the manuscript. He will act as

WHAT IS ALREADY KNOWN?

- Relative adrenal insufficiency is common in children with fluid refractory shock.

WHAT THIS STUDY ADDS?

- Salivary cortisol estimation is feasible in children. There was a trend towards having poor survival and catecholamine refractory shock among those with absolute or relative adrenal insufficiency.

guarantor. SR written the protocol, recruited patients and helped in analysis and manuscript writing. SA supervised the study and revised the manuscript. AS helped in recruiting patients and manuscript writing. VB analyzed the samples and revised the manuscript. The final manuscript was approved by all authors.

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