RESEARCH PAPER

Trier Social Stress Test in Indian Adolescents

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Objective: To test the Trier Social Stress Test for children (TSST-C) in a cohort of Indian adolescents.

Design: Cohort study

Setting: Holdsworth Memorial Hospital, Mysore, India.

Participants: Adolescent children (*N*=273, 134 males; mean age 13.6 yrs) selected from an ongoing birth cohort; 269 completed the test.

Intervention: Performance of 5-minutes each of public- speaking and mental arithmetic tasks in front of two unfamiliar 'evaluators'.

Outcome measures: Salivary cortisol concentrations were measured at baseline and at regular intervals after the TSST-C. Continuous measurements of heart rate, finger blood pressure, stroke volume, cardiac output and systemic vascular resistance were carried out before, during and for 10 minutes after the TSST-C using a finger cuff.

Results: Cortisol concentrations [mean increment (SD): 6.1 (6.9) ng/mL], heart rate [4.6 (10.1) bpm], systolic [24.2 (11.6) mmHg] and diastolic blood pressure [16.5 (7.3) mmHg], cardiac output [0.6 (0.7) L/min], stroke volume [4.0 (5.6) mL] and systemic vascular resistance [225 (282) dyn.s/cm^5] increased significantly (*P*<0.001) from baseline after inducing stress.

Conclusions: The TSST-C produces stress-responses in Indian adolescents of a sufficient magnitude to be a useful tool for examining stress physiology and its relationships to disease outcomes in this population.

Keywords: *Cortisol, Stress, Validation studies.*

Repeated exposure to psychological stress may result in adult-onset chronic diseases [1,2]. A deranged hypothalamic-pituitary-adrenal axis (HPAA) response to stress, leading to altered release of cortisol, and altered autonomic nervous system activity resulting in cardiac-sympathetic dysfunction, are the major factors determining this association. Individuals vary in stress-responses, and, thus, in their risk susceptibility [2,3]. Higher HPAA sensitivity in Indians may contribute to their high chronic disease risk [4]. Studying stress-responses in relation to disease risk, especially in younger individuals, may help to understand the underlying mechanisms and to intervene early in the lifecourse. However, the utility of the existing experimental psychological stressors in this population is unknown.

The Trier Social Stress Test for children (TSST-C), developed in Germany for European populations, is commonly used to study stress-responses in children [5]. We aimed to determine whether TSST-C, modified to suit local purposes, is useful for studying the HPAA and cardiovascular stress-responses in Indian children.

METHODS

Adolescent children were recruited from the Parthenon birth cohort [6], which was established to study the effect of maternal and developmental factors on offspring risk factors. 663 women attending the antenatal clinic of Holdsworth Memorial Hospital (HMH), Mysore, India delivered normal singleton babies during 1997-1998. At 13.5 years of age, 273 of the 545 children available for follow-up were selected from those living within Mysore (*N*=354) to achieve equal representation from four birth weight categories (134 boys). Willing families were approached in the chronological order of the children's date of birth until the target number was achieved.

Protocol (***Web Fig.* 1**): The robustness of a stress-module is assessed by its ability to induce strong cortisol reactivity [7,8]. The TSST-C involves 5-minutes each of public speaking and mental arithmetic tasks performed in front of an evaluative panel. A perception of negative assessment of the participants' self image by others (social evaluative threat) has been shown to trigger strong cortisol response [7-9].

We invited the cohort children for these tests as part of a routine cardiovascular assessment. The details were given before they confirmed participation. On the test morning, the children underwent detailed anthropometry. The tests were conducted between 2.00 PM and 3.30 PM. A standard lunch was provided approximately 1½ hours before the test to avoid postprandial variations in cortisol secretion. Subsequently, they spent a relaxed time with their family. A baseline (pre-test) salivary sample was collected 10 minutes before the test, after they watched a calming video for 5 minutes in a standing position.

The children were tested individually. The investigator explained the procedure to the child and gave 10 minutes to prepare an imaginative story following a lead. The lead was modified from the original to make it locally more identifiable (**Web *Table* I**). He/she was then accompanied to the test room which they had not seen previously, and was asked to stand in front of a microphone, facing a video camera. A male and a female staff member, previously unknown to the children, acted as 'judges'. They indicated that the child's performance will be evaluated for its quality, and will be video-recorded. The judges remained neutral throughout the test, and did not give positive feedback or encouragement, by words or by gesture, which was crucial to increase the stress-response.

First task consisted of public speaking (story). The male judge asked the child to complete the story in free speech, lasting 5 minutes. If they spoke uninterruptedly, the judge tried to make the situation more difficult (***Web Fig.* 1**). If they remained speechless, the judge gave prompts and hints to continue, as disengagement from the task was likely to decrease the stress-response [9]. Second task involved mental arithmetic (maths). The female judge asked the child to serially subtract '3' from '501' as fast and accurately as possible, for 5 minutes. Our pilot trials had shown that this series enabled the children to give enough right answers to sustain their interest, as well as having the scope for frequent errors. If they made a mistake, they were asked to start again from the beginning. The difficulty of the task was reduced or increased depending on the child's performance (***Web Fig*. 1, *Web Table* I**). It was ensured that they looked at the panel continuously during these tasks, by prompting if necessary. Tests were stopped immediately if the children seemed upset.

Systolic and diastolic blood pressure (BP), cardiac output, stroke volume, heart rate and systemic vascular resistance (SVR) were measured continuously before, during and for 10 minutes after the TSST-C by a non-invasive, portable hemodynamic monitoring system using appropriately sized finger cuffs (Nexfin, BMeye, Amsterdam, Netherlands). The beat-to-beat values were averaged over 5 minutes for the pre-test video-viewing (baseline), story, maths, and immediate post-stressor periods.

A salivary sample was collected at the end of the tasks. The judges commended the children for their performance. Children joined their family members in a separate room subsequently, but there was no contact with the untested children or their companions. Further samples were taken at 10, 20, 30 and 60 minutes after the TSST-C to measure the cortisol response. Another calming video was played before the final salivary sample was collected. The samples were then transferred

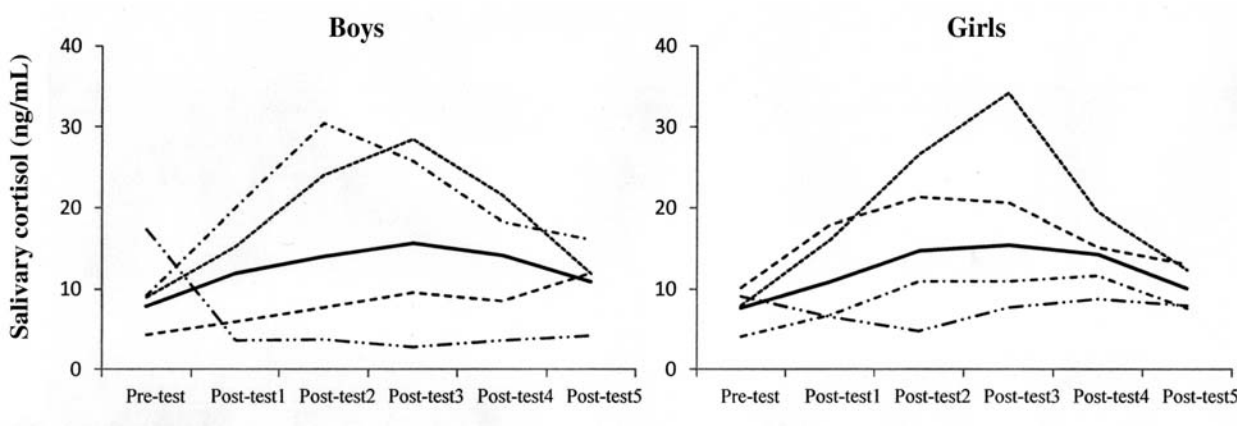

FIG. 1 *Stress-induced cortisol response in the study subjects.*

TABLE I GENERAL CHARACTERISTICS, AND CORTISOL AND CARDIOVASCULAR PROFILE IN THE STUDY SUBJECTS

	*All (N=273)***	*Boys (n=134)*	*Girls (n=139)*	*P*[#]
Age (yr)	13.6 (0.2)	13. 6 (0.2)	13.6 (0.1)	0.5
Height (cm)	154.2 (7.0)	154.7 (8.2)	153. 7 (5.7)	0.2
BMI (kg/m^2)	17.8 (2.9)	17.0 (2.4)	18.6 (3.1)	<0.001
Pubertal stage (N) 2	22 (8.0%)	3 (2.3%)	19 (13.9%)	
3	109 (41.0%)	33 (25.6%)	76 (55.5%)	
4 and 5	135 (50.8%)	93 (72.1%)	42 (30.7%)	<0.001
Obesity/overweight (N)	34 (12.5%)	10 (7.5%)	24 (17.3%)	0.01
Baseline				
Cortisol concentrations (ng/mL)*	6.6 (4.9,9.0)	6.8 (4.7,8.9)	6.6 (5.2,9.1)	0.97
Systolic BP (mmHg)	100.7 (11.7)	101. 3 (11.7)	100.1 (11.6)	0.4
Diastolic BP (mmHg)	69.3 (7.7)	70.3 (8.2)	68.5 (7.2)	0.07
Heart rate (bpm)	106.4 (12.2)	104.4 (11.2)	108.7 (12.8)	0.005
Cardiac output (L/min)	4.6 (0.8)	4.6 (0.8)	4.5 (0.8)	0.2
Stroke volume (mL)	43.6 (7.9)	45.0 (7.4)	42.1 (8.2)	0.004
SVR (dyn.s/cm^5)	1492 (225)	1486 (233)	1499 (218)	0.7
*Post-stress cortisol concentrations (ng/mL)**[$]				
0-min	9.0 (5.9,14.1)	9.2 (5.9,14.5)	8.8 (5.9,14.0)	0.6
10-min	12.2 (7.9,18.9)	12. 0 (7.9,19.6)	12.4 (8.2,18.5)	0.6
20-min	12.9 (8.3,20.7)	13.4 (8.2,21.2)	12.5 (8.7,20.7)	0.7
30-min	11.9 (8.1,18.8)	11.9 (7.7,18.7)	12.0 (8.1,19.2)	0.6
60-min	8.7 (6.2,12.3)	9.1 (6.4,12.9)	8.3 (6.1,12.0)	0.5
TSST-C cardiovascular parameters-story[$]				
Systolic BP (mmHg)	125.0 (15.9)	123.4 (16.5)	126.7 (15.2)	0.1
Diastolic BP (mmHg)	85.9 (9.7)	85.8 (10.2)	86.1 (9.2)	0.8
Heart rate (bpm)	109.8 (14.3)	104.7 (11.5)	115.0 (15.1)	<0.001
Cardiac output (L/min)	5.2 (0.9)	5.0 (0.9)	5.4 (0.9)	<0.001
Stroke volume (mL)	47.8 (8.4)	47.8 (8.1)	47.7 (8.8)	0.9
SVR (dyn.s/cm^5)	1748 (393)	1829 (426)	1666 (338)	0.001
TSST-C cardiovascular parameters-Maths[$]				
Systolic BP (mmHg)	124.8 (16.0)	124.0 (16.5)	125.6 (15.5)	0.4
Diastolic BP (mmHg)	85.7 (10.4)	86.2 (10.8)	85.2 (10.0)	0.5
Heart rate (bpm)	112.3 (14.5)	108.0 (12.1)	116.8 (15.5)	<0.001
Cardiac output (L/min)	5.3 (0.9)	5.1 (0.9)	5.4 (0.9)	0.004
Stroke volume (mL)	47.5 (8.6)	47.6 (8.1)	47.3 (9.0)	0.8
SVR (dyn.s/cm^5)	1682 (359)	1760 (402)	1604 (290)	0.001

*Values given are mean (SD) or *geometric mean (IQR); SVR: Systemic Vascular Resistance; [#]P value for the difference between boys and girls using independent t-tests; [$]P<0.001 for differences between baseline and post-test values in all children using paired-t tests. ** n= 266 for pubertal stage, and baseline and past-stress cortisol concentrations; N=249 for all TSST – C parameters.*

to a –20^0 C freezer. The children remained standing for 10 minutes after the TSST-C and during post-test video-viewing to make the conditions uniform with the test period.

All participants returned the next day for detailed cardiometabolic investigations, including blood sampling. The pubertal status was assessed using Tanner's method [10], and was classified as the stage of breast development (girls) or genital development (boys). The socio-economic status (SES) of the family was determined using the Standard of Living Index designed by the National Family Health Survey-2 [11].

The HMH ethics committee approved the study; informed written consent from parents and assent from children were obtained.

Cortisol assay: Salivary samples were thawed and centrifuged at the end of the study. The supernatant

liquid was stored at -20^0 C before sending it for analysis at KEM Hospital Research Centre, Pune, on dry ice. The samples were thawed and centrifuged again and the supernatant was transferred to new vials in Pune before assaying. Cortisol concentrations were measured using an ELISA method (Alpco Diagnostics, Salem, NH) as per the manufacturer's instructions. All samples from a child were analyzed in the same batch. Standard curves were established for each run, based on the calibrators provided by the manufacturer (range:1-100 ng/mL). High and low controls were included with each run to ensure quality control. The assay sensitivity was 1 ng/mL; inter- and intra-assay coefficients of variation were 10% and 6.6%, respectively.

Statistical methods: Salivary cortisol concentrations were log-normalized for analyses. The cortisol stress-response was calculated by subtracting the pre-test value from the post-stress values. The cardiovascular stress-response was calculated as the difference between the pre-test and the TSST-C averages. Differences between boys and girls in cortisol and cardiovascular parameters was analyzed using independent t-tests. Paired t-tests were used to analyze the difference between baseline and the post-stressor values.

RESULTS

Two children refused to perform in front of the judges and the test was stopped in two other children as they were upset; the TSST-C was completed in 269 children. Adequate pre- and post-test salivary samples were available for 266 children and complete cardiovascular responses were available in 249 children. None of the participants reported negative after-effects of stress, and all returned for blood sampling the next day.

In general, girls were heavier than boys, and had higher heart rate, while boys had greater stroke volume at baseline (***Table* I**). There was no difference in baseline cortisol concentrations between boys and girls.

Cortisol concentrations increased consistently after inducing stress in all, except in 13 children in whom the concentrations decreased (***Fig.* 1**). The mean (SD) increment from baseline was statistically significant [6.1 (6.9) ng/mL; ***Table* I**]. Cortisol responses were similar in boys and girls (P=0.5). More advanced puberty was associated with lower responses in girls (P=0.04), but not in boys (P=0.8). Girls who had attained menarche had significantly lower cortisol response than pre-menarchal girls (5.6 *vs* 9.9 ng/mL, P=0.02).

Mean values for cardiovascular parameters increased significantly from baseline during story and mental arithmetic tasks (***Table* I**). The responses were greater in girls than boys for systolic BP, heart rate, cardiac output and stroke volume and less for SVR. In both sexes, more advanced puberty was associated with lower heart rate and cardiac output during TSST-C (P<0.05). The menarchal status in girls was not associated with cardiovascular responses.

There was no association between SES and cortisol and cardiac responses to stress. The stress-responses were similar in obese/overweight children and those with normal BMI.

DISCUSSION

This study, conducted to test the effectiveness of using a well-known European stress test in Indian adolescents, showed that both endocrine and cardiovascular stress responses of a similar magnitude to those seen in other populations can be stimulated in Indian conditions. There were no residual negative psychological effects of the stressor.

A major limitation was that our study was conducted only in urban children. As the cognitive performance was better in our urban children cohort than rural children [12], their orientation towards a stressful situation may also have differed. Thus, our findings may not be applicable to the rural population. Another limitation was that we did not know about any background stresses in the children's lives, which may have influenced their stress-responses.

This is the first time that the TSST-C has been used to study stress-responses in India. The children tolerated the test well and all, including those who did not complete the test, returned the next day for an invasive investigative procedure. This suggests minimal or no residual effect of their stressful experience. In common with other studies [7,13], we modified the TSST-C protocol to suit our population, in which it also elicited strong stress-responses. These were highly variable suggesting that the test could be used to identify children vulnerable to the effects of stress. Our findings in relation to gender and pubertal status, especially in girls, are consistent with earlier studies [14,15].

A number of biological and environmental factors determine individual variations in stress-reactivity [3]. A test that identifies individual differences in physiological stress-responses, particularly HPAA response, is a vital requirement for research aimed to study stress physiology [8]. The TSST-C, in which a combination of public speaking and mental arithmetic tasks maximises participant motivation by increased uncontrollability (eg. forced to make repeated errors) and social evaluative threat, has been shown to stimulate reliable

WHAT IS ALREADY KNOWN?

- TSST-C is a valid test for eliciting cortisol responses to stress in European children.

WHAT THIS STUDY ADDS?

- TSST-C is a useful test to examine cortisol and cardiovascular stress-responses in Indian children.

cortisol response in children and adolescents [7,8,13]. Though other modules such as isolated public speaking tasks, situations that trigger negative emotions and threat of social separation/rejection, exposure to novel situations and induction of mild physical pain also trigger cortisol responses in adolescents, the TSST-C produces them more consistently [7]. Our experience suggests that it is very crucial that the protocol is followed exactly, and that the 'judges' are trained and monitored during the study to ensure that they remain impassive and do not give in to the normal human desire to encourage or reassure the children.

We conclude that a modified TSST-C is a useful test to examine stress-responses in Indian adolescents. This method can be used effectively to establish the links between stress-responsiveness and markers of disease development in Indian children.

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