

CORTISOL IN SALIVA – A MARKER FOR INCREASED ANXIETY IN CHILDREN

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ABSTRACT

Objectives: Cortisol, called also "stress hormone" participates in organism's response to stress situations and enters into complex interactions with the hormonal and immune system of a man. The purpose of the study is to follow the cortisol level in saliva as dependent on the anxiety rate in children.

Methods: A screening investigation of 170 children was carried out to evaluate anxiety rate with the help of the revised children's manifest anxiety scale. 71 children (41 with high and 30 with low anxiety rate) were selected to measure the cortisol in the saliva by ELISA method with Saliva Cortisol Enzyme Immunoassay Kit, product of Salimetrics, USA.

Results: The results show a dependence between the subjective psychological test for anxiety evaluation in infant age and the cortisol in saliva. Saliva is used as a non-invasive diagnostic indicator for the changes in cortisol levels.

Conclusion: Cortisol is an objective oral biomarker that can be used for evaluation of risky oral environment and its connection to the general psychological status of children.

Key World: Cortisol, stress hormone, anxiety rate, saliva, ELISA, psychological test

INTRODUCTION:

Oral based diagnostics" is a scientific field developed very actively in recent years. (1,2). Cortisol, known as a stress hormone, participates in the response of the body to stress conditions and enters into complex interactions with the hormonal and immune system of a man. It participates in the most complex hormonal system, known as hypothalamic-pituitary-adrenal axis (3, 4, 5).

Stress is defined as a nonspecific reaction of the body to any request for adjustment or adaptation, performed in a stereotyped manner on the base of identical biochemical changes.(6, 7). Anxiety is one of the many symptoms of the reaction of men to stress situations.(8).

Homeostasis in oral environment is the most important

condition for maintaining oral health. Imbalance that can occur with chronic stress on all mentioned above levels affects the oral environment.(9, 10).

Evidence is available that cortisol exhibits higher levels in abused children, children raised outside family environment, predisposed to obesity, diabetes and etc.(11, 12). Given the immature psychology of children, the methods used for evaluation of anxiety in childhood are subjective and very suggestive. The combination of these tests with the study of an objective parameter as salivary cortisol will provide more precise information about the stress influence on oral environment and somatic health of children.

Purpose: *By examining cortisol in saliva, to study the relationship between that hormone and the degree of anxiety and somatization in children.*

MATERIALS AND METHODS:

Purpose of the Study

170 children aged between 11 and 14 (86 boys and 84 girls), selected randomly from two secondary schools in Ruse were evaluated by a psychological test in two parts: a RCMAS-test and a test for somatization (13). Children filled out scales (questionnaires). The raw score of the test was converted into T-standard evaluation (X50; SD10) by linear transformation of the data. The results ranged from 32 to 77 points. All 170 children were selected into two groups:

- „low anxiety” - with standard T-evaluation”45 (N=30);

- „high anxiety” - with standard T-evaluation”56 (N=41).

Tools

(1) RCMAS (*Revised Children's Manifest Anxiety Scale* - Reynolds & Richmond 1978) is among the most popular self-descriptive methods for evaluating of child anxiety(14). The Bulgarian version of RCMAS (Kalchev, 2006) was used. The test consists of questions divided into 4 groups, evaluating: anxiety, social concerns, physiological reactions, nervousness (tension) (13).

(2) *Scale for somatization* - BASC (*Behavior*

Assessment System for Children- Reynolds & Kamphaus 1992). Somatization is defined as a tendency to experience and distress in response to psycho-social stress and seek of medical help for this. The scale consists of 8 questions, evaluating sensibility and frequent complaints from relatively minor somatic problems, especially psychic difficulties (13).

Samples of saliva

A sample from morning saliva on empty stomach at least half an hour after teeth washing and 10 minutes after washing with distilled water was taken for the study of cortisol. Samples were frozen (- 20°C).

Quantitative determination of cortisol in saliva.

ELISA with „Salivary cortisol KIT” of Salimetrics_{LLC} - USA was used to quantify the cortisol in saliva (15).

Statistical methods. Statistical processing was made by SPSS program, version 16 (SPSS Inc. Chicago USA). $P < 0.05$ was accepted as importance level of zero hypothesis.

RESULTS:

1. Distribution of studied children with high and low anxiety by gender and age. The distribution by gender and age of children is shown in table 1.

Table 1. Distribution by gender and age of children with high and low anxiety

Parameters		Children with low anxiety	Children with high anxiety
		N 30	N 41
Gender	♂	17 (56,66%)	20 (48,8%)
	♀	13 (43, 33%)	21(51, 2%)
Age		12,34±1,11 years (11-14 years)	12,26±1,21 years (10-14 years)

The results show that children of both groups are evenly distributed by gender and do not differ by age, which is a key factor for the objectivity in comparing both groups with other indicators.

2. Evaluation of cortisol in saliva in studied children with different degree of anxiety

2.1. The quantities of cortisol in saliva in the studied children, distributed by gender are shown in the following table.

Table 2. Distribution by gender the average values of salivary cortisol (µg./dl) in studied children

Children	n	mean±SD	t p
Boys ¹	37	0,102±0,007	t _{1,2} = - 0,792 p=0,431
Girls ²	34	0,112±0,011	
Total	71	0,107± 0,551	

The average amount of salivary cortisol in the studied children is 0,107±0,551 µg/dl, and it did not differ significantly between boys and girls.

When comparing the average values of salivary cortisol with similar studies it is seen that the results are close to the values quoted by the manufacturer of the test for salivary cortisol.(Salimetrics_{LLC}). The study of salivary cortisol on 285 children from 8 to 11 years shows the values ranged from 0,084 - 0,839 µg/dl, and on 403 children from 11 to 18 years the values varied between 0,021- 0,883 µg/dl. (15). Our results are 0,016 - 0,365 µg/dl salivary cortisol in 71 studied children.

2.2. Quantity of salivary cortisol according to the degree of anxiety. The results are shown in Table. 3:

Table 3. Average values of salivary cortisol in children with low and high anxiety

Children	N 71	Cortisol µg./dl	t p
With high anxiety ¹	30	0,095±0,049	t _{1,2} =1,961 (P<0,05)
With low anxiety ²	41	0,122±0,062	

The comparison of cortisol levels in children with high and low anxiety tends to be with higher average values in the second group.

2.3. Correlation of indicators of anxiety and somatization and the level of cortisol in saliva. The level of cortisol was defined as a dependent variable. The data were analyzed using multiple regression analysis in two versions:

- as independent variables (predictors) a general indicator of anxiety (RCMAS) and somatization was introduced;

- the level of cortisol regressed over the components of RCMAS and somatization.

Step algorithm (stepwise method) was used for analysis with probability error of 0,05. Single correlations (r of Pearson) of the indicators of anxiety and somatization and the level of cortisol were calculated. The results show differences supported by relativity $p < 0.05$.

Table 4. Correlation of indicators of anxiety and somatization and the level of cortisol in saliva

(N=71)	Level of cortisol Coefficient of correlation
GENERAL INDICATOR OF ANXIETY (RCMAS)	r = 0,208
Anxiety	r = 0,077
Social concerns	r = 0,278*
Physiological reactivity	r = 0,288*
Nervousness	r = -0,013
SOMATIZATION	r = 0,255*

According to the results obtained in the **first model** and after accounting the relationship between the general indicator of anxiety and somatization, the effect of somatization on cortisol with scandalized regression coefficient Beta of 0,255 ($p < 0,05$) and 5,1% explained dispersion (adjusted value $R^2 = 0,051$) proved to be significant.

In the **second model** (in which the components of RCMAS were added instead of the general indicator) a significant effect is seen only on *Physiological reactivity* on cortisol (Beta=0,288 ($p < 0,05$) and 7,0% explained dispersion; (adjusted value $R^2 = 0,051$). *Social concerns*, although with coefficient of correlation close to that of the physiological reactivity, showed lack of significance as predictors ($p > 0,05$).

In both models a significant predictor is *somatization* (when the relationship with the general anxiety is accounted) or *physiological reactivity* (when the relationships of other components of RCMAS and somatization are accounted).

DISCUSSION

The study of cortisol in saliva is made for the first time in our country. The average values obtained in healthy children are close to the values presented in other similar

studies (15). The results of multi-regression analysis indicate the dependency of cortisol on somatization and physiological reactivity. In evaluating of such a result, the fundamentally different approach for evaluation based on self-description in the first case and physiological reactions in the second should be taken into consideration.

The observed increase of cortisol in saliva of „high anxiety” children and the additional analysis of the dependency of the hormone on the physiological reactivity and somatization proved the possibility of salivary cortisol to be used as an objective indicator of anxiety in children.

CONCLUSIONS:

1. The cortisol of saliva in healthy children with high and low anxiety is within the limits ($0,107 \pm 0,551 \mu\text{g./dl}$), and there is a tendency for higher values in children with high anxiety and dependency of cortisol on somatization and physiological reactivity.

2. The cortisol in saliva is an indicator of the reliability of the Bulgarian version of the scale showing children's anxiety (RCMAS) and somatization, especially for the evaluation of physiological reactions of children under stress.

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REFERENCES:

1. Tabak LA. A revolution in biomedical assessment: the development of salivary diagnostics. *J Dent Educ.* 2001 Dec;65(12):1335-9.
2. Malamud D, Tabak LA, Saliva as a diagnostic fluid. *Ann NY Acad Sciences* 1993; 1-694.
3. Gozansky, W.S., Lynn, J.S., Laudenslager, M.L., and Kohrt, W.M. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. *Clin Endocrinol (Oxf)*, 2005, 63,3, 336-41.
4. Schwartz, E. B., Granger, D. A., Susman, E. J., Gunnar, M. R., Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development*, 1998, 69,6, 1503-1513.
5. Johnson, L.R., ed. *Essential Medical Physiology*, 3rd ed. San Diego, Elsevier Academic Press, 2003, pp. 499-502.
6. Hans Selie - Stress without distress, 1982 ed. Science and art, 135 pages
7. Cohen, S., R.C. Kessler and L.U. Gordon, *Measuring Stress: A Guide for Health and Social Scientists*, Oxford University Press: 1997; 13(1):p67
8. Ilana Eli - Oral Psychophysiology: Stress, Pain, and Behavior in Dental Care, 214 p;
9. Breivik T, Thrane PS, Murison R, Gjermo P. Emotional stress effects on immunity, gingivitis and periodontitis. *Eur J Oral Sci.* 1996 Aug;104(4 (Pt 1)):327-34.
10. Brewer-Smyth, K., Wolbert-Burgess, A., & Shults, J. Physical and sexual abuse, salivary cortisol, and neurologic correlates of violent criminal behavior in female prison inmates. *Biological Psychiatry*, 2004,55(1), 21-31.
11. Andrews RC, Herlihy O, Livingstone DE, Andrew R, Walker BR. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. *J Clin Endocrinol Metab.* 2002 Dec;87(12):5587-93.
12. Epel ES, McEwen B, Seeman T, et al. Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. *Psychosom Med.* 2000 Sep-Oct;62(5):623-32.
13. Kalchev p. Scale for anxiety in childhood and adolescence, 2006 ed. East West pages 258
14. Reynolds CR, Richmond BO. What I think and feel: a revised measure of children's manifest anxiety. *J Abnorm Child Psychol.* 1978 Jun;6(2):271-80.
15. Salimetrics/Products and Services [Internet] Available from: <http://www.salimetrics.com/>.

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