DETERMINATION OF SALIVARY CORTISOL IN HEALTHY CHILDREN AND ADOLESCENTS

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Abstract- Recognized as a reliable tool for assessing the hypothalamus-pituitary-adrenal (HPA) axis, measurement of salivary cortisol plays an important role in both the clinical and research settings. To establish a normative data, which forms the basis for the usage of this valuable parameter, we gathered 8:00 h saliva samples from 94 healthy individuals aged 6-14 years. Cortisol levels were measured by radioimmunoassay technique, using Orion Diagnostica's coated tube technology. Based on mean±2SD, we established a normal range for salivary cortisol concentrations in this age group: 1.69 - 12.81 nmol/L. Considering a confidence interval for upper and lower limits, there was an upper limit equal to 11.42 -14.29 nmol/L and a lower limit of 1.21 - 2.25 nmol/L. Regarding the results of this study, cortisol levels were age dependent, and although with a low correlation coefficient, there was a positive correlation between cortisol levels and weight and height. There was no correlation with BMI and no sex difference was found.

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Key words: Salivary cortisol, normal range, children, adolescents, hypothalamus-pituitary-adrenal axis

INTRODUCTION

Cortisol, the most important glucocorticoid, is synthesized by the adrenal cortex. In serum, over 90% of cortisol is bound to a carrier protein (Cortisol binding globulin or transcortin). Cortisol measurement is indicated in suspected over or under production of cortisol by the adrenal cortex and also in some psychiatric disorders (*e.g.*, mood disorders) (1-3).

In recent years the advantages of salivary cortisol measurement over plasma testing have been appreciated and it has been popularly used as a reliable alternative tool for investigations of hypothalamus-pituitary-adrenal (HPA) axis functions.

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It has been proved that salivary cortisol is directly correlated with plasma free cortisol and thus reflects the biologically active fraction of this hormone (1, 2, 4-7). It has been shown that under conditions of stimulation and suppression, the changes in plasma cortisol are accurately and immediately and in greater magnitude reflected in salivary cortisol (2, 4, 8-12). Thus salivary cortisol measurement is an appropriate parameter in dynamic endocrine tests.

Salivary cortisol exhibits a clear diurnal variation and circadian rhythmicity with a time course closely parallel to that of plasma cortisol (2,13-15). Moreover, cortisol concentration in saliva does not depend on salivary flow rate (2, 4, 8, 9, 16) or other variables such as cigarette smoking (17). While plasma free cortisol measurement through blood sampling is oppressive, annoying and stressful and therefore can alter HPA axis activity, and also requires advanced techniques that are not easily available, salivary cortisol sampling is a simple, non-

invasive, and stress-free procedure that can be performed at home without the need of paramedical staff (18,19). As going through changing environmental conditions (such as temperature, motion and growth of organisms) does not alter concentration of cortisol in the saliva samples, even mailing saliva samples for cortisol assay seems to be acceptable (5).

There are some points to be taken into account while performing saliva sampling to help ensure accurate results; appropriate controls should be taken when taking saliva samples as contamination of saliva with some substances, like formula and breast milk, can interfere with the results; samples should not be refrozen, as concentrations of cortisol is significantly lower in refrozen samples. Despite these facts, salivary cortisol determination could provide an easily accessible index of HPA function under circumstances where blood sampling may be ethically or technically difficult to accomplish (especially for studies on children or those requiring serial samplings).

Since the existence of normative data forms the basis for usage of this valuable parameter in clinic, as well as researches, we performed this study in healthy children and adolescents (6-14 years old) to determine 8:00 am salivary cortisol levels in normal subjects under normal circumstances. We also aimed to see how sex, age, weight, height, and body mass index (BMI) affect the cortisol levels.

MATERIALS AND METHODS

We recruited 100 normal subjects (58 female and 42 male, age range 6-14 yr) randomly from local schools in Tehran, Iran. A complete medical examination was performed for each candidate and only the ones with no apparent disease and no history of endocrine, nutritional or growth problems were included in our study. Subjects' history, physical examination, height and weight were all recorded by means of a standardized information sheath. Six subjects were excluded from the study because of either missing samples or data. A written consent was obtained from each subject and/or their parents.

Saliva samples were obtained at 8:00 am before meal. All participants were asked to wash their mouths properly before sampling. Stimulation of saliva flow was done, for those who had problem in giving sample, using uncoated, sugar-free chewing gum. Saliva samples (at least 2 ml) were collected in 10 ml plastic, screw cap tubes and upon arrival in the laboratory were centrifuged and stored frozen at -20°C until adequate number of samples were ready for analysis.

Salivary cortisol was measured by the SPEC TRIA cortisol test based on the widely used radioimmunoassay technique. The sensitivity of the method using saliva samples is 0.8 nmol/L. The specificity of the assay was defined by a 45.3% cross-reactivity of the antiserum (at the 50% binding level) with prednisolone, 0.2% with corticosterone and < 0.1% with cortisone and dexamethasone.

Statistical analysis was performed using SPSS (10.0) statistical software program. The relationship between salivary cortisol and sex, age, weight, height and BMI was assessed using *t* test, non-parametric Spearman's correlation and Pearson's correlation.

RESULTS

Figures 1 and 2 and table 1 show the distribution of the height and weight of the studied population.

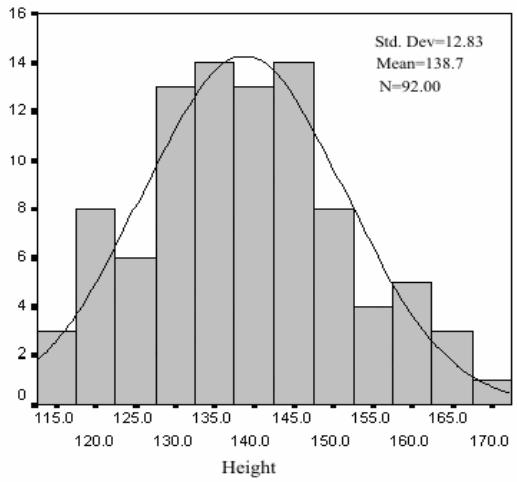

Fig. 1. Description of the height (cm) in studied population. Salivary cortisol levels were positively correlated with body height.

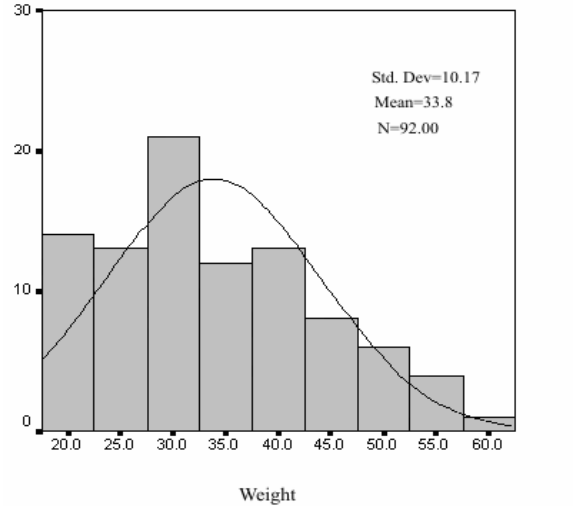

Fig. 2. Description of the weight (kg) in studied population. Salivary cortisol levels were positively correlated with weight.

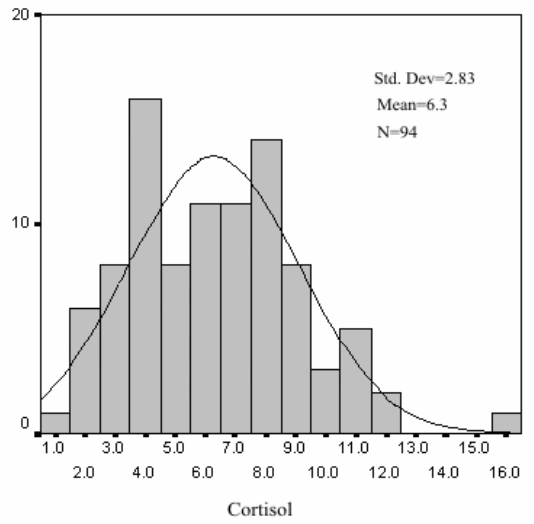

Fig. 3. Description of salivary cortisol levels in studied samples.

Salivary cortisol levels in healthy children and adolescents aged 6-14 yr

As previously described 8:00 am cortisol levels were measured in saliva from 94 healthy individuals aged 6-14 yr. Normal range for salivary cortisol concentrations in the studied subjects (based on mean±2SD) was 1.69-12.81 nmol/L.

Considering confidence interval for upper and lower limits, we achieved an upper limit of 11.42 - 14.29 nmol/L and a lower limit of 1.21- 2.25 nmol/L, which can be applied to the whole population. Figure 3 and table 1 show the distribution of salivary cortisol levels in studied samples.

Relationship between salivary cortisol levels and sex, age, weight, height and BMI

We found no significant sex difference in salivary cortisol levels (P= 0.59, t test). On the other hand, there was a significant positive correlation between age and salivary cortisol levels with a correlation coefficient of 0.25 (P= 0.013, nonparametric Spearman's correlation). Salivary cortisol levels at 8:00 am were also positively correlated with body weight (kg) and height (cm), with correlation coefficients equal to 0.24 (P= 0.022, Pearson's r correlation) and 0.29 (P= 0.004, Pearson's r correlation), respectively. It seems that there was no correlation with BMI (P= 0.27, Pearson's r correlation).

Table 1. Description of salivary cortisol levels, height and weight in studied population

Variable	Population No.	Mean	St Dev	Median	Min.	Max
Cortisol (nmol/L)	94	6.28	2.83	5.9	0.87	16.10
Height (cm)	92	138.7	12.83	138	114	170
Weight (kg)	92	33.8	10.17	32	18	61

Abbreviations: St Dev, standard deviation; Min, minimum; max, maximum.

DISCUSSION

It has not been long that salivary cortisol measurements have been proven to play a great role in determining how well the HPA axis functions. To appreciate the advantages of this precious index, we established normal range of salivary cortisol in the basal state in our study, which could establish diagnostic value of salivary cortisol measurements for the studied population.

Regarding the results of this study, salivary cortisol levels vary with age, weight, and height, but not with BMI throughout childhood and adolescence; although not with a very high correlation coefficient, the higher the weight and height one has, the higher morning salivary cortisol is measured, and there is also an increase in morning salivary cortisol levels as the subject ages (the older the child, the higher 8:00 am salivary cortisol levels). These results are in agreement with the results from Kiess *et al.* study (4). Unlike the result from the study conducted by Laudat *et al.* on adults (1), but like the result from Kiess *et al.* study on children and adolescents, there was no significant sex difference in salivary cortisol levels among the subjects of our study.

It should be noted that there is no claim that these results could assess all of factors influencing the salivary cortisol levels in the light of the limited number of healthy individuals participated in our study. Therefore, as salivary cortisol measurements could provide considerable advantages in assessing HPA axis functions and abnormalities, we suggest further studies on larger samples of the population to realize the use of this advantageous index as a routine paraclinical measure.

Acknowledgement

Dr. F. Mostafavi initially conceived the study and participated in its design and coordination. Dr. M.T. Haghi Ashtiani carried out the immunoassays. Dr. E. Safarzadeh participated in the sequence alignment and design of the study, gathered samples and data, performed the statistical analysis and drafted the manuscript.

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