

STUDYING THE VARIATIONS OF SALIVARY CORTISOL LEVELS IN SUBJECTS EXPOSED TO STRESS

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ABSTRACT. In this paper work are presented the results of an experimental study, realized on a batch of subjects representative for the magistrates in Constanta County, including 47 judges and 33 prosecutors, 38 men and 42 women, with ages between 20-60 years old and seniority in magistracy work of 1-25 years. During this study were measured the level of salivary cortisol, the blood pressure, the heart rate and were made ECG and Holter recordings. The study provides the variation of the salivary cortisol. The samples were collected early and late working hours, while the trial judge conducted specific activities in the courtroom, and the prosecutor had conducted hearings of persons in a file. This study investigated the role of work stress in generating these patterns, analysing cortisol output in relation to job demands and job control. The high level of determined parameters are explained by effects of stress reaction in neuropsychological activities with special request. There were analyzed the types and the frequency of stressors and the ways of preventing and fighting against them.

Keywords: salivary cortisol, professional stress, stress factors, stress related manifestations, magistrates.

INTRODUCTION

In modern society, stress is one of the major factors influencing many diseases. Numerous epidemiological and experimental studies have associated stress with the development and course of viral infections (Cohen et al.,1991), gastric ulcers (Das et al.,1993), cardiovascular diseases (Malyszko et al.,1994), cancers (Chen et al.,1995, Geyer ,1996) and others diseases (Chrousos et al.,1995). The hypothalamic-pituitary-adrenal (HPA) axis is vital in the initiation of the stress response (Stratakis et al.,1995), but the molecular link between stress hormones and long-term consequences of chronic stress are still mostly unknown. Monitoring stress-related gene expression is one of the most recent combinations of morphologic and quantitative approaches to stress evaluation. Selye's last main contribution to the stress concept (Szabo et al.,1985) was the recognition that, despite our different psychologic and cerebral reactions, both negative and positive stressors (distress and eustress) elicit virtually identical corticoid (catecholamine) responses. Certain recent experiments suggest that, depending on the conditions, ACTH may cause a predominant secretion of one or the other type of corticoid. However, be this as it may, the "growth hormone", or somatotrophic hormone (STH), of the pituitary increase the inflammatory potential of connective tissue very much as the proinflammatory corticoids do; hence, it can sensitize the target area to the actions of proinflammatory corticoids. Among the derailments of the general adaptation syndrome (Selye,1946) that may cause disease, the following are particularly important: (t) an absolute excess or deficiency in the amount of adaptive

hormones (for example, corticoids, ACTH, and STH) produced during stress; (tt) an absolute excess or deficiency in the amount of adaptive hormones retained (or "fixed") by their peripheral target organs during stress; (ttt) a disproportion in the relative secretion (or fixation) during stress of various antagonistic adaptive hormones (for example, ACTH and antiprogesterone corticoids, on the one hand, and STH and proinflammatory corticoids, on the other hand); (tttt) the production by stress of metabolic derangements, which abnormally alter the target organ's response to adaptive hormones (through the phenomenon of "conditioning"); and (ttttt) finally, we must not forget that, although the hypothalamic-pituitary-adrenal mechanism plays a prominent role in the general-adaptation syndrome, other organs that participate in the latter (for example, nervous system, liver, and kidney) may also respond abnormally and become the cause of disease during adaptation to stress. Researches proved that the magistrates belong to one of the most exposed and vulnerable to occupational stress professional category, with negative effects on their work ability and health status (Kirby,1995, Mack et al.,2004, Roach Anleu et al.,2005). In countries such as New Zealand, judges are being included in the high-risk professionals category, likely to become addicted to alcohol and drugs (Kirby,1995). Since 1980, the attention, concern and studies of the researchers, psychologists and doctors on the stress of the magistrates have had seen a steady growth, aiming at developing projects and training programs and risk management of distress and health impairment in this occupational category (Cooper et al.,1978, Derevenco et al.,1992, Kirby,1995, Mack et al.,2004, Roach Anleu

et al.,2005). Studies on the causes, characteristics and management solutions of stress in the magistrates work have remained relatively low compared to the importance of the phenomenon (Brațe,2002, Wisner,2006, Site). Practical implementation of the proposed actions was limited in scope of countries with high living standard as Australia, USA, Canada, England etc. In Romania, the drafts of study and stress management of the magistrates are neglected.

MATERIALS AND METHODS

There was considered for study a sample of 80 magistrates, representative for Constanta County, including 47 judges and 33 prosecutors, 38 men and 42 women aged 20-60 years and seniority in magistracy between 1-25 years, from 3 sections of activity, court, tribunal and court of appeal. Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. It is increased under stress situations, physical exercise and external administration of ACTH. Measurement of cortisol levels in general can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia and carcinoma. Most circulating cortisol is bound to cortisol binding globulin or transcortin and albumin. The free cortisol, which is considered the active part of blood, is about 1-2%. In the absence of appreciable amounts of the cortisol binding proteins in saliva, salivary cortisol is considered to be free and shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night. The determination of salivary cortisol levels were made using the ELISA method (Enzyme linked

immunosorbent Assay). Random male and female samples were taken in the early morning and had an absolute range of: 5 - 21.6 ng/ml. The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in calibrators, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of cortisol in the sample. A set of calibrators is used to plot a calibration curve from which the amount of cortisol in patient samples and controls can be directly read. To process this analyze is necessary approximately 1 ml of saliva per duplicate determination. We have collected 4-5 ml of saliva into a clean glass tube without force or inducement and before eating, drinking or brushing the teeth. The subjects simply rinsed their mouth with water before collection. It's recomandated not to use blood-contaminated specimens. After, the samples were stored at 4°C for up to 24 hours. We have considered all human specimens as possible biohazardous materials and we have taken appropriate precautions when handling. We placed the specimen tubes into a freezer and allowed to freeze. When ready to use, the specimens have been thawed and centrifuged. We collected the supernatants and we poured into freshly labelled tubes.

The following compounds were tested for cross-reactivity with the Direct Cortisol Saliva ELISA kit with cortisol cross-reacting at 100%.

Steroid	% Cross Reactivity
Cortisol	100
Prednisolone	13.6
Corticosterone	7.6
Deoxycorticosterone	7.2
Progesterone	7.2
Cortisone	6.2
Deoxycortisol	5.6
Pednisone	5.6
Dexamethasone	1.6

RESULTS AND DISCUSSIONS

Following the survey regarding stress factors at the magistrates from the circumscription of Constanta Court of Appeal, it has been derived that there are many stress factors with negative effects over labour capacity and state of health. Among the professional stress factors the most encountered in the activity of prosecutors and judges are the increasing number of files to settle under the conditions in which the number

of prosecutors and judges has remained constant or has decreased following the retirements or the departures from the system. Furthermore, other Professional stress factors are increasing complexity of the cases had for settlement by prosecutors and judges under the framework of improving the methods and means used by the persons in dispute with the law; the performance of some activities in an extended schedule and/or outside the normal working hours (criminal

investigations performed by prosecutors at various events specific to the activity and trial sessions performed way after the working hours or outside the working hours, sometimes even during night hours); the permanent contact with paper from the files for settlement, filers, stored most of the times due to lack of space in insalubrious warehouses, etc. The aim of

this study was to identify the existency of correlations between the variations of salivary cortisol levels and the exposure to stressful factors at work. It is a randomized study which included 47 judges and 33 prosecutors, 38 men and 42 women aged 20-60 years and seniority in magistracy between 1-25 years, from 3 sections of activity, court, tribunal and court of appeal.

Tabel 1

Years	Total		Men		Women	
	Number	%	Number	%	Number	%
21-30	30	17	10	33	20	67
31-40	82	47	34	41	48	59
41-50	26	15	11	42	15	58
51-60	38	22	15	39	23	61
Total	176	100	70	40	106	60

Tabel 2

Seniority(years)	Total length of service		Magistracy		Current position	
	Number	%	Number	%	Number	%
Total	176	100	176	100	176	100
<5	26	15	48	28	69	39
6-10	39	22	46	26	45	25
11-15	43	24	40	23	31	18
16-20	19	11	22	12	19	11
>20	49	28	20	11	12	7

In Tables 1 and 2 it is outlined the sample structure by gender, age groups and seniority in the magistracy and the current position. During this study were monitored the following variables: heart rate, systolic blood pressure and the level of salivary cortisol. Early morning before starting the working hours, were obtained basic heart rate's levels, systolic blood pressure and salivary cortisol. These values were considered reference points for all variables. During the working day the heart rate and the systolic blood pressure were monitored every 60 minutes using ambulatory methods and the ranges for cortisol in saliva were established after the trial judge conducted specific activities in the courtroom, and the prosecutor had conducted hearings of persons in a file.

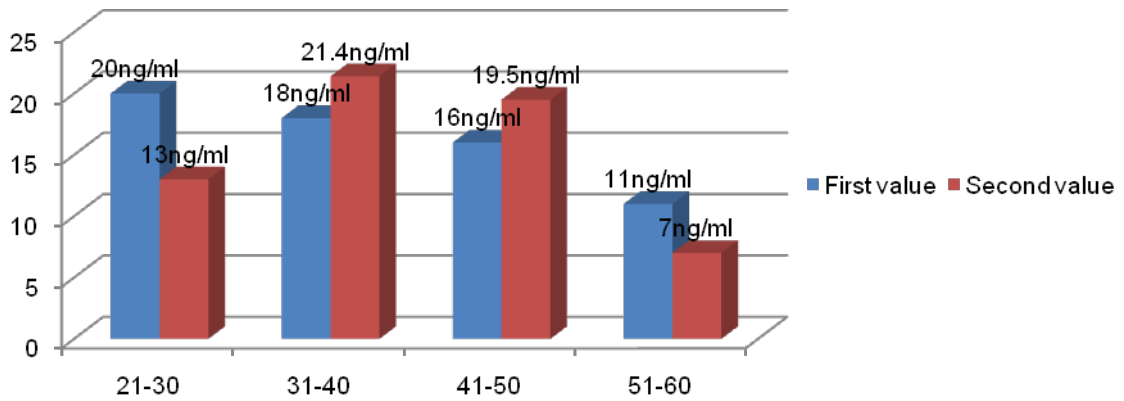
There are some differences between the age groups concerning the variation of salivary cortisol levels with more than 1 ng/ml between two successive measurements during activity or increasing the concentration of salivary cortisol by more than 15% from expected values for a certain time of day. There were no significant differences between two successive determinations of salivary cortisol levels for the extreme age groups, between 21-30 years and 51-60 years. This observation could call into question the relationship of the age with the adjustment and work experience as magistrate. The types and frequencies of the stressors vary depending on the position and work seniority as magistrate. With reference to all the magistrates, the level of salivary cortisol did not consistent increased or decreased in frequency with the age increasing.

Between the sections there are some differences concerning the variation of salivary cortisol levels, with doubtful statistical significance, but possible: higher variations of salivary cortisol levels are at tribunal compared to court.

Compared with the prosecutors, the judges have felt the existence of extraprofessional stress situations, with a significantly higher variation of salivary cortisol levels. The groups differ in their overall cortisol excretion and in cortisol reactivity to acute daily stressors. Compared to judges, prosecutors had a diurnal pattern of cortisol excretion with relatively lower morning and higher levels after specific activities like hearings of persons in a file. Subject's daily activities and their locations were associated with diurnal rhythm differences. For the group of senior judges with management positions compared to the rest of the judges, is being reported higher variation of salivary cortisol levels. For the prosecutors, are not being found statistically significant differences in the frequency of salivary cortisol ranges between the officials and those without management positions.

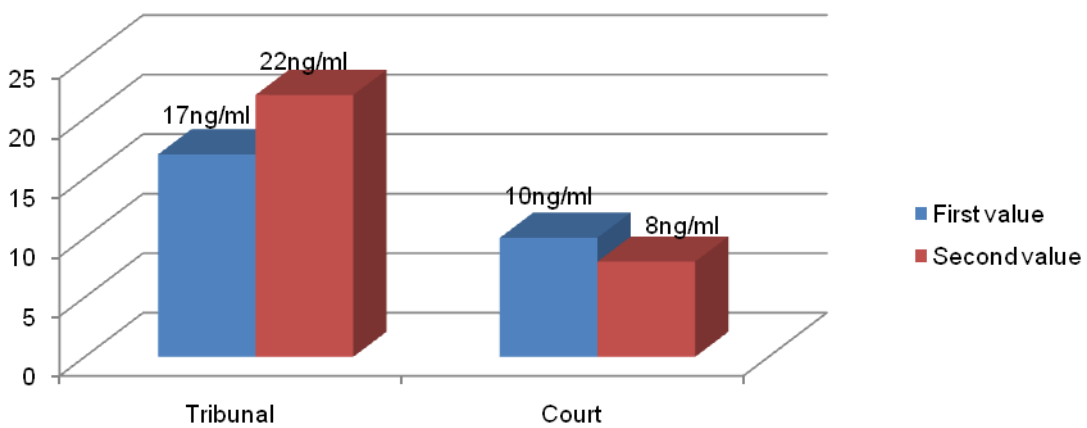
We observed that work stress is related differently to cortisol responses for men and women. We have found that the variations of salivary cortisol levels over the working day are more relevant for women than for men. On the work day, women showed larger increases than men. Cortisol output over the early part of the day may be particularly sensitive to the influence of chronic stress and its anticipation, especially in women. For the men, high degrees of neuropsychological stress, effort reward imbalance and over-commitment were

not significantly associated with higher levels of cortisol.



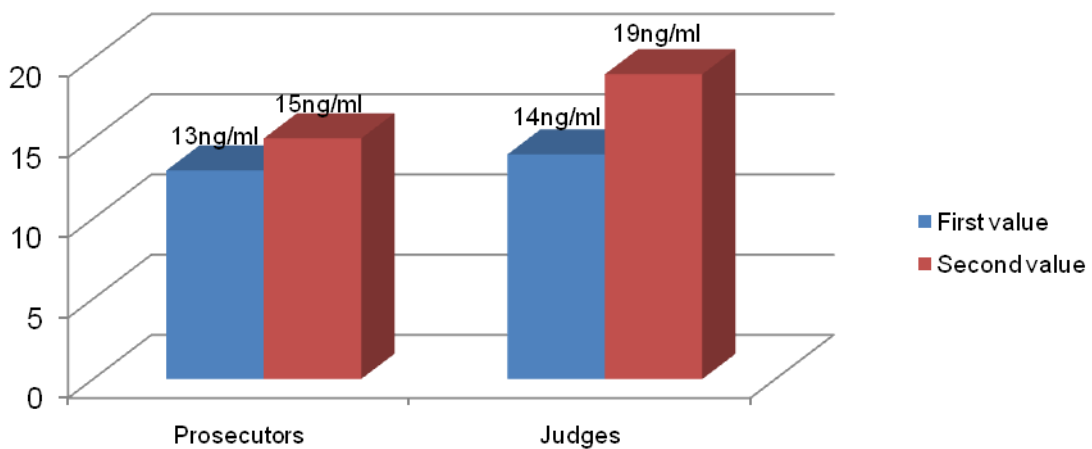
Variations of salivary cortisol regarding groups of age

Fig. 1 Variations of salivary cortisol levels regarding age groups



Variations of salivary cortisol regarding the sections

Fig. 2 Variations of salivary cortisol levels regarding the sections



Variations of salivary cortisol per categories of magistrates

Fig. 3 Variations of salivary cortisol levels per categories of magistrates

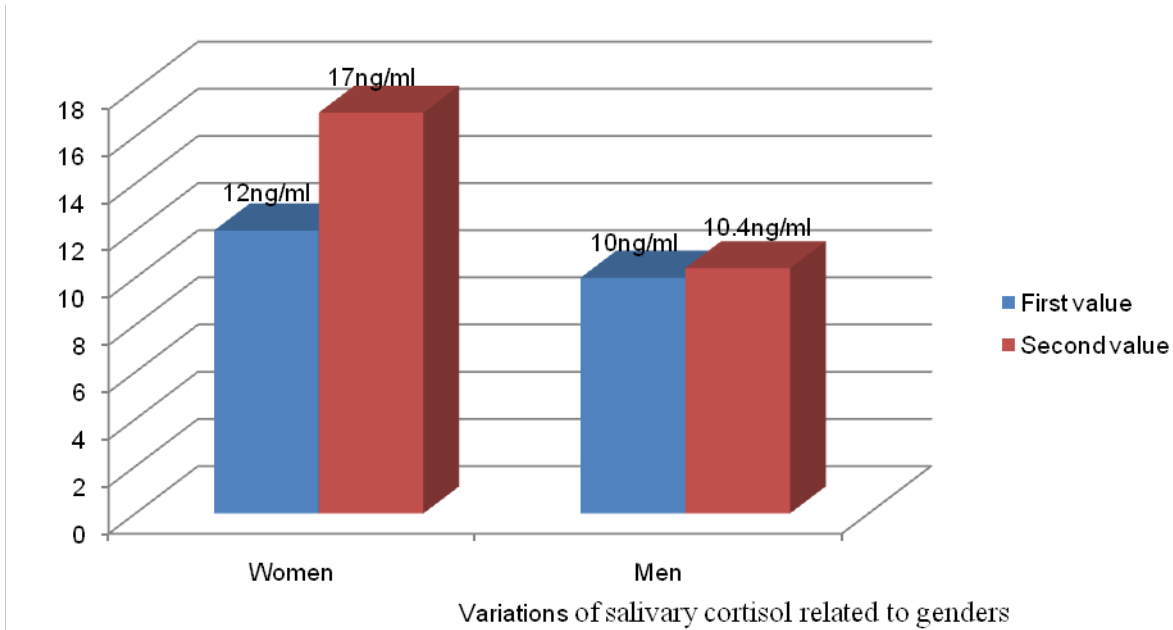


Fig. 4 Variation of salivary cortisol levels related to genders

CONCLUSIONS

Chronic stress causes multiple biochemical and physiological changes in the human organism. The present paper provides an up-to-date overview of recent methodological developments, novel applications as well as a discussion of possible future applications of salivary cortisol determination. Neuropsychological factors are of significance to the level of salivary cortisol. The study emphasises the benefits of taking physiological measurements of stress in labour medicine studies. We notice a relation of direct proportionality between the higher levels of salivary cortisol and the existence of stress factors at work. An interesting fact over which our survey draws attention to is the greater frequency and variability of manifestations at judges than at prosecutors, being correlated to the stress factors evaluated in a previous survey. Salivary cortisol levels are correlated with stress reactions in activities neuropsychological with special request. Studying levels of salivary cortisol could be used as a prognostic tool but also as a guiding tool of the evaluation of the various stress related manifestations which is useful for the individual and collective medical care. In conclusion, the satisfactory precision of the analysis and the simple non-invasive sampling procedure suggest that saliva may be used for cortisol measurements in situations where blood sampling is difficult to perform.

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