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Salivary cortisol, dehydroepiandrosterone-sulphate (DHEA-S) and testosterone in women with chronic migraine

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Abstract Hypothalamus-pituitary-adrenal (HPA) axis activity was monitored in 20 women with chronic migraine (CM), previously affected by medication overuse headache (MOH), in comparison to healthy women (20 subjects) by measuring salivary cortisol, testosterone, dehydroepiandrosterone-sulphate (DHEA-S) levels, and their ratios, one week after the end of the MOH rehabilitation procedure. The participants were instructed how to collect saliva samples at home, a procedure that was performed twice a day (08:00 a.m. and 8:00 p.m.). Morning and evening levels of cortisol were significantly increased in CM patients with respect to controls. With regard to the cortisol/DHEA-S ratio, an inverse marker of psycho-physical well-

being, CM women showed significantly higher values than controls. Moreover, testosterone/cortisol ratios (anabolic/catabolic index of physical performance) were significantly lower in CM patients than in controls. In the present study, CM appears not to be associated with an impairment of cortisol and DHEA-S circadian fluctuation; however, CM patients present alterations in HPA axis function that might contribute to metabolic and psychological alterations that have also been associated with CM.

Key words Chronic migraine • Medication overuse headache • Salivary hormones • Cortisol/DHEA-S ratio • Testosterone/cortisol ratio

Introduction

Chronic migraine (CM) is the most disabling form of headache in the second edition of the International Classification of Headache Disorders (ICHD-II) [1]. The failure of its treatment is often due to a superimposed medication overuse headache (MOH), a new form of secondary headache [2, 3], which has been defined as an interaction between a medication used excessively and a susceptible patient [1].

Migraine is a disorder affecting a larger proportion of women than men [4] and neuroendocrine research has focused mainly on the relationship between CM and female sexual hormones levels [5, 6]. Although CM is a frustrating and stressful condition, little information is available on the adrenal hormones and their regulation during the disease.

Studies on neuroendocrine patterns are often limited to plasma samples. The current possibility to determine androgens and adrenocortical hormones (testosterone, cortisol and dehydroepiandrosterone-sulphate (DHEA-S)) in saliva

offers evident advantages in the sample collection, avoiding the “stress” of blood taking [7–10]. Therefore, this study investigates the salivary levels of cortisol, testosterone and DHEA-S in 20 CM women previously affected by MOH, in comparison with age and body mass index (BMI)-matched controls. Moreover, the ratios cortisol to DHEA-S and testosterone to cortisol were calculated, respectively, as general markers of well-being and fatigue [11, 12].

Subjects and methods

Patient population

We selected from 4536 outpatients admitted over a 12-month period (July 2004–June 2005) at the Regional Referral Headache Centre at S. Andrea University Hospital 58 women patients affected by CM and MOH.

CM diagnosis fulfilled the 2004 IHS criteria [1] and MOH the following revision of IHS criteria for 8.2 *Medication-overuse headache* [2].

The enrolled patients attended an in-hospital rehabilitation procedure ranging from five to ten days, to recover from MOH [3]. Salivary samples were collected at a time when the patients had been asymptomatic and had not taken any anti-migraine medication for at least 14 days. None of the selected patients presented a previous chronic or episodic tension headache history. None of these patients had received any immunosuppressive/corticosteroidal drug in the previous six months. The main haematological and haematochemical parameters of all subjects were within the normal range for our laboratory. Sixteen of the 36 eligible woman declined to give salivary samples. The remaining 20 women voluntarily agreed to participate in the study and gave their informed consent. The selected women, all non-smokers, were not consumers of any vasoactive drug that could influence cortisol secretion (i.e., anti-hypertensives, antidepressants, thyroid agents, etc.). Twenty female age-matched healthy women from the hospital staff were enrolled as the control group. We matched cases and controls for age and BMI. BMI was considered because it may affect the concentration of hormone-binding globulins and testosterone [5, 13].

All participants were fully informed concerning the procedure and gave their written consent. The study protocol was approved by our Institutional Ethic Board and informed written consent was obtained also from controls. The recommended principles of the Declaration of Helsinki, September 1989, were closely observed during this clinical research.

Subject somatic characteristics are summarised in Table 1.

Table 1 Somatic characteristics of the subjects. Data are shown as mean value \pm SEM

Group	Age (years)	BMI (kg/m ²)
Control (<i>n</i> =20)	48.7 \pm 3.3	23.2 \pm 1.0
Chronic migraine (<i>n</i> =20)	49.6 \pm 4.1	23.7 \pm 1.9

Experimental study design

In order to evaluate the diurnal fluctuation of cortisol and DHEA-S, the participants were instructed how to collect saliva samples at home. The samples of saliva were collected twice a day (8:00 a.m. i.e., within 1 h of awakening, and 8:00 p.m.) for two weeks after analgesic drug withdrawal. The selected patients, all triptan abusers, attended the rehabilitation procedure in the outpatient regime for 5 days. The treatment consisted in infusion therapy with tiapride, ketorolac, ademetionine sulphate, reduced glutathione, granisteron and ranitidine [14].

The salivary samples were kept in the home refrigerator until the participants' return to the laboratory.

Salivary sampling procedure

Samples of saliva were collected by the Salivette (Sarsted, Italy) sampling device, which allows quick and hygienic saliva recovery from a polyester wool swab by centrifugation at 3000 rpm for 5 min.

Measurement of cortisol, DHEA-S and testosterone

For each sample, duplicate measurement was performed on 50–100 μ l of saliva by means of commercial immunoenzymatic kits (Diametra, Italy) for direct salivary assay of cortisol, testosterone and DHEA-S. Inter-assay coefficient of variation was <10%, and intra-assay coefficient of variation <7%, with a minimum detectable concentration of cortisol of 0.5 ng/ml, of testosterone of 5 pg/ml and of DHEA-S of 25 pg/ml.

Statistical procedure

Analysis of variance (ANOVA) was applied, followed by a multiple comparison test: statistical significance was set at $p < 0.05$ [15].

Results

Diurnal fluctuation of salivary cortisol and DHEA-S levels

As shown in Figure 1, the CM group presents significantly higher levels of cortisol compared to controls, both in the morning and in the evening. All the subjects, CM and controls, present cortisol levels significantly higher in the morning compared to evening measures. Figure 2 presents data on DHEA-S. There are no statistically significant differences between CM and control subjects, whereas the physiological DHEA-S diurnal fluctuation was significantly present in both groups.

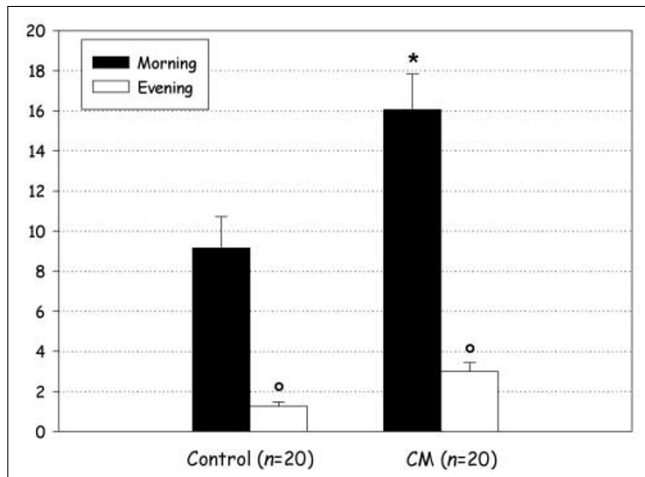


Fig. 1 Morning and evening salivary cortisol levels (mean value±SEM). Statistical analysis: ANOVA: F -ratio=4,67, p =0.0351; post-hoc Fisher's LSD Multiple-Comparison Test: * p <0.05 vs. morning control value; ^o p <0.01 vs. respective morning value

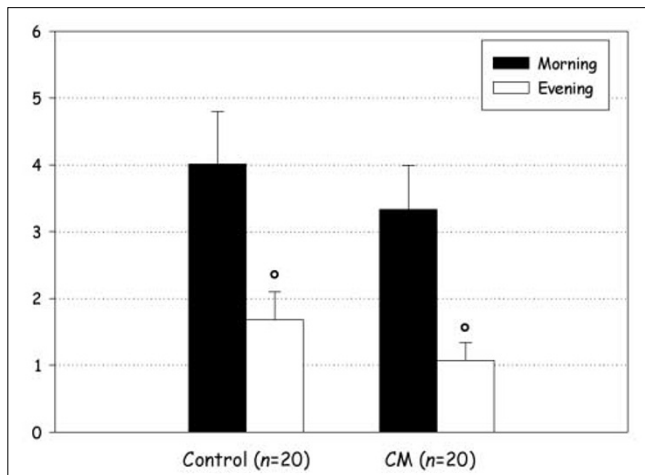


Fig. 2 Morning and evening salivary DHEA-S levels (mean value±SEM). Statistical analysis: ANOVA: F -ratio=0,00, p =0.9510; post-hoc Fisher's LSD Multiple-Comparison Test: ^o p <0.05 vs. respective morning value

Morning salivary testosterone levels

Salivary testosterone was measured in the morning samples only. No significant differences were recorded between the control group (46.89 ± 4.17 pg/ml) and the CM group (47.74 ± 4.96 pg/ml).

Cortisol/DHEA-S ratio

Data on cortisol/DHEA-S ratios, calculated from morning values, are reported in Figure 3 (upper part). A significant

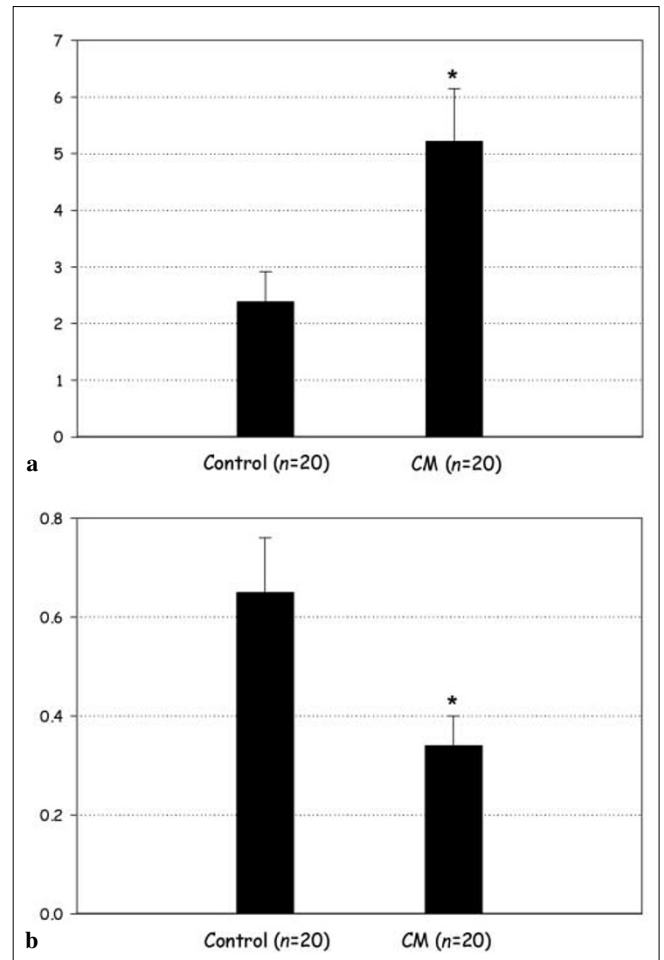


Fig. 3 Upper: cortisol/DHEA-S ratio (mean value±SEM). Statistical analysis: Student's t -test: $t=2,65$, $p=0.0067$; * p <0.01 vs. control. Lower: testosterone/cortisol ratio (mean value±SEM). Statistical analysis: Student's t -test: $t=2,41$, $p=0.0117$; * p <0.05 vs. control

increase in cortisol to DHEA-S ratios was found in CM women in comparison with controls.

Testosterone/cortisol ratio

As shown in Figure 3 (lower part), testosterone/cortisol ratios, calculated from morning values, were significantly lower in CM patients than in control subjects.

Discussion

Previous studies from our group and others showed that saliva allows an easy, stress-free means for monitoring

changes of adrenal function in physiological and pathological conditions [8–11, 16–18].

The cortisol, DHEA-S and testosterone levels, and accordingly, the cortisol/DHEA-S and testosterone/cortisol ratios, obtained from the saliva appear to be more reliable measurements because salivary levels reflect better than those in plasma the hormone serum fraction that is biologically active [11, 12].

A dysregulation of cortisol secretion pattern has been associated with different pathologies [16, 19]. This study reports that CM appears not to be associated with impairment of circadian variation of cortisol and DHEA-S: the physiological rhythm was maintained in all the subjects, with morning levels significantly higher than in the evening.

Levels of cortisol in saliva of CM patients were consistently higher as compared to healthy subjects. This aspect is of interest, as experimental data suggest that chronic exposure to high levels of corticosteroids can contribute to produce neurotoxic effects [19]. Several mechanisms have been considered to explain corticosteroid-induced neurotoxicity, including metabolic vulnerability of neurons due to oxygen radical generation and impairment of neuronal defences against neurologic insults [20]; in addition, cortisol hypersecretion is regarded as important in the pathophysiology of major depression [21] and has been associ-

ated with cognitive dysfunction [22] and also with loss of bone density [23]. A number of potential actions have been recently postulated for DHEA-S on cognition and well-being [7]. DHEA-S might moderate the action of glucocorticoids and protect neurons from the neurotoxic effects of glucocorticoids [23]. In this study CM patients had DHEA-S levels not significantly lower than controls; however the cortisol to DHEA-S ratio is significantly higher than controls. A higher cortisol to DHEA-S ratio was associated with lower cognitive performances and greater mood disturbances [7], suggesting that CM is associated with a negative index of well-being. Moreover, the lower testosterone to cortisol ratio measured in CM patients in comparison with controls confirms the presence of tiredness, a symptom frequently reported by the CM patients.

Further studies are required to elucidate the possible clinical relevance of HPA axis dysfunction in CM. However, it is clearly important that psychopathological and metabolic factors are controlled in future investigations on HPA axis function in CM patients, as migraine is often associated with different disorders, which are often highlighted as trigger factors of migraine attacks but may also appear as a reaction to migraine attacks.

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