SALIVARY CORTISOL AND METABOLIC SYNDROME COMPONENT’S ASSOCIATION

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ABSTRACT - Background: Actually the lifestyle exposes the population to several risk factors related to alimentary habits and less physical activity that contributes to chronic diseases appearance worldwide. Aim: To analyze the association between salivary cortisol and the components of metabolic syndrome. Methods: This is a cross-sectional study. As part of it, 28 individuals aged 30-59 years presenting three or more of the following findings: CA: ≥88 cm for women and ≥102 cm for men; SBP>130 mmHg and DBP>85 mmHg; GL>100 mg/dl; TG>150 mg/dl; HDL<40 mg/dl for men and <50 mg/dl for women. Was performed analysis of salivary cortisol (by radioimmunoassay) from 25 salivary samples collected throughout the day, for evaluating changes in the circadian rhythm of this hormone (8AM, noon and 8PM). Results: 28 evaluated individuals had a mean age of 51.9±7.5 years, mostly women (64.3%) and a mean of BMI 33.6±3.2 kg/m². The cortisol level from the 8AM averaged 18.7±4.8 ng/dl. Individuals with FPG>110mg/dl, have significantly lower average levels of cortisol than ones with FPG <110 (12.8±5.2 vs. 17.3±4.2). Significant correlations were HOMA vs. WC (r=0.446; p<0.005), WC vs. FG (r=0.446; p<0.005) and TG vs. HDL (r=0.441 p<0.005) and FG (r=0.440; p<0.005). Conclusion: Morning salivary cortisol in subjects with chronically elevated blood glucose can represent a downregulation of the hypothalamic-pituitary adrenal axis. This is an important finding not yet well investigated.

INTRODUCTION

The current lifestyle exposes the population to several risk factors related to eating habits and lack of physical activity, contributing to the emergence of chronic diseases. Among these, cardiovascular, diabetes and obesity have become common causes of premature death. Cardiovascular disease is the final event of several systemic alterations caused by other diseases such as hypertension, obesity, diabetes and hypercholesterolemia, which make up the so-called metabolic syndrome (MS). It is a complex set of cardiovascular risk factors, related to abdominal fat and insulin resistance, with high risk of morbidity and mortality.

According to The Third Report of the National Cholesterol Education Program - NCEP-ATP III, diagnosis of MS is necessary to alter three of the five risk factors: abdominal obesity, abdominal circumference ≤102 cm (men) or ≤88 cm (women); triglycerides (TGL) ≥150 mg/dl; HDL<40 mg/dl (men) or <50 mg/dl (women); systolic blood pressure ≥130/85 mmHg and fasting glycemia ≥100 mg/dl, regardless of the presence of diabetes.
Another important metabolic marker is salivary cortisol, which serves as a biomarker of stress. It is synthesized from cholesterol and its production is stimulated by the adrenocorticotrophic hormone (ACTH) which is regulated by the corticotropin releasing factor and excreted in urine, blood plasma and saliva. The action of cortisol affects several physiological systems such as immune function, glucose regulation, vascular tone, and bone metabolism. Its production has circadian rhythm that depends on ACTH, with maximum levels in the morning decreasing throughout the day. ACTH and cortisol are secreted independently of the circadian rhythm as a reaction to physical and psychological stress. In individuals exposed to constant chronic stressors, excess of cortisol is very harmful to health.

The relationship between salivary cortisol and SM components evidences important stress response markers of the systemic inflammatory process associated with MS and its components.

The present study aims to demonstrate the results of the association between salivary cortisol and the components of MS.

METHODS

All selected participants signed the Informed Consent Form and individually received information about the study procedures. The confidentiality and confidentiality requirements of the information collected were complied with in accordance with Resolution No. 466/2012, which establishes the guidelines for human research. The project was approved by the ethics committee of the institution, under number: 10/05153. The main study was registered in the Brazilian Registry of Clinical Trials, ReBEC, under number: RBR-9wz5fc. The authors committed themselves to maintaining the confidentiality of the data.

This is a cross-sectional study based on secondary data extracted from the study on the effects of different lifestyle modification interventions on physical, metabolic and behavioral aspects involved in the metabolic syndrome through the MERC (Research Modification Style Group of Life and Cardiovascular Risk of Catholic University, RS, Brazil). From this study, 28 gender-independent individuals, aged 30-59 years, were analyzed. As an inclusion criterion, they had to present three or more of the following findings that characterize SM: abdominal circumference of ≥88 cm for women and ≥102 cm for men; systolic blood pressure >130 mmHg and diastolic >85 mmHg; fasting glucose >100 mg/dl; triglycerides >150 mg/dl; HDL cholesterol <40 mg/dl for men and <50 mg/dl for women. Those who did not present all the necessary records in the main study database were excluded.

The assessment included the measurement of body weight, height and waist circumference. The body weight was verified by means of a scale with capacity for 160 kg, duly calibrated, with the patient barefoot and with the least possible clothing. A vertical anthropometer was used to measure height. Abdominal circumference was assessed through the abdominal perimeter (cm), measured at the site of maximum extension of the abdominal region. The instrument used was an inelastic millimeter tape measuring 180 cm in length.

The values of systolic and diastolic blood pressure were measured according to the recommendations of V Brazilian Guideline for Hypertension.

For blood collection, volunteers were previously instructed to fast for 10-12 h. The collection of saliva was performed by the volunteer for later delivery to the research group. The evaluation of salivary cortisol was measured from samples collected throughout the day to evaluate changes in the circadian rhythm of this hormone, being 8 h (C8), at noon (C12) and at 20 h (C20). All collections were done before meals. They were used small rolls of cotton, previously prepared and sterilized for individual use. After centrifugation, the supernatant was separated for routine analysis.

The blood biochemical markers analyzed were: lipid profile (HDL-c, total cholesterol (CT), triglycerides (TG)) whereas the determination of LDL-c was performed indirectly - LDL-c=(TG/5+HDL-c)/CT, glycemic profile (fasting glucose, fasting insulin and HOMA). For the analysis of biochemical markers, 10 ml of blood were collected from each participant before and after the intervention, totaling 20 ml. Serum and plasma aliquots were separated for freezer storage at -80° C.

The IMC² was calculated according to the World Health Organization’s recommendation for nutritional status evaluation. The cutoff points for the classification of the individuals regarding BMI (kg/m²) were: normal 25 kg/m²; overweight from 25-29.99 kg/m²; obese ≥30 kg/m².

Statistical analysis

The Kolmogorov-Smirnov test was used to verify the normal distribution of the continuous data. The data were presented quantitatively (mean±standard deviation). To determine the relationship between continuous variables, the Pearson and Chi-square correlation test were used to measure the association between categorical/dichotomous variables. Student’s T test was used to evaluate the difference between means. The level of significance was set at p=0.05 (bi-flow). For the analysis of the data the SPSS statistical package, version 22.0.0.0 was used.

RESULTS

We evaluated 28 individuals with MS, with a mean age of 51.9±7.5 years; the majority were female (64.3%) with an average BMI of 33.66±3.27 kg/m². The 8 h salivary cortisol level had a mean of 16.70±4.87 ng/ml in the 12 h it was of 10.19±4.23 ng/ml and in 20 h of 4.74±2.30 ng/ml. The cortisol drop throughout the day can be analyzed in Figure 1. The reference value of the 8 h salivary cortisol used was 13.5-23.5 ng/ml.

![FIGURE 1 – Salivary cortisol levels (ng/dl) in the 24 h period](image)

The data results of the patients and variables studied can be seen in Table 1.

| TABLE 1 - Characteristics of the sample |
|-----------------------------------------|
| Variables | Mean±SD(n=28) |
| Age | 51.86±7.46 |
| Weight (kg) | 91.72±12.34 |
| Body mass index (kg/m²) | 33.65±3.27 |
| Abdominal circumference (cm) | 109.04±8.39 |
| Triglycerides (mg/dl) | 196.86±94.71 |
| High density lipoprotein - HDL (mg/dl) | 47.29±11.73 |
| Glucose (mg/dl) | 107.57±36.42 |
| Systolic blood pressure (mmHg) | 133.68±16.06 |
| Diastolic blood pressure (mmHg) | 91.36±13.05 |
The results regarding the correlation between salivary cortisol and the components of SM can be seen in Table 2.

**TABLE 2 - Correlation between salivary cortisol and components of metabolic syndrome**

| Variables                  | Salivary cortisol  | 8 AM | Noon | 8 PM |
|----------------------------|--------------------|------|------|------|
|                            | r      | p*   | r    | p    | r    | p*   |
| Abdominal circumference     | -0.133 | 0.499 | -0.097 | 0.624 | 0.037 | 0.0852 |
| Systolic blood pressure     | 0.108  | 0.583 | -0.140 | 0.478 | -0.186 | 0.344 |
| Diastolic blood pressure    | 0.000  | 0.997 | -0.181 | 0.356 | -0.264 | 0.174 |
| High density lipoprotein - HDL | -0.191 | 0.331 | -0.090 | 0.647 | -0.224 | 0.252 |
| Triglycerides              | 0.264  | 0.175 | 0.054  | 0.786 | 0.006  | 0.097 |
| Glucose                    | 0.052  | 0.792 | -0.203 | 0.301 | -0.197 | 0.314 |
| Body mass index            | -0.117 | 0.552 | -0.043 | 0.829 | 0.048  | 0.0808 |

* Pearson correlation

Figure 2 shows the division of patients regarding the presence or absence of each component of MS; in this way, individuals with fasting glycemia > 110 mg/dl had significantly lower mean cortisol levels than those with fasting glycemia <110 mg/dl.

**FIGURE 2 - Relationship of morning cortisol to fasting glycemia (Boxplot chart showing mean±SD, p<0.05)**

Insulin and HOMA measurements were available only in a subset of the study (n=24). Table 3 shows the various variables and correlations between the components of SM, HOMA and morning salivary cortisol.

**TABLE 3 - Correlation between the components of Metabolic Syndrome and Homestatic Model Assessment (HOMA)**

| Variables                  | HOMA Total sample | r    | p    |
|----------------------------|-------------------|------|------|
| Cortisol                   | 24                | -0.049 | 0.818 |
| Abdominal circumference    | 24                | 0.465  | 0.022 |
| Triglycerides              | 24                | 0.473  | 0.019 |
| Insulin                    | 24                | 0.0910  | <0.0001 |
| Fasting glycemia           | 24                | 0.678  | <0.001 |
| Abdominal circumference    | Total sample      | r    | p    |
| Fasting glycemia           | 28                | 0.446  | 0.017 |
| Diastolic blood pressure   | 28                | 0.375  | 0.050 |
| Body mass index            | 28                | 0.730  | <0.001 |
| Triglycerides              | Total sample      | r    | p    |
| High density lipoprotein - HDL | 28                | 0.441  | 0.019 |
| Fasting glycemia           | 28                | 0.440  | 0.019 |

When comparing the components of MS with salivary cortisol levels at 8AM, noon and 20PM, there is an important relationship between fasting glycemia and salivary cortisol. Individuals with fasting glycemia > 110 mg/dl have significantly lower salivary cortisol levels than those with glycemia <110 mg/dl.

Currently, the relation of the hypothalamic-pituitary-adrenal axis and alterations in glucose metabolism has been the subject of clinical studies.

**DISCUSSION**

The dysfunction of the adrenal pituitary hypothalamic axis is associated with obesity and MS and, consequently, BMI; increased abdominal circumference and visceral fat are associated with low levels of salivary cortisol in the morning.
In this study, the relationship between the components of MS and their association with insulin resistance can be seen in Table 3. Although no significant correlation between insulin resistance measured by HOMA-
1 IR and morning cortisol has been demonstrated, other authors suggest that salivary cortisol tends to increase with high BMI and correlate with hip circumference in men and with systolic pressure in women significantly. Even so, the study concluded that these data do not support a strong relationship between cortisol levels and SM components.

Analyzing the relationship between salivary cortisol and obesity, a study of 82 subjects showed that there was no difference in the level of morning salivary cortisol between non-obese, obese and weight-loss individuals. However, men who lost weight have a lower level than non-obese men; this relationship in women was not observed. The observation that visceral fat loss may reduce hypothalamic-pituitary-adrenal axis activity contrasts with the findings that weight loss associated with restriction of caloric intake increases the activity of the hypothalamic-pituitary axis. From this it can be inferred that fasting and weight loss may increase circulating levels of cortisol, possibly to increase appetite in an attempt to "defend" itself from loss of body mass.

In fact, current literature shows inconsistent relationships between cortisol levels and metabolic parameters. While one study shows a tendency to increase salivary cortisol in higher BMI, another shows an inverse correlation between morning salivary cortisol levels. In this study, the correlation between cortisol and BMI was not significant, most probably because the sample did not have patients with very high BMI (no patient with BMI >40 kg/m²).

Previous studies have reported that individual components of MS and activity of the hypothalamic-pituitary-adrenal axis are related; however, few studies have examined the components individually. Specifically, elevated hip circumference has been associated with high rates of morning cortisol and related to stress reactivity as well as decreasing cortisol variability throughout the day. Evidence suggests that hypertension is associated with morning cortisol levels and/or cortisols stress reactivity. Studies of the BMI association and central adiposity with cortisol have been misleading with some reporting that people with high BMI and adiposity rates have high cortisol under-curves, but others report that people with high BMI and waist circumference have low levels of cortisol at dawn and a decrease in the rate of daily drop in cortisol levels.

CONCLUSIONS

Salivary cortisol in the morning in individuals with chronically elevated glycaemia may represent a counter-regulation of the hypothalamic-pituitary-adrenal axis, being important marker and not well investigated till now.

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