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Original Research Article

An Observational Study to Identify Serum Cortisol, Salivary Amylase and Salivary Nitric Oxide as Objective Markers for ED

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Conflict of interest: Nil

Abstract:

Aim: The aim of the present study was to identify serum cortisol, salivary amylase and salivary nitric oxide as objective markers for ED.

Material & Methods: A cross sectional study was conducted among 50 patients presenting in the Department of Surgery for one year with complaints of erectile dysfunction. The IIEF and IIEF-5 were administered as valid tools to identify the situation among them in last 4 weeks and last 6 months respectively.

Results: In our study the patients were of age 20 years to 49 years and mean serum testosterone level was 462.08 ng/ml. The mean serum cortisol and salivary alpha amylase values calculated in sample collected between 8 am to 10am were 12.36 U/ml and 768.8U/ml respectively. Based on IIEF-5, we didn't found any case of severe ED in our study. The patients of mild, mild to moderate and moderate ED were 5, 20 and 13 in numbers. On basis of history, clinical examination and blood profile (normal blood sugar, lipid profile, TSH) all our patients could be grouped into Psychogenic ED. There was no significant correlation between age of patients and serum testosterone levels. We found a negative correlation between IIEF-5 score (spearmen's coefficient - 0.32) and IIEF score (spearmen's coefficient -0.08) with serum cortisol but was statistically not significant. The salivary amylase values were found to have statistically significant negative correlation with between IIEF-5 score (spearmen's coefficient -0.45) and IIEF score (spearmen's coefficient -0.38). We measured salivary nitric oxide levels by Berkeley test strip in 10 patients. 2 of them had mild ED, 4 were of mild to moderate ED and 4 had moderate ED. We didn't fount any significant co-relation between salivary NO levels and IIEF-5 score in our study (chi square test=1.13, p value=0.88).

Conclusion: Salivary amylase is a good non-invasive objective marker in evaluating patients of stress related psychogenic erectile dysfunction. Serum cortisol is not a good indicator of chronic stressful situation like psychogenic ED. However, outcome needs to be studied in a larger sample size for better assessment of results.

Keywords: Psychogenic Erectile Dysfunction, Salivary Alfa Amylase, Erectile Dysfunction Marker.

Introduction

Erectile dysfunction (ED) is defined as inability to achieve and maintain erection sufficient to permit satisfactory sexual intercourse for at least 3 months. [1] International index of erectile function (IIEF) is a brief, multidimensional and validated self-administered tool to assess erectile function without identifying the cause. [2] But the results are highly variable according to the patient's mood, state of mind and attitude.

Psychological stress impairs homeostasis in many aspects, including immune dysregulation [3] with significant variability according to age and sex. [4] The use of easily accessible biomarkers is essential to assess these changes in fragile subjects, like children, especially in a context of exposure to stressful experiences during childhood, where invasive sampling procedures may increase psychological stress. Saliva is a promising medium that has been used to evaluate both acute and chronic stresses. [5] Several salivary immune biomarkers are now considered to be sensitive and reliable readouts of mental stress in adult patients, [6] primarily cortisol, alpha-amylase (sAA), secretory immunoglobulin IgA (sIgA), and opiorphin (OPI).

The human salivary alpha amylase (sAA) is produced mostly in the parotid glands, some in the submandibular glands, and small portions also in the sublingual and small salivary glands. The enzyme is exceptionally stable at high pH and temperature ranges. Salivary amylase (sAA) was also recently suggested as an indicator of

sympathetic nervous system (SNS) activation but significantly different from cortisol. [7] Typical sAA concentration reaches a peak in the late afternoon or evening. [8] sAA showed positive correlations with heart rate, pain intensity, and cortisol, suggesting HPA and SNS coordination. [9]

In the current stressful life, ED is a growing public health problem mostly among young adults. The anxiety and stress causes increased sympathetic activity reflected by flaccid penis in those situations. The human body have two different stress response systems- Hypothalamopituitaryadrenal (HPA) axis and Autonomic i.e. sympathetic nervous system (SNS) and para sympathetic nervous system (PNS). [10] The integrity of HPA axis in maintaining stress related homeostasis can be assessed by its well described marker serum cortisol secreted from adrenal cortex.

Also, nitric oxide (NO) is a well understood significant mediator of smooth muscle relaxation response for engorgement of erectile tissue in muscle. NO is released by cholinergic and non-cholinergic nerves appearing to be controlled by PNS. [11] Hence the aim of the study was to identify serum cortisol, salivary amylase and salivary nitric oxide as valid objective markers for diagnosing Psychogenic ED.

Material & Methods

A cross sectional study was conducted among 50 patients presenting in the Department of Surgery, Netaji Subhas Medical College and hospital, Bihta, Patna, Bihar, India for one year with complaints of erectile dysfunction. The IIEF and IIEF-5 were administered as valid tools to identify the situation among them in last 4 weeks and last 6 months respectively.

Exclusion Criteria

Those < 18 years or >50 years of age, unmarried, history of coronary artery disease/ diabetes mellitus, history of previous perineum surgery, trauma over back or pelvic trauma, smoker or tobacco chewer, having active teeth or gum infection, chronic steroid intake or on antidepressants were excluded from the study.

Methodology

One ml of peripheral blood sample was collected and immediately sent to laboratory for assessing serum cortisol level by chemilluminescense method. After washing mouth with tap water patient was asked to spit 3-4 times to collect one ml of unstimulated saliva in a sterile plastic container and sent for biochemical assessment of salivary amylase by spectrophotometry. The Berkeley test strips were used for measuring salivary nitric oxide level as depleted, low, threshold and high. The saliva collection strip was placed over the tongue of patient for 5 seconds and then test strip was approximated over collection pad for 10 seconds giving colour change. The colour on strip was correlated to identify the level of salivary no.

Method for Salivary Amylase Measurement:

To measure the salivary amylase level, amylase ASX-CNPG3 reagent supplied by Maxchen pharmaceuticals was used. The reagent was supplied to read linearity upto 2000U/L in serum and urine. As amylase content in saliva is many times higher than serum some dilution of saliva sample was required. We used 1:200 dilution factor of saliva sample into distilled water. As per manufacturer's instruction 1ml of working reagent was incubated at 37 0 c with 25 µml of diluted salivary sample for 1 minute. The optical density was read under colorimeter at 405nm wavelength per minute during 3 minutes against distilled water.

Statistical Analysis

The data was evaluated with appropriate statistical test (spearmen's correlation coefficient and chi square test).

Results

Based on IIEF-5, we didn't found any case of severe ED in our study. The patients of mild, mild to moderate and moderate ED were 5, 20 and 13 in numbers. On basis of history, clinical examination and blood profile (normal blood sugar, lipid profile, TSH) all our patients could be grouped into Psychogenic ED. There was no significant correlation between age of patients and serum testosterone levels. We found a negative correlation between IIEF-5 score (spearmen's coefficient -0.32) and IIEF score (spearmen's coefficient -0.08) with serum cortisol but was statistically not significant. The salivary amylase values were found to have statistically significant negative correlation with between IIEF-5 score (spearmen's coefficient -0.45) and IIEF score (spearmen's coefficient -0.38).

Table 1: Descriptive statistics

	Mean	Range
Age	28.6 year	20-49
Serum testosterone	462.08 ng/ml	326-751
Serum cortisol	12.36 U/ml	6.23-18.35
Salivary amylase	768.8 u/ml	362-1325

In our study the patients were of age 20 years to 49 years and mean serum testosterone level was 462.08 ng/ml. The mean serum cortisol and salivary alpha amylase values calculated in sample collected between 8 am to 10am were 12.36 U/ml and 768.8U/ml respectively.

Table 2: Correlation of EF and IIEF with various study parameters

	Spearmen's correlation coefficient	P value
IIEF-5 with serum cortisol	-0.32	0.06
IIEF with serum cortisol	-0.08	0.57
IIEF-5 with serum testosterone	-0.12	0.42
IIEF with serum testosterone	-0.008	0.96
IIEF-5 with salivary amylase	-0.45	0.003
IIEF with salivary amylase	-0.38	0.01

Table 3: Berkely test results (total n=10)

	Mild ED	Mild to Moderate ED	Moderate ED
Depleted	1	2	2
Low	0	1	1
Threshold	1	1	1

We measured salivary nitric oxide levels by Berkeley test strip in 10 patients. 2 of them had mild ED, 4 were of mild to moderate ED and 4 had moderate ED. We didn't fount any significant co-relation between salivary NO levels and IIEF-5 score in our study (chi square test=1.13, p value=0.88).

Discussion

In acute stress situations, sAA secretion may have additional distinct peaks. [12] IgA is a secreted glycoprotein, part of the adaptive immune system, that acts synergistically with other inherited mucosal defense factors, such as alpha-amylase, lactoferrin, and lysozyme. [13] Similarly, to cortisol, sIgA peaks in the morning and then gradually declines till the evening. Acute stress increases sIgA release, whereas chronic stress has an inhibitory effect, emphasizing the ability of the immune system to protect the body against disease. [14]

In our study the patients were of age 20 years to 49 years and mean serum testosterone level was 462.08 ng/ml. The mean serum cortisol and salivary alpha amylase values calculated in sample collected between 8 am to 10am were 12.36 U/ml and 768.8U/ml respectively. Based on IIEF-5, we didn't found any case of severe ED in our study. The patients of mild, mild to moderate and moderate ED were 5, 20 and 13 in numbers. On basis of history, clinical examination and blood profile (normal blood sugar, lipid profile, TSH) all our patients could be grouped into Psychogenic ED. There was no significant correlation between age of patients and serum testosterone levels. We found a negative correlation between IIEF-5 score (spearmen's coefficient - 0.32) and IIEF score (spearmen's coefficient -0.08) with serum cortisol but was statistically not significant. The salivary amylase values were found to have statistically significant negative correlation with between IIEF-5 score (spearmen's coefficient -0.45) and IIEF score (spearmen's coefficient -0.38). The psychogenic ED can be identified by its sudden onset, situational nature and presence of morning erection. In our study we followed these criteria for identifying the patients of psychogenic ED and excluded organic causes of ED in them. It has been reported in various studies that prevalence of ED increases with increasing age (>40 years), involving both organic and psychogenic causes. [15] Most of the patients in our study were of younger age. Particularly for Psychogenic ED, the possible mechanism could be direct inhibition of spinal erection centre by brain or excessive sympathetic outflow or elevated peripheral catecholamine levels that increase the smooth muscle tone to prevent its necessary relaxation. [16] The male sexual response is based on the balance between the excitatory and inhibitory central nervous system impulses. The IIEF is a validated multidimensional self-administered questionnaire for assessing erectile function without differentiating the cause. It is constituted of 15 questions covering erectile function (Q1,2,3,4,5,15), orgasmic function (Q9,10), sexual desire(Q11,12), intercourse satisfaction(Q6,7) and overall satisfaction(Q13,14).The IIEF-5 is a five points questionnaire for identifying erectile dysfunction.

But patient filling the questionnaire may have tendency to exaggerate or undermine his problem. [17] The patient builds his own world of anxiety, distress and depression, increasing the stress level and thus resulting in psychogenic ED. Some stress related parameter could be used to assess the erectile dysfunction in these patients. It has been seen in some studies that the subjects exposed to various psychogenic stressful conditions were found to have increased SAA levels. [18] In a study, the subjects exposed to 'Trier social stress test or TSST' were found to have raised SAA

compared to those who had not taken the test. [19] We measured salivary nitric oxide levels by Berkeley test strip in 10 patients. 2 of them had mild ED, 4 were of mild to moderate ED and 4 had moderate ED. We didn't fount any significant co-relation between salivary NO levels and IIEF-5 score in our study (chi square test=1.13, p value=0.88). The HPA axis shows adaptability to recurrent stimulus and therefore in chronic stress the marker does not show increase similar to acute stress. This could be the reason of not getting significant correlation between ED and serum cortisol values. Schommer has assessed the response of HPA axis and SNS activity in repeated stress and found the fact that HPA axis quickly habituates. [20] The role of nitric oxide in penile smooth muscle cell relaxation leading to erection is well understood and reported. We didn't found any significant correlation between salivary nitric oxide and erectile dysfunction in our study. But our study group was small (n=10) in which we measured salivary nitric oxide by commercially available nitric oxide test strips, thus limiting the results. Also the relation between salivary NO and plama NO2-(marker of endothelial nitric oxide synthetase) is lacking as described by Clodfelter et al. [21]

Conclusion

Salivary amylase is a good noninvasive objective marker in evaluating patients of stress related psychogenic erectile dysfunction. Serum cortisol is not a good indicator of chronic stressful situation like psychogenic ED. However, outcomes need to be studied in larger sample size for better assessment.

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