

Salivary Cortisol Level Pre and Post MRI Scanning

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Abstract:

The measurement of salivary cortisol level has become a reliable method for studying the adrenal cortical function and it is response to different intrinsic and extrinsic factors such as medications and stressful factors.

The aims of this study is to investigate if there is difference between sex and the effect of time of testing on salivary cortisol level, pre and post was scanning, data for cortisol, Salivary Flow Rate (SFR) and PH of saliva were analyzed.

Non-stimulated salivary samples from 24 subjects (8 males, 16 females) pre and post scanning with Magnetic resonance imaging (MRI) was collected and the diurnal variation was taken into consideration for that all the pre and post scanning samples collected at the morning. Salivary cortisol was measured by ELISA technique, PH of saliva was measured by PH meter and salivary flow rate by specific equation.

The results shows there was significant difference in the level of salivary cortisol, SFR and PH of saliva pre and post examination and there was positive correlation with regard to cortisol level and PH of saliva pre and post scanning, just the salivary flow rate showed negative correlation, in addition the results revealed significant difference with regards to the sex of the participant as well as positive correlation between salivary PH and SFR in pretest phase and positive correlation between salivary cortisol level, SFR in post scanning phase.

From the results of this study we can conclude that the exposure to MRI scanning have an effect on Hypothalamic pituitary –adrenal axes and predispose to significant changes in cortisol level post scanning and this difference must be taken into consideration in concern to effect of raising the level of cortisone on other variables.

Key words: Salivary cortisol, Magnetic resonance imaging (MRI).

مستوى هورمون الكورتيزول في اللعاب قبل وبعد إجراء فحص الرنين المغناطيسي
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الخلاصة:

أصبح قياس مستوى الكورتيزول في اللعاب طريقة موثوق بها لدراسة وظيفة الغدة الكظرية ومدى تأثرها بمختلف العوامل الداخلية والخارجية مثل الأدوية والعوامل المجردة.

الهدف من هذه الدراسة هو قياس مستوى هورمون الكورتيزول في اللاعب قبل وبعد إجراء فحص الرنين المغناطيسي ومدى اختلاف مستوى الكورتيزول بين الجنسين.

تم إختيار (24) شخصا (8 ذكور، 16 إناث) وتم قياس مستوى هورمون الكورتيزول وكمية اللاعب وقاعدته قبل وبعد المسح بواسطة الرنين المغناطيسي وقد أخذ بعين الاعتبار تباين مستوى الكورتيزول خلال اليوم، وأن جميع العينات قد أخذت صباحا وتم قياس المتغيرات تحت الدراسة. تم قياس هورمون الكورتيزول بواسطة تقنية الإيلايزا وقياس مستوى درجة الحموضة بواسطة جهاز قياس الحموضة كذلك تم حساب كمية إفراز اللاعب وفق معادلة خاصة.

أظهرت الدراسة وجود فروق ذات دلالة إحصائية في مستوى الكورتيزول ومستوى إفراز اللاعب وتغير حموضة اللاعب قبل وبعد إجراء فحص الرنين المغناطيسي كذلك إختلاف نتائج قراءات العوامل تحت الدراسة بين الجنسين كما أظهرت النتائج وجود إرتباط ذو دلالة إحصائية إما إيجابية أو سلبية بين بعض هذه المتغيرات.

من خلال النتائج نستنتج أن إجراء فحص الرنين المغناطيسي يؤثر بصورة محسوسة على مستوى الكورتيزول وبعض المتغيرات الأخرى ويجب أن يؤخذ هذا المتغير بنظر الاعتبار بما يخص تأثير إرتفاع مستوى هورمون الكورتيزول على عوامل أخرى.

الكلمات المفتاحية: كورتيزول اللاعب، الرنين المغناطيسي.

Introduction:

The classical endocrine stress system is a hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis involves a complex neural system involving many different anatomical structures and neurochemical events that are required to activate and/or inhibit the HPA axis. The cortisol is one of the most widely used biological markers of stress, since elevations often occur in response to novel and unpredictable situations^[1].

Cortisol is the major glucocorticoid produced in the adrenal cortex, has a circadian rhythm, and with levels peaking in the early morning and dropping to lowest levels at night and it is Levels rise independently of circadian rhythm in response to stress^[2,3].

Studies consistently report high correlations between serum and salivary cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels^[4].

Most subject underwent magnetic resonance imaging (MRI) presented with different unpleasant feelings, ranging from

claustrophobia to severe anxiety and phobic reactions, subsequently these emotional reactions can produce a wide range of complications, such as hyperventilation, tachycardia, sweating, light-headedness, and nausea, often leading to premature termination of the scan^[5].

It is likely that some of the subjects underwent MRI also manifest a neuro-endocrine response to the experience given that the MRI is stressful to many subjects. In particular, the psychological stressors have been shown to influence the hypothalamic-pituitary-adrenal (HPA) axis and the HPA axis is an important regulatory system that acts through hormone cascades to sub serve both behavioral and physiological responses to stressful conditions^[6]. Many authors indicates that most psychological and physical challenges produce heightened salivary cortisol concentrations^[7]. Author's paris, JJ *et al*^[8] suggested that women may be more vulnerable to aberrant hypothalamic pituitary-adrenal (HPA) axis responses to stress than men.

The salivary flow measurement is frequently used as biomarker for the evaluation of oral and systemic diseases^[9].

There are different methods for measuring the saliva where some measure directly from the salivary glands, others measure the saliva present in the mouth, or the whole saliva^[10]. The saliva can be obtained with or without stimulus (resting and the stimulated salivary flow), for stimulated salivary flow the paraffin and citric acid are mainly used, while non-stimulated is obtained in the absence of any stimulus^[11]. The most common methods for collecting the saliva are: suction, fluid synthetic swab, cotton pledged, hydrocellulose micro- sponge or passive drooling^[12]. The saliva volume depending on the type and intensity of stimulation. A good salivary flow protects against dental caries, erosion, abrasion and candidiasis^[13]. Kiess, W. and Pfaeffle, R.^[14] reported that the saliva is a readily available fluid can be used to monitor the presence and concentration of drugs, hormones, antibodies and other molecules. Schmidt NA, *et al*^[15,16] recorded that the saliva has superiority to urine, plasma for assessment of free and bound cortisol but specific considerations must be taken into account like the time of sampling, food intake and highly specific immune assay device have to be used.

In contrast to more traditional biological specimens (e.g., urine or plasma), analytic measurements in oral fluid seem to afford researchers an opportunity to gather measures of the confluence of bio-behavioral processes during, and in response to, naturalistic, and contextually meaningful events with direct relevance to variation in individuals' everyday interpersonal demands and experiences. Theorists speculate this embedded bio-social-ecological approach is a conceptual advance that will extend our understanding of why some individuals are placed at risk by, while others are resilient to, similar circumstances, adversities, and experience^[17].

Saliva contains a variety of host defense factors. It influences calculus

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formation and periodontal disease. Different studies have been done to find exact correlation of salivary biomarkers with periodontal disease. With a multitude of biomarkers and complexities in their determination, the salivary pH may be tried to be used as a quick chairside test^[18].

The aim of this study is to estimate the salivary cortisol level, salivary flow rate and PH of saliva in response to MRI examination and if any difference in these parameters regarding the sex of the subject, as well as to assess the correlation between these parameters pre and post scanning.

Materials and Methods:

A total of twenty four subjects were included in this study by random sample technique. The participants were chosen from subjects attending to Al-Karama Teaching Hospital and private clinics for MRI scanning. Questionnaires was used to identify the socio-demographic data, smoking statues, medical history of the participants and if she/he received any medications known have an effect on the parameters under study at the last three days before scanning day and any subject who was received any of these medications was ruled out of study.

The non-stimulated saliva samples were obtained from the participants, the PH of saliva was measured by PH meter and the salivary flow rate was determined by measuring the total amount of saliva (volume/ml) and dividing this amount by period of time through which the sample was collected:

$$\text{SFR (ml/min)} = \frac{\text{Saliva Volume (ml)}}{\text{time of collection (min)}}$$

Salivary cortisol was measured by ELISA according to the instructions of manufacturing company (SAIMETRICS, COMPANY) as follow:

First, Elisa plate and diluents were prepared, then 25 µl /min of standard, control and

samples were pipette into wells in duplicate, then 25 µl/min of assay diluents were added into wells. The conjugate dilutions were prepared than 200 µl/min of conjugate were added into each well, Elisa plate was mixed for five minutes and incubated at room temperature for 55 minutes, the plate then washed for four times.200 µl/min of TMB solution was added for each well, mixed, for 25 minutes in dark room, lastly 50 µl/min of stop solution was added to each well ,mixed for three minutes then read the results by using spectrometer at 450 nm within 10 minutes and calculate the result (SAIME-TRICS, COMPANY).

Statistical analyses:

Data were expressed as number and percentage, significant differences were assessed by the paired student’s t test, P-value of ≤0.05was considered significant.

Results:

The primary aim in this study is to estimate the hormonal responses as reactionary factor to an MRI environment. The results showed that the level of salivary cortisol hormone was rose after the MRI examination but salivary PH and SFR were decreased, further more we assessed the correlation between different parameters, where the result showed positive correlation for some of them and negative for others.

Study group of 24 patients with mean age of 22.3 years (SD±1.7) distributed according to their sex as shown in table-1 and figure-1, the result showed that 33.3% of cases were males and 66.7% were females.

Table-1: Sex distribution of studied group

Gender	No.	%
Female	16	66.7
Male	8	33.3
Total	24	100

Date of acceptance: 9-5-2016

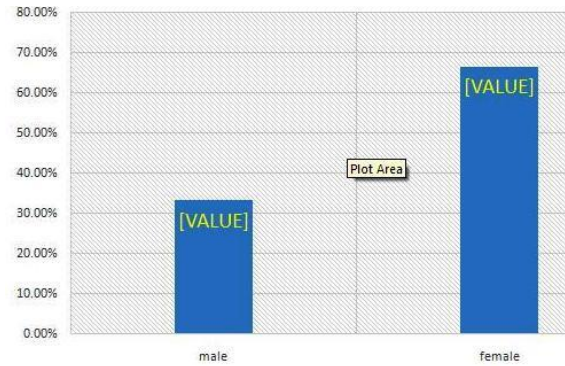


Figure-1: Sex distribution of studied group

The results in table-2 showed that the mean value of SFR and PH of saliva were decreased post scanning, while the level of salivary cortisol was increased and this reported difference statistically significant.(p value=0.001).

Table-2: Mean of studied variables pre and post scanning.

Variables	Mean	No.	S.D	P-value
SFR (pre)	1.7	24	0.1	0.001
SFR (post)	1.2	24	0.1	
S.PH (pre)	7.0	24	0.2	0.001
S.PH (post)	6.5	24	0.2	
S, Cortisol level (pre)	0.15	24	0.13	0.001
S, Cortisol level (post)	0.2	24	0.1	

Table-3 revealed that there is a negative correlation between pre and post scanning value with regard to salivary flow rate and positive correlation for other tested variables and this correlation statistically significant(p value=0.001).

Table-3: Correlation between value of studied variables pre and post scanning.

Pre and post exposure of tested variables	No.	R=	p-value
SFR	24	-0.79	0.001
S. PH	24	0.78	0.001
S. Cortisol	24	0.96	0.001

Table-4, Figure-2 depicts the difference (pre and post scanning) in concentrations of salivary cortisol, SFR and PH of saliva across all males and females participants.

The difference in concentrations of salivary cortisol were statically not significant while the difference of SFR and PH of saliva were significant statistically (p=0.001).

Table-4: The difference in mean of studied variables (pre and post scanning) according to the sex of the patients.

Variables	Gender	No.	Mean difference (pre and post scanning)	S.D	P-value
SFR	Female	16	-0.7	0.1	0.001
	Male	8	-0.15	0.05	
S.PH	Female	16	-0.6	0.07	0.001
	Male	8	-0.3	0.01	
S. Cortisol	Female	16	0.07	0.03	0.8
	Male	8	0.08	0.04	

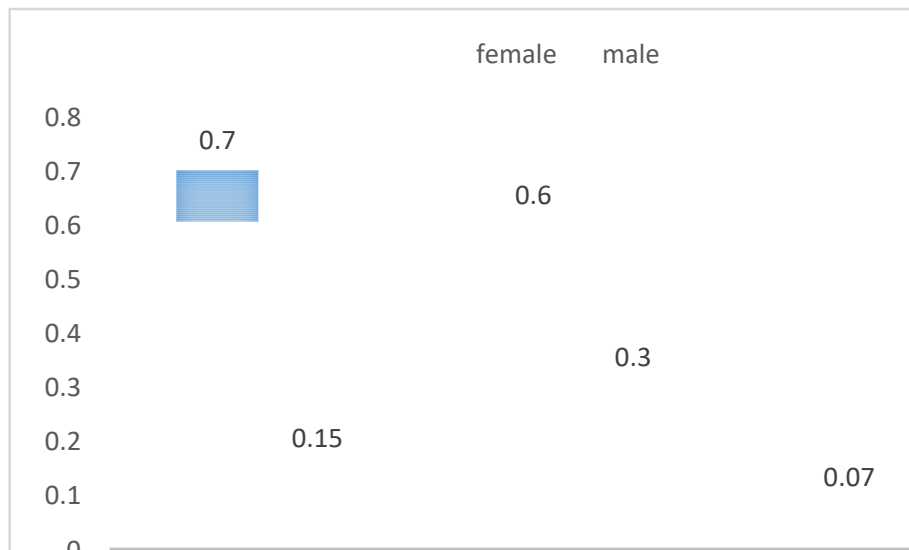


Figure-2: The mean difference (pre and post scanning) of SFR, salivary PH and s.cortisol level between male and female patents.

Table-5 showed that there was negative correlation between SFR and salivary PH and this correlation statistically not significant (p-value=0.8) while there was a positive correlation between other tested variables and the significant difference was reported only between salivary PH and salivary cortisol level (p-value=0.001).

Table-6 showed that there is a negative correlation between SFR and S. cortisol with statistical significant difference (p-value=0.001) and there were a positive correlation between other tested variables and the significant difference was reported between (S.PH and S. Cortisol) and (salivary cortisol and SFR) (p-value≤0.05).

Table-5: Correlation between tested variables for pre scanning state.

		SFR	S.PH	S. Cortisol
SFR	Pearson Correlation	1	-0.05	0.1
	Sig. (2-tailed)		0.8	0.4
S.PH	Pearson Correlation	-0.05	1	0.6
	Sig. (2-tailed)	0.8		0.001
S. Cortisol	Pearson Correlation	0.1	0.7	1
	Sig. (2-tailed)	0.4	0.001	

N=24

Table-6- correlation between tested variables for post-scanning state.

		SFR	S.PH	S. Cortisol
SFR	Pearson Correlation	1	0.08	-0.6
	Sig. (2-tailed)		0.7	0.001
S.PH	Pearson Correlation	0.08	1	0.7
	Sig. (2-tailed)	0.7		0.001
S. Cortisol	Pearson Correlation	-0.6	0.7	1
	Sig. (2-tailed)	0.001	0.001	

N=24

Discussion:

The present study revealed significant difference pre and post examination with regards to variables under study with sex variation, then the present study revealed that the subjects undergoing MRI examination exhibit increased salivary cortisol, SFR and PH post examination significantly higher post examination than pretest. The present findings are consistent with other study that have investigated the effect of novel environments and various stress-induction tasks on cortisol levels where suggest that MRI induce an elevation in cortisol as stressful environment, Some of the factors in the MRI environment that may lead to stress and post-scan elevations in cortisol include machine noise, uncomfortable temperatures in the magnet bore and inability to change positions during the examination^[2]. Katz *et al.*^[19] suggest that anticipatory anxiety in patients undergoing MRI stems from fear of the unknown and apprehension about what the test might reveal. Because the imaging carried out for this study was for research purposes,

concerns about diagnostic findings would be expected to be minimal. Physical discomfort associated with MRI therefore is the most plausible source of the post-scan cortisol elevations in scanner-naive participants.

Moelker and Pattynama^[20] have highlighted acoustic noise concerns in MRI. For example, MR-related acoustic noise has been described to interfere with functional MR acquisition in both direct and indirect ways, such as the noise itself directly increasing blood flow in auditory regions of the brain or the indirect effect of scanner noise on attentional mechanisms leading to cerebral blood flow changes in attention-related cortices and this may affect HPA axes also, however a meta-analysis investigating acute effects of various types of stressors on humans, revealed that noise exposure failed to elicit a significant cortisol response^[21].

The results of this study also are consistent with Tessner. D.K *et al.*^[22], whom hypothesized that the scanning environment can induce cortisol elevations and are congruent with the well-established effects

of acute stressors on activity of the hypothalamic-pituitary-adrenal (HPA) axis.

Dantendorfer *et al.*^[23] found that cortisol hormone significantly elevated compared to post-imaging suggesting that anticipation of the MRI examination increased anxiety levels.

Erin M. Eatough^[24] mentioned that the timing of salivary sample collections may overlap with the start of the recovery phase during the MRI.

A research done by Dickerson *et al.*^[21] confirmed increased salivary cortisol on exposure to mildly stressful experiences, such as competitive challenges, cognitive tasks, public speaking/verbal interaction tasks, emotion induction procedures (e.g. watching a film with disturbing scenes), or exposure to disturbing noise.

Schulteiss OC *et al.*^[25,26] revealed that the stressful experience results in an elevation of cortisol hormone level and a reduction in testosterone level, although there have been studies reporting different changes in these hormones, or no changes at all^[27].

The reason for the discrepancies between the results of different studies may be due to a substantial inter-individual variability in hormonal responses to psychosocial stress, and because of this variability, studies with small sample sizes may report different results from those conducted with larger sample sizes. Inter individual variability in responsiveness does not simply represent noise in the system but it is a crucial element for understanding the relation between hormones and psychosocial stress; yet the determinants of this variability are still poorly understood.

Dickerson and Kemeny^[21] reported that although there is some evidence that men and women differ in their hormonal responses to stress (e.g. women exhibit greater cortisol increases following stress but this evidence is far from being unequivocal. The variability with which psychosocial

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stress can affect testosterone and cortisol secretion in individuals of the same sex is also poorly understood. A common explanation for this variability is that hormonal responses to psychosocial stimuli depend in large part on the subjectivity with which these stimuli are interpreted, and that this subjective interpretation can vary dramatically in relation to individuals' personalities and context.

For many years dental investigators have been exploring changes in salivary flow rate and composition as a means of diagnosing and monitoring a number of oral diseases, the normal non-stimulated salivary flow rate from 0.3 to 0.5 mL/min, and flow rates between 0.10 and 0.01 mL/min are considered hypo salivation. The finding in this study showed that the salivary flow rate was significantly decreased after MRI examination, the most likely explanation for this changes is due to anxiety and fear mostly associated with such examination especially if it is for the first time, Ghezzi *et al.*^[28] and ship *et al.*^[29] reported there are multiple causes of salivary hypofunction, including oral disorders, systemic diseases, prescription and non-prescription medications, chemotherapy, head and neck radiotherapy, psychogenic factors and decreased mastication.

Conclusion:

In summary, the present study revealed that the exposure to MRI scanning can result in elevation of cortisol level, in turn, this elevation can effects the levels of many others hormones, or even affect the cognitive performance in humans.

Researchers to put in their mind this changes in cortisol level post scanning with MRI, because this heightened may be the confounder to other variables under study.

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