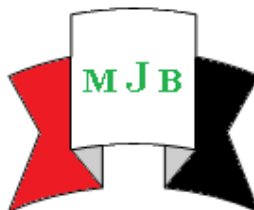


The Comparison Study between Serum and Salivary Steroid Hormones in Young Ages

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Abstract

Saliva is composed of a complex mixture of mucins, enzymes, antibodies, electrolytes, and hormones, all of which serve to begin the process of digestion and protect the oral mucosa. Saliva testing is proving to be the most reliable medium for measuring hormone levels. Hormone levels in saliva accurately represent the amount of hormone delivered to receptors in the body, unlike serum, which represents hormone levels that may or may not be delivered to receptors of the body. (30 male and 35 female) serum and saliva samples were collected for young student age (18-22) year. Samples were collected from November 2012 to February 2013 of Tikrit University students. All samples were normal individual who no personal or family history of any diseases. Serum and saliva (Testosterone, Progesterone, Estrogen and Cortisol) were determined by using AccuBind ELISA Microwells. All serum steroid hormones were increased when compared with saliva in male but in female serum steroid hormones were increased rather than saliva except serum testosterone which decreased. Measurements of salivary hormone levels are of clinical importance if they accurately reflect the serum hormone levels or if a constant correlation exists between salivary and serum hormone levels.

Key words:- Serum steroid hormones, Saliva steroid hormones, Testosterone, Progesterone, Estrogen and Cortisol.

دراسة المقارنة بين الهرمونات الستيرويدية في مصل ولعاب الشباب

الخلاصة

يتكون اللعاب من خليط معقد من الميوسين ، والانزيمات ، والأجسام المضادة ، الشوارد ، والهرمونات ، وكلها تعمل على البدء في عملية الهضم وحماية الغشاء المخاطي للفم . اختبار اللعاب يبرهن على أنه الوسيلة الأكثر موثوقية لقياس مستويات الهرمون . مستويات الهرمون في اللعاب تمثل بدقة كمية الهرمون التي يتم تسليمها إلى المستقبلات في الجسم ، على عكس المصل ، التي تمثل مستويات الهرمون التي قد تسلم أو لا يتم تسليمها إلى المستقبلات في الجسم. تم جمع عينات (٣٠ ذكر و ٣٥ أنثى) من مصل دم و لعاب لطلاب شباب بعمر (١٨-٢٢) سنة من نوفمبر ٢٠١٢ وحتى فبراير ٢٠١٣ من طلاب جامعة تكريت . وكانت جميع العينات لأشخاص طبيعيين الذين ليس لديهم تاريخ شخصي أو عائلي من أي أمراض. تم قياس تركيز (التستوستيرون والبروجسترون، الاستروجين والكورتيزول) في مصل الدم واللعاب وذلك باستخدام AccuBind ELISA Microwells وتبين بان هناك زيادة في جميع هرمونات الستيرويدات في المصل مقارنة مع اللعاب في الذكور والاناث ما عدا التستوستيرون في المصل.

Introduction

Blood components enter the watery fluid of the salivary duct by one of three processes: active transport, ultrafiltration or passive diffusion. Saliva can be analyzed as part of the evaluation of endocrine function. The factors that affect drug availability in saliva are generally true also for salivary hormones. The majority of hormones enter saliva by passive diffusion across the acinar cells. Most of these hormones are lipid-soluble (i.e., steroids). Small polar molecules do not readily diffuse across cells and instead enter saliva through the tight junctions between cells ^(1, 2). Measurements of salivary hormone levels are of clinical importance if they accurately reflect the serum hormone levels or if a constant correlation exists between salivary and serum hormone levels. For neutral steroids which diffuse readily into saliva, salivary hormone levels represent the non-protein-bound (free) serum hormone levels. Salivary cortisol levels were found to be useful in identifying patients with Cushing's syndrome and Addison's disease ^(3, 4), and also for monitoring the hormone response to physical exercise and the effect of acceleration stress ^(5, 6). Contrary to cortisol, salivary cortisone levels do not accurately reflect serum cortisone levels. Cortisone is a neutral steroid and therefore readily diffuses into saliva; however, cortisol is converted to cortisone by an enzyme present in the salivary glands (11 β -hydroxysteroid dehydrogenase). Thus, cortisone levels in saliva are higher than in serum and do not bear any diagnostic significance ⁽⁷⁾. Testosterone and dehydroepiandrosterone have also been identified in saliva. Salivary concentrations were found to be 1.5-7.5% of the serum concentrations of these hormones ⁽⁸⁾. Estradiol can be detected in saliva in concentrations that are only 1-2% of serum concentrations. These concentrations are similar to the serum concentrations of free estradiol, which can diffuse into saliva. Salivary estradiol levels followed the same trends as serum estradiol levels during a menstrual cycle ⁽⁹⁾. Salivary progesterone levels can be useful for the

prediction of ovulation, demonstrating a correlation of 0.75 with serum progesterone levels, and salivary estradiol and progesterone levels can be used for the evaluation of ovarian function ^(10, 11). The stability of hormones in saliva is important as well for accurate evaluation. Hormones in saliva can be degraded, among other ways, by enzymes native to saliva, enzymes derived from oral micro-organisms, and enzymes derived from leukocytes that enter the oral cavity from the gingival sulcus. In addition, molecules that reach saliva by passive diffusion across cells, like unconjugated steroids, may be subjected to enzymatic degradation within the salivary glands, prior to entering saliva ^(1, 2 & 7). The aim of this study to compare between Serum and Salivary Steroid Hormones in Young Ages.

Materials and Methods:-

The study include (30 male and 35 female) serum and saliva samples were collected for young student age (18-22) year. Subjects were asked to spit the saliva into a 10 ml tube until a 2.5 ml sample was collected in the morning at 8:00-10:00 AM in the same time of venipuncture from the same person. The specimen was dated and stored in the freezer until analysis. Saliva specimens were collected from all female students during their follicular phase of menstrual cycles. Samples were collected from November 2012 to February 2013 on Tikrit University students. All samples were normal individual who no personal or family history of any diseases. Serum (Testosterone, Progesterone, Estrogen and Cortisol) were determined by using AccuBind ELISA Microwells (competitive enzyme immunoassay kit) (Monobind Inc., USA). Results were analyzed statistically using (T) tests and the statistical program Minitab. Averages were compared in calculations of the characteristics of the application Duncan's Multiple Range Test by probability level $P \leq 0.05$.

Results

The mean levels of serum and saliva were listed in (Table 1), which show a highly significant increased ($p \leq 0.01$) in serum male testosterone (7.29 ± 1.54) when compared with saliva (0.81 ± 0.21) with negative correlation ($r = -0.228$, $P \leq 0.01$) (fig.1), but slightly increased in saliva (0.7 ± 0.15) rather than serum (0.57 ± 0.15) in female testosterone with positive correlation ($r = 0.194$, $P \leq 0.05$) (fig.2). Serum male progesterone (0.60 ± 0.21) was increased rather than salivary progesterone (0.15 ± 0.03) with positive correlation ($r = 0.096$, $P \leq 0.05$) (fig.3), while Serum female progesterone (0.92 ± 0.18) was increased when compared with salivary progesterone (0.22 ± 0.03) with negative correlation ($r = -$

0.109 , $P \leq 0.01$) (fig.4). Serum male estrogen (32.6 ± 16.7) was increased when compared with salivary estrogen (0.52 ± 0.10) with negative correlation ($r = -0.198$, $P \leq 0.01$) (fig.5), while Serum female estrogen (104.3 ± 57.7) was increased when compared with salivary estrogen (0.95 ± 0.23) with positive correlation ($r = 0.081$, $P \leq 0.01$) (fig.6). Serum male cortisol (10.9 ± 4.23) was increased when compared with salivary cortisol (1.74 ± 0.45) with negative correlation ($r = -0.401$, $P \leq 0.01$) (fig.7) and Serum female cortisol (12.15 ± 4.35) also was increased rather than salivary cortisol (1.78 ± 0.50) with negative correlation ($r = -0.073$, $P \leq 0.01$) (fig.8).

Table 1:- Measurement of steroid hormones in serum and saliva

Steroid hormones	Male		P	r	Female		P	r
	Serum	Saliva			Serum	Saliva		
Number of subject	30	30			35	35		
Testosterone (ng/ml)	7.29 ± 1.54	0.81 ± 0.21	* $P \leq 0.01$	-0.228	0.57 ± 0.15	0.7 ± 0.15	** $P \leq 0.05$	0.194
Estrogen (pg/ml)	32.6 ± 16.7	0.52 ± 0.10	$P \leq 0.01$	-0.198	104.3 ± 57.7	0.95 ± 0.23	$P \leq 0.01$	0.081
Progesterone (ng/ml)	0.60 ± 0.21	0.15 ± 0.03	$P \leq 0.05$	0.096	0.92 ± 0.18	0.22 ± 0.03	$P \leq 0.01$	-0.109
Cortisol ($\mu\text{g/dl}$)	10.9 ± 4.23	1.74 ± 0.45	$P \leq 0.01$	-0.401	12.15 ± 4.35	1.78 ± 0.50	$P \leq 0.01$	-0.073

*Highly significant, **Significant

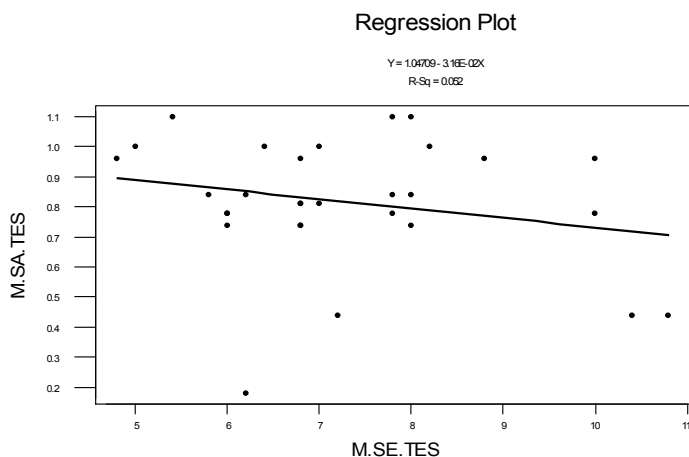


Fig.1:- Correlation between serum and salivary male testosterone.

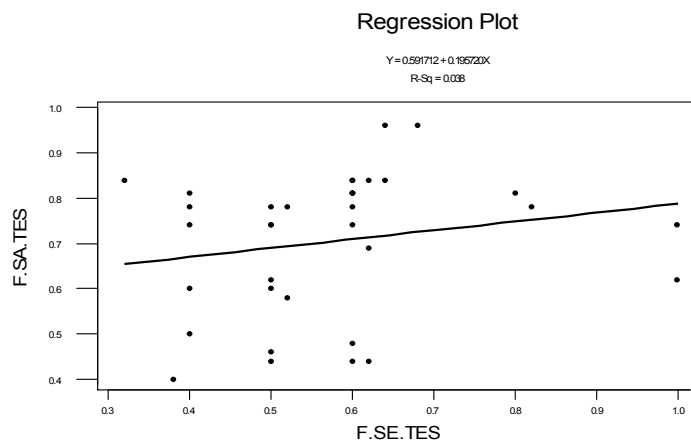


Fig.2:- Correlation between serum and salivary female testosterone.

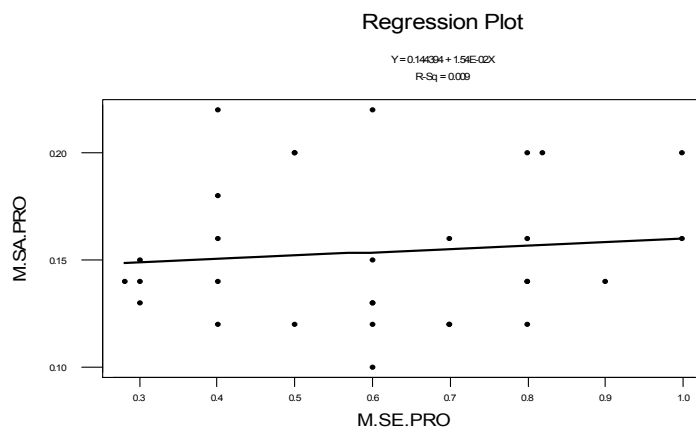


Fig.3:- Correlation between serum and salivary male progesterone.

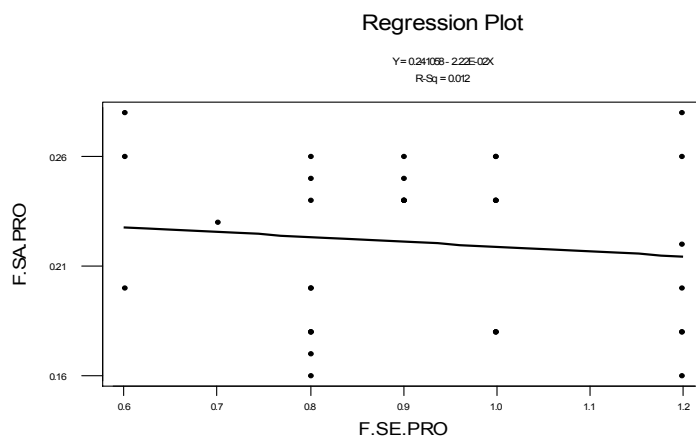


Fig.4:- Correlation between serum and salivary female progesterone.

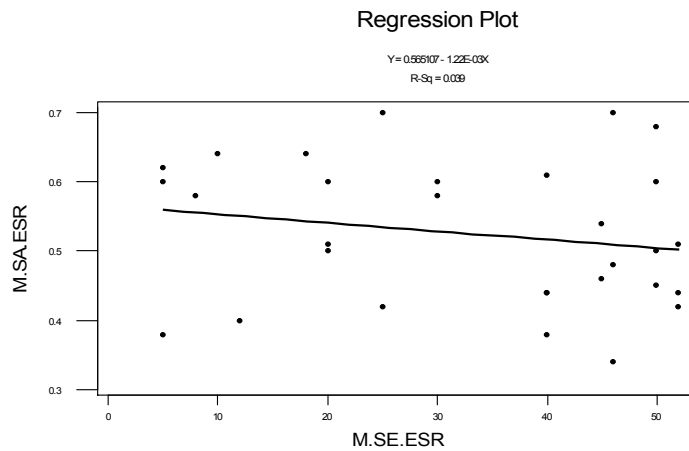


Fig.5:- Correlation between serum and salivary male estrogen.

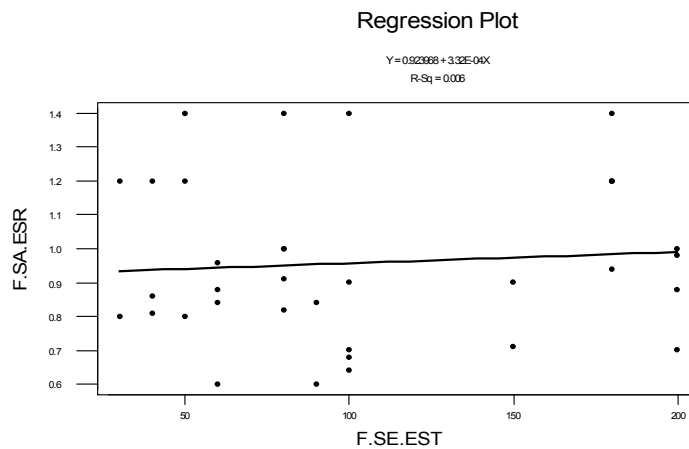


Fig.6:- Correlation between serum and salivary female estrogen.

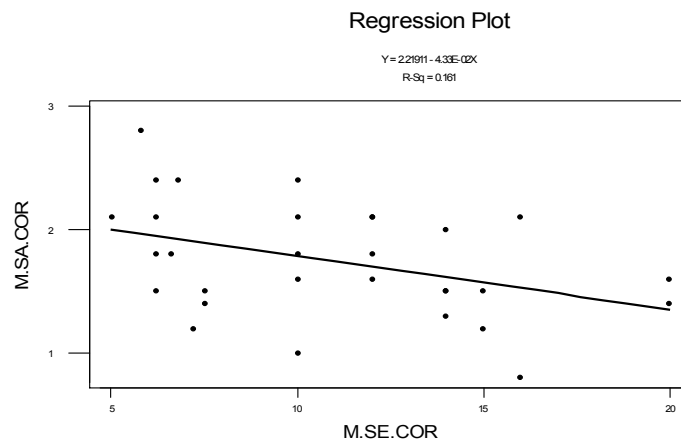


Fig.7:- Correlation between serum and salivary male cortisol.

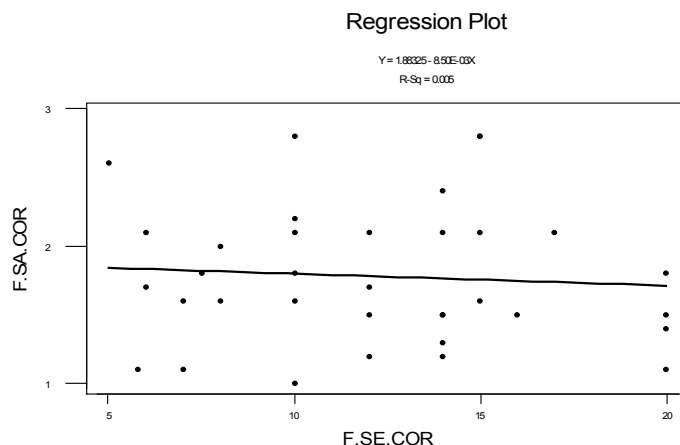


Fig.8:- Correlation between serum and salivary female cortisol.

Discussion

In this study, all serum steroid hormones were increased when compared with saliva in male but in female serum steroid hormones were increased rather than saliva except serum testosterone which decreased, therefore there was a positive correlation between serum and salivary progesterone in male, while there was a negative correlation between serum and salivary testosterone, estrogen and cortisol. However, there was a positive correlation between serum and salivary testosterone and estrogen, while a negative correlation between serum and salivary progesterone and cortisol in female. These results were in agreement with previous studies⁽¹²⁻¹⁶⁾. In the human body the blood supply and the salivary glands are separated by a porous membrane. This membrane allows small molecules to pass, provided the molecules are not polar in nature. Therefore very small and non-polar molecules can cross through this membrane by passive diffusion. This phenomenon applies to steroid hormones, which appear first in the blood supply in both carrier-protein bound (inactive) form and biologically active free-fraction. The biologically active free-fraction migrates through the salivary gland membrane, while the inactive carrier-protein bound fraction is

unable to do so. Salivary samples are easily collectable, non-invasive, and pose no risk to the health of people. Therefore, salivary samples can be easily applied in investigation of the health and development of children. Due to the non-invasiveness and easiness of sample collection, salivary samples may help research the development of children⁽¹⁷⁾. Passage of neutral steroids from blood into the salivary ducts is about 10 times faster than the flow rate of saliva. Because of the rapid passive diffusion of steroids into the saliva ducts, saliva hormone levels are not altered significantly when the flow of saliva is increased with stimulants such as chewing gum⁽⁷⁾.

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