Determination of Testosterone and Cortisol Levels in Sera and Saliva of Women Patients with Ovarian Tumors (Case – Control Study)

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ABSTRACT

It has been reported that measurements of salivary hormone levels are of clinical importance if they reflect the hormone level in the serum, or if a constant correlation exists between salivary and serum hormone levels[1]. The goal of the present study was to test the possibility of using vibration in the cortisol and testosterone levels in saliva as an indication of that which take place in serum of patient with ovarian tumors.

Women patients with benign and malignant ovarian tumors (n=16) aged from (20-39) years attending various hospitals in Baghdad were included in the present study. Age matched healthy women (n=8) were also included to be used as normal control.

The study involved measurement of the level of testosterone (T) and cortisol (C) in the serum and saliva specimen of the above studied groups. The Anabolic/Catabolic Index (ACI), which was represented by T/C ratio, was estimated in these specimens. The results showed no variation in testosterone levels ($p>0.01$), while highly significant increase in cortisol levels ($p<0.001$), and a significant decrease of T/C ratio ($p<0.05$) were found upon malignancy.

Out of the correlation results that have been drawn between the sera and saliva levels of different biochemical parameters included in the present study, a conclusion can be achieved that is saliva can not be used as a test fluid instead of sera as far as the patients and the different parameters included in the present study are concerned.

Key Words: Ovarian tumors, Testosterone, Cortisol, T/C ratio, Anabolic/Catabolic Index.

تعيين مستوى التستوستيرون والكورتيزول في مصول ولعاب النساء المصابات باورام المبيض (دراسة حالة - شاهد)

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الملخص

إن قياس مستويات الهورمونات في اللعاب ممكن أن يكون له أهمية سريرية إذا كان انعكاس لمستوى ذلك الهورمون في مصل الدم أو في حالة وجود علاقة ارتباط ما بين مستوى الهورمون في اللعاب مع مستواه في الدم (1). من هنا جاءت فكرة البحث في إمكانية استخدام التغيير في مستويات هرموني الكورتزول(C) والتستوستيرون (T) في اللعاب كمؤشر عما يحدث في مصل الدم.

شملت الدراسة (16) عينة من مريضات مصابات بأورام بالمبيض حميدة وخبيثة تم إدخالهم إلى مستشفيات مختلفة في بغداد وبمعدل عمري يتراوح من (20-39) سنة وقد جمعت من النساء الطبيعيات كمجموعة شاهد (8) عينات في نفس المديات العمرية.

تضمنت هذه الدراسة قياس مستوى هرموني الكورتزول والتستوستيرون وحساب النسبة T/C في المصل واللعاب لمجاميع الدراسة ،حيث أظهرت النتائج عدم وجود تغير يذكر بمستوى التستوستيرون ($p>0.01$) بينما لوحظ حدوث ارتفاع معنوي بمستوى الكورتزول ($p<0.001$) وانخفاض معنوي في النسبة T/C ($p<0.05$) في مصل ولعاب النساء في حالة الإصابة بالأورام الخبيثة .

من خلال نتائج العلاقة المرسومة بين المصل واللعاب لمستويات المعايير الكيميائية الحيوية المختلفه المتضمنة في هذه الدراسة في المصل واللعاب نستنتج إنه لا يمكن استخدام اللعاب كسائل اختبار بدلاً من المصل لقياس هذه المعايير المختلفة في الحالات المرضية المشموله في هذه الدراسة.

الكلمات المفتاحية: أورام المبيض والتستوستيرون والكورتزول ونسبة التستوستيرون إلى الكورتزول ومعيار الابتناء/ التقويض.

Introduction

Generally there are two major processes involved in cell growth; proliferation and differentiation. Cancer is a disease of abnormal growth, when the growth and development of normal cells lose control, tumor cells begin to emerge.[2] Tumors of the ovary are common forms of neoplasia in women, among cancers of the female genital tract, the incidence of ovarian cancer ranks below only carcinoma of cervix and the endometrium. Ovarian cancer accounts for 6% of all cancers in the female and is the fifth most common form of cancer in women in the U.S.A.[3, 4] In addition, because many of these ovarian neoplasms cannot be detected early in their development, they account for a disproportionate number of fatal cancers, being responsible for almost half of the deaths from cancer of the female genital tract.[5] It is the seventh most common malignant tumor among the women in Iraq. The Iraqi cancer registry estimated that in Iraq there is a threefold increase in the incidence of this disease during the last two decades.

Medical researches suggest that cancers of the reproductive organs may be affected by naturally occurring steroid hormone produced by the endocrine system. [6]

Testosterone is a powerful anabolic hormone that stimulates and controls the development of muscle, bone, skin, sex organs and spermatogenesis, and most other masculine physical features. Recently, scientists have discovered that testosterone also aids mental function, enhancing both visual and perceptual skills. Common causes of increased serum testosterone levels include polycystic ovaries, adrenal tumors and adrenal hyperplasia. It was suggested that testosterone may have important anabolic effect on protein metabolism. [6]

Cortisol is a defense hormone protecting the organism against any abrupt changes in the physiological equilibrium by affecting carbohydrate, protein and lipid metabolism (catabolism) ,as well as electrolyte balance, in many tissues it inhibits DNA, RNA, and protein synthesis and stimulates the degradation of these macromolecules.[7] While short-term elevations of cortisol represent a normal adaptive response to life-threatening situations, injury and/or illness, prolonged cortisol elevations are extremely unhealthy and harmful. Chronically-high cortisol levels have been linked to depression, osteoporosis, obesity, heart disease, cancer and diabetes.[7,8] Generally measuring

cortisol levels does not provide sufficient information that is due to its biphasic response to stress[9].

An adequate balance of both the anabolic hormones (testosterone and DHEA) and catabolic hormones (cortisol and estrogen) which have just the opposite effect are essential for healthy muscle growth, tissue repair, immune response and metabolism. [8, 10]

Generally there is no universally accepted biomarker for anabolic metabolism. Most clinicians only encounter the terms anabolic and catabolic in relation to wasting syndromes that affect AIDS and cancer patients. The ratio of serum testosterone (T) to cortisol (C) has been used as an anabolic /catabolic index (ACI). The ACI test measures and reports one important indication of anabolic drive; it is a snapshot view of repair and rebuilds activity. This test is looking at the macro view, which assesses the body's ratio of damage to repair. This ratio in saliva was proposed as an anabolic/catabolic index also[11].

Human whole saliva is a mixed fluid comprising secretions from major and minor salivary glands, a serum transudation from the gingival cervices as well as components from oral microorganisms, leukocytes and epithelial cells[12]. It has been used as a source of non invasive investigation of metabolism. At present saliva represents an increasingly useful auxiliary means of diagnosis[13]. The value of salivary biomarker for diagnostic and prognostic assessment has become increasingly well established in clinical medicine, pharmacology and dentistry [14].

The aim of this study was to test the possibility of using the variation in the cortisol and testosterone levels in saliva as an indication of that which takes place in serum.

Material and Methods

Patients:

A case-control design was used in which newly diagnosed patients with serious ovarian tumors were compared with age matched healthy controls.

The inclusion criteria were:

1- Females patients with benign serious ovarian tumors.
2- Females patients with malignant serious ovarian tumors.

The exclusion criteria were:

1- Abnormal psychological stress.
2- Infectious disease & immunological – associated disease or presence of any major systemic diseases.

3- Smoking .
4- Alcholol abuse.
5- Thyroid dysfunction, adrenal disorders, hyperprolactinemia or any other pathological hormone parameter.

Two groups of ovarian tumors patients were used through out this study. The first group consisted of (8) premenopausl patients with benign serious ovarian tumor, cyst adenoma, (age=20-39 years). The second group consisted of (8) premenopausl patients with malignant serious ovarian tumor, cyst adenocarcinoma, (Age=22-37 years).These two groups were matched with a control group consisted of (8) premenopausl healthy women as confirmed by medical examination performed by the specialist (Age=19-37 years). The samples were taken from the Medical City Hospital and AL-Habebia hospital, they were histologically proven under the supervision of specialists: - Dr. Nawal Alash, Dr. Raji Al-Hadithi and Dr. Luay Edwar.

Chemicals:

All chemicals and reagents used throughout this work were of highly analar grade. CIS bio International kits were used for hormonal assays.

Samples:

Blood samples:

Five milliters of blood samples were obtained from patients undergoing hysterectomy by venipuncture just before surgery, and from healthy women as controls. Blood samples were left for 30 min. at room temperature, after coagulation, sera were aspirated by centrifugation at (3000xg) for 10min. Sera were aspirated and stored in capped sterilized tubes at (-20) °C until time of use.

Saliva samples:-

Saliva samples were collected at the morning from the patients and healthy women after thoroughly rinsing the mouth with water, the saliva was centerifuged after collection and the supernatant was stored at (-20) °C until used for different investigation.

Methods:

Free testosterone and cortisol levels were measured in sera and saliva of normal, benign and malignant ovarian tumors patients by radioimmunoassay method (RIA) [15, 16].

The T/C ratio was also calculated:-

T/C ratio = testosterone level in sera or saliva (ng/ml) / cortisol level in sera or saliva (ng/ml)

Statistical analysis:

The data throughout this work was reported in the form of (mean value ± the standard deviation). Quantitative differences between groups were determined by student T-test, where difference is considered as highly significant when ($P<0.001$), significant when ($p<0.05$), and non significant when ($p>0.01$). The linear regression curve was used to test the correlation between the levels of the hormones in the serum and saliva samples.

Results and discussion

This work was devoted to measure the changes in testosterone (as an anabolic state) and cortisol (as catabolic state) in the patient groups compared to that of control group.

The results in table (1) show that there was no significant variation in testosterone levels in the two groups of ovarian tumors patients when compared with that of control group in serum($p>0.01$) and saliva($p>0.01$). While a highly significant increase in cortisol levels in the two groups of ovarian tumors patients was observed when compared with that of the control group in serum($p<0.001$) and saliva($p<0.001$) .

Table (1). The testosterone and cortisol levels in sera and saliva

	serum		**saliva**	
Groups**	Testosterone (ng/ml)*	Cortisol (ng/ml)*	Testosterone (ng/ml)*	Cortisol (ng/ml)*
Normal	0.7 ± (0.1)	50 ± (1)	0.04 ± (0.002)	7.8 ± (1)
Benign	0.67± (0.04)	121.5±(12.5)	0.039 ± (0.003)	12.85±(0.25)
Malignant	0.72 ± (0.1)	230 ± (20)	0.0395±(0.0025)	20 ± (2)

* Mean value ± (S.D) ** The number of samples (saliva and serum) for each group was (8)

The T/C ratio was estimated in sera and saliva samples from patients with benign and malignant ovarian tumors and control group. The results in table (2) revealed a significant decrease of T/C ratio in ovarian cancer patients compared with that of benign patients ($p<0.05$) in sera and saliva, and in sera and saliva of normal patients ($p<0.05$) respectively.

Table (2). The T/C ratio in sera and saliva

Groups	Sample size	Serum T/C *	Saliva T/C*
Control	8	0.014 ± (0.002)	0.0052 ± (0.0008)
Benign	8	0.0056 ± (0.0008)	0.0032 ± (0.0004)
Malignant	8	0.0034 ± (0.0006)	0.002 ± (0.0003)

*Mean value ± (S.D)

From table (2) the results in normal group (high T/C ratio) reflect the presence of anabolic state. While upon transformation to malignant group (low T/C ratio) the results reflect that the metabolic pathway changes upon malignancy to catabolic state. The terms "anabolic" and "catabolic" refer to the specific effect that these hormones have on the growth, maintenance and repair of cells inside the body[17]. The transformation of the T/C ratio towards the catabolic state in the patients with malignant tumor was in agreement with the increase of the RNase activity reported in patients with ovarian cancer which is necessary to hydrolyze the large number of RNA molecules that are accumulated in the cancer cell that mediate the increase of proteins synthesis[17], and also with previous study which reported increasing the DNase activity in ovarian cancer, that is necessary to hydrolyze the large number of the DNA molecules that are accumulated in the cancer cell[18]. The transformation of a normal cell into a cancer cell is a multistep process that involves altering not only the mechanisms that regulate cell replication but also those that control the invasiveness of the cell and its ability to subvert the body's defense mechanisms[19]. The change in this ratio was in agreement with the increase in energy requirement of the cancer cells.

To test the possibility of using the variation in the cortisol and testosterone levels in saliva as indication of that in serum, the correlation between cortisol and testosterone levels and T/C ratio in sera and saliva were studied. Figs. 1, 2, 3 (A,B,C) show the correlation between testosterone, cortisol, and T/C ratio in serum and saliva**.**

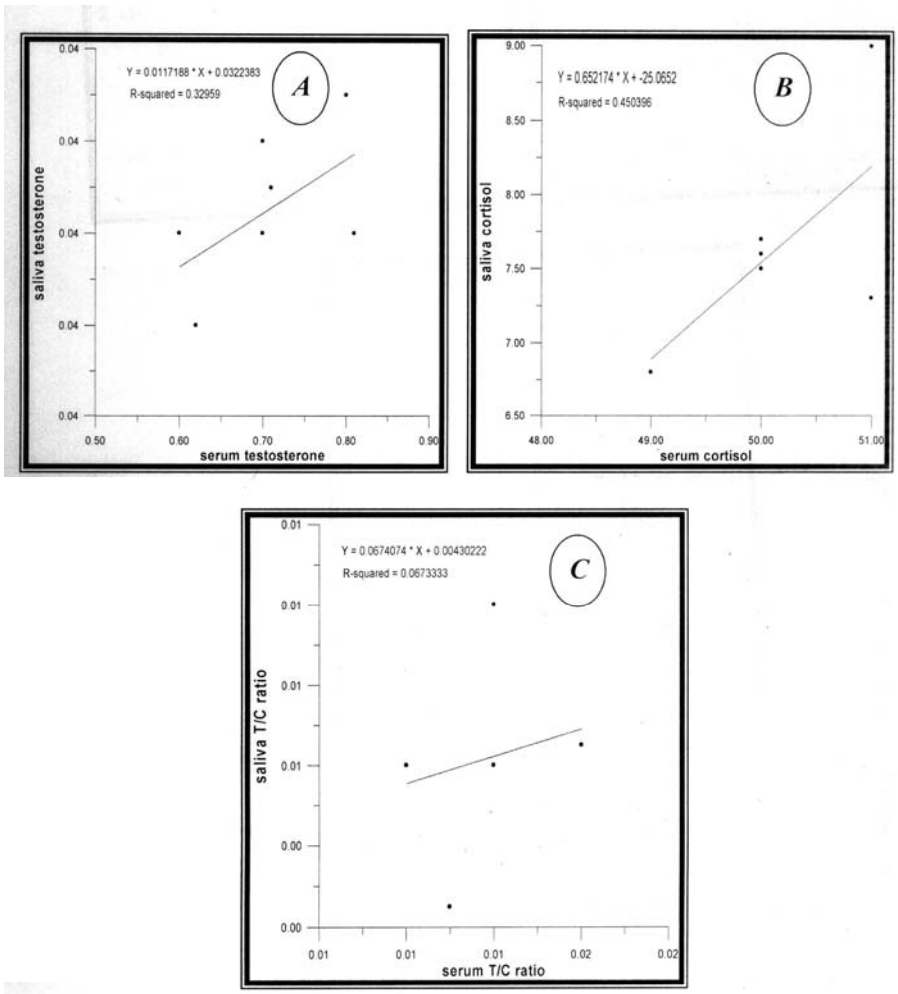

Fig. (1). The correlation between serum and saliva hormones levels in control individuals.

A: testosterone B:cortisol C: T/C ratio

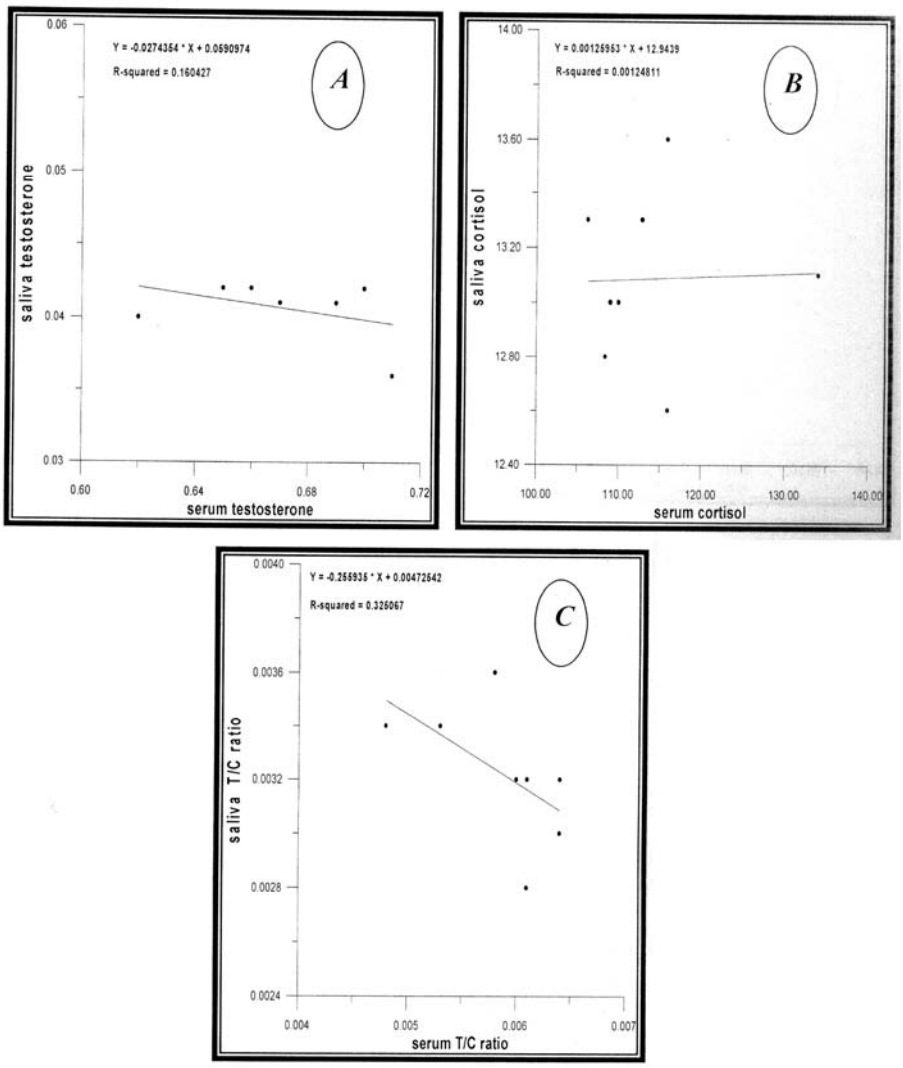

Fig. (2). The correlation between serum and saliva hormones levels in patients with benign tumor.

A: testosterone B: cortisol C:T/C ratio

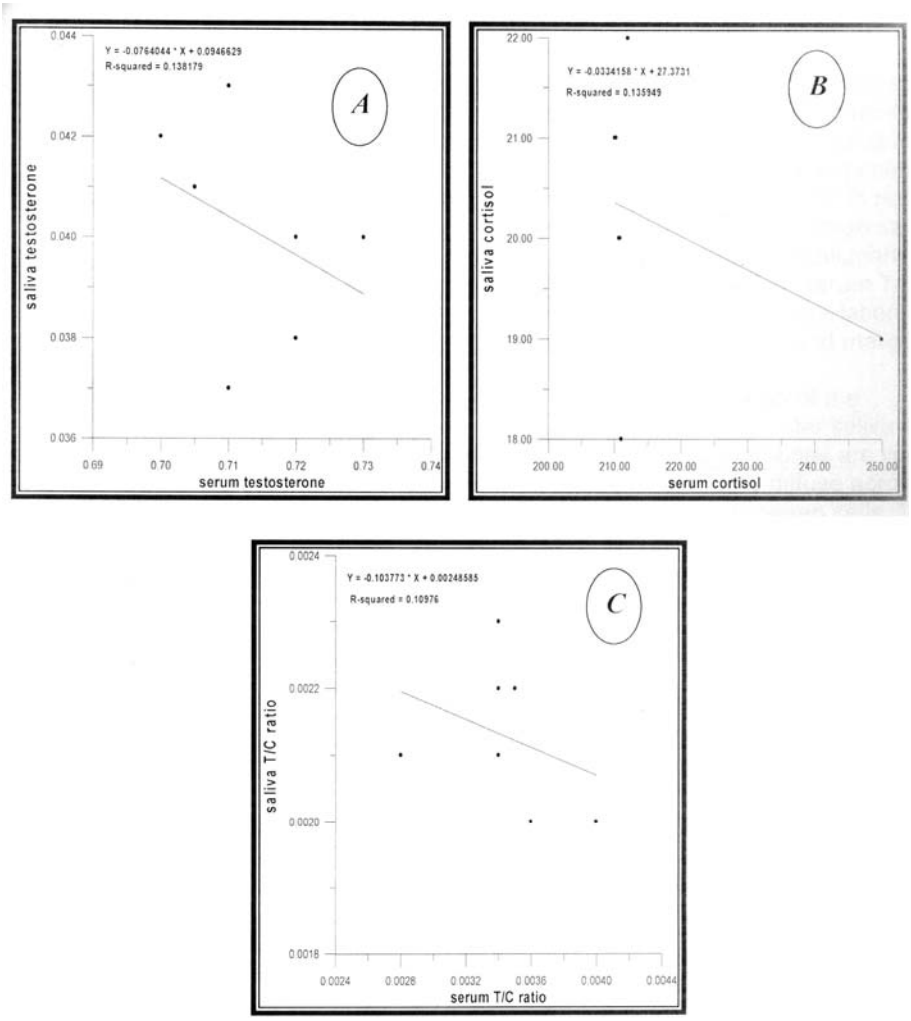

Fig. (3). The correlation between serum and saliva hormones levels in patients with malignant tumor.
A: testosterone B: cortisol C: T/C ratio

It is clear that there is a weak positive correlation between serum testosterone and saliva testosterone(r = 0.329) in normal samples, while this correlation changes to a negative weak correlation in patients with benign samples (r=-0.16) and in patients with malignant samples (r=-0.138). A weak positive correlation between serum cortisol and saliva cortisol (r=0.450) in normal samples, and a very weak positive correlation in patients with benign samples (r=0.0012) while a negative weak correlation in patients with malignant samples (r=-0.135). A very weak positive correlation between serum T/C ratio and saliva T/C ratio (r=0.067) in normal samples, while this correlation change to a negative weak correlation in patients with benign and malignant samples (r =- 0.325) and (r = -0.109) respectively.

It has been reported that saliva can be analysed as part of the evaluation of endocrine function. 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 (ultrafiltration;). The molecular-weight cut-off for ultrafiltration is 100-200. This relatively small molecular size prevents many hormones from entering saliva from serum by means of ultrafiltration. In addition, active transport does not appear to facilitate hormone transfer into saliva. 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 (20).

From the results of the present work, it is clear that there is no correlation between the variation of hormones levels in serum and in saliva. So a conclusion can be drawn that the saliva can not be used instead of serum to measure the variation in the cortisol and testosterone levels in patients with the diseases the present study deals with.

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