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# POSSIBLE USE OF SALIVA AS A DIAGNOSTIC TOOL IN HYPOTHYROIDISM

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**ABSTRACT**
Hypothyroidism is caused by inadequate supply of or response to, thyroid hormones throughout the body and considered the number one endocrine system problem. There has been increasing interest in the diagnosis based on saliva analyses. This study aims to assess the use of saliva samples for the diagnosis of hypothyroidism disease, by estimating thyroid hormones ($T_3$, $T_4$ and TSH) in saliva. The present study included 90 subjects, 60 of them were hypothyroid patients with age ranged between (20-64) years, and 30 subjects as control with age between (20-53) years. Serum and saliva samples were collected from study groups; and the levels of thyroid hormones (TSH, $tT_4$, $fT_4$, $tT_3$ and $fT_3$) were determined by an enzyme linked immunosorbent assay (ELISA). The present study found that serum TSH levels are significantly higher ($P<0.001$) in patients group as compared to control. Whereas, serum levels of $tT_4$, $fT_4$, $tT_3$ and $fT_3$ in patients groups were significantly low ($P<0.001$) as compared to control. However, there are no significant differences ($P>0.05$) in salivary TSH, $fT_4$ and $fT_3$ levels among study groups, but the values of hormones are parallels to their values in serum. The current study revealed that although salivary levels are parallel to their levels in serum, but does not reflect their concentration in serum in any clinically useful way.

**KEYWORDS:** hypothyroidism, thyroid hormones, serum, saliva.

## INTRODUCTION

Thyroid hormones act on nearly all cells in the body with various functions including; development, growth and increasing the basal metabolic rate, affecting protein synthesis and regulating the metabolism of protein, lipids and carbohydrates with an alteration in oxygen consumption (Yen, 2001; Sinha and Yen, 2014). Thyroid disease is the second most public disorder of the endocrine system that can affect any system of the body. It is amongst the most prevalent of medical conditions, especially in women (Beastall *et al.*, 2006; Babu and Patel, 2016). Thyroid disorders are commonly separated into two major categories, hyperthyroidism and hypothyroidism (DeRuiter, 2002; Babu and Patel, 2016). Hypothyroidism is caused by inadequate supply of or response to, thyroid hormones throughout the body and considered the number one endocrine system problem and it affects hundreds of millions around the world (Jonklaas *et al.*, 2014; Rowe *et al*., 2016). It most frequently reflects a disease of the gland itself (primary hypothyroidism), but can also be central hypothyroidism; either caused by pituitary disease as secondary or hypothalamic disease as tertiary (Dillman, 2000; Cooper and Ladenson, 2011).

Saliva is an exocrine secretion of salivary gland. It is an important biological fluid in oral physiology (Holsinger and Bui, 2007). According to several data it mirrors general health condition thus reflecting various systemic changes in the body (Chiappelli *et al.*, 2006; Nagler, 2008). There has been increasing interest in diagnosis based on saliva analyses, because saliva has a simple and non-invasive collection method and relatively stress free, painless, and possible several times a day to provide accurate and reliable assessments of diurnal fluctuations of, the unbound and biologically active, form of certain hormones and drugs. Oral fluid sampling is safe for the operator, with minimal risks of cross-contamination, and easy and low-cost storage (Bonne and Wong, 2012; Javaid *et al*., 2016).

Another reason that makes saliva interesting for diagnostic purposes is the linkage with traditional biochemical parameters which appear in the circulation in various forms (Tiwari, 2011). In addition to its oral indications, the analysis of saliva provides important information about the functioning of various organs within the body. In this respect, endocrine research certainly occupies a central role (Chiappin *et al.*, 2007; Tiwari, 2011). Therefore, this study aims to assess the use of saliva samples for the diagnosis of hypothyroidism disease, by estimating thyroid hormones ($T_3$, $T_4$ and TSH) in saliva.

## MATERIALS & METHODS

The present study was carried out on 60 (4 males and 56 females) Iraqi hypothyroid patients their age ranged (20-64) years, were rounded up from Nuclear Medicine and Radiation Therapy Department, Educational Oncology Hospital. Beside 30 (3 males and 27 females) volunteers subjects who were considered as control, their ages and gender were matched with patients, their age ranged between (20-55) years. Serum and saliva samples were

collected from study groups. All patients had no complained of other chronic or systemic diseases, and pregnant women were excluded from the study. Approximately (4 ml) of human blood was collected intravenous from patient and control groups; also, un-stimulated saliva (3 ml) was collected from studies groups. Both of them centrifuged at 3000 rpm for 10 minutes. Serum of blood was immediately separated and the supernatant of saliva aspirated immediately, both divided into aliquots and kept at -20 °C until used. The diagnosis of hypothyroidism was based on the clinical features and biochemical test. The clinical features include weight gain, cold intolerance, bradycardia, fatigue and visible or palpable thyroid. The biochemical test comprise determined the levels of serum TSH, $tT_4$ and $tT_3$ by using commercially available enzyme linked immunosorbent assay (ELISA) kits (Monobind, USA); $fT_4$, and $fT_3$ by using ELISA kit (Human com., Germany). Detection of thyroid hormones (TSH, $fT_4$ and $fT_3$) in saliva was done by using human ELISA kits (MyBiosource; USA). The Statistical Analysis System- SAS (2012) program was used to identify effect of difference factors in study parameters. The outcome quantitative variables were normally distributed, and therefore conveniently described by mean, standard error (SE) and tested for statistical significance by t-test with least significant difference (LSD)**.**

**RESULTS & DESCUSSION**

The results presented in this study are based on the analysis of 60 hypothyroidism patients compared with 30 individuals as controls. The age of patients ranged between (20- 64) years with a mean age of (39.88 ± 1.423) years. However, the majority (43.33%) of patients are at the age group of (35-45) years. Furthermore, there is a significant female's predominance among patients group. Males/females ratio is (1:14) and no statistically significant differences ($p>0.05$) in age or gender existed between patients and controls groups. The results of this study revealed that serum TSH levels are significantly higher ($P<0.001$) in hypothyroid patients (10.26 ±0.36 µIU/ml) as compared to control group (5.99 ± 0.2552 µIU/ml). On the other hand, serum levels in patients of $tT_4$, $fT_4$, $tT_3$ and $fT_3$ (3.54 ± 0.17µg/dl, 0.891 ± 0.04 µg/dl, 0.572 ± 0.02 ng/dl, and 1.227±0.06pg/ml) are significantly low ($P<0.001$) as compared to control for $tT_4$, $fT_4$, $tT_3$ and $fT_3$ (6.12 ± 0.37 µg/dl, 1.489 ± 0.08 µg/dl, 0.813±0.04 ng/dl and 2.206 ± 0.16 pg/ml) respectively, these results illustrated in the table (1).

Regarding salivary TSH, $fT_4$ and $fT_3$ levels, the current result found that there are no significant differences ($P>0.05$) between study groups of patients and controls in TSH (1.37 ± 0.168 and 0.96 ± 0.22 ng/ml), $fT_4$ (10.42± 1.49 and 14.73 ± 2.57 ng/ml) and $fT_3$ (20.75 ± 2.13and 25.11 ±3.25ng/ml) respectively, but the values of hormones are parallels to their values in serum, tables (2).

**TABLE 1:** Serum thyroid hormones levels in study groups

| Hormones in serum (Mean ± SE) | TSH (µIU/ml) | tT4 (µg/dl) | fT4 (µg/dl) | tT3 (ng/ml) | fT3 (pg/ml) |
|---|---|---|---|---|---|
| Patients N= 60 | 10.26 ±0.36 | 3.54 ± 0.17 | 0.891 ± 0.04 | 0.572 ± 0.02 | 1.227 ± 0.06 |
| Control N= 30 | 5.99 ± 0.25 | 6.12 ± 0.37 | 1.489 ± 0.08 | 0.813±0.04 | 2.206 ± 0.16 |
| t-test *(P-value)* | P<0.001** | P<0.001** | P<0.001** | P<0.001** | P<0.001** |

**: Highly Significant (P<0.001); SE: Standard Error

**TABLE 2:** salivary thyroid hormones levels in study groups

| Hormones in saliva (Mean ± SE) | TSH (ng/ml) | fT4 (ng/dl) | fT3 (ng/ml) |
|---|---|---|---|
| Patients N= 60 | 1.37 ± 0.168 | 10.42± 1.49 | 20.75 ± 2.13 |
| Control N= 30 | 0.96 ± 0.22 | 14.73 ± 2.57 | 25.11 ± 3.25 |
| t-test *(P-value)* | 0.524 $^{NS}$ | 0.324 $^{NS}$ | 9.27 $^{NS}$ |

NS: Non Significant; SE: Standard Error

The results of serum TH levels are in accordance with the observations of the previous researchers (Mortoglou and Candiloros, 2004; Joshi (2011); Senthilkumaran *et al.*, 2015 and Hasan *et al.* 2016), who demonstrated that hypothyroidism patients have elevated serum level of TSH and decrease levels of $T_4$ and $T_3$ than that in controls. Consistency, Li *et al*. (2014) revealed that there are significant differences between controls and hypothyroid patients in levels of $tT_4$, $fT_4$, $tT_3$, $fT_3$ and TSH. On the other hand, previous Iraqi study done by Sultan and Jumma in (2015) showed a significant increase in the level of TSH and significant decrease $T_4$ level among overt hypothyroid patients in comparison with control, while the lowering in $T_3$ levels was not significantly than control. Jayan and colleagues (2015) suggested that elevation in serum TSH is an early and sensitive indicator of decreased thyroid reserve and in conjunction with decreased $fT_4$ and $ft_3$ is diagnostic of primary overt hypothyroidism.

It is well known that patients with low thyroid hormone levels have increased TSH levels because of the negative feedback relationship between the different hormones. Therefore, the results of the thyroid function tests for overt hypothyroidism are characterized by a low serum $T_4$ level and an elevated serum TSH level. The majority of hypothyroidism cases result from primary thyroid failure. Consequently, pituitary gland responds to that failure by secreting more TSH, raising serum TSH levels fairly before there is a detectable decline in circulating thyroid

hormones $T_4$ and $T_3$. Thus, elevated TSH level is the earliest and most definitive indicator of hypothyroidism. As thyroid failure progresses, $T_4$ and $T_3$ levels eventually become very low or even undetectable, and the TSH level increases, afterward, the levels of $fT_4$ and $fT_3$ reduced (DeRuiter, 2002). **C**oncerning the salivary TH levels in this study, the findings are consistent with serum TH levels in that there are also elevation in TSH and lowering in $T_4$ and $T_3$ levels among patients group as compared to controls but statistically non-significant. Correspondingly, Gotovtseva and Korot'ko (2002) measured $tT_4$, $fT_4$, $tT_3$, $fT_3$ and TSH concentrations in serum and saliva and found coincidence between them. Likewise the results are coinciding with Putz *et al*. (1985) and Chiappin *et al*. (2007) showed that salivary thyroid hormone values are consistent with serum values.

In contrast to present results Elson and coworkers (1983), collected serum and saliva from 32 euthyroid volunteers and observed that salivary $T_4$ and $T_3$ levels were higher than serum levels, about ten folds higher than anticipated. Likewise Shames and Shames (2015) demonstrated that TSH level in saliva was high, not like normal results on blood testing. Furthermore, Vining *et al.* (1983) revealed that the concentration of thyroxin in saliva probably does not reflect their concentration in plasma in any clinically useful way. Thyroxin enters saliva via the ultra-filtration route or by contamination of the saliva by plasma or gingival fluid. The concentration of conjugated steroids, thyroxin, and protein hormones in saliva probably does not reflect their concentration in plasma in any clinically useful way, because of the proposition that plasma proteins may cross the salivary glands and carry bound $T_4$ into the saliva that may be strongly affected by protein binding in saliva (Vining *et al.,* 1983). However, all evidence suggests that plasma proteins are too big to cross the salivary membranes, and the reason for their presence in variable, trace amounts in saliva is contamination with blood (from minor abrasions in the mouth) or gingival fluid; even trace contamination with saliva may easily outweigh any contribution due to passive diffusion of the free fraction from plasma across the salivary glands (Chiappin *et al.,* 2007). So, measurement of $T_4$ in saliva as an index of $fT_4$ in plasma was complicated by the presence of $T_4$- binding proteins in saliva. The protein binding of $T_4$ in whole saliva is probably due to trace contamination of saliva with plasma or gingival fluid is likely to cause a large variability in the concentration of hormone in saliva (Vining *et al.,* 1983). Conducted study confirms the fact that hypothyroidism recognized by elevated serum TSH and decline $T_4$ and $T_3$ hormones. Although salivary levels of TSH and unconjugated $T_4$ and $T_3$ hormones parallel to their levels in serum of hypothyroid patients, but probably does not reflect their concentration in serum in any clinically useful way.

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