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Interleukin-1 β , interleukin-6 and tumour necrosis factor-alpha levels in blood and saliva in hypothyroidism accompanied with periodontitis

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ABSTRACT

Relationship between thyroid dysfunction and periodontal disease has been mediated through an immune response. Cytokines are implicated in the initiation, consequences of immune response and a crucial role in the pathogenesis of thyroid disease, directly target thyroid follicular cells; and in the development and progression of periodontitis. This study aimed to detect cytokines levels which known to be associated with periodontitis in serum and saliva, to test the hypothesis that hypothyroidism influences the levels of biomarkers of periodontitis. Samples were collected from sixty patients with hypothyroid age ranged (20-64) years, thirty of patients were without periodontitis (group I) and 30 with periodontal disease (II); moreover, 30 subjects considered as control (group III) with age (20-53) years. Detection of cytokines was performed by ELISA. The results showed a significant elevation in serum and salivary levels of IL-1 β ($P < 0.001$) among patients' groups (I and II) as compared to group III, as well IL-1 β increase significantly in group II ($P < 0.001$) than in group I and also than group III. There are non-significant differences ($P > 0.05$) in serum level of IL-6 and TNF- α and salivary levels of TNF- α among all study groups. Nevertheless, the salivary level of IL-6 is increased significantly ($P < 0.05$) in group II as compared with group I and group III, and their non-significant differences ($P < 0.05$) between groups I and III. The present finding proposed that hypothyroidism might encourage periodontitis development; as well as serum and salivary levels of IL-1 β , with salivary IL-6 may represent important biomarkers for the early detection of periodontitis in hypothyroid patients.



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INTRODUCTION

Hypothyroidism is a clinical condition that results from insufficient production or action of T₄ and T₃, leading to a total decrease in metabolic processes.

Autoimmune thyroid diseases (AITD) like Hashimoto thyroiditis (HT) are complex diseases in which autoimmunity act against auto-antigens.

They occur due to loss of tolerance to auto-antigens thyroid peroxidase (TPO) and thyroglobulin (Tg) which leads to the infiltration of the gland (Wiersinga, 2016).

Periodontitis is a type of oral infections. It is a disease of the upholding texture of the teeth caused by the flaming host response to bacteria in the gingival plaque, or by gram-negative bacteria lead to the destruction of periodontal ligaments and alveolar bone with pocket formation, recession or both (Gomes *et al.*, 2016). Recently, the relationship between the endocrine system especially thyroid dysfunction and periodontal disease has been mediated through the immune system. The decrease in serum TSH levels within the normal range

among hypothyroid patients following non-surgical periodontal treatment leads to a reduction in inflammatory markers responsible for periodontal as well as hypothyroid condition (Bhankhar *et al.*, 2017).

Cytokines are small proteins synthesized by a variety of cell types and significantly contribute to the immune system. Their major function is to regulate immune response and set autoimmunity (Tawfig, 2016; Khan *et al.*, 2015). They are involved in the beginning, consequences of immune response and vital role in the pathogenesis of AITD, directly target thyroid follicular cells by modulating epithelial cell growth and function. Various cytokines like IL-1 β , IL-6, TNF- α and others are found in follicular cells (Khan *et al.*, 2015). It is worthy of mentioning that various studies have demonstrated the presence of pro-inflammatory IL-1 β , IL-6 and TNF- α in thyroid follicular cells (TFC); cytokines are generated in the thyroid by intrathyroidal inflammatory cells, in special lymphocytes and thyroid follicular cells (TFC) themselves and can thus act in a cascade to boost the autoimmune style (Ajjan *et al.* 1996; Ganesh *et al.*, 2011).

Moreover, there is a suggestion that a rise making of pro-flaming cytokines, like IL-1 β , TNF- α , IL-6, play an important part in the evolution and sequence of periodontitis due to the liberation of inflammatory cytokines is neatly linked to a higher tendency to bacterial infection, rise by a change in the immune response (Amrutiya and Deshpande, 2016; Tawfig, 2016). Local make of IL- β and IL-6 can be looked as regulate marker award notification about the periodontal condition, and high levels of crevicular IL-1 β and IL-6 may be a valuable tool in earmark prospect for the soon revelation of periodontal disease (Alwan, 2015).

This study aimed to measure levels of some cytokines known to be associated with periodontitis in serum and saliva, to test the hypothesis that hypothyroidism influence levels of salivary biomarkers of periodontitis.

Subjects and Methods

Sixty Iraqi hypothyroid patients (4 males and 56 females) enrolled in this study, their age (20- 64) years, they were from Nuclear Medicine and Radiation Therapy Department, Educational Oncology Hospital. Total patients divided into two groups; 30 patients with no periodontitis [group I] and 30 patients were diagnosed with periodontitis [group II] by specialized dentists in the Department of Periodontics, College of Dentistry, Baghdad University. Beside 30 subjects (3 males and 27 females) as apparently healthy control [group III], their ages and gender were matched with patients (20-55) years.

Approximately (4 ml) of human venous blood was collected from patient and control groups; also, unstimulated saliva (3 ml) was collected from studies groups. Both of them centrifuged; then serum of blood and supernatant of saliva were immediately separated and kept at -20 °C until used.

The diagnosis of hypothyroidism was made by a specialized endocrinologist and based on the clinical features and biochemical tests that based on elevated serum levels of TSH, low T₄ level, and low or normal T₃ as compared with control.

Assessment of cytokines in serum levels was performed by ELISA Kits (Komabiotech, Korea). Estimation of cytokines in saliva was performed by using ELISA Kits (MyBiosource, USA).

Statistical Analysis: Statistical analyses were done using SAS (2012) program. The variables were expressed as a mean \pm standard deviation; the significance of differences in mean was assessed using the Student's t-test and ANOVA test. Analyses where the P-value was <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Results presented in this study are based on the analysis of 60 hypothyroid patients (30 patients associated periodontitis and 30 patients with no periodontitis) contrast with 30 controls. The age of patients was (20-64) years with a mean age of 39.88 \pm 1.423 years; while for control the age was (20-53) years with a mean age 42.23 \pm 1.657 years. No statistically important distinction ($p > 0.05$) for age or gender found between two study groups.

The current result observed that serum IL-1 β level in hypothyroid group without periodontitis [group I] and hypothyroid with periodontitis group [group II] (240.71 \pm 13.89 and 277.83 \pm 21.49 pg/ml) respectively, are increased significantly ($P < 0.001$) as compared with control [group III] (80.58 \pm 13.75 pg/ml); furthermore, level in group II (277.83 \pm 21.49 pg/ml) is significantly higher ($P < 0.001$) than level of group I (240.71 \pm 13.89).

Moreover, salivary levels of IL-1 β in groups I and II (43.43 \pm 3.98 and 64.52 \pm 2.98 pg/ml) increased significantly as compared to the level of group III (27.68 \pm 2.01 pg/ml). Furthermore, there are significant differences ($P < 0.001$) between two patients' groups I and II (43.43 \pm 3.98 and 64.52 \pm 2.98 pg/ml), where the level in group II of hypothyroid patients with periodontitis is higher than the level in group I without periodontitis, as demonstrated in table (1).

These results are in agreement with a result of Marchiori and coworkers (2015) who showed elevate in the serum grade of IL-1 β in hypothyroid

Table 1: Serum and salivary IL-1 β level in study groups

Cytokine (Mean \pm SE)	Serum IL-1 β (pg/ml)	Salivary IL-1 β (pg/ml)
Group I Hypothyroidism	240.71 \pm 13.89 B	43.43 \pm 3.98 B
Group II Hypo. with Periodontitis	277.83 \pm 21.49 A	64.52 \pm 2.98 A
Group III Control	80.58 \pm 13.75 C	27.68 \pm 2.01 C
LSD	36.08	14.578
ANOVA (<i>P-value</i>)	P<0.001**	P<0.001**

A, B, C: Different letters in the same column represent significant differences; SE: Standard Error; **: Highly Significant (P<0.001)

Table 2: Serum and salivary IL-6 level in study groups

Cytokine (Mean \pm SE)	Serum IL-6 (pg/ml)	Salivary IL-6 (pg/ml)
Group I Hypothyroidism	14.63 \pm 3.15	2.42 \pm 0.59 B
Group II Hypo. with Periodontitis	23.05 \pm 6.97	5.14 \pm 1.03 A
Group III Control	20.42 \pm 3.08	2.77 \pm 0.51 B
LSD	11.27	1.23
ANOVA (<i>P-value</i>)	0.701 ^{NS}	0.016*

A, B: Different letters in the same column represent significant differences

Table 3: Serum and salivary TNF- α level in study groups

Cytokine (Mean \pm SE)	Serum TNF- α (pg/ml)	Salivary TNF- α (pg/ml)
Group I Hypothyroidism	18.84 \pm 3.77	17.19 \pm 2.22
Group II Hypo. with Periodontitis	29.54 \pm 6.20	27.75 \pm 5.81
Group III Control	26.05 \pm 2.54	24.25 \pm 4.70
LSD	12.04	11.05
ANOVA (<i>P-value</i>)	0.473 ^{NS}	0.411 ^{NS}

NS: Non-Significant

Patients. As well as, Kammoun-Krichen *et al.* (2012) revealed elevation in the level of IL-1 β in hypothyroid patients and suggested the possibility of using it to distinguish between thyroid diseases. On the other hand, the present result is disagreement with the result reported by Phenekos and colleagues (2004) who found a decrease in IL-1 β level in Hashimoto patients than Graves's disease (GD) and control.

Actually, it is famed that IL-1 β effect the work of thyroid cells by reducing the term of Tg and TPO, that intensity of IL-1 β alter thyroid epithelial hardship of human thyrocytes by changing the expression, localization and regulation of cross proteins, supported the remarkable part played by IL-1 β in thyroid pathogenesis (Kammoun-Krichen *et al.*, 2012).

About IL-1 β in patients group with periodontitis, Raunio *et al.* (2007) and Hajishengallis (2014) reported that the rise in serum IL-1 β might be demonstrated both by seep of the locally generated IL-1 β from the inflamed periodontal region and by systemic output in reply to the systemically sparse antigen capacity. On the other hand, previous Iraqi study (Al-Ghurabi, 2013) showed that the serum IL-1 β level was significantly higher in patients with periodontitis as compared with the healthy control group, hence concluded that IL-1 β might play a major role in pro-inflammatory response in the serum of patients with periodontitis.

The possible explanation of this elevation among patients with periodontitis may be imputed to the existence of more locally cytokine production. However, Rathnayake and coworkers (2013) revealed that there is an elevation in salivary IL-1 β level in subjects with periodontitis than control,

and suggest that salivary IL-1 β is a good biomarker of periodontitis can discriminate periodontitis from a healthy control.

The current results of IL-6 serum level revealed that there are no important differences ($P>0.05$) between patients groups (I and II) and group III of control (14.63 ± 3.15 ; 23.05 ± 6.97 and 20.42 ± 3.08 pg/ml), respectively. Correspondingly, about salivary levels of IL-6, this study found that there is no important variation ($P>0.05$) between groups I and III (2.42 ± 0.59 and 2.77 ± 0.51 pg/ml) correspondingly. Furthermore, the level in group II of hypothyroid patients with periodontitis (5.14 ± 1.03) is increased significantly ($P<0.05$) than the level in group I of patients with no periodontitis (2.42 ± 0.59), also increased than group III of control (2.77 ± 0.51), as shown in table (2).

The non-significant differences in mean serum level of IL-6 between patients' groups and control group are consistent with other previous studies reported by (Monea *et al.*, 2014; Mikos *et al.*, 2014) who indicated that there was no statistical variation in the IL-6 in hypothyroid than control. Conversely, the result obtained by Marchiori *et al.* (2015) indicated that cytokine serum level is elevated in patients with the hypothyroid disease and decreased gradually after treatment while Kiziltunc *et al.* (1999) and Kammoun-Krichen *et al.* (2012) demonstrated that serum IL-6 decreased significantly in the hypothyroid group and increased significantly in hyperthyroidism than control group.

In general, the level of IL-6 decreased because of decreasing the intrathyroidal making of IL-6 and reduces TNF- α level in serum. TNF- α induces IL-6 liberation by osteoblasts, increase grade of this cytokine may share to the increased manufactured of IL-6 there is providing backup for the concept of a cascade impact of these cytokines by explaining a positive linkage between IL-6 and TNF- α (Kiziltunc *et al.*, 1999). Nevertheless, the non-significant increase serum IL-6 levels in group II than group I of patients may be due to IL-6 is produced locally in the infected periodontal tissue then enter the systemic circulation. This result is confirmed by other reports also revealed non-significant increase IL-6 level in serum of periodontitis as compared to control (Al-Rassam and Tahab, 2014; Talvan *et al.*, 2016).

Furthermore, the result of salivary IL-6 level in hypothyroid patients (group II) with non-significant differences than control (group III), is compatible with results of Olavegogeoascoechea *et al.* (2016) use GCF to measure IL-6 level in hypothyroid patients and found that there were no significant differences with control. Conversely, there is disagreement with the result of Monea *et al.* (2014)

who reported that mean values of salivary IL-6 were statistically higher in hypothyroid subjects as compared to controls. Moreover, the result about salivary IL-6 in patients with periodontitis corresponds with other studies showed that IL-6 in saliva significantly higher in periodontitis than healthy subjects (Ebersole *et al.*, 2013; Javed *et al.*, 2014).

The results of this study that elucidated in the table (3) revealed that there is no considerable variation in serum level of TNF among study groups (I, II and III), (18.84 ± 3.77 ; 29.54 ± 6.20 and 26.05 ± 2.54 pg/ml) respectively. In addition, there are no significant differences in the salivary level of TNF among study groups I, II and III (17.19 ± 2.22 ; 27.75 ± 5.81 and 24.25 ± 4.70 pg/ml) respectively.

This finding is concordant with the result of Drugarin *et al.* (2000) who observed non-significant differences in serum TNF- α in subjects with overt hypothyroidism autoimmune thyroiditis compared with healthy controls, in which this cytokine was barely detectable. As well, Karanikas *et al.* (2004) demonstrated that there was no effect of thyroid hormone on cytokine generation style by T cells, as they noticed that patients with rising values of anti-TPO-Ab had a significantly rising proportion of cells making TNF- α than healthy individuals. On the other hand, this result is inconsistent with the result of Monea *et al.* (2014) who revealed that serum TNF- α concentration for patients with hypothyroidism was 15 times significantly higher than control.

At variance with the current result, Monea and colleagues (2014) showed a significant increase of salivary TNF- α level in hypothyroid patients, and they suggested that this condition of cytokine secretion due to thyroid dysfunction consequently from the circulation spread in the body including the periodontal tissue. However, Teles *et al.* (2009) observed that there is no considerable variation between patients and control groups for TNF- α , concluded that mean salivary levels of TNF- α could not discriminate between periodontal health and disease.

The contribution of TNF- α in the pathogenesis and maintenance of thyroid diseases is as yet unclear. This is mainly due to conflict results reported on the detection of TNF- α mRNA and TNF- α within thyroid tissue. TNF- α was not present in the supernatants of thyrocyte and fibroblast cell cultures tested under basal conditions. Moreover, IL-1 had only a low enhancing effect on TNF- α production in fibroblasts. Thyrocytes contained only a few copies of TNF- α message, which could not be increased by various stimuli (Aust *et al.*, 1996).

An anticipated the TNF- α in serum and saliva is higher in hypothyroidism associated periodontitis group than hypothyroidism group. The role of this cytokines in periodontitis patients without systemic disease was confirmed as reported by Chauhan and his colleagues (2016) who found a significant increase in the serum and saliva TNF- α in periodontitis patients than control, and suggested that the level of TNF- α in saliva may be used as standing by the marker. To clarify the insignificant differences, it is possible that local periodontal production of receptors of TNF- α in serum is not sufficient for a detectable change in serum concentrations (Matić-Petrović *et al.*, 2016).

CONCLUSION

The present finding proposed that hypothyroidism might encourage periodontitis development; as well as serum and salivary levels of IL-1 β , with salivary IL-6 may represent important biomarkers for the early detection of periodontitis in hypothyroid patients.

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