Evaluation of the Level of Interleukin-6 and C-Reactive Protein in Type 2 Diabetic Patients with Candida Sp.

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Abstract
Background and objective: Type 2 diabetes is a serious and chronic disease prevalent in the world, so the current study was designed to reveal the role caused by the cumulative high level of glucose in the blood and to study some immunological variables and blood tests in patients with type 2 diabetes. Methods: the study included 57 patients and they were divided into two groups based on the percentage of glycated hemoglobin, the first group included (diabetes typeII patients with have oral candidiasis ,HbA1C ≥7) and the second group included (diabetes typeII with don't have oral candidiasis ,HbA1C ≥7) while the third group included the healthy group It included 31 individuals, Their ages range between 20-60 years, all patient samples that were taken had a glycated hemoglobin percentage of more than 7. People suffering from chronic diseases such as pressure, heart disease, cancer and immunodeficiency were excluded. Result In type 2 diabetic patients with have Candida and with dont have Candida, elevated IL-6, CRP and elevated WBCs were observed in type 2 diabetic patients with Candida, while LYM was elevated in type 2 diabetic patients without Candida. Conclusion: it was concluded from the study that high blood sugar level and lack of control causes a decrease in the body's immunity and therefore causes severe inflammatory cases, as well as the appearance of oral candidiasis in abundance as a result of weak immunity.
Keywords: diabetes type 2; Oral candidiasis; C-reactive protein; Interleukin-6

1. Introduction

Diabetes Mellitus: It is a chronic disease condition that affects the fitness of a person's life. According to the World Health Organization (WOH), there were more than 171 million people suffering from diabetes in 2000, and it may reach 366 million in 2030, and 2019 statistics show that more than 13.9% of adults suffer from diabetes in Iraq [1,2]. The World Health Organization classifies diabetes into three main types: type 1 diabetes, type 2 diabetes, and gestational diabetes [3]. Each type has its own causes, and none of these types produces sufficient amounts of the hormone insulin by the beta cells of the pancreas [4]. In the first type, autoimmune destruction of the beta cells of the pancreas occurs, while in the second type, the cause is insulin resistance in the cells it infects, and that these cells cannot respond to insulin, which results in the need for more than normal levels of the hormone insulin [5].
Diabetes has short-term and long-term complications, including short-term complications: (ketoacidosis or hyperosmotic coma due to high blood osmotic pressure, hypoglycemia), and if the patient does not receive adequate care, these complications appear [6]. As for the long-term complications, they are diseases of the circulatory system, including (cardiovascular disease and doubling the risk of diabetes), and complications include chronic renal failure, nepharopathy, and retinopathy, which can lead to blindness, as well as neuropathy, and damage to capillaries, which may lead to blindness. It slows the healing of wounds, especially on the feet, which may lead to amputation [7].
Oral candidiasis : It is a chronic fungal disease that affects the oral mucosa and is caused by Candida infection [8]. This fungus causes infections of the tongue and oral mucosal sites and is characterized by excessive fungal growth and invasion of surface tissues [9]. Oral candidiasis is more common in people of extreme ages or those with a wide variety of underlying diseases [10]. Oral candidiasis is frequently seen in patients with poor glycemic control and it has already been shown that an increased load of oral candidiasis is associated with increased levels of glucose in saliva [11]. Because the high salivary glucose content helps overgrowth of Candida in the oral cavity [12]. It has been proven that uncontrolled diabetes and fungal infections make life very uncomfortable and can lead to serious infections. Type 2 diabetics suffer from poor oral health and this leads to increased rates of disease [13].

2. Materials and Methods

Study samples

The current study was conducted in specialized

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clinics in the city of Kirkuk, and 57 blood and oral swab samples were collected from people with Type 2 diabetes and oral candidiasis, taking into account the exclusion of samples of patients suffering from pressure, heart disease and other chronic diseases during a period ranging from December 15, 2021, until April 20, 2022. The number of samples was (males 26, females 31), and the average age of patients ranged from (30-60) years.

Information was collected from the patient, including gender, age, duration of diabetes, weight, height, and whether there are chronic diseases. A total of 31 blood samples and oral swab were collected from healthy people without diabetes and without oral candidiasis and chronic diseases. Diabetes was diagnosed through HbA1c test in the laboratory of the specialized clinic. Oral candidiasis was diagnosed in the laboratories of the College of Education for Pure Sciences, Kirkuk University. The samples of diabetic patients were divided according to the incidence of oral candidiasis into:

1. First group: (30) patients with type 2 diabetes and oral candidiasis, 14 males and 16 females.
2. The second group: (27) patients with type 2 diabetes who do not have oral candidiasis, 12 males and 15 females.
3. The third group: (31) control sample, which included healthy people, 15 males and 16 females

Collecting blood samples and isolating serum

Blood samples were collected from patients with diabetes during the fasting period (8-12 hours). By means of wine syringes, (5) ml of venous blood was withdrawn after sterilizing the area, as (2) ml was placed in a tube containing an anticoagulant (EDTA) ethylene diamine tetraacetic acid, in order to perform the required tests, and (3) ml of the blood sample was placed in Gel Tube, as the blood components were separated by a Centerifuge centrifuge at a speed of 3000 rpm and for a period of 15 minutes to perform the immunological examinations, After that, the blood serum was withdrawn using a micropipette and transferred into the Abendrov tubes and kept at a temperature of (-20 °C) for the purpose of conducting the required tests later.

Oral swabs collection

Oral swabs were collected using Sterial transport medium swab by wiping the roof of the tongue and mucosa, then aseptically immersed cotton buds in sterile culture tubes containing transport medium and transported as soon as possible to the laboratory and incubated at 37°C for 24 hours.

Ethical statements

The Iraqi Ministry of Health and Environment approved the study conducted in accordance with the ethical standards of the Helsinki Declaration.

3. Statistical Analysis

The data was statistically analyzed using a computer, depending on the spss program, and ANOVA was used according to the F-test to test the significance or non-significance of the groups used in the study at a probability level = 0.05 and a probability level of 0.01 and the averages were compared according to Duncan's multi-range test.

4. Results

The results of Table (1) showed that there were significant differences at the probability level (0.0004**) that the average levels of C-reactive protein between the type 2 diabetes group without have oral candidiasis had high levels of significant difference (9.640 ± 2.672) compared to the type 2 diabetes group with have oral candidiasis (6.920 ± 2,250) when compared with healthy controls (2,140 ± 1.636).

The results indicated in Table (1) that there was a significant difference at the probability level (0.049*) that the average interleukin-6 level between the type 2 diabetes group with have oral candidiasis had significant differences levels (30.76 ± 5.72)) compared to the type 2 diabetes group without have oral candidiasis. (30.49 ± 1.61) when compared with the healthy group (27.41 ± 2.29).

The results in Table (1) indicated that there was a significant difference at the probability level (0.0005**) that the white blood cell count among the diabetic type2 group with have oral candidiasis had a severe high level of significant difference (9.183±3.187) when compared with the diabetic type2 group without have oral candidiasis (7.712 ± 2.712) and the healthy group (6.303 ± 0.644).

The results in the table (1) showed that there was no significant difference at the probability level (0.593) in the rate of red blood cell count between the diabetic group with oral candidiasis and the group without diabetes with oral candidiasis, respectively (4.694±1.559 million cells/µl) (4.495± 1.189 million cells/µl) when compared with the healthy control group (4.599±0.778 million cells/µl)

The result in the table (1) showed that there was no significant difference at the probability level (0.613) in the rate of lymphocytes between the type 2 diabetes group with oral candidiasis and the type 2 diabetes group without oral candidiasis, respectively (2.563 ± 1.154) (2.793 ± 0.905) when compared with the group Healthy subjects (2.757±0.627).

Table (1) shows the comparison between the type 2 diabetes group with oral candidiasis and the type 2 diabetes group without oral candidiasis with the control group.

Group Para Meters	Patients with type 2 diabetes with have oral candidiasis	Patients with type 2 diabetes who do not have oral candidiasis	control group	P-Value
	N=30 (Mean ± SD)	N=27 (Mean ± SD)	N=31 (Mean ± SD)	
C-reactive protein (mg/dl)	6.920±2.250 a	9.640±2.672 a	2.140±1.636 b	0.0004**
Interleukin-6 (pg/ml)	30.76±5.72 a	30.49±1.61a	27.41±2.29b	0.049*
White blood cells (103 /µL)	9.183±3.187 a	7.712±2.712 b	6.303±1.879 b	0.0005**
Red blood cells (106 / µL)	4.694±0.766 a	4.495±0.615 a	4.599±0.778 a	0.593ns
Lymphocytes (103 /µL)	2.563±1.154a	2.793±0.905a	2.757±0.627a	0.613ns

The results of the current study indicated in the table (2) that there were significant differences at the probability level (0.003**) in the average levels of C-reactive protein concentration that the males and females of the type 2 diabetes group without oral candidiasis had severe high levels of significant differences (8.57 ± 1.86) (10.38 ± 2.37) for males and females of the type 2 diabetes group with oral candidiasis (7.20 ± 1.89) (6.67 ± 1.92) respectively when compared with males and females of the healthy group (3.04 ± 1.05) (0.94 ± 0.29), respectively.

The results of the study in Table (2) showed that there was a significant difference at the level of probability (0.053*) that the average levels of interleukin-6 concentrations among the males of the type 2 diabetes group with oral candidiasis had high levels of significant differences (32.76 ± 4.32) compared to the type 2 diabetes group. Non-infected with oral candidiasis (30.89 ± 5.14) when compared with the healthy group (25.41 ± 3.52), while it was found that the females in the type 2 diabetes group who were not infected with oral candidiasis had significantly higher levels (30.46 ± 4.12) than the type 2 diabetes group infected with oral candidiasis (29.00±4.86) when compared with healthy controls (28.86±5.92).

Table (2) Comparison of C-reactive protein and interleukin-6 between males and females of the type 2 diabetes group with have oral candidiasis and the group of type 2 diabetes without have oral candidiasis with the control group

Group	Number	Sex	C-reactive protein (mg/dl)	Interleukin-6 (pg/ml)
Patients with type 2 diabetes with have oral candidiasis	14	Male (Mean ± SD)	7.20±1.89b	32.76±4.32a
	16	Female (Mean ± SD)	6.67±1.92b	29.00±4.86ab
Patients with type 2 diabetes who do not have oral candidiasis	12	Male (Mean ± SD)	8.57±1.86ab	30.89±5.14ab
	15	Female (Mean ± SD)	10.38±2.37a	30.46±4.12ab
control group	15	Male (Mean ± SD)	3.04±1.05c	25.41±3.52c
	16	Female (Mean±SD)	0.94±0.29d	28.86±5.92bc
P- Value			0.003**	0.053*

The results of the current study in the table (3) show that there are significant differences at the probability level (0.002**) in the average white blood cell count that the males and females of the type 2 diabetes group with oral candidiasis have high levels of significant differences (8.800 ± 3.599) (9.519 ± 2.856).) respectively for males and females of the type 2 diabetes group without oral candidiasis (8.017 ± 2.740) (7.413 ± 2.678) respectively when compared with males and females of the healthy group (6.973 ± 1.825) (5.638 ± 1.801), respectively.

The results of the current study show in Table (3) that there are no statistically significant differences at the probability level (0.902) in the rate of lymphocyte counts between males and females of the type 2 diabetes group with oral candidiasis and males and females of the type 2 diabetes group without Oral candidiasis, respectively (2.514±1.091) (2.613±1.218) (2.900±0.942) (2.687±0.869) When compared with the males and females of the healthy group (2.833 ± 0.852) (2.681 ± 0.402) respectively.

Table (3) Comparison of blood parameters between males and females of the type 2 diabetes group with have oral candidiasis and the type 2 diabetes group without have oral candidiasis with the healthy group

Group	Number	Sex	White blood cells (103 /µL)	Red blood cells (106 / µL)	Lymphocytes (103 /µL)
Patients with type 2 diabetes with have oral candidiasis	14	Male (Mean± SD)	8.800 ±3.599ab	4.741±0.838a	2.514±1.091a
	16	Female (Mean± SD)	9.519 ±2.856a	4.647±0.721a	2.613±1.218a
Patients with type 2 diabetes who do not have oral candidiasis	12	Male (Mean± SD)	8.017 ±2.740ab	4.649±0.570a	2.900±0.942a
	15	Female (Mean± SD)	7.413±2.678bc	4.342±0.619a	2.687±0.869a
control group	15	Male (Mean±SD)	6.973±1.825cd	4.882±0.765a	2.833±0.852a
	16	Female (Mean± SD)	5.638±1.801d	4.316±0.724a	2.681±0.402a
P- Value			0.002**	0.494ns	0.902ns

5. Discussion

The result in Table (1) showed that elevated CRP was associated with the development of insulin resistance, type 2 diabetes, and persistent levels of hyperglycemia [14]. It is a non-specific biochemical marker of chronic inflammation and a substitute for other inflammatory cytokines such as interleukin-6 and interleukin-8. During well-established infections, the positive relationship between C-reactive protein and interleukin-6 is shown [15]. Increasing the level of inflammatory cytokines in the peripheral circulation causes an elevation of C-reactive protein in liver cells and adipose tissue, as it rises within 48 hours of inflammation and decreases within hours after the absence of stimulation[16].

As for the rise in C-reactive protein in patients with type 2 diabetes with have oral candidiasis, it may be caused by an increase in white blood cells because it is related to the conditions in which these cells are elevated.It appears in both men and women without have oral candidiasis, and it was found that the rise in females increases compared to males and may be due to the higher percentage of body fat or obesity rates in women more than men. It was found that oxidative stress causes an increase in C-reactive protein in a non-acute manner [17].

The results in Table (1) and Table (2) showed an increase in the level of interleukin-6 in the type 2 diabetes group with have oral candidiasis and the type 2 diabetes group without have oral candidiasis when compared with the healthy group. And the rise that occurs in the group of type 2 diabetes with oral candidiasis may be due to the high concentration of glucose in the blood and this leads to an increase in glucose in saliva, which leads to the activation of the growth of fungi in mouth. Fungi are able to attach to biotic and abiotic surfaces and invade tissues. Once the biofilm of Candida albicans comes into contact with the mucosa, it can trigger an innate and adaptive immune response, a process dependent on Toll-Like Receptors (TLRs) such as TLR2 and TLR4. These receptors stimulate inflammatory cytokines, which leads to a rise in interleukin 6 [18].

The increase in white cells in type 2 diabetic patients with have oral candidiasis compared to the non-diabetic group without have oral candidiasis and the control group, and these results did not agree with what was indicated Amin et al., Which concluded that there was no significant difference between the type 2 diabetic group with Candida albicans and those without Candida albicans with the control group [19].

This rise may be due to lack of control of blood glucose and lack of control of HbA1c and lipid-modifying therapies [20]. And the resulting rise in males may be due to the large number of patients' anxiety, and that psychological pressure and what results from it and exercising abnormally, these reasons lead to an increase in white blood cells. In women, it may be due to weight gain [21].

There was no significant difference in red blood cell count between the group of type 2 diabetic patients with oral candidiasis and the group of type 2 diabetics without oral candidiasis with the control group. The decrease in the number of red blood cells in the case of anemia, people with type 2 diabetes have weak immunity and this increases the load of oral candida [22]. This decrease occurs in diabetic kidney patients because the kidneys secrete a hormone called erythropoietin, which stimulates the bone marrow to produce red blood cells In the case of nephropathy, the small blood vessels that filter waste from the body are damaged, and substances such as protein begin to leak into the urine, and the amount of erythropoietin produced by the kidneys is reduced, which leads to anemia Other causes of anemia include less O2 sensitivity due to autonomic neuropathy or the use of inhibitors of the renin-angiotensin-aldosterone system, the inhibitory effect of inflammatory cytokines [23].

The results of the study did not agree with the findings [19], which indicated that there was no significant difference in the rate of lymphocyte counts in diabetic patients compared to healthy controls. Lymphocyte numbers are essential for the innate and adaptive immune response and are affected by inflammation, stress and infection [24]. Weak innate immunity leads to an increase in infectious organisms among different organisms that cause opportunistic infections in the oral cavity and are considered members of the most common genus Candida. Malnutrition in patients with type 2 diabetes leads to a decrease in the number of lymphocytes, which leads to the emergence of oral candidiasis [25].

6. Conclusions

The rise in type 2 diabetes in the blood leads to the growth of many microorganisms, including the oral candida fungus, which grows due to the high glucose of saliva because the fungal biofilm when it comes into contact with the sugar-rich mucous membrane leads to an overgrowth of fungi in the mouth , The rise in type 2 diabetes in the blood and the growth of fungi cause a decrease in the body's immunity, and this leads to the secretion of inflammatory cytokines in the body in a large manner indicative of weak immunity and the presence of inflammation, Among the cytokines are interleukin-6, and C-reactive protein, as well as high white blood cells, one of the indicators of weak immunity.

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