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Investigation The Role Of Oral Candidiasis And IL 17 Gene Polymorphism Rs 2275913 In Type 2 Diabetes Mellitus In Iraqi Arab Patients.

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Abstract
Type 2 diabetes mellitus is a major health concern distrusted globally and affected by several genetic and immunologic factors. Oral fungi and cytokine gene diversity may also play a significant role. The current study aims to investigate the association of oral candidiasis and IL-17A gene polymorphism with T2DM in Iraqi Arab patients. 75 T2DM patients and from both genders were enrolled; in addition, 75 healthy people of matched age and sex were also considered a control. TETRA-ARMS-PCR, Vitik^2, and ELISA techniques were used in this study. The results showed that the serum level of IL17A was significantly higher in T2DM than in the control group (86.7 ± 20.0; p=0.002). The genetic results showed that A allele of IL17A was slightly increased frequent (40%), GG VS AA+GA was significant as an etiological genotype with patients (OR:3.36, IC:1.6- 6.7; p,0.006). In addition, Candida albicans was the most frequent fungus isolated from T2DM patients. The current study is the first to diagnose species with less frequent oral presence, including Cryptococcus neoformans, Candida ciferrii, and Trichosporon spp.In conclusion, the present study indicated IL17A gene polymorphism rs 2275913 significantly affects developing T2DM. Moreover, increased levels of IL17A were identified as a susceptibility marker of T2DM. And increased levels of IL17A for oral candidiasis patients.
Keywords: oral candidiasis, IL 17 gene polymorphism, rs **2275913**, type 2 diabetes mellitus.

Introduction:

Normally, the human body employs unique defense systems to ward off invasion by bacteria, viruses, fungi, poisons, and parasites. It is difficult for pathogens to infiltrate this defensive mechanism under normal circumstances, but various conditions and disorders impair the immune system's function. (Nicholson.,2017) Unfortunately, diabetes impairs the host's immunological response. Along with the danger of natural barrier disruption caused by neuropathy, Type II diabetic Mellitus (T2DM) may impair cellular immunity, Due to a lack of insulin and hyperglycemia (Martins et al.,2017).

Monea et al. (2017) have indicated that yeast colonization of the oral cavity directly influences an individual's proclivity to develop type 2 diabetes. According to this hypothesis, oral yeast colonization may influence the pathway in which the immune response handles subsequent responses to glucose intake.

According to the American Diabetes Association, infections are a significant concern for people with diabetes since their immune systems cannot combat invading microorganisms. (Jafar et al .,2016). Candidiasis has increased significantly in recent decades throughout the world and is a leading cause of morbidity and mortality, especially among critically ill patients (Rodrigues et al ., 2019). Candida spp. is a commensal that people carry in their mouths, digestive systems, and vagina. It may induce opportunistic infection on a superficial to systemic level in immunocompromised people, hospitalized patients, and otherwise healthy persons. (Gürsoy et al .,2018It can induce infection due to its virulence or pathogenic features, and All spacies of candida spp can cause opportunistic infections (Mohd et al ., 2020).

Numerous reasons have been proposed to explain the increased Candida spp. Susceptibility in T2DM patients, depending on the nature of the local or systemic infection (Sherry et al .,2017). Yeast adherence to epithelial cell surfaces, increased salivary glucose levels, decreased salivary flow, microvascular degeneration, and diminished neutrophil candidacidal activity are all known host factors for candida colonization and eventual infection (Mohd et al ., 2020). Inflammation is commonly inferred as a crucial etiological factor that plays a vital role in the production of insulin resistance that contributes to T2DM significantly. T2DM was associated with increased circulating acute-phase inflammatory markers and insulin tolerance rates (Raffone et al., 2020). Candida components are primarily mediated by phagocytic cells, and T helper (Th) cells are divided into antigen-specific subsets, eventually leading to pathogen clearance (Hofs et al .,2016).

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Interleukin-17 (IL-17) is produced by Th17 cells and acts as a pro-inflammatory cytokine. IL-17 consists of six members (from IL-17A to IL-17F) involved in the pathogenesis of numerous inflammatory diseases (Ghaznavi et al .,2020). In addition, the G-197A (rs2275913) promoter polymorphism of IL17A has been implicated in the pathogenesis of various inflammatory diseases.

Aim of the study :

Association study between rs2275913 genetic polymorphism and serum levels of IL-17A with oral candidiasis in Iraqi Arab patients.

Subject and Methods

study subject.

This study was conducted between February and November 2020 on Arab Iraqi T2DM patients from the Endocrinology and Diabetes Center in Baghdad. This research enrolled 75 patients and 75 healthy as control. Collection of samples, including oral swabs and whole blood. Oral swabs were collected using AIMS transport media, and the samples were divided into two groups before being examined under a microscope and cultured on media. It was collected via EDTA and Heparin tubes, and serum was collected via gel tubes.

isolation and identification of Candida spp in the Oral cavity:

Several techniques for isolating Candida spp throughout the oral cavity are usable, including using a smear via swab (Smitha et al .,2011). Each smear collected from patients and healthy individuals from the oral cavity is transported to the lab via AIMS transport media for identification. The VITEK 2 Compact system identification Kit was used to identify Candida spp.

Genotyping study.

Using NCBI, Gene SNP Geneview, we found single nucleotide polymorphisms (SNP) in the IL-17A gene with a minor allele frequency (MAF) of less than 5% that had been reported in the literature and had clinical significance. IL17A genotype was done via Tetra allele refractory mutation system-polymerase chain (TETRA-ARMS) (You et al.,2008). The reaction was carried out in a 20 L volume that included DNA (4 L), outer primers (1 L), inner primers (2 L), and DW (10 L).The primers were in the sequence (outer primer: forward AATGGAAAATCAAGGTACATGACACC, revers primer: GATGGATGAGTTTGCCTGCT) and in the ratio (1:2, Frw: Rev).The inner primers dependent on the detected SNP are as follows (outer primer: TTCCCATTTTCCTTCAGACGG, revers primer: CCCCAATGAGGTCATAGAAGAATCTATT).

(Muhsen et al .,2019) The annealing temperature was determined through a gradient modified by researchers. And then followed the PCR program, which included one step of pre-denaturation at 95 degrees Celsius for 5 minutes, followed by cycling steps in a total of 30 cycles: Denaturation at 95 C^0 for 30 seconds, annealing at 57 C^o for 40 seconds, extension at 72 C^0 for 40 seconds, and final extension at 72C^0 for 5 minutes. The amplicon of PCR included 404 pbs total gene, allele G 193pbs, and allele A 260.

2-4. statistical analysis

SPSS version 23 was used for statistical analysis (IBM; Armonk, NY). Continuous variables were expressed as mean + standard deviation, while categorical variables were expressed as a percentage of the total. The Student's t-test and the Pearson's 2 test were employed to determine differences and intergroup significance. Furthermore, depended on genotype analysis via the med calculator, which depended on OR and 95% confidence intervals. Moreover, inserted the control group under hardy Weinberg equilibrium.

Results

study subject

The demographic and clinical features of participating studies are shown in (Table 1). The findings revealed a significant difference in mean age, mean serum glucose concentration, disease duration in the group (> 10 years), increased inherited disease in family history, associated smoking with the disease, and related disease with females between T2DM cases healthy. Accordingly, in the following

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Parameter		Cases	Healthy	p-value
Gender(M:F)		32(42.7):43(57.3)	42(56.0):33(44.0)	0.245:0.256
family History	Yes	44(58.7)	NA	NA
	No	31(41.3)	NA	NA
Duration of disease	<5	10(13.3)	NA	NA
	5-10	21(28)		
	>10	44(58.7)		
Smoking	yes	22(29.3)	2(2.6)	NA
	no	53(70.7)	73(97.4)	NA
Age	20-45	8(10.7)	[a]72(96)	**<0.001**
	46-70	[a]67(89.3)	3(4)	**<0.001**
Glucose (mg/dl)		[a]186.4±33.7	93.9 ± 1.6	**<0.001**

analyses, we choose these parameters as the main variables.

Table (1): patients' demographic and risk factors in T2DM.

Association studies

Serum levels of IL17A with the relapse of T2DM.

Following that, we measured the concentration of serum levels for IL17A, and the recorded data that was higher significant (p= 0.002) than healthy(table 2).

Table (2) : Serum levels mean of interleukins in T2DM patients and healthy

Groups	percent	Mean ±SD	p-value
Patients	50	[a]86.7±20.0	0.002**
Healthy	50	25.8±11.5	

As shown by the ROC curve, it was used to estimate the predictive value of IL17A serum levels. The cut-off value is 42.3, with a sensitivity of 100% and specificity of 99%. the AUC value of IL17A serum levels for predicting T2DM 1.00 (0.79-1.9) with high significance (fig: 1)

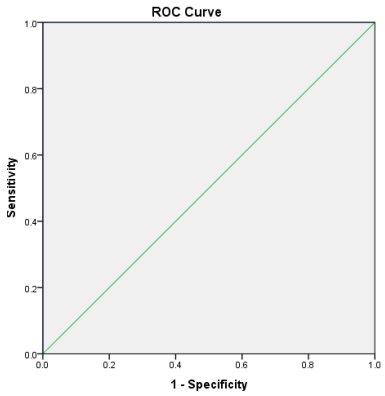

fig 1: ROC curve for IL17A serum levels to predict the occurrence of T2DM

Association between IL17A gene polymorphism(rs: 2275913 G>A) and T2DM risk.

The genotype and allele distribution for IL17A gene polymorphism under different genetic models are shown in (table 3).

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Table 3; genotype frequency of IL17A gene polymorphism in cases and healthy.

Models	Genotype	Patients	Control	OR(95%CI)	P-value
rs: 2275913 G>A, HWE: 0.483 Control.					
Co-dominant	GG	19(25.3)	40(53.4)	0.29(0.14 to 0.59	<0.001**
	GA	37(49.4)	25(33.3)	1.90(1.00 to 3.76	0.045*
	AA	19(25.3)	10(13.3)	2.20(0.94 to 5.13	0.066^{NS}
Dominant	GG	19(25.3)	40(53.4)	3.36(1.6 to 6.7)	<0.006**
	AA+GA	56(74.7)	35(46.6)		
Recessive	GA+GG	56(74.7)	65(86.7)	0.45(0.19to 1.05)	0.066^{NS}
	AA	19(25.3)	10(13.3)		
Allele	A	75(50.0)	45.0(30.0)	0.42(0.26 to 0.68)	<0.001*
	G	75(50.0)	105.0(70.0)		

We found IL17A rs 2275913 G > A was positive with risk heterozygote GA under the co-dominant model that reported (OR:1.9, p<0.045), negatively risk disease with homozygote AA (OR: 2.20 .P<0.066) and positively with (AA+GA) under the dominant genetic model in (OR:3.36, P< 0.006). Thus, g allele frequency is highly significant (p <0.001) with the risk of disease. These results were significant with T2DM patients compatible with health.

Distribution of oral fungal types on T2DM patients compared with healthy.

We found in the distribution of oral fungal types that the increased frequency of Candida albicans was significant (p < 0.003) compared with healthy and reported increased frequency in cryptococcus laurentii (12%) in patients. Firstly, we isolated rare types of oral fungal as Cryptococcus neoformans and Candida ciferrii from patients with (2.7,1.3%) respectively (Fig (2).

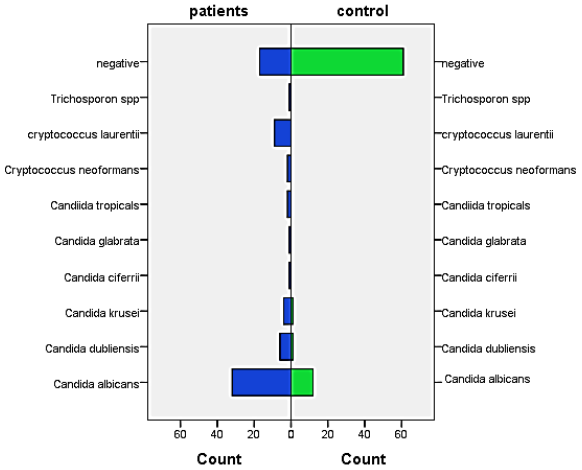

Fig (2): Distribution of oral fungal types.

Cross over-analysis.

As shown in table (4), we discovered a significant relationship between oral fungal variation and serum levels of IL17 A. with (130±14.8) to 32 frequency of Candida albicans, and various types of fungal in T2DM patients, and a positive increase of concentration of IL17A with other types of fungal. We discovered that IL17A rs 2275913 G > A was positively associated with risk heterozygote GA in the co-dominant model(OR:1.9, p0.045), negatively associated with homozygote AA in the co-dominant model (OR:2.20, p0.066), and positively associated with (AA+GA) in the dominant genetic model (OR:3.36, P=0.006). The frequency of allele with illness risk is very important (p <0,001). These findings were important for health-compatible T2DM individuals.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Interleukin	Patients			Control		
IL17A	Genotypes (rs 13)	%	Mean ±SD	%	Mean ±SD	P-value
	GG	25.3	102.0±15.5	53.3	24.2±8.9	0.001
	GA	25.3	105.9±12.4	33.3	26.1±8.0	0.003
	AA	49.4	102.7±17.7	13.4	21.7±6.3	0.001

table (4): Association between oral fungal and serum level of IL17A(pg/dl)

Types of Fungal	%	Mean ±SD (IL17A .pg / dl)	P-value
Candida albicans	40	[a]130±14.8	0.042*
Candida dubliensis	8	[c]107±10.5	
Candida krusei	2.7	94.0±18.1	
Candida ciferrii	1.3	82.0±2.4	
Candida glabrata	1.3	[b]120± 11.4	
Candiida tropicals	1.3	[b]120±11.0	
Cryptococcus neoformans	2.7	[C] 109±1.2	
cryptococcus laurentii	12.0	98±16.7	
Trichosporon spp	1.3	112±10.4	
negative	29	107±11.5	

Table(4): Association of genotype with the level of IL17A.

Distribution of genotype depended on levels of IL17 A, showed in a table significant AA genotype (49.4%)with level (102.7±17.7), and highly significant in GA heterozygote with patients compared healthy(p. 0.003) table (4).

Discussion:

Several studies highlighted risk factors such as age, smoking status, duration of disease, and family history. In this study, subgroup analyses showed positive disease with smoking patients, hyperglycemia, and high frequency with age (40-70 years). Satter et al,(2019) reported that T2DM was identified in those over the age of 80 years. However, when T2DM was discovered in adolescence, it was more than a decade lower. Finally, among younger women with T2DM, the hazard ratios for most outcomes were quantitatively higher. Studies have shown a relationship between cigarette smoking and a higher risk of type 2 diabetes (Maddatu et al .,2017). IL17A plays a critical role in inflammation and is associated with various diseases, including type 2 diabetes. (Zhou et al .,2016). There is evidence that interleukin (IL)-17A has a significant role in periodontitis and type 2 diabetes mellitus (DM) (Techatanawat et al .,2020).

To our best knowledge, we confirmed a significant role of IL17A gene polymorphism rs2275913 G>A with T2DM. It is the first scientific study to determine this role at the molecular level. Many studies investigation IL17A and ischemic stroke (Huang et al .,2017).and gastric cancer (Zhou et al .,2016).overall, we committed an association between the rs2275913 G>A, A allele, and T2DM risk in all genetic models. Otherwise, oral candidiasis is a common infection in diabetics, and several cofactors have been implicated in the infection's etiology. When compared to healthy, the prevalence of C.albicans with T2DM risk was significant (p 0.003). That is consistent with the findings of the Sampath et al. (2019) study. Significant associations were noted between oral candidal carriage amongst people with diabetes. Furthermore, although the Candida carriage rate and density were statistically higher in diabetics than in healthy individuals (Zomorodian et al.,2016), we isolate oral candidiasis (C.neoformans, C.lauterii, C. ciferrii, and Trichosporon spp) only rarely in Iraq.Lao et al (2016) The most frequent infection was yeast infection (56/120, 46.7 %), which included candidiasis (31/56, 55.4 %) and cryptococcosis (25/56, 44.6 %). Candidiasis was mostly seen in the urinary system

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

(12/31, 38.7%). More than half of the cryptococcosis cases (16/25, or 64.0 percent) had pneumonia as a presenting symptom. IL17A is TH17's main inflammatory cytokine, and it plays a role in diabetes mellitus etiology (Hetta et al.,2017). Statistically, we found a significant increase of IL17A (130±14.8) with oral candidiasis in diabetes mellitus. We reported an increase in the level of IL17A with infection fungi that including (Candida dubliensis (107±10.5), Candida glabrata, Candida tropical (120±11.4), and Cryptococcus neoformans (109±1.2). Although the role of the oral commensal microbiome in the production of oral protective immunity is unknown, mouth bacteria are known to play important roles in disease development (Dutzan et al., 2017). not found a publisher on oral fungal and relatives with IL17A.

conclusion :

In conclusion, our study demonstrated that C.albicans infection was higher in patients of T2DM, particularly in patients suffering increased concentration of glucose .on the other hand, there was a significant increase in levels of IL17A in patients oral candidiasis. Also, the genetic result demonstrated that the AA genotype could be considered an etiological fraction under dominant genetic models .for validation of the result, we need to increase the size sample.

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ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

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