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Evaluation The Systemic Immune Responses Among Diabetic Mellitus Patients Suffering From Odontogenic Infections Inbabylon Province/ Iraq

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Abstract

Background: Patients with diabetes mellitus (DM) suffer more frequent infections than those with no DM. The progress of the infections is also more complex in the patient group.

This work aims to provide evidences of the systemic immunological aspect among diabetic patients suffer from odontogenic infection. Our study considered study of systemic immune parameters among odontogenic infection patients suffering from diabetes mellitus. The immune parameters include antibodies such as serum IgG, IgM and IgA and cytokines such as the proinflamatory cytokine IFNγ, IL-6 which represent as regulatory cytokine act as both pro-inflammatory and anti-inflamatory cytokine, cellular immunity represented by CD4

CD8 T cells and Tcells are in each of DM patients, DM-odontogenic infection patients and normal control subjects are also with the odontogenic infected DM free patients.

Methods

Systemic immune parameters were measured usingradial immunodiffusion plates for enzyme-linked immunosorbent assay (ELISA), IgM and IgG & IgAfor IFN-γ, IL-6, CD-4 AND CD-8 T-cell receptors.

Results: The result shows that the patients with DM recorded significantly higher IgG level (884.74 ±244.15) ng\ml than in control group (443.04±171.18) ng\ml, (P≤0.05).(

DM patients with dental infection record higher IgM (126.91±34.83) ng\ml than in control group (37.51±16.62) ng\ml, (P≤0.05).

The result recorded non-significant differences in IgA antibodies concentrations among normal control(59.26±25.86 ng\ml) versus DM control subjects, (83.06±36.94ng\ml). Odontogenic infection causes significant elevation in serum IgA concentration in each of both DM patients and DM free patients (200.66±68.03 ng\ml, 129.2±64.54 ng\ml) respectively (P≤0.05).

The IL-6 cytokineamountincreased significantly more than in DM control patients versus normal control subjects (38.115±23.80 pg/ml, 10.68±5.06 pg/ml) respectively (P≤0.05). Odontogenic infection causesimportant elevation in Il-6 concentration in each of DM patients and DM free patients (66.99±33.256 pg/ml, 41.94±18.85 pg/ml)(P≤0.05).

Odontogenic infection cause significant elevation in IFN-γ concentration both in DM free and in DM patients (84.17±41.73 pg/ml , 82.07±43.07 pg/ml)

in compare with control groups in DM free and in DM patients (18.59±9.22pg/ml, 32.36pg/ml ±17.24) respectively.

The elevation in IFNγ in odontogenic DM free infected patients and in odontogenic DM patients was almost approximately similar.

CD-4 T-Cell concentration in each of odontogenic infected, DM control and odontogenic DM patients (4.64±2.12 ng/ml, 4.22±1.21 ng/ml, 4.49±1.79 ng/ml) were significantly higher than control group (2.11±1.37 ng/ml) (p≤ 0.05).

CD-8 T-Cell concentration in each of odontogenic infected and DM control and odontogenic DM patients (2.57±1.1 ng/ml, 1.88±0.9 ng/ml, 2.8±1.13 ng/ml) were significantly higher than control group (0.72±0.28 ng/ml) (p≤ 0.05).

Conclusion: IgM antibodies which represent as part of innate immunity do not depressed in DM patients.

Other humoral immune response parameters represented by IgG, and IgA were high in diabetic patients with odontogenic infection as in patients without DM, this result reflect that the class switching mechanism was not depressed in DM patients.

Further study for specificities and avidity determination of these antibodies in DM odontogenic infested patients required.

Our result recorded significant elevation in CD-4 T cells in DM control patients in comparing with normal control subjects control group, this result indicate there were stimulation and induction in producing such types of cells. This may cause depletion in the deposit of naïve T-cell and may lead to affect the number of antigen specific T-cells.

Similar results recorded for cellular immunity depicted by CD-4 & CD-8 T-cells. Also, theT-cells elevation number may combine disturbance in function and cytokine production.

As we recorded as high IL-6 which represent as both proinflamatory and anti-inflamatory cytokine in the same time and non significant elevation in the level of proinflamatory cytokine IFNγ indiabetic patients, and the elevation in IFNγ in odontogenic infected patients and in odontogenic DM patients was almost approximately similar.

Keywords: Odontogenic infection, Diabetes mellitus, immune Response, cytokine, antibodies and CD4 & CD-8 T-cells.

Introduction

Odontogenic origin orofacial infections are characterized by a long plagued mankind.Their spread could be managed in waysincluding impaired host defense, functional abnormalities of the host andthe virulence of microorganism.Frequent infections are a systemic complication of diabetes occurringbecause of the impaired host defense (Kamat et al., 2015).Diabetes is a big health problem universally caused by chronic rise in glucose levels in the blood because the beta cells (β cells) in the pancreas are not ableof creating enough insulin or is unable to use insulin correctly by cells in the body. Hyperglycemia in diabetes could causeimmune response dysfunction making it unable to control the invasion of the pathogens in diabetic subjects. So, diabetic patients seem to be more infection-susceptible (Berbudi etal.,2020).In the clinical experience, patients with (DM) could bevulnerable to facial cellulitis and deep neck infections due to odontogenic infections (Ko et al., 2017).These frequent infections are systemic diabetes complication occurringdue to an impaired host defense (Kamat et al., 2015).Other potential reasons could be theflaws in immunity, ahigh microorganismadherence to diabetic cells, higher resting values of TNF-K, IL-6 and IL-8 compared to nondiabetic controls. However there are normal concentrations ofserum antibody in people with DM (Geerling et al., 1999).

The present study aim to evaluate some systemic immune parameters in diabetic patients include antibodies such as IgM as a part for innate immunity, IgG and IgA; cytokines such as interferon gamma and IL-6 and cellular immunity represented by CD-4 and CD-8 cluster of differentiation in order to get some explanation that why diabetic patients are more vulnerable to some infection like odontogenicinfections and measure the same immune parameters in DM free odontogenic patients, and get evidence for the immune response in diabetic patients against odontogenic infection.

Serum antibodiesconcentrations correlate positively with antigens of infectious agents. Findings of Ebersole et al., in 1995 indicated that human antibodies IgM, IgG & IgA reactivate with specific outer membrane antigens bands ofA. There is a positive relation between actinomycetemcomitans (i) and the level of serum antibody. There is also an assiation (ii) with the number of infected teeth, and (iii) isthe variation in the disease severity measured by the teeth frequency with deep pockets (Ebersole et al., 1995).

Cytokines orchestrate the type of immune response is created. IFNγ is immunoregulatory, proinflamatory cytokine, antiviral, antiproliferative, high MHC class I and MHC II and an activation ofmacrophage,IFNγ is produced by T-cell and NK cells. commonly seen in

infectious diseases. Also T-cells utilization the cytokines for variation in the T-cells subsets. Th-1 is generated in the presence of interferon gamma.Evaluation of IFN-gamma production can be monitor the T-cells function(Brooks et al., 2013).

IFN-γ is important to modulate the immune response, in particular the host defense against cancers and intracellular pathogens. In addition to NK cells, T cells, T-helper 1 (Th1) $CD4^+$ T cells and NK T cells produceIFN-γ $CD8^+$.IFN-γ support Th1 variation, boost macrophage function, encourage the leukocyte migration to the site of infection, and increase the main histocompatibility complex expression to get the better T-cell recognition of infected or malignant cells. Humans andmice deficiencies in IFN-γ signaling are vulnerable to tumors and infections with intracellular pathogens like mycobacteria. Yet, uncontrolled IFN-γ protein production is critical to the overexpression of IFN-γ and host in mice promoting autoimmunity. So, tight IFN-γ expression regulation plays a big role to balanceinflammation with immune tolerance (Mah and Cooper., 2016).

Interleukin 6 (IL-6) is an inflammation marker. It is a pleiotropic cytokineand adipocytes, endothelial cells and fibroblastproduce it and activatemonocytes leukocytes. IL-6 mainly is the mainacute-phase inflammatory response regulator. Yet, there is one of the main role of IL-6 in the transformation from acute to chronic inflammation.In patients with DM1, the IL-6 concentration seems normal or higher in comparison to the healthy subjects. In addition, there arestudies showing that IL-6 participates to initiate and accelerate the chronic inflammation process contributing to improve micro- and macrovascular complications in diabetic people(Wegner et al., 2013).

IL-6 save as markers for disease is detectable d in the patients with septic shock sera tracking their existenceas a prognostic sever sepsis value of. IL-6 contributestoTh-17 production(Brooks et al., 2013).

$CD4^+$cluster of difference seems on the surface of CD4 in the outer layer of immune cells incldуing T helper cells, macrophages andmonocyte and (Brooks et al., 2013). T cells play an important role to achieve a regulated effective immune response to pathogens. Naive $CD4^+$T cells could be activated following the interaction with antigen-MHC complex and differentiated into specific subtypes that rely on the microenvironment cytokine scene. In addition to the the classical T-helper 1 and T-helper 2, other subsets were seen, such as T-helper 17, follicular helper T cell,regulatory T cell, and T-helper 9, each with a profile of cytokine.The effector functions of these cells are mediated by the cytokines secreted by the differentiated cells (Luckheeram et al., 2012).

The CD8 co-receptor expression occurrs on the outer layer of thecytotoxic T cells, asnatural killer cells, dendritic cells and cortical thymocytes. The CD8 molecule signals cytotoxic T cell population (Leonget al., 2003).

In addition, CD8+ T cells seems cytotoxic CD-8 T-cells killingviruses and intracellular bacteria. Furthermore, the majority of cytotoxic CD8 T cells release the cytokines IFN-γ, TNF-β andTNF-α contributingfor thedefense of thehost (Janeway et al., 2001).

Understanding immune responses status in diabetic patients with or without odontogenic infection thrucomparing such responses with DM free patientswith or without odontogenic infection may provide an evidences for improvement the immune system to face and control such infections.

Materials and Methods

Patients and control groups

Patients of all age groups were clinically diagnosed to have moderate to severe odontogenic infection; patients have DM with odontogenic infection.

Control Group

Subjects without DM and without odontogenic infection. Subject with DM and without odontogenic infection.

Odontogenic Patients: Patients with odontogenic infection for diabetic patients and diabetic free patients.

Both patients and control groups entered the department of oral and maxillofacial surgery of Al-Hilla surgical teaching hospital, the study samples collected from both sexes during the study was conducted from October 2016 to March 2017.

Blood Collection and Storage

Blood samples were collected by sterile one-use syringes, from each group, blood samples were centrifuged for 15 minutes at1000´g, the supernatant were gathered and saved at -20°C. Then,the assay was carried out during the first month after storage.

In Vitro Quantitative of Antibody and Cytokines

In vitro quantitative determination of IgG,IgM and IgA using radial immunodiffusion plate (LTA/ Italy). Interferon gamma, IL-6 inserum of patients group and control group were

measured usingSandwich-ELISA as in company manufacture instructions (ElabscienceBiotechnology Co., Ltd).

Serological Markers of Cell-mediated Immunity

Serum soluble CD4 and CD8 were measured asmarkers for cell mediated immunity among control and tests groups using ELISA techniques as in company manufacture instructions(ElabscienceBiotechnology Co., Ltd).

Statistical Analysis

Statistical analysis comprise F-test and post hoke test for determinethe significant of the differences between means between groups, standard deviation using online Interactive Statistic, and graphs were done using online GraphPad Prism- software version 8.4.3(471).CA.USA.

Results:

Humoral ImmuneResponse

Results shows that the IgM results also recorded elevation in IgM concentration in patients with DM with dental infection,

We recorded elevation in IgG concentration in DM patients with dental infection as well as in dental infection patients without DM ($p \leq 0.05$).

The result recorded significant elevation in IgA and the concentration washigher than those with dental infection without DM ($p \leq 0.05$), (table-1) higher thanthose with dental infection without DM ($p \leq 0.05$). Although the IgM and IgA concentration in DM control group higher than its concentration in healthy control group but still the elevation was non significant and the IgG concentration in both groups closely similar(table-1& Fig.1).

Table-1: Immune response parameters concentrations (IgG,IgM,IgA,IL-6,IFN-γ,CD-4 T-Cell and CD-8 T-Cell)

Immune response parameter	(Mean ± St.dev) Control group	Odontogenic Infected patients	(Mean ±St.d) DM subjects (control)	(Mean± St.d) Test group (DM+dental infection)
IgM	37.51±16.62	108.1±52.82	75.72±35.45	126.91±34.83
IgG	443±171.18	811.1±225	449.6±224	884.74±244.2

IgA	59.26±25.86 ng\ml	129.2±64.54 ng\ml	83.06±36.94 ng\ml	200.66±68.03ng\ml
IL-6	10.68±5.06(pg/ml (	41.94±18.85(p g/ml)	38.115±23.80(pg/ (ml	66.99±33.256(pg/ml (
IFN-γ	18.59±9.22 pg/ml	84.17±41.73 pg/ml	32.36±17.24 pg/ml	82.07±43.07 pg/ml
CD-4 T-Cell	2.11±1.37pg/ml	4.64±2.12 ng/ml	4.22±1.21 ng/ml	4.49±1.79 ng/ml
CD-8 T-Cell	0.72±0.28pg/ml	2.57±1.1ng/ml	1.88±0.9ng/ml	2.8±1.13ng/ml

N=10 for each group; St.dev: Standard deviation.

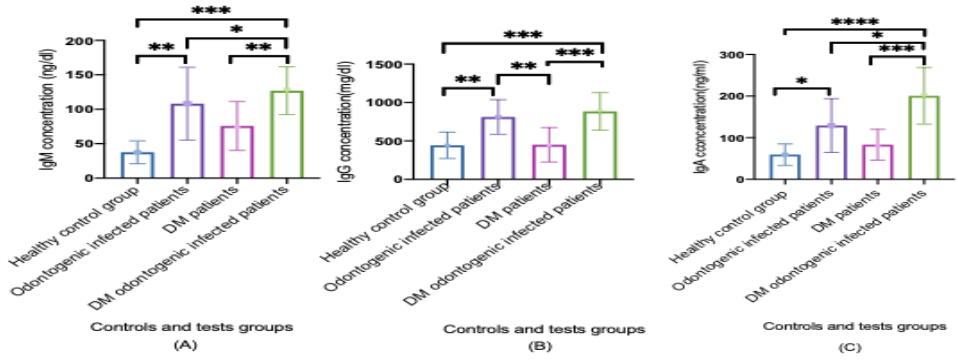


Fig. 1: Antibodies concentration (A:IgM; B:IgG & C: IgA) in each of the healthy control group; odontogenic infected patients; Diabetes mellitus (DM) patients , Diabeticmellitus odontogenic infected patients. Bars graphs represent group mean± St.dev. n=10 for the control and patient groups. *p:≤ 0.05; p**:≤ 0.00; p***:≤ 0.000; p****≤ 0.0000.

Cytokines Profile

There was a significantlyInterleukin-6 concentration in DM patients than in the control group (p≤0.05). There was elevation in DM infected patients compared with DM control group (p≤0.05). While the concentration of IL-6 in in DM patients did not shows significant variances in comparisonto the infected patients without DM (p≤0.05).Interferon –γ concentration was higher in DM patients than in normal control group, however the

differences were non-significant (p≤0.05).Interferon –γ concentration was higher in DM odontogenic infected patients comparing with DM non infected patients and no significant differences between infected patients with and without DM group (p≤0.05) were found (table-1, Fig. 2).

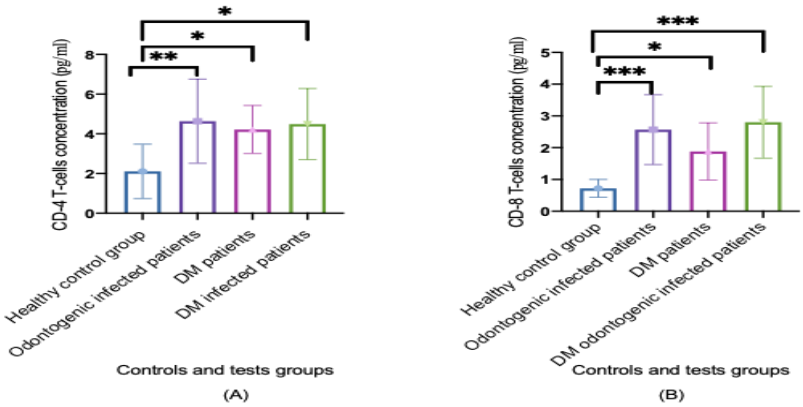
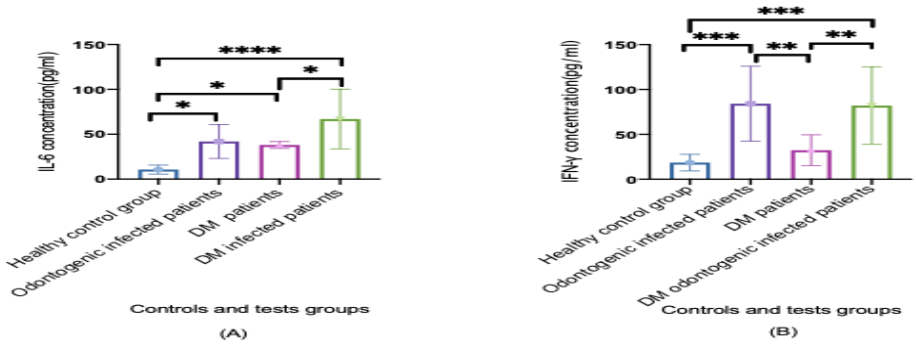


Fig. 2: Cytokine concentration (A:IL-6; B:IFN-) in each of the healthy control group; odontogenic infected patients; Diabetes mellitus (DM) patients , Diabeticmellitus odontogenic infected patients. Bars graphs represent group mean± St.dev. n=10 for either group. *p:≤ 0.05; p**:≤ 0.00; p***:≤ 0.000; p****≤ 0.0000.DM patients did not shows significant differences in comparing with infected patients with out DM (p≤0.05).

3.4. Serum CD4 & CD8T-Cell



CD-4 T-Cell and CD-8 T-Cell concentration in each of infected and DM control and DM with dental infection were significantly higher than those of the control group (p≤ 0.05). (table-1, Fig. 3).

Fig. 3: CD4T-cells & CD8 -T cells concentration in each of the healthy control group; odontogenic infected patients; Diabetes mellitus (DM) patients, Diabeticmellitus odontogenic infected patients. Bars graphs represent group mean± St.dev. n=10 for either group. *p:≤ 0.05; p**:≤ 0.00; p***:≤ 0.000; p****≤ 0.0000.

Discussion:

Patients with diabetes are more vulnerable to develop various oral infections such as fungal and bacterial infections. Lower salivary flow rate and no antimicrobial effects could be the lesson for these infections. Also, a poor metabolic control andan impaired defense mechanism and could help the development of the infection(Rohani, 2019).

Results regarding humoral immune defense against orofaisialodontogenic infected patients results recorded significant elevation in IgM, IgG and IgA in compare with control groups (Healthy control & DM control group). Both IgM and IgA responses were higher in DM odontogenic than in odontogenic DM free patients while IgG almost in closed concentration.Regarding control groups there were non significant differences in each of IgM, IgG and IgA between healthy control group and DM control group.IgM represent as natural antibody and represent asa part of the innate immune system present in healthy individuals, this reflect that this part of innate immunity dose not depressed in DM patients.Our results were in agreement with result of Geerlings and Hoepelman in 1999 recordingserum antibody concentrations andhumoral adaptive immunity in those with DM are normal responding to vaccination with pneumococcal vaccine and nondiabetic controls. (Geerlings and Hoepelman, 1999).

The important point in our results that both of IgM and IgA concentration was significantly higher in odontogenic diabetic patients than in odontogenic infected patients with no diabetes. There were explanation for this result, first there were percent of glaycation antibodies in diabetic infected patients, the glaycation present depend on glucose level in the blood, the glycation of immunoglobulin may have no affect or harm the function of antibodies (Casqueiro, 2012).This situation may be induce the body to produce more antibodies in order to provide adequatehumoral systemic immune response.

While our results show no differences in IgG concentration between DM odontogenic infected and DM free odontogenic infected patients, however this antibody may be percent of glycation.Study of Mo et al., 2018 demonstrated that glycation can affect the function of a

representative IgG1 mAb by binding with antigen binding site.Humoral immunity in type 2 diabetic patients seem in itself in the high value of B-lymphocytes, more plasma cells, higher antibodies (IgM, IgG, IgE), increased quantity of circulating immune complexes (CIC). Some studies confirmed that the CIC concentration in blood is in correlationwith complement system and themetabolic disease courseseverity (Pietropaolo et al., 2008& Ovsenyan et al., 2008).

Research papers demonstrated that there serum antibody level inthe humoral adaptive immunity in patients with DM responding vaccination with hepatitis B and pneumococcal (Geerlings & Hoepelman., 1999) andCovid-19 vaccines(Dispinseri et al., 2021) as well as nondiabetic controls.

Study of Azizova et al., 2009 divided glysemia and diseases in to 3 groups, in the stage of compensation, subcompensation and decompensation. The biochemical marker level of glycated hemoglobinwas identified and we calculated immunological parameters IgA, IgM, IgG, CIC and the antibody.Circulating immune complex andimmunoglobulines grewand phagocytic number reduced in all three stages of the disease,and there was a higher level low-avid antibodies. The disease duration negatively affects o cellular immunity, in particular its functional activity. IgA, IgG, CIC and T-lymphocytes levels in the patientswith disease for1 yearseemed significantly lower than with those for6, 10 years and more.Yet,those withdisease for6–10 years and over 10 years have noticeable IgG.

Disease long durations increase the ofIgA, CIC andIgG.They prove that antibody folding disorders in DM with conformational levels changes directly depending on disease seriousness.In contrast, the T-system immunity parameters decreased.T-peripheral blood lymphocytes in patients for less than 12 monthsis near this parameter in blood ofnearly healthy people(Azizova et al., 2009).

The immune response cytokines orchestrate is created. IFNγ is immunoregulatory, proinflamatory cytokine, produced by T-cell and NK cells. commonly seen in infectious diseases. T-cells use the cytokines for variation in to the T-cells subsets. Th-1 is generated in the presence of interferon gamma, Th-17 is produced in the presence of IL-6 and TGF-B

IL-6 is one of the cytokines can save as markers for disease and is detectable in the sera of septic shock along patients with TNF-alpha and IL-1 and tracking their existenceis a possible prognostic value of sever sepsis.

T- cell function can be monitored by the ability of T- cell to produce IFN-gamma

(Brooks et al., 2013).

IL-6 concentration was higher in DM control group than normal control group, our result agree with the finding of Wegneret al., 2013 investigating the level of IL-6 was higher in DM1 patients than in the control group (Wegner et al., 2013).T- helper created cytokine having increased potential in diabetogenesis is IL-6. IL-6 levels increased in generalin T1D subjects such as the long term diabetics. Yet, it the IL-6 levels are not outcome or disease progressionpredictive. Also, discordancedemonstration between humans and mice transitional studies showed that blocking IL-6 in young non-obese diabetic (NOD) mice prohibits the disease onset. Although not studied in this work, the IL-6 inhibition have prevented the creation of Th17 cells (Wegner et al., 2013).

Both pro- and anti-inflammatory functions of IL-6the promoting functional beta-cell compensation for keeping the insulin secretion and glucose homeostasis. The rise of IL-6 identied for the activation pathways facilitating the energy turnover and enhancing insulin sensitivity. Promoting functional beta-cell compensation keeps this secretionsuitable andkeeps glucose homeostasis (Quet al., 2014).

Also there was significant elevation in IL-6 concentration in each of odontogenic infected in DM patient and DM free patients.

The elevation in IL-6 and IFNγ was in parallel with the elevation of the concentration of CD-4 T cell, these reflect the elevation in TH-2 cells which produce these cytokines (Brooks et al., 2013).Wagner, 2011 also mentioned for increasing IL-6 in type-1 diabetic patients.

TNF-α and IL-6 are important in leukocyte function against pathogens, and taking exogenous insulin in diabetes could raise the immune cell activity for theprotection against pathogens (Moazezi & Hosseinian, 2014).

Yet, the AGEs could potentially reduce the activity of the therapeutic protein interferon alfacon-1 (Mironova et al., 2008).

Results of CD-4 T-Cell concentration shows significant differences between control versus control with DM, odontogenic infected DM patients and odontogenicinfected DM free patients , while the differences between each of infected DM versus infected DM free, DM non infected and infected DM patients were non significant ($P \leq 0.05$),

Diabetic type 1 was autoimmune disease, in past to be mediated by T-cells (Roep, 2003).Recently, data collected explain that the DM type 2 is also auto immune disease mediated by T-cells response (Xia et al., 2017).

In T2DM, Treg cells suppressing Th1,Th2, and Th17 response improves insulin resistance. Treg could prevent the inflammatory response. The suitable balance between proinflammatory (Th17 orTh1) and anti-inflammatory (Treg) subsets of T cells plays a role in maintaining the host immunity and control inflammatory damage. Also, the Treg cells are reduced in patients with T2DM. In addition, its Treg/Th17 ratio and Treg/Th1 ratio was reduced in patients with T2DM. Additional studies show that T2DM. BothTh1 andTh2 associated with insulin resistance and chronic inflammation in T2DM(Xia et al., 2017).

T cells is important in diabetogenesis similar to other cells. Various types of T cells, Th1, Th17 and now Th40 are identified in this disease. Each of these category contributes to the general disease establishingdebilitating inflammation. To control the inflammatory process without decreasingundesired immune suppression requires surgical interferences. It is possible that no treatment option is totally successful with a focus on any one cell type diminishing the ability of comprehending the completeform of the disease process(Wagner, 2011).

CD-8 T-Cell concentration in each of odontogenicinfected, DM control and odontogenic infected DM patients were significantly higher than healthy control group ($p \leq 0.05$).Dental infection patients induce significant elevation in serum CD8 T-cells concentration in patients comparing with control group (Al-Mahdi & Abood, 2021), while odontogenic infection induce higher elevation in CD8-Tcells concentration (Abd, 2017).

Findingsof Kumaret al., 2015 reveal that that pulmonary TB complicated with type 2 DM is linked to a changed cytokine-producing and cytotoxic molecule-expressingCD8+ Tcellsrepertoire. It couldcontribute to higher pathology (Kumar et al., 2015).

Conclusions

Humoral immune response represented by immunoglobulin IgG, IgM and IgA were high in diabetic patients with odontogenic infection as in patients without DM. Further study for avidity determination of these antibodies required.Similar results recorded for cellular immunity represented by CD-4 & CD-8 T-cells, the increase of T-cells may combine disturbance in operations and cytokine production. As we recorded as high IL-6 which

represent as both proinflamatory and anti-inflamatory cytokine in the same time and non-significant elevation in the level of proinflamatory cytokine IFNγ indiabetic patients, and the elevation in IFNγ in odontogenic infected patients and in odontogenic DM patients was almost approximately similar.

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