Type 1 Diabetes Mellitus (T1DM): Induced by Environmental, genetic and immunity factors

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Abstract: T1DM is progressively common among children and youths. The combination of environmental factors and immunological disturbances form a sensitivity risk factor that induces T1DM, especially in the presence of genetic factors. The current study focuses on the role of relationship between immune system, viruses and genetic factors with T1DM occurrence. 230 T1DM patients were sampled, based on absolute dependence on insulin with another 130 healthy individuals as control group. Indirect Enzyme-Linked Immuno Sorbent Assay (ELISA), Immuno fluorescent and Polymerase Chain Reaction (PCR) - SSP technique were used for detection of HLA-DR3 and DR4. Significant differences in mean values was observed between the T1DM patients and control group in the quantitative measurement of anti-Cytomegalovirus (CMV) antibody (Ab), rubella, CSV-B, Glutamic Acid Decarboxylase (GAD), insulin receptor (IR) Ab, IL-4, IL-10, CD4, CD8, and the ratio of CD4:CD8 (P-value < 0.05). The results of this study indicate that there are overload of immune responses (cellular &molecular-genetic) among T1DM patients. The presence of human leukocyte antigen (HLA) genes showed significant relationship to viruses that induce T1DM. It also showed an existing link between T1DM occurrence, HLA genes and environmental factors (viruses and consumption cow milk during infancy).
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1. Introduction

Diabetes is a complex metabolic disorder affecting various body organs [1]. It is a diseases associated with high blood glucose level resulting from defects in insulin production, insulin action or both [2]. While,T1DM is characterized by an absolute lack or severe deficiency of insulin [3]. Numerous factors are known to induce T1DM. Several factors such as environmental, inherent, and psychological (nervousness, tension and phobia) are among the factors known to induce T1DM [4]. Other known factors include consumption of cow milk, vaccination and chemical agent [5]. However, factors that induce T1DM are not just limited to those mentioned above, because according a study by WHO, 1999 there are still other factors which are yet to be identified [6]. Genetic factors, disturbance in immune system and environmental factors (viral infection) are three risk factors forming a horror triangle in the human body that can induce T1DM [7, 8]. Disturbance in both cellular and molecular-genetic immunity immune responses are implicated with T1DM [9]. According to Orban et al, 2007, the tendency to develop abnormal antibodies in T1DM patients is genetically inherited to some extent, even though the details are not fully understood [10]. A study also reported according to Yanai, 2011 that anti-GAD antibodies usually appear to be high among T1DM patients[11]. The immune system produces Ab against antigenic part of CSV-B virus, and this virus is the most common childhood viral infection. According to Van-Belle et al, 2011, part of GAD produced in the human pancreas is similar to the part of CSV-B virus that is found to be common in childhood and this similarity had been known to confuse the immune system [12]. Anti- insulin receptors antibodies are other autoantibodies that are found in high level in the blood circulation of T1DM patient [11]. However, the autoreactive CD4+ and CD8+T cells, autoantibody-producing B+ lymphocytes and activation of the innate (natural) immune system can lead to damage in β-cells. These disturbance and damage may further lead to changes in immunological parameters that can be observed in T1DM patients. Some of these changes relates to complications of the disease [13, 14]. Furthermore, T lymphocytes and macrophages produce cytokines are known to attack pancreatic beta cells resulting in insulin deficiency [15]. In some

patients, changes in some cytokines such as high level of IL-4, with relatively low IL-10 even after many years may also mean that such patients are under threat of developing other autoimmune insults [16].

Environmental factors such as viral infection and consumption of cow milk during infancy are important factors known to trigger T1DM according to a report by Patelarou et al, 2012. Consumption of cow milk during infancy is one of the theories that is believed to be responsible for increase in T1DM [17]. This according to studies is because cow milk induces T1DM similar to bovine serum albumin (17-amino acid "ABBOS" peptide) and this is also present in the structure of β-cells in the pancreas [18]. Viral infections are other environmental factors that are related to T1DM[19]. The most common types that triggers the T1DM are rubella, Coxsackie B, cytomegalovirus, adenovirus and mumps [20]. These viruses can induce diabetes through direct infection of beta cells [21, 22] or increasing expression of cytokines that stimulate auto reaction of lymphocytes against beta cells [22].

Genetically, genes such as HLA-DR and DR4 are other factors that are responsible for T1DM. HLA-DR3 and DR4 are located on major histocompatibility complex (MHC) region and this is considered as a high risk factor for T1DM. A study which was conducted among Europeans revealed that, the Europeans are more prone to T1DM than the US population. Some alleles, such as DRB1*3906, have also been linked with T1DM especially in young ages [23]. Although there have been increases in conventional T1DM cases, but the actual cause of the ailment is still not fully understood. Several theories concerning the causes of T1DM have been put forward, but most of those theories and studies are not very clear or proven. This therefore calls for needs for further research on the causes of the T1DM. In Erbil city, there are no known studies conducted that focus on the relationship between viral infection and immunological parameters especially the parameters that are considered in this research and the role of the presence of HLA genes on these parameters among T1DM patients. In this study, the relationship of viral infections with T1DM in Erbil city of Kurdistan region of Iraq will be investigated by identifying the common types of viral infection which are related to the T1DM that causes damage to the insulin producing cells. In addition, the study also aims to define the presence of HLA genes in Kurdish ethnicity that is more related to T1DM. Furthermore, the study is also aimed to understand the immunological changes that lead to more complication by comparing the results with non-diabetic individuals.

2. Material and Methods

This study is concerned with T1DM patients at the LaylaQassim Centre for Diabetic Patients, Erbil, Iraq. The ethical approval and permission to perform the work was granted by the local scientific committee ofHawler Medical Institute. Furthermore, consent was obtained from each participant prior to the study. Kurdish T1DM patients were selected randomly. Non-Kurdish individuals, such as Arabs and Turkmanis were excluded from the study. The diagnosis of T1DM followed the 1997 criteria of the American Diabetes Association (ADA). The molecular tests were carried out at the Forensic Department, Ministry of Health, Baghdad. The blood glucose tests were carried out at Nanakali Hospital for Blood Diseases, Erbil. Testing for cytokines by ELISA was conducted at Zanko Private Laboratory for Advanced Immunological Investigations, Erbil. The interpretations of tests were performed at the Department of Bioprocess, Faculty of Chemical Engineering, University Technology Malaysia (UTM), JohorBahru, Malaysia.

Study population: Two hundred and thirty T1DM patients comprising 108 males and 122 females, aged between 3 and 40 years, were selected randomly for this study in addition to 130 healthy subjects (non-diabetic patients) made up 68 females and 62 males were also included in this study as the control group. The control group consisted of Kurdish volunteers with age ranges similar to those of the selected patients.

Blood collection: Peripheral blood samples were collected by intra-venous puncture and aspiration from the cubital vein. From each patient, 10 ml of blood was drawn and divided into three parts. The first part was processed immediately for the estimation of CD4+ and CD8+ lymphocytes. The second sample was left to clot, and after centrifugation at 1000g for 10 minutes, sera were aspirated and preserved frozen till they were used for measuring the glucose levels, anti-CMVAb, anti-rubella Ab, anti-CSV-B Ab, anti-GAD Ab, estimation of interleukin-4 and interleukin-10. The third part was used for the HLA study where genomic DNA (1-2 µg) was extracted from peripheral blood leucocytes using DNAzol(BAGHEALTH CARE) and stored at 4°C until ready for typing. Confidentiality of patients' information was assured by coding all the test samples, and the master list was available only to the investigators.

ELISA technique:A. Measurement of interleukins: The IL-4 and IL-10 were measured using an ELISA kit as instructed by manufacturer (Koma Biotec Inc., Seoul, South Korea). Briefly, the microtite ELISA plate was washed three times with wash buffer and after blotting on dry paper towels, 100 µl of the diluted test sample or control sample was added on to the first monoclonal antibody to IL-4 or IL-10 (MAbI)

coating the plate wells and incubated for 2 hours. This was washed 3 to 4 times and 100 ul of the second anti IL-4 or IL-10 biotinylated monoclonal antibody (MAb2) and again incubated for 2 hours. After washing, the diluted Streptavidin-HRP conjugate and color development solution were added. The reaction was stopped using the stop solution and the color was read spectrophotometrically at 450 nm.

B. Detection and measurement of autoantibodies: The estimation of anti Human Insulin Receptor β) ISR-β (antibodies followed the procedure of (Catalog No. CSB-E08142h-96T) Briefly, the microtite ELISA plate was washed three times with wash buffer and after blotting on dry paper towels, 50 µ l of the diluted test sample or control sample was added into the first monoclonal antibody to Human Insulin Receptor β) ISR-β ((MAbI) coating the plate wells and incubated for 2 hours. This was washed 3 to 4 times and 50 ul of the substrate A and substrate B again incubated for 15 minutes. After washing, the diluted Streptavidin-HRP conjugate is added, and color development solution is added. The reaction was stopped by the stop solution and the color read spectrophotometrically at 450 nm. For anti-GAD antibody we used the kit from Euroimmun Company 2011, catalog (EA 1022-9601 G). Briefly procedure was as follow;, 25 µl of the calibrators, negative and positive controls or patient samples were transferred into the individual microplate wells according to the pipetting protocol, and covered the frame and incubated for 1 hour at room temperature. After incubation, the microtite ELISA plate was washed three times with wash buffer and after blotting on dry paper towels, 100 µl of GAD (biotin-labelled GAD) added into each of the microplate wells and mixed well. The plate was covered the frame and incubated for 1 hour at room temperature. after incubation the plate washed and 100 µl of enzyme conjugate (peroxidase-labelledavidin) was added into each of the microplate wells and the frame was covered and incubated for 20 minutes at room temperature (+18°C to +25°C) on a microplate shaker set at 500 rpm. The plate washed again and 100 µl of chromogen/substrate solution was added into each of the microplate wells.The plate was incubated for 20 minutes at room temperature and it was protected from direct sunlight. The reaction was stopped by the stop solution and the color read spectrophotometrically at 450 nm. The sera were separated for the determination of glucose.

C. Detection and measurement of antiviral Ab.: The quantitative test by ELISA technique was used to detect the presence of anti CMV, Rubella and CSV-B. Catalogue No. BC-1089 for CMV, and No. BC-1081 was used from BioCheck, Inc. USA, Company, while the procedure of CSV-B IgG antibodies followed the procedure of catalogue No. COB-116, ELISA Kit/. DIACHECK. Switzerland Company. ELISA-based determination of serum IgG antibody (I.U./mL) against CMV, rubella, and CSV-B were used. The concentration of antibodies at the cut-off absorbance were: 15 I.U./mL (absorbance 2 at λ 450nm), 1.2 I.U./mL (absorbance 1.2 at λ 450nm) and 100 I.U./mL (absorbance 1.5 at λ 405nm) against CMV, rubella, and CSV-B respectively. The serum antibody concentration was calculated according to the following equation.

$$\text{Serum antibody } \left(\frac{\text{I.U}}{\text{mL}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of cut-off}} * \text{Concentration of cut-off}$$

Immunofluorescence technique: For studying the lymphocyte sub-populations,lymphocytes were separated from fresh heparinised blood, then carefully 1 ml of blood transferred into the separation medium which made of equal volumes of both ficoll-hypaque and RPMI 164 and centrifuged at 1500g for 30 minutes in a refrigerated centrifuge[24]. After centrifugation, the cell band layer was aspirated and transferred into sterile tube and washed 3 times by phosphate buffer saline (PBS) pH 7.2. Their lymphocytes were finally suspended in RPMI and four 50 ulaliquotes were transferred into sterile tubes. Fluorescein isothiocyanate (FITC)-conjugated human monoclonal antibodies to CD4 and to CD8 which can detect or calculated by fluorescent microscopy. Then 50 µl of cold PBS PH 7.2-7.4was added to suspension which was incubated on ice for 30 minutes and then washed with 1 ml of cold PBS. After washing, the mixture was centrifuged at 2000 RPM for 15 minutes at 2-8 ◦C. This step was repeated twice, and after the second washing the supernatant was aspirated and the cell pellets were transferred to a microscope glass slide and a drop of glycerol was added and cover slip was laid over the content of the slide. The slide was examined under fluorescent microscope (Olympus-Japan) by using phase contrast visible illumination in conjunction with immuno-fluorescence. One hundred lymphocytes subsets was counted under a face-contrast microscope, then the same field was examined by fluorescent illumination of the same field in order to count the percentage of CD4+ and CD8+ lymphocytes precisely(Puchleim, Germany).

PCR-SSP technique: The HLA-gene study started with the isolation of the DNA with the EXTRA-GENE kit [25]. Briefly, the procedure was included; adding 500 µl of blood to 900 µl of lysis-buffer in a nuclease – free, 1.5 ml micro-centrifuge tube. After centrifugation for 1 minute at 8000 rpm, the sediment is washed twice then the leukocyte sediment is re-suspended in 240 µl aqua dest and 120 µl of the extraction-buffer is added, mixture vortexed, then it was washed and centrifuged twice for 5 minutes at

13000 rpm. The supernatant transferred into a new nuclease-free 1.5 ml centrifuge tube. The sediment was then washed twice in 96% and then in 70% ethanol. The tube was placed upside down on a filter paper for about 5 minutes. The DNA pellet is dissolved in 100 µl of aqua dest, for complete DNA dissolution. The tube is then incubated at to 56°C for about10 minutes and the DNA obtained is stored at -20°C. For determining the DNA concentration, five µl of the DNA solution was diluted with 495 µl distilled water and the optical density was determined at 260 nm in UV spectrophotometer (Schimadzu-Japan) using distilled water (D.W) as a reference. The contaminating proteins were measured at 280 nm and the quotient A260/A280 was calculated. Amplification was performed by the prealiquoted and dried reaction mixture which already contained allele-specific primer sets, internal control primers and nucleotides. The HLA PCR-SSP plates were removed from -20 C and the 10x per –buffer was thawed at room temperature. In an eppendorf reaction tube, a master mix was prepared for each typing test. A master mix was contained Aqua dest, 10 xPCR- buffer, taq-polymerase (5u/ µl) and the DNA solution (50-100 ng/ µl). The contamination control was performed, using the master mix without the DNA solution was prepared first and 10 µl of this mix was pipetted for the contamination control. Following that, 10 µ1 of the mix was pipetted into each of the dried primer mixes in the plate. The PCR plate semi tubes were tightly closed with the reactive caps. The plate was shake down-wards to ensure dissolve the dried reaction mixes at the bottom of the plate. The plate was transferred to the thermocycler and PCR program was started. Gel electrophoresis was used for separation of the amplification products by electrophoresis via a (horizontal) agarose gel. For documentation, visualize the PCR amplification was used an UV transilluminator (220-310 nm). The images captured by using software computer and saved for comparing with the special map in the kit of HLA-DR [26].

3. Results

The demographical analysis of this study shows in (Table1). This table shows that the number of females who have T1DM was higher than males. Moreover, the result of Chi-square test(χ^2) shows that there are no significant difference between T1DM patients and control group in gender ($\chi 2$=0.018, p= 0.89). Family history revealed that 73.5 % of diabetic had a previous family history for diabetic compared to the 30% in the control group Table1. The result of statistical analysis shows that there is a significant difference in the mean value of serum glucose between T1DM patients and control group. The (Mean ± SE) for T1DM patients was (237.79 ± 6.13 mg/dl) and in the control group was (118.42 ± 0.83 mg/ dl) *(P <0.05)*.

The statistical findings further revealed that most T1DM patients were suffer from diabetes at ages less than five years. The distribution of patients and control groups according to duration of diabetes (year) groups is shown in Table 2. In the Table, it appears that most patients 137 (59.6%) had a duration of disease between (1- 5 years) and only a very low frequency 1 (04 %) was suffered from disease for 31 - 35 years. On the other hand, the result of one-sample t-test revealeda significant difference in the duration of diabetes among these two groups (P = 0.05).

Table 1: Demographical picture of the studied groups

Demographical Parameters	Diabetic Patients (n=230)	Healthy Control (n=130)	*P-value*
Age(years) [Mean ± SE]	15.57 ± 0.69	11.95 ± 0.61	*0.17*
Ratio of Female: Male	122:108	68:62	0.893
Positivity Family History	169 (73.5%)	39 (30%)	*<0.05*
Random Plasma Glucose (mg/ dL) [Mean ± SE]	237.79 ± 6.13	118. 42 ± 0.83	<0.05

Table 2: Distribution of patients with T1DM according to duration of disease.

Duration of diabetes	Number	%
1 – 5	137	59.6
6 – 10	53	23
11 – 15	21	9.1
16 – 20	11	4.8
21 – 25	3	1.3
26 – 30	4	1.7
31 – 35	1	0.4
Total	230	100

The data obtained showed that fewer patients have had the disease for long periods, as a majority of 59.6% of the patients have acquired the disease within the past five years (table 2.), and 82.1 % of them acquired the disease during the first 15 years of their lives. This (82.1 %) was distributed as follow; (32.6 %) were between 0-5 years, 33.5 % between (6-10) years and (17 %) were between 11-15 years.

The study of environmental factor related to T1DM is presented in Table 4. This table shows the distribution of patients with T1DM who were fed with cow milk in the early ages of their lives.

Statistical analysis amongst participants who consumed cow milk during infancy revealed the followings: (38.69 %) as against (50.8 %) for patients with T1DM and control group. Moreover, The chi-square test (χ^2) shows that there is significant difference between T1DM and non-diabetic individuals in the using of consumption cow milk during infancy, (χ^2) = 4.93 with (P =0.026)}.

The result of this study reveals the presence of large amount of anti-viral Ab in the blood circulation of T1DM patients and control group. The result of statistical analysis indicated that the (Mean ± SE) for CMV of T1DM patients was (1.11 ± 0.02 IU/ml) compared to the control group (0.79 ± 0.02 IU/ml), and shows high significant differences between these two groups (*P-<0.05*).

Table 3: Distribution of patients with T1DM according onset of disease.

Onset of diabetes	Number	%
0 – 5	75	32.6
6 – 10	77	33.5
11 – 15	39	17
16 – 20	19	8.3
21 – 25	12	5.3
26 – 30	7	3
31 – 35	1	0.4
Total	230	100

Table 2.4: Consumption cow milk during infancy among T1DM patients.

Consumption cow milk during infancy	T1DM patients		control group		*P-value*
	Freq.	%	Freq.	%	
No	141	61.31	64	49.2	0.026
Yes	89	38.69	66	50.8	
Total	230	100.0	130	100	

While, for rubella the (Mean ± SE) for T1DM patients was (0.60 ± 0.01 IU/ml) as compared to the control group (0.38±0.01 IU/ml), and shows high significant differences between these two groups (*P<0.05*), and for CSV-B for T1DM patients was (0.35 ± 0.01 IU/ml) as compared to the control group (0.26 ± 0.01 IU/ml), and shows high significant differences between these two groups (*P<0.05*) (Table 5).

Table 5:The presence of anti viral and GAD IgGAb among T1DM and control group.

Types of virus Ab.		Most frequency age group 0 - 10 years	Mean ± SE (I.U/ml)	P-Value
Anti CMV IgG	Patient	45.65 %	1.11 ± 0.02	0.05
	Control	35.38 %	0.79 ± 0.02	
Anti RubellaIgG	Patient	26.95 %	0.60 ± 0.015	0.05
	Control	49.23 %?	0.38 ± 0.015	
Anti CSV-B IgG	Patient	6.15 %	0.35 ± 0.01	0.05
	Control	6.15 %	0.26 ± 0.01	

On the other hand, both of T1DM patient and control group recorded high positive result for multi-viral infections. There were15.6% of T1DM patients who were infected with all three types of viruses as compared to 0.0 for control group, (33.9%) were infected with two types of viruses as compared to (16.2%) for control group,(43.9%) had at least one type infection as compared to (46.9%) for control group, while (6.5%) did not record infection with any of these three viruses as compared to (36.9%) for control group (Table 6).

4. Discussions

The findings of this study from the demographic point revealed the following: T1DM is more common among young ages, it is important to be mentioned that the patients who participated in this study were 19 years and above and they were diagnosed of T1DM from their early age. This implies that those in their young ages in Kurdistan suffer more from T1DM and this finding agrees with that of the National Diabetes, (2012) from the Diabetes UK, (2010) report, that the peak of diagnosis of T1DM is between 10-14 years.Furthermore, T1DM can be observed among male and females, and there is no significant difference in spread of T1DM between the gender [27]. Findings of this study show that T1DM is common amongst both of males and females with a very little difference. This confirm with the result by Patterson CC *et al.* (2007), and the small difference in this study may be due to randomize sampling or in the Kurdish society females were more expose to psychological stress which is one of the causes of T1DM. Another important issue related to T1DM is the family history with diabetes. The finding about the family history in this study agrees with other studies that diabetes mellitus is common among families who

have a history of diabetes among parents, sibling, and relatives [28]. The results of RBS reveal a difference between the serum glucose between T1DM patients and non-diabetes individuals and this is in line with that of Papanas, (2010), which mentioned that the glucose in the blood circulation of diabetes patients is already higher than non-diabetes individuals.

The finding of this study about consumption cow milk during infancy one of signals that may be related to causes of growing of T1DM, this result in line with[12]. This is because that induce T1DM in cow milk is similar to bovine serum albumin (17-amino acid "ABBOS" peptide) in the cow milk and the structure of β-cells in the pancreas [18]. The result of this study are in line with others that recorded the relationship between consumption of cow's milk and T1DM in the countries who show increase of T1DM in age group 0-14 years [29]. The second environmental factor related to T1DM is viruses that are suspected to induce T1DM. These results show the epidemiology of these viruses in Erbil city. According to Shu, et al., (2010) CMV is one of the most common viruses around the world. Furthermore, according to Albaitushi, (2011) CMV is considered the commonest among T1DM patient in Iraq. The result of this study reveal a frequent incidence of rubella in Erbil city and this supports the report of the Ministry of Health, Kurdistan region of Iraq in 2011, in which 85% of children under age 1 were vaccinated against rubella in Erbil city. This implies that non-vaccinated children recording higher values before 2011 as the quality of health services in Kurdistan increased considerably after 2003 when the economic sanctions imposed on Iraq which destroyed the infrastructure of the citylifted. In this study, the findings of reveal that (25.7%) of T1DM patients are infected with CSV-B, while the result by Dezayee, (2012) [30] is (29.37 %). In addition, this study revealed that of T1DM having positive result for all viruses (CMV, rubella, and CSV) or when they are infected with one or two types of these three viruses. Based on these results we can infer that these three viruses are greatly responsible for increases in TIDM in Erbil city, and this is in line with the common believe that viral infections is one of the causes that triggers T1DM through disturbance of immune responses or direct destruction [31]. Furthermore, the positive result recorded for cytomegalovirus, rubella, and CSV among age group 0-10 years old. These infections become highly effective or dangerous because of the incomplete immune system among patients of these ages [32], and as it is mentioned earlier the mechanism of how viruses induced T1DM is not fully known [33].

In this study, the first finding of the immune responses (genetic & molecular) related to T1DM was observed for anti-GAD Abs. The difference between mean value of anti-GAD antibody in the blood circulation of T1DM patients and control group is indicating damage of β-cells in the pancreas [34-37]. The explanations for high level of anti-GAD antibodies among T1DM patients related to:

1. The anti-GAD antibodies are more common among T1DM patients [11].
2. The presence of viral infection induced T1DM, because there are high number of T1DM patients who participate in this study having viral infection [12]..
3. The overload of cellular immunity have been related to T1DM [38] and the result for activity of cellular immunity of this study shows that there are overload of in the immune response in the cellular immunity
4. In this study, the age group was between 3-40 years and the anti-GAD antibodies can be observed among T1DM even in above of 30 years age also which known as: Latent autoimmune diabetes in adults (LADA) [39]. This is one of another reason that the level of anti-GAD antibodies was high in this study.

However, the result of this study confirms by another Iraqi study among Arab population that recorded significant differences of the presence of anti-GAD antibodies between T1DM patients and control group [40, 41], while[42] linked the presence of anti-GAD antibodies with the presence of HLA-DR3, DR4 and family history with diabetes. The second finding of immune responses (genetic & molecular) related to T1DM was the presence of high level of anti-insulin receptor antibodies in the blood circulation of T1DM patients. The result of anti-insulin receptor antibody is goes along with the reports that mention the presence of high level of antibodies in the blood circulation of T1DM patients [34, 43-46]. It also indicates the loss of function of the body of T1DM patients [47].

Cellular immunity response is other immune responses related to T1DM.The result of this study shows that there are overload of cellular immune system activity, and mostly this type of activity demonstrated among T1DM patients [48]. However, based on the statistical analysis results, the mean value of the positive results of IL-4 and IL-10 of T1DM patients is higher among patients in the age group 0-10 years, while it is lower among patients in the age group of 21-30 years. On the other hand, the level of IL-4 was higher among those who have been suffering from diabetes for 31-35 years, while IL-10 level was higher among who have had diabetes for 21-25 years.This means the over secretion of IL-4 and IL-10 in the T1DM patient's blood circulation can be harmful for β cell and cause damage. It is considered as one of the signals for damaging β cells in pancreas

[48]. While, the level of IL4,10were in normal range among control group. The results of this study show that IL-4 and IL-10 in the blood circulation of T1DM patients and control group are agrees with the result of Amirshahrokhi, Dehpour*et al*. (2008) which mentioned that the overload of cellular immune activities demonstrated among T1DM patients. While, the increasing ratio of CD4:CD8 is another indicator showing that the cellular immunity activity is related to T1DM. The significance difference between the mean of CD4:CD8 of T1DM and control group is an important signal for autoimmune disturbance in the body. Both of CD4 and CD8 cells were implicated in the T1DM. The ratio is significantly higher in T1DM patients than other non-diabetes,The malfunctioning of these cells lies the failure in differentiating between own body and other foreign cells. The result of this study confirms with the final reports of the studies of Phillips, 2009, and Ferreira, 2010, which state that the ratio of CD4 and CD8 are directly related to developing of diabetes.

This study is also focused on a specific genes feature of Iraqi Kurdish population. In this regard, the current study revealed that the HLA-DR3 has the highest frequency among T1DM patients (61.3 %) as against (35.4%) for healthy group. While for HLA-DR4 is (73.5 %) in TIDM patients s compared to (30 %) in the control group.This explains that these alleles can induce T1DM, (i.e. chance of acquiring disease is more than those of the same population who lack this type of alleles). The present study reveals that 39.56 % of HLA-DR3 has positive result among age group 0-10 years of T1DM patients, while this amount for HLA-DR4 was (41.30). There were other Iraqi study that relied the use of serotype technique rather than genotype and showed the effect of HLA-DR3, DQ2, DQ3 genes on T 1DM in Iraqi Arab patients. [49]In a study by Kawabata et al., (2002), theyfound thatDR4 and DR3 genes arerelated with T1DM. In Iraq incident of HLA-DR3 with 27.25% is higher than HLA-D4 with 21.25%. However, in Saudi Arabia 30.90% of population have HLD-DR3, and 25.20% have HLA-DR4 [50]. While, the presence of both of HLA-DR3 (60%) and HLA-DR4 (41%) was higher in Iraqi Arab T1DM patients than non-diabetic individuals with HLA-DR3 (10%) and HLA-DR4 (6.6%). Similarly in another study the presence of these genes were around 30% in T1MD patients while in healthy group was 0 (HLA-DR3) and 13% (HLA-DR4)[51]. The result of this study supports researchers who believe that HLA-D3 and DR4 are related to T1DM. In the other words, the presence of these genes enhances viral infection to induce T1DM [52].

Generally, viral infection such as CSV B, cytomegalovirus, adenovirus, and mumps are more responsible in inducing T1DM in the presence of HLA-DR3 or/and DR4 genes [53, 54]. Finally, the cases of T1DM that are not related to HLA genes, may be due to stressful situations, such as psychological effects of wars and environments which may stimulate/induced the development of T1DM resulting in an increased in the incidence of this disease.

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