



## ESTIMATION CELL MEDIATED IMMUNITY IN SOME IRAQI PATIENTS WITH TYPE 1 AND 2 DIABETES MELLITUS.

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### Abstract

Cell-mediated immunity was estimated in diabetic patients by evaluation a level of CD4 and CD8 in type 1 and 2 diabetic patients. Of the 33 patients with type 1 diabetes mellitus 17 were males and 16 were females, as well as the type 2 diabetes mellitus patients were made up of 17 males and 16 females. Both groups attend the outpatient Clinic in the National Diabetes Center, Al-Mustansiriya University. Besides that, the results of this study recorded that the mean of age among patients with type 1 diabetes mellitus was  $23.42 \pm 4.25$  y whereas mean of age among second group was  $51.27 \pm 7.83$  y with highly significant difference was noticed between them. In addition, the result of this study indicated that duration of disease among type 1 diabetic patients was  $7.15 \pm 2.55$  y while  $11.45 \pm 6.49$  y among second group with highly significant difference was noticed between them. For CD4 lymphocytes, there was a highly significant decrease ( $P < 0.001$ ) in the percentage means of the CD4 cells in type 2 diabetic patients, as compared with type 1 diabetic patients. For CD8 lymphocytes, there was a decrease in the percentage of these lymphocyte in the peripheral blood of the type 2 diabetic patients, as compared with type 1 diabetic patients with highly significant importance was recorded between them. In conclusion, this study proved that impaired cell mediated immunity is prevalent in type 2 diabetic patients, thus the change level of cytotoxic T-lymphocytes (CD8) and helper T- lymphocytes (CD4) might be responsible for depressed immune response in patients with type 2 diabetes.

**Key words: Cell mediated immunity, Diabetes Mellitus, CD4, CD8.**

CD8 CD4

±

±

±	±
CD8	CD4
	(P<0.001)
	CD4
	CD8

## Introduction

Diabetes mellitus, often simply referred to as diabetes—is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (1). There are three main types of diabetes: Type 1 diabetes: results from the body's failure to produce insulin, and presently requires the person to inject insulin (Also referred to as *insulin-dependent* diabetes mellitus, *IDDM* for short, and *juvenile* diabetes), Type 2 diabetes: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. (Formerly referred to as *non-insulin-dependent* diabetes mellitus, *NIDDM* for short, and *adult-onset* diabetes) and Gestational diabetes: is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM (2, 3). T cells or T lymphocytes belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity(4). T helper cell ( $T_H$  cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and activation of cytotoxic T cells and macrophages, among other functions. These cells are also known as  $CD4^+$  T cells because they express the CD4 protein on their surface. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of Antigen Presenting Cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response (5). Cytotoxic T

cells ( $T_C$  cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as  $CD8^+$  T cells since they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body (6). Patients with diabetes mellitus have an increased incidence of infections caused by bacteria, virus, and fungi (7). Immune deficiencies are often invoked to explain their increased incidence of infections and morbid complications. Additionally several types of functional abnormalities have been demonstrated in polymorph nuclear leukocytes, particularly when the patients are in ketoacidosis (8).

Studies of cell-mediated immunity (CMI) have demonstrated abnormal cellular immunity in patients with diabetes mellitus (9, 10). CMI appears to be important in host defenses against certain infections, especially those caused by fungi, virus and bacteria (10), therefore the purpose of this study was to investigate CMI in diabetic patients by determination a level of CD4 and CD8 in type 1 and 2 diabetic patients.

## Subjects and Methods

A total of 66 diabetic patients comprising as 33 patients from both sex have type 1 diabetes their age range (17-34 year) and 33 patients from both sex have type 2 diabetes with age range (36-64 year). All diabetic patients who attended the outpatient clinic in the National Diabetes Center, Al-Mustansiriya University for monthly clinical examination and laboratory investigation which including certain hematological and biochemical tests. A blood sample was taken from each person of the patients by vein-puncture. First the area was wiped with 70% alcohol and left to dry up, then

a disposable syringe (10 ml) was used to withdrawn 10 ml of the blood (5 ml as a minimum volume). From the previously collected sample, 5ml was transferred to EDTA-containing collecting tube. The tube was labeled with the required information about the participant; this part was used for CD4 and CD8 assay.

## Methods

### Immunological test

#### CD4 and CD8 T-cell assay.

##### Principle

Is an immune-enzymatic assay based on the specific capture of the TCD<sub>4</sub> or TCD<sub>8</sub> lymphocytes, with paramagnetic micro particles coated with capture antibodies (11).

##### Procedure

- 1- Mixing the blood sample and a suspension of paramagnetic (coated with anti-pan T antibodies for the capture of T lymphocytes). Dispense the mixture in the wells of a micro titration plate associated with a magnetic frame.
- 2- After aspiration of the residual blood, a peroxidase – labeled monoclonal antibody with anti-CD<sub>4</sub> (or anti-CD<sub>8</sub>) specificity is added to the sample in the well (separate measurement for TCD<sub>4</sub> and TCD<sub>8</sub> cells for a same sample).
- 3- On completion of the labeling step, the peroxidase immobilized in the antigen – antibody complexes is revealed by incubation in presence of substrate, after the unbound fractions have been removed (Washing).
- 4- After the reaction stopped, the measurement of the absorbance values of the wells is performed with a spectrophotometer at 450nm. The absorbance values are proportional to the number of TCD<sub>4</sub> and TCD<sub>8</sub> cells in the blood samples (11).

##### Statistical analysis

Data have been analyzed statistically using SPSS program version 10. Analysis of quantitative data was done using t-test and ANOVA (analysis of variance). Acceptable level of significance was considered to be below 0.05 (12).

##### Results and Discussion

Patients with diabetes mellitus appear to have an increased incidence of infections with a wide variety of pathogens. cell-mediated immunity appears to be central in host resistance against certain infections, particularly those caused by fungi, virus and bacteria, in addition cell-mediated immunity have demonstrated

abnormal in patients with diabetes mellitus, for that reason the aim of study was to examine cell-mediated immunity in diabetic patients by assessment a level of CD4 and CD8 in type 1 and 2 diabetic patients.

The distribution of studied groups according to sex and age are shown in tables 1a and b. Of the 33 patients with type 1 diabetes mellitus 17 were males and 16 were females, as well as the type 2 diabetes mellitus patients were made up of 17 males and 16 females. Besides that, the results of this study recorded that the mean of age among patients with type 1 diabetes mellitus was  $23.42 \pm 4.25$  y whereas mean of age among second group was  $51.27 \pm 7.83$  y with highly significant difference was noticed between them as presented in tables (2, 3). These results coincide with the studies done by (13) who establish that 23.5y was the mean age for type 1 diabetes mellitus patients and (14) reported that the mean age was 51 y for type 2 diabetes mellitus patients. Also on other hand (15) registered that the mean age for type 1 and 2 diabetes mellitus was 24y and 52.6y respectively. Furthermore, 24 of the type 1 diabetic patients were above 20 years of age while 7 of the type 1 diabetic patients were below 20 years, on the other hand 17 of the type 2 diabetic patients were above 50 years of age while 12 of the type 2 diabetic patients were below 50 (40-49) years are shown in tables 1a and b. This results agreed with the study of (16) who has recorded 70% of the type 1 diabetic patients were above 20 years of age, additionally (17) found that 52% of the type 2 diabetic patients were above 50 years of age. Type 1 diabetes is less common than type 2 diabetes. This type of diabetes can occur at any age, but usually first develops in childhood or adolescence. Type 1 diabetes accounts for 5 - 10% of all diabetes cases (18). Besides that, type 2 diabetes is typically recognized in adulthood, usually after age 45 years. It used to be called adult-onset diabetes mellitus, or non-insulin-dependent diabetes mellitus. These names are no longer used because type 2 diabetes does occur in younger people, and some people with type 2 diabetes need to use insulin (3).

Besides that, the result of this study indicated that duration of disease among type 1 diabetic patients was  $7.15 \pm 2.55$  y while  $11.45 \pm 6.49$  y among second group with highly significant difference was noticed between them as pointed out in tables (2, 3). This is similar to that in the study of (16) who found that their duration of

disease among patients with insulin-dependent diabetes mellitus was 7.55 y. It is also similar to the findings of (17) who established that their

duration of disease among patients with non-insulin-dependent diabetes mellitus was 11.85 y.

**Table 1a: Age and sex distribution of Type 1 diabetes mellitus group.**

Type 1 diabetes mellitus patients			
Parameter	sex		
Age (year)	Male	Female	Total
(<20)	5	2	7
(20-29)	12	12	24
(>30)	0	2	2
Total	17	16	33

**Table 1b: Age and sex distribution of Type 2 diabetes mellitus group.**

Type 2 diabetes mellitus patients			
Parameter	sex		
Age (year)	Male	Female	Total
(30-39)	2	2	4
(40-49)	7	5	12
(50-60+)	8	9	17
Total	17	16	33

In the field of cellular immune response, the present study also highlights the role of cellular immune response in diabetes mellitus in particular cytotoxic T-lymphocytes (CD8) and helper T- lymphocytes (CD4).The percentage of CD8 and CD4 in patients with type 1 and 2 diabetes mellitus have been estimated at tables (2,3) and the results revealed that :

For CD4 lymphocytes: there was a highly significant decrease ( $P<0.001$ ) in the percentage means of the CD4 cells in type2 diabetic patients, as compared with type 1 diabetic patients.CD4 (TCD4/ $\mu$ L) levels in type 2 diabetic patients (mean  $\pm$  SD) was  $1182.36 \pm 158.82$  while  $1846.06 \pm 359.79$  in type 1 diabetic patients .

For CD8 lymphocytes : there was a decrease in the percentage of these lymphocyte in the peripheral blood of the type 2 diabetic patients, as compared with type 1 diabetic patients. CD8( TCD8 / $\mu$ L) levels in type2 diabetic patients (mean  $\pm$  SD) was  $1131.03 \pm 343.53$  while  $1664.45 \pm 326.34$  in type 1 diabetic patients with highly significant importance was recorded between them. These results is concurrence with (9) they found a highly significant decrease in mean percentage of T-cytotoxic (CD8) and T-helper (CD4) in type2 diabetic patients than type 1 diabetic patients, also this finding was also detected by (19) and

(20) they reported that the percentage number of circulating

T-lymphocyte (cytotoxic CD8 and helper CD4) were reduced significantly in type2 diabetic patients as compared to type 1 diabetic patients.

The cytotoxic T-lymphocytes (CD8) and helper T-lymphocytes (CD4) exert a prominent role in the disease pathogenesis. So the reduced number of CD8 T cells and CD4 T cells in peripheral blood of type2 diabetic patients may indicate that these patients are suffering from hypimmune responsiveness and have an increased incidence of infections with a wide variety of pathogens than type 1 diabetic patients who manifested a normal immune response (i.e. percentage of CD4 and CD8 within normal range).

This study demonstrates abnormalities in cell mediated immunity in the peripheral T lymphocyte subset (CD4 and CD8) in patients with type 2 diabetes mellitus when compared with type 1 diabetes mellitus patients .A significant difference was also detected in numerical peripheral blood T lymphocytes (CD4 and CD8) between type 2 diabetes mellitus and those with type 1 diabetes mellitus. The evidence of an imbalance in cell mediated immunity found in patients with type 2 diabetes mellitus in this study suggests a possible relationship between long duration of disease

and abnormal cellular immunity. In conclusion, this study shows that impaired cell mediated immunity is prevalent in Type 2 diabetic patients, therefore diabetes mellitus can act as a stressor affecting immune modulation and outcome of

infection, as well as the altered levels of cytotoxic T-lymphocytes (CD8) and helper T-lymphocytes (CD4) might be responsible for lowered immune response in patients with type 2 diabetes.

**Table 2: Descriptive Statistics for Type 1 and 2 diabetes mellitus patients**

parameter	Type1 diabetes mellitus patients			Type 2 diabetes mellitus patients		
	No.	Mean	Std.Dv	No.	Mean	Std.Dv
Age (Year)	33	23.42	4.25	33	51.27	7.83
CD4 (CD4/ $\mu$ L)	33	1846.06	359.79	33	1182.36	158.82
CD8 (TCD8/ $\mu$ L)	33	1664.45	326.34	33	1131.03	343.53
Duration of disease (year)	33	7.15	2.55	33	11.45	6.49

**Table 3: t-test for Type 1 and 2 diabetes mellitus patients**

Type 1 diabetes patients	Type 2 diabetes mellitus patients	t-test	p-value	C.S
Age (Year)	Age	17.605	P<0.001	HS
CD4 (TCD4/ $\mu$ L)	CD4	8.948	P<0.001	HS
CD8 (TCD8/ $\mu$ L)	CD8	5.800	P<0.001	HS
Duration of disease (Year)	Duration of disease	3.907	P<0.001	HS

\*HS: Highly Significant

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