Evaluation of T-lymphocytes in Peripheral Blood of Diabetic Patients

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Abstract
Our study includes the estimation of absolute number of T-lymphocytes by using (E-rosette) test in diabetic patients with hyperglycemia. The study included the collection of (20)blood samples from individuals Type I ,II diabetes mellitus, ten of them were of type one I others of type II, with age range between (8-55)years and of both sexes. Blood samples from healthy individuals as control samples were used as well. The study showed a significant decrease in absolute numbers of T-lymphocytes in diabetic patients when compared with control and also a significant reduction in the absolute number in type I and type II diabetic patients compared with control, our result suggest that there is a defect in T-lymphocytes numbers in diabetic patients with hyperglycemia.

Key Words: Hyperglycemia; Diabetes; E-rosette.

Introduction
Type I (insulin-dependent) diabetes mellitus is an autoimmune disease with defective glucose metabolism resulting from islet of Langerhans destruction. The hyperglycemia of type I diabetes may actually be a late phase of the disease, because it is preceded by a clinically quiescent period during which autoantibodies to islet cells are produced and subtle decreases in insulin production develop **[1,2].**
Type 2 diabetes results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency **[3] .**
Human T-lymphocytes play a central role in the regulation of the immune response. Functionally distinct subpopulations of lymphocytes have been defined by monoclonal antibodies recognizing cell-surface markers. The major subdivision of human peripheral T-lymphocytes is between those cells bearing the CD4 (T4) antigen and those that express the CD8 (T8) antigen. T4+ cells, comprising 55-70% of peripheral T-lymphocytes, proliferate in response to soluble antigens, induce B-lymphocytes to secrete Igs, and stimulate precytotoxic cells to become cytotoxic (Knotek *et al*., 1991),**[1]**. T4+ cells recognize antigen in the context of MHC class II (HLA-D)gene products (Ledbetter *et al*.,1981). In contrast, T8 antigen-bearing cells, comprising 20-35% of circulating peripheral blood T-lymphocytes, exert suppressive and cytotoxic activities and recognize antigen in the context of class I (HLA-A, -B,and -C) gene products **[4].**
Patients with diabetes mellitus have an increased incidence of infections caused by bacteria, virus, and fungi **[5]**. 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 polymorphnuclear leukocytes, particularly when the patients are in ketoacidosis**[6]**.
The Erythrocyte rosette (E-rosette) test, is a technique used to characterize T-lymphocytes using sheep red blood cells (SRBCs). The principle is that the suspected receptor bearing cells are mixed with the signal cells which carry the corresponding receptor building substance on their surface. That substance is either naturally occurring on it or artificially coupled to it. The receptor bearing cell will then bind the signal cells around their surface and form rosettes. Human lymphocytes can be classified according to their surface markers, thymus derived (T) lymphocytes may be identified by spontaneous rosette formation with sheep red cells **[7]** .
Studies of cell-mediated immunity (CMI) have demonstrated abnormal cellular immunity in patients with diabetes mellitus
[8,**9]**. cell- mediated immunity appears to be important in host defenses against certain infections, especially those caused by fungi, virus and bacteria **[9]**, therefore the purpose of this study was to investigate CMI in diabetic patients by determination the the absolute number of T-lymphocytes in diabetic patients in general and type I and type II diabetic patients.

Materials and methods
- **Measurement of sugar in fasting state:**

Fasting Sugar:
Serum glucose was determined according to instructions of Biocon, Germany which is an enzymatic and colorimetric method(GOD, POD)**[10]**.

- **White Cells Count:**

Total leukocytes count=Number of WBCs in 1mm3 = Total leukocytes in 4 sqmm/4
TLC=X200**[11]**

- **Differential White Cells Count:**

A small drop of EDTA-anticoagulant blood is placed and spreaded on a slide, then the film fixed and stained with leisman's stain **[12]** .

- **Separation of lymphocytes**

The differential gradient density centrifugation method employed by **[13]** was used. ten (10) ml of heparinised blood was diluted 1: 2 with phosphate buffer saline ,This was layered in 7 ml aliquots on 3 ml of Ficoll-Histopaque density gradient in 10 ml tissue culture tubes. The mixture was centrifuged at 1800 rpm 30 minutes at room temperature, lymphocytes harvested by gentl aspiration removing of lymphocyte layer at the Ficoll-diluted plasma interface.

- **Viability test:**

Lymphocyte viability was tested by trypan blue exclusion test.

- **Sheep red blood cells**

Blood was collected from jegular vein and mix with equal volume of Alsever's solution. They were washed three times in Hanks and resuspended in the appropriate medium just before testing.

- **Active E-rossate:**

1- 0.25ml of lymphocytes suspension +0.25ml sheep RBC

2- (1ml) of lymphocyte suspension ($1*10^6$ cells/ml) and (0.5%) SRBCs suspension were mixed in polystyrene tubes.

3- The tubes were incubated in 4°C at 30 min.

4- The tubes were centrifuged ~t 125 g 5 min. The cells were gently resuspended in the tubes before counting. At least 200 cells were counted. Lymphocytes with at least 3 bound erythrocytes were considered as RFCs **[14]**

Statistical analysis:

Comparisons of means were analyzed statistically, using one way Analysis Of Variance (ANOVA) of probability $P \leq 0.05$,all statistical analysis was performed using SPSS 19.0 software.

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.

The results showed a significant decrease in the absolute number of T-lymphocytes (E-rosette) for diabetic patients in general compared to the control as shown in Table (1).see Fig(1).

Table (1) The absolute number of E-rosette forming cell in diabetic patients

T-lymphocytes	Subject	No. of patients	Mean ± S. D. cell/ ml	Extreme Values cell/ ml	Sig.
T-Lymphocytes (E-rosette)	Diabetic patients Control	20 20	373±110 1065±253	164-536 495-1364	0.000*

*$P \leq 0.05$

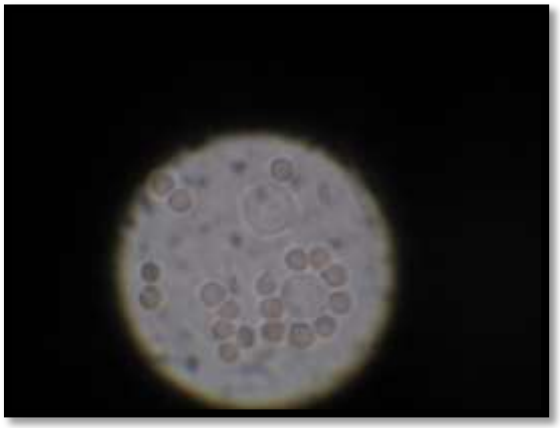

Fig (1) peripheral blood T-lymphocytes forming E-rosette from diabetic patients

The results also showed a significant decrease in the numbers of T-lymphocytes (E-rosette) in type I and type II diabetic patients compared with control, as shown in Table (2).

Table (2)The absolute number of E-rosette forming cell in diabetic patients Type I, II

T-lymphocytes	Subject	No. of patients	Mean ± S. D. cell/ ml	Extreme Values cell/ ml	Sig.
T-Lymphocytes (E-rosette)	IDDM NIDDM Control	10 10 20	395±88 360±124 1065±253	310-536 164-531 495-1364	0.000* 0.000*

*$P \leq 0.05$

Alterations in T- lymphocytes are a common finding in both type I and type II diabetes. Autoimmune phenomena in type I diabetes, the stage of the diabetic disorder and metabolic effects of therapeutic interventions may also affect actual distribution of lymphocyte phenotypes **[15]**.

The study by**[16]** showed that almost all of the juvenile-onset type have a significant reduction of the leukocyte migration index after exposure to pancreatic antigen was present**[17].** have shown a reduced transformation response to PHA in poorly controlled diabetics and have related this finding to the metabolic situation.

As for rosette-forming ability **[9]** pointed out that this is an energy-dependent process, suppressed by metabolic inhibitors: metabolic decompensation could therefore be relevant in the interpretation of the lower values of peripheral T-lymphocytes found in diabetic patients.

Our results seem to differ from those of **[17]** who did not find any difference in T-lymphocyte percentage between insulin-dependent diabetics (IDD) and normal controls, mean while our data suggest a significant difference, as far as T-lymphocyte percentage in peripheral blood is concerned, between diabetic patients and control.

The study by **[18]** showed a significant decrease in number of T-cell and NK cells. Some studies **[19,20,21].** have shown that is no significant change in total T-cell population in type II diabetics and control group. **[22]** Reported reduced level of activated T-lymphocyes (CD_{25}^{+}) in patients with type II diabetes mellitus compared to healthy controls.

The study of **[21]** showed decrease cytokine level, decrease in blast cell transformation mitogen-induced proliferation and reduced IL_2 receptors on lymphocytes and deficiency of T-cells with CD_3.

These results agreed with **[23]** who reported that glutamine is both an oxidative substrate an important source for synthesis of pyramidine and purine nucleotides and amino sugars in lymphocytes, glutamine is known to be required for both lymphocytes proliferation and cytokine production, glutamine oxidation decreased in diabetic lymphocytes. Also the study of **[24]** was agreed with these results who noticed that a high proportion of apoptotic lymphocytes in diabetic cases may explain the impaired immune function in poorly controlled diabetic patients.

It have been reported that decreased lymphocyte transformation abnormalities may exist in membrane receptors for mitogen in these cells or may reflect intracellular defects in metabolism could well be one of the mechanisms for the impaired immune function observed in diabetic type 2 patients **[23,25]**.

In our study all patients suffering from with hyperglycemia, where the sugar level around the somatic cells is very high, leading to changes in metabolic pathway and ways to pull glucose from blood stream ,in addition to the absence of sufficient amount of insulin which allows the entry of glucose in to the cell, all biological processes like reproduction and immune response which includes cytokine production in T lymphocytes will be changed. In Juvenile onset diabetes the high glucose level effecting on development and differentiation of T lymphocytes in thymus gland during maturition.

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تقدير الخلايا اللمفاوية التائية في الدم المحيطي لمرضى داء السكر

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الملخص

تضمنت دراستنا الحالية تقدير الاعداد المطلقة للخلايا اللمفاوية التائية بأستخدام تقنية (E-rossate) لمرضى داء السكر والذين يعانون من الارتفاع المزمن لمستويات السكر في الدم (hyperglycemia)، تضمنت الدراسة اختبار (20) عينة دم لمرضى داء السكر، (10) لمرضى النمط الاول والباقي لمرضى النمط الثاني وبعمر تراوح بين (8–55) سنة ومن كلا الجنسين واستخدمت عينات دم لاشخاص معافيين كعينة سيطرة .اظهرت النتائج وجود انخفاض معنوي في الاعداد المطلقة للخلايا اللمفاوية التائية لمرضى داء السكر بشكل عام مقارنة بعينة السيطرة ،كما اظهرت النتائج وجود انخفاض معنوي في اعداد الخلايا اللمفاوية التائية لمرضى النمط الاول والثاني مقارنة بعينة السيطرة لذلك اقترحت دراستنا وجود خلل في اعداد الخلايا اللمفاوية التائية لمرضى داء السكر والذين يعانون من حالة (hyperglycemia).