Study of Cell Mediated Immune Response Represented By T-Lymphocytes Transformation and Proliferation in Diabetic Patients Type 2

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الخلاصة:

شملت هذه الدراسة قياس الاستجابة المناعية الخلوية المتمثلة بعملية التحول والانقسام في الخلايا اللمفاوية التائية لـ 60 مريض مصاب بداء السكري النوع الثاني بعمر (30−70) سنة ومن كلا الجنسين من المرضى الراقدين في مستشفى المحمودية العام ومقارنتها مع مجموعة سيطرة مكونة من 30 شخص من الاصحاء وبنفس المرحلة العمرية.

تم اختيار العينات بعد أجراء فحص سكر الدم الصيامي على المجموعتين وقد أظهرت النتائج انخفاض معنوي في نسبة عملية التحول والانقسام للخلايا اللمفاوية في مرضى داء السكري من النوع الثاني مقارنة مع مجموعة السيطرة.

Abstract:

This study included the measurement of T- Lymphocytes transformation and proliferation for 60 diabetic patients type 2 in the range of (30-70) years of the both sexes who are entered out patients clinic of AL- Mahmodia hospital. The results compared to a control of 30 healthy individuals in the same age range.

The results showed a significant decrease percent of T-Lymphocytes transformation in diabetic patients in compared to the control group.

The selection procedure was confirmed only after performing a fasting blood sugar (FBS) for both groups.

Introduction:

Diabetes is a chronic disease that occurs either when the pancreas dose not produce enough insulin or when the body can not effectively use the insulin it produces Insulin is a hormone produced by the pancreas regulates blood sugar [2, 9].

Hyperglycemia or raised blood sugar is a common effect of uncontrolled diabetes and over time leads to serious damage to many of body's systems especially the nerves & blood vessels. [9, 12]

Type 1 diabetes: (previously known as insulin–dependent, juvenile or childhood- onset), it characterized by deficient insulin production & requires daily administration of insulin.
Type 2 diabetes: (formerly called non –insulin- dependent or adult onset) results from the body's ineffective use of insulin. Its symptomsinclude excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, these symptoms are often appears slowly, as a result the disease may be diagnosed several years after onset, once complications have already arisen [10, 12].

Type 2 diabetes is a serious & costly disease affecting 90- 95% of people around the world, the chronic complications of diabetes include accelerated development of cardiovascular disease end stage renal disease, loss of visual acuity and limb amputations [1, 9, 12].
Immunological disturbances of cell mediated origin are believed to initiate from T- Lymphocyte dysfunction. Resent *invitro* studies implicated that in type 2 diabetes mellitus inappropriate immune responses may result from the defects in the action of insulin that is required for the function of T- Lymphocytes [7].

Mature lymphocytes recirculate via blood and lymph through lymphoid tissue in a relatively quiescent state until stimulated to proliferate during, for example bacterial or viral infection. T- Cell blastic transformation stimulated by phytohaemagglutinin (PHA) and plasma levels of immunoglobulins are markedly reduced in patients with diabetes mellitus and effect reversed by insulin administration [4, 7].
The aim of the present study is to investigate the cell mediated immune response represented by T.lymphocytes transformation in diabetic patients type 2.

Materials and Methods:

Solutions:

1- **Glutamic acid solution:** 25 gm glutamic acid + 100 ml distilled water
2- **Antibiotic Solution:** 1 gm streptomycin sulphate (SDI) + 1000000 IU. Crystallized penicillin (SDI) + 100 ml distilled water.
3- **Hepes Solution:** Prepared by flow company to use as a buffer to keep the PH (7.4- 7.5), Add ass 1: 100 ml of the complete culture media.
4- **Fixative solution:** 3 volumes absolute methyl alcohol + 1 volume of glacial acetic acid.
5- **Hypotonic Solution:** 2.85 gm Kcl + 5000 ml distilled water.
6- **Sodium bicarbonate solution (0.75) %:** 7.5 gm NaCo3 + 100 ml distilled water.
7- **Complete RPMI- 1640 media:** [(10.4 gm) RPMI- 1640 media powder + 2 gm analar sodium bicarbonate + 1L. deionized distilled

water] sterilized by filtration using membrane filters (0.22 Mm) then 10 ml antibiotic solution added and 10 ml glutamic acid solution and 10 ml hepes solution (prepared previously) . 100 ml fetal calf serum was added and incubated at 37°C for 24 hrs. then kept in refrigerator until use [8].

Sample Procedure:

1- Control group:

Blood samples from the peripheral blood were taken from a group of 30 healthy individuals aged (30-70) years. The samples collected in sterile tubes contain heparin as anticoagulant agent.

2- The cases:

Blood samples from the peripheral blood of 60 patients in the range of (30-70) years of the both sexes, all the subjects were in the category of type 2 diabetes mellitus who are entered out patients clinic of AL- Mahmodia hospital during (June- October 2009).

The selection procedure was confirmed only after performing a fasting blood sugar (FBS) and then distinguished the samples into diabetic and non diabetic cases.

Lymphocyte Transformation Assay:

This test includs the measurement of lymphocyte transformation and proliferation stimulating by phytohaemagglutinin (PHA) [8].

2 sterile siliconized tubes were used for each blood sample each one contained 2.5 ml of complete RPMI- 1640 media and 250 Ml of heparinized blood sample. 250 Ml of PHA added for one tube & left the other tube without PHA as a control.

The 2 tubes incubated at 37 °c for 72 hrs., after incubation they centrifuged at (2000 r/m) for 10 min.

Then amount of hypotonic solution was added with vibration and incubated at 37 °c for 50 min. then centrifuged as above,(3-8) drops of fixative agent then added to the precipitate with continuous vibration, at last 5 ml of the same fixative agent were added to the precipitate and cooled at 4 °c for 10 min., The precipitation carried for 3-4 times until had a colorless suspension, drops of the same fixative agent were added for the last precipitate with vibration then 5-7 drops of the last product of the cell suspension were fall on a clean slide 60 cm. elevation to make a smear. Smear was dried and stained by Giemsa stain for 15 min. and washed with distilled water and examined after dried by microscope at power 10* 100 using oil immersion. The stimulated cells percentage was calculate by the following formula:

$$sensitive\ cells\% = \frac{number\ of\ sensitive\ cells}{200 Lymphocyte\ and\ Lymphoblast} * 100$$

Statistical analysis:

The statistical analysis was performed using t test to compare mean values of T.lymphocytes transformation in diabetic patients type 2 with the control group. Values of $p<0.005$ were considered as statistically significant [7].

Results and Discussion:

The results of T- Lymphocytes transformation stimulated by PHA *invitro* test showed a significant ($p< 0.005$) decrease in diabetic patients when compared to the control who had normal mean of T-Lymphocyte transformation percent as showed in (Table -1)

Case	number	Mean of T- Lymph. Trans.	P
Diabetes II Patients	60	48.310	< 0.005
Control	30	59.223	

Table-1: Lymphocyte transformation percent in diabetic and control cases

This result agreed with [4] 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 well known to be required for both lymphocytes proliferation and cytokine production, , glutamine oxidative decreased in diabetic lymphocytes.
Also the study [5] was agreed with this result 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 [4, 6].

Production of IL. 2, IL. 6 and IL. 10 is dose and time- dependently suppressed by elevation in glucose concentration, high glucose levels also inhibit proliferation of peripheral mononuclear cells [4].

Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses and inappropriate T-Lymphocyte function which is vital in this pathogenic condition has a link with insulin defect [3, 7].

Adenosine deaminase plays a crucial role in lymphocyte proliferation and differentiation and shows its highest activity in T-lymphocytes, It reported that an elevation in adenosine deaminase levels in diabetic subjects when compared to controls, the high plasma adenosine deaminase activity might be due to abnormal T- lymphocyte response or proliferation may point towards a mechanism that involves its release into circulation, its elevation could be due to altered insulin related T- lymphocyte function. This may help in predicting immunological dysfunction in diabetic individuals [7].

The results also showed that the prevalence of diabetes type 2 in females is higher than males as showed in (Table-2) and figure (1).

Sex	Number	%
Female	37	61.7
Male	23	38.3
Total	60	100

Table-2: Distribution according to sex in diabetic patients type 2

This result agreed with [11] who reported to the high percentage of female with diabetes than male, and, [12] which projected that 55% of diabetes deaths are in women, and [1] who noticed that the prevalence of diagnosed type 2 diabetes is slightly higher in female than in male

The combined effect of a greater number of elderly female than male in most populations and the increasing prevalence of diabetes with age is the most likely explanation for these observations [11].

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