The Possible Role of Peripheral Blood Lymphocytes in the Pathogenesis of Diabetes Mellitus

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Abstract

Background: Diabetes mellitus (DM) is disorder with world wide distribution with severe complications and high mortality rate.
Aim: Our study shed light on the possible role of peripheral blood lymphocytes (PBL) B-cells and T-cells and subsets (T-helper (CD4) and T-cytotoxic (CD8)) in the pathogenesis of DM.
Patients and Methods: In this study, 40 patients with type-1 DM, and 40 patients with type-2 DM, with 22 age matched control group were included. Immunocytochemistry for PBL for each group were done to detect the level of B-cell (CD19), T-cell (CD3), T-cell subsets CD4 and CD8.
Results: The results showed a significant difference between control group and DM-1 in CD8, while DM-2 showed a significant differences in CD3, CD8 and CD19. A significant difference also seen between type-1 and type-2 DM in CD3 and CD19 markers, while CD4 and CD8 were not significant.
Conclusion: This study may explore the role of the immune cells, T-cells (CD3, CD4 and CD8) and B-cells in the pathogenesis of DM especially type -1 DM as it is the prototype of autoimmune disease.
Key words: Diabetes mellitus (DM), type-1 DM, type-2 DM, T-cell, B-cell, T-helper, T-cytotoxic, CD3, CD4, CD8, CD19.

الخلاصة

المقدمة: الداء السكري منتشر في كافة أنحاء العالم ويسبب مضاعفات خطيرة ووفيات عالية.
الهدف: الدراسة تهدف إلى تسليط الضوء على الدور المحتمل للخلايا اللمفاوية المحيطية كل من خلايا بي اللمفاوية وتي اللمفاوية والخلايا ذات التعليم بمعلمات سي ٤ وسي دي ٨ في امراضية داء السكري.
طريقة العمل: شملت هذه الدراسة ٤٠ مريض مصاب بداء السكري من النوع الأول و٤٠ مريض بداء السكري من النوع الثاني و٢٢ شخص صحيح كمجموعة سيطرة. تم تصبيغ الخلايا اللمفاوية المحيطية لكافة المرضى بطريقة التصبيغ الخلوي المناعي الكيمياوي بمعلمات خلايا تي (سي دي ٣، سي دي ٤ وسي دي ٨) ومعلمات خلية بي (سي دي ١٩).
النتائج: أظهرت النتائج بان هناك اختلاف ذات قيمة بين مجموعة الأصحاء ومرضى النوع الأول من داء السكري بمستوى خلايا تي من السي دي ٨، بينما كان هناك اختلاف ذات قيمة في اختلاف مستويات خلايا تي من النوع سي دي ٣و سي دي ٨ وخلايا بي اللمفاوية بين مرضى النوع الثاني والاصحاء. كذلك أظهرت الدراسة بان هناك فرق ذات قيمة بين مستوى الخلايا اللمفاوية بين مرضى النوع الاول ومرضى النوع الثاني لداء السكري لخلايا تي (سي دي٣) وخلايا بي (سي دي١٩) بينما لم يكن هناك أي أختلاف ذات قيمة بين خلايا تب من النوع سي دي ٤ وسي دي ٨.
الاستنتاجات: هذه الدراسة بينت الدور المحتمل للخلايا اللمفاوية تي و بي وانواع خلايا تي (سي دي٤ وسي دي ٨) في امراضية داء السكري في نوعيه الأول والثاني وخصوصا النوع الاول حيث يعتبر من أمراض المناعة الذاتية.

Introduction

Diabetes mellitus (DM) is a disorder in which the pancreas either does not produce enough, or does not properly respond to insulin. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes, the body either fails to properly respond to its own insulin, does not make enough insulin, or both. This causes glucose to accumulate in the blood, often leading to various complications [1- 5]. It is of 2 types, type-1 DM (formerly called insulin-dependent diabetes or juvenile-onset diabetes); more than 90% of the insulin-producing cells of the pancreas are permanently destroyed. The pancreas, therefore, produces little or no insulin [4]. Only about 10% of all people with diabetes have type-1 disease. Most people who have type 1 diabetes develop the disease before age 30. Scientists believe that an environmental factor—possibly a viral infection or a nutritional factor in childhood or early adulthood—causes the immune system to destroy the insulin-producing cells of the pancreas. A genetic predisposition may make some people more susceptible to the environmental factor [5].Type-1 DM can occur in adult, which diagnosed as type 2, but ultimately recognized as late onset, slow progressing, immune mediated type-1 DM called LADA (latent autoimmune diabetes in adult) [6]

In type 2 diabetes, type-2 DM (formerly called non-insulin-dependent diabetes or adult-onset diabetes), the pancreas continues to produce insulin, sometimes even at higher-than-normal levels. However, the body develops resistance to the effects of insulin, so there is not enough insulin to meet the body needs [3].

Type 2 diabetes was once rare in children and adolescents but has recently become more common. However, it usually begins in people older than 30 and becomes progressively more common with age. About 15% of people older than 70 have type 2 diabetes [5].

Type-1 diabetes (type-1 DM) is a prototypical organ specific autoimmune disease, caused by T-cell induced autoimmune destruction of the insulin producing-cells of the islets of Langerhans of the pancreas [7]. Autoimmune diabetes exhibits two clearly distinguishable stages: (a) a clinically occult phase termed insulitis, featuring infiltration of autoreactive T lymphocytes and other inflammatory cells into the islets; and (b) an overt diabetes phase, when extensive destruction of cells results in a deficiency in insulin production and ultimately hyperglycemia. The silent insulitic state, reflected by circulating autoantibodies called Islet Cell Antibody (ICA) [8], can persist for long periods of time before the clinical onset of the disease, suggesting that immunoregulatory controls are able to keep autoreactive T cells in check [3, 9]. On the other hand, type-2 DM has not been related to any immune dysfunction [5].

In this study, we are trying to shed light on the role of some immune cells and their possible role in the disease process of DM.

Patients and Methods

In this study, 40 patients with type-1 DM, and 40 patients with type-2, with 22 age matched control group. Patients age range from 13-55 years and have been selected by specialist consultant in endocrinology from Al-Kadhimiya Teaching hospital, Baghdad, and according to specific features have been agreed on, as patient should have DM at least for 2 years, no complications, and well controlled glucose level.

Blood has been withdrawn from each patient, and lymphocytes (PBL) have been isolated for phenotyping by immunocytochemistry with the following markers CD3 for total T-lymphocytes, CD4 for T-helper, CD8 for T- suppressor/cytotoxic cells, CD19 for B-cells, (all from Sigma). The staining methods done as in Kadhim *et al* [10] and briefly: the cells stained with the primary antibody of each marker for 1 hour then washed and secondary conjugated antibody applied to cells then washed and a freshly prepared substrate and chromogen were added for 15-20 min then washed and counter stained with hematoxylin, lastly a mounting media and a cover slip were added. Examination of the cells under microscope with high power X40 was done, counting the positive cells (brown) and negative cells (blue) and making a percentage for each.

Statistical analyses have been done to check the significant difference in the percentage of cell phenotypes in 3 groups using SPSS.

Results

Lymphocytes phenotyping revealed the significant difference between control group and DM-1 in T-helper (CD4) and a highly significant difference between control group and DM-1 in T-cytotoxic (CD8) as in table1.

Table 1. Showing the differences between type-1 DM and the control group

Cells	Groups	N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
T lymphocyte (CD3)	DM-1	40	68.150	6.290	0.995	0.524^{NS}
	Control	22	69.227	6.421	1.369	
T-helper (CD4)	DM-1	40	45.625	7.682	1.215	0.040*
	Control	22	41.591	6.314	1.346	
T-cytotoxic (CD8)	DM-1	40	21.750	2.924	0.462	0.000**
	Control	22	26.409	3.404	0.726	
B lymphocyte (CD19)	DM-1	40	14.100	2.827	0.447	0.627^{NS}
	Control	22	14.455	2.558	0.545	

NS: no significant difference; * P< 0.05; ** P< 0.001

Results of Immunostaining of the immune cells in DM-2 showed a significant differences in T-lymphocyte (CD3), T-cytotoxic (CD8) and B-lymphocyte (CD19) as in table 2. A significant difference was also seen between type-1 and type-2 DM in CD3 AND CD19 markers, while CD4 and CD8 were not significant as in table3.

Finally, figure (1) shows that there is a significant increase in total CD3 and CD19 cells in DM-2, while a significant decrease in CD8 in DM-1.

Discussion and Conclusion

This study showed significant differences in the phenotype of different peripheral blood lymphocytes in patients with type-1 and type-2 DM and the control group. However, the differences between type-1 and type-2 is significant in all markers except for CD4 and CD8 which is in agreement with study of Kis et al., 2006 [11], whom showed no significant difference in level of CD4 and CD8 in different patient sets including type-1 DM. This may reflect the idea of the role of immune system in type-1 DM

rather than type-2 as type-2 DM appears to involve the progressive failure of pancreatic beta-cells as a result of several dysfunctional effects, glucose toxicity, islet amyloid formation, and impairment of both the responsiveness to insulin (insulin resistance) and insulin secretion [12].

Regarding the difference between the phenotype of blood lymphocytes in type-1 DM and the control group, there are significant differences in the level of CD4 and CD8, while no significance seen in the level of CD3 and CD19, and this may be explained as there is no difference in the total level of T-cell but the level of T-cell subsets as CD8 showed a significant difference. The same may be true for the B-cell (CD19) as the level of specific B-cell could be increased that is B-cell with specific autoantibodies that play role in the disease process of type-1 DM rather than total B-cells [9].

The highly significant difference in other markers CD8 may explore the role of these cells in the pathogenesis of the type-1 DM and as a cytotoxic and suppressor function of these cells which is a sign of autoimmunity [13].

Type-2 DM group of patients and the control group, the results showed some differences but no highly significant as in CD3, CD8 and CD19 and not significant in CD4. This may reflect the idea that immune system has little or no role in the pathogenesis of type-2 DM [5].

Conclusion

This study shed light on the role of some immune cells and their phenotype and may prove that these cells play a role in type-1 DM rather than type-2 DM.

Table 2. Showing the differences between type-2 DM and the control group

Cells	Groups	N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
T lymphocyte (CD3)	DM-2	40	73.450	5.826	0.921	0.011*
	Control	22	69.227	6.421	1.369	
T-helper (CD4)	DM-2	40	44.900	7.200	1.138	0.076 NS
	Control	22	41.591	6.314	1.346	
T-cytotoxic (CD8)	DM-2	40	23.600	5.334	0.843	0.030*
	Control	22	26.409	3.404	0.726	
B lymphocyte (CD19)	DM-2	40	16.300	2.963	0.468	0.017*
	Control	22	14.455	2.558	0.545	

NS: no significant difference; * P< 0.05

Table 3. Showing the differences between type-1 DM and type-2 DM

Cells	Groups	N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
T lymphocyte (CD3)	DM-1	40	68.150	6.290	0.995	0.000**
	DM-2	40	73.450	5.826	0.921	
T-helper (CD4)	DM-1	40	45.625	7.682	1.215	0.664 NS
	DM-2	40	44.900	7.200	1.138	
T-cytotoxic (CD8)	DM-1	40	21.750	2.924	0.462	0.058 NS
	DM-2	40	23.600	5.334	0.843	
B lymphocyte (CD19)	DM-1	40	14.100	2.827	0.447	0.001**
	DM-2	40	16.300	2.963	0.468	

NS: no significant difference; * P< 0.05; ** P< 0.001

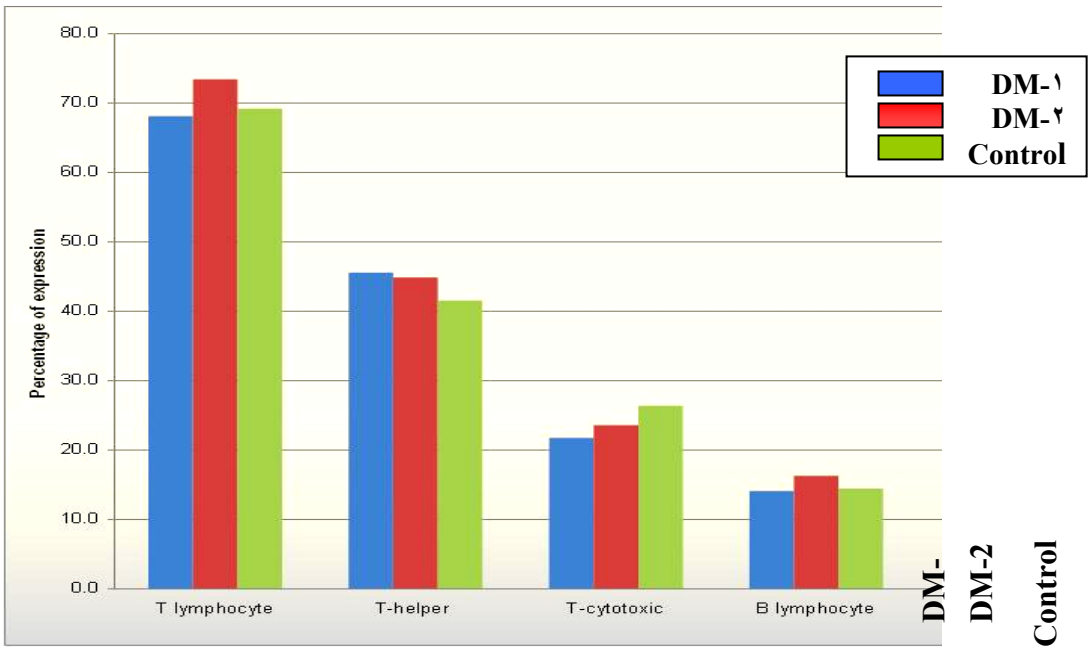

Figure 1. showing the percentage of different phenotyping of lymphocytes in the three groups

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