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The Role of T-helper 1 and T-helper 2 Cytokines in the Immuno-pathogenesis of Type 1 Diabetes Mellitus

Qasim Dawood Yasir Altameemi[1]*, Mohannad Khadim Alazzam **

Abstract---*It is believed that there is a strong evidence for the role of genetic susceptibility and immunological disturbances in the development of type 1 diabetes mellitus (T1DM) which is also called Insulin Dependent Diabetes Mellitus (IDDM), thus one approach to investigate diabetic patients is to study their first degree relatives. The aim of the present study to investigate the relationship of diabetes in near siblings with the diabetes children. The case control study was performed on 270 children aged between 2 to 13 years old. They were divided into 3 groups viz. diabetics, siblings, and control (each n=90) matched for age and gender. Fasting and random blood were evaluated for the glucose estimation and haemoglobin A1C (HbA1C). Serum cytokines profile also performed along with oral glucose tolerance test. The mean levels OGTT was higher among diabetic siblings than healthy group. The mean levels of IFN-γ and IL-2 was significantly higher among diabetic children compared to their siblings and control group. Oppsite treade was observd in serum IL-10 levels. These results confirm the theory that the balance between T-helper 1 (Th1) and T-helper 2 (Th2) is critical in the development of T1DM and T1DM is a Th1 mediated disorder while Th2 cytokines (especially IL-10) may play a protective role against the development of T1DM. The higher mean levels of OGTT among diabetic siblings may reflect their potential to develop T1DM in the future.*

Keyward---*T-helper 1 cells, T-helper 2 cells, T1DM.*

1. Introduction

Diabetes mellitus is metabolic syndrome, which is common, chronic and characterized by hyperglycemia as a feature of cardinal biochemical, diabetes are majorly classified as type 1 and type 2 diabetes mellitus. Type 1 Diabetes mellitus is characterized by deficiency of insulin secretion due to pancreatic beta cell damage, which is also called insulin dependent diabetes or juvenile diabetes (Ramin and David, 2007). The natural history of type 1 diabetes mellitus includes preclinical autoimmunity of β cell which progresses to insulin secretion defect; clinical diabetes onset; transient remission;

[1]**Asst. Prof. and Consultant Pediatrician , College of Medicine, Wasit University, City: Kut_____, Iraq: (52001). Email: qdawood@uowasit.edu.iq*
Tel. number (with in code): +964 771 674 6135
*** Tutor and Specialist Pediatrician , College of Medicine, Wasit University, City: Kut_____, Iraq: (52001). Email: mohannadAlazzam@uowasit.edu.iq*
Tel. number (with in code): +964 780 166 7686
Corresponding Author
Qasim Dawood Yasir Altameemi
Email: qdawood@uowasit.edu.iq
Tel. number (with in code): +964 771 674 6135

establishment of acute and chronic complications associated with diabetes and decrease in life expectancy (Evertson and Jennifer, 2009). The onset may occur at any age, but is predominantly in childhood with a median age of 7-15 years. The type diabetes mellitus may be found associated with autoimmune diseases such as thyroiditis, celiac, multiple sclerosis and addison disease (Ramin and David, 2007). The T1DM incidence is rapidly increasing in specific regions and directing toward the early age of onset (15). In total diabetes affected around 15 million population the T1DM accounts for about 10% (Ramin and David, 2007). Diabetes is estimated to be in 2030 that almost about 552 million people will be diabetic (Rabinovitch et al., 2002). T- lymphocytes and macrophages have an important role in mediating cell destruction and cause Type 1 diabetes. Both activated T- cells and macrophages operate and act together through the release of soluble factors called cytokines, which persuade the type and degree of immune responses (Hussain, 1996).

Type 1 diabetes have an effect on children or adults, however, it was traditionally termed as "juvenile diabetes" because a majority of these diabetes cases were originate in children. About 5–10 % of diabetic patients suffering from the Type 1 form and can arise at any age, but is frequently observed in adolescence and early adulthood. A percentage of affected patients (<10%) are classified as type 1B, with no evidence of autoimmunity and the pathogenesis in these cases is considered idiopathic (Paschou et al., 2018). The etiology of T1D is unidentified; however, genetic, immunologic, and environmental factors contribute to the pathogenesis of disease (Whiting et al., 2011; Morran et al., 2015).

In Type 1 diabetes, insulin deficiency consequences from autoimmune destruction of the insulin-producing cells in the pancreatic islets of Langerhans owing to the activation of auto-aggressive T-helper (Th) lymphocytes and macrophages (Galleri et al., 2012). Th1 and Th 2 cells are functionally different subpopulations of T-helper cells and distinguished by their cytokine profiles and actions. Th1 cells are a chief source of interferon-gamma (IFN-γ) and also a major component of cellular immune response; while, Th 2 lymphocytes generate interleukin-4 (IL-4) and are more effective in stimulation of humoral immune system (Szablewski et al., 2014). The autoimmune responses that lead to insulin-dependent diabetes mellitus (IDDM) have a genetic basis; but, environmental factors can exert profound effects on the genetic predisposition (Zhang et al., 2018).

The rising evidence of autoimmune diseases such as T1D suggests altered Th1/Th2 balance and associated cytokines which plays an important role in the pathogenesis. However, there is disagreement in the literature about T1D being a Th 1 or Th 2 - mediated autoimmune disease, or both (Rabinovitch, 1994; Young et al, 2009). Th1 type cytokines (IL-2 and IFNγ) associate with T1 D, while Th 2 (IL-4 and IL-10), Th 3 [transforming growth factor beta (TGF-β)], and T regulatory cell-type cytokines (IL-10 and TGFβ) associated with protection from T1 D (Rabinovitch and Suarez-Pinzon, 2014; Yarde et al., 2014). Consequently, elucidating the role of Th1 and Th2 subpopulations in T1D may facilitate in identify specific immune parameters.

There is a strong evidence for the role of genetic susceptibility and immunological disturbances in the development of type 1 diabetes mellitus (T1DM) which is also called Insulin Dependent Diabetes Mellitus (IDDM), thus one approach to investigate diabetic patients is to study their first degree relatives. The aim of this study was to elicit the confounding effects of chronic hyperglycemia and insulin therapy upon the immune system through determination of cytokines profile of humoral and cellular mediated immunity in IDDM children and their siblings and to compare the results with a healthy control group.

2. Material and method

Ethical clearance

The ethical permission was taken from the ethics committee of Wasit University, College of Medicine, Iraq. The study was carried out at the diabetic center of AL-Zahraa teaching hospital in Kut, Iraq for the period between March 2012 to March 2014.

Patient enrollment

In the present study, we randomly selected 270 children age ranging between 2 to 13 years old with mean age 8.9 years. The 270 children were divided into 3 groups, each consisting of 90 children matched for age and sex, 1st was diabetics, 2nd was siblings and 3rd was control.

Blood biochemistry

The blood was collected from the individuals and subjected for serum separation. The separated serum was used for laboratory investigations, including fasting blood glucose (FBG), random blood glucose (RBG), haemoglobin A1C (HbA1C), profile of cytokines, including interferon gamma (IFN-γ), interleukins 2, 4, and 10 (IL-2, IL-4, IL- 10) by ELISA method (VIDAS® system, Biomerieux, Italy). Oral glucose tolerance test (OGTT), were also performed for control and diabetic individuals.

Statistical analysis

Please tell me which method was followed by you and software name Chi-square test was carried out to determine the relative importance of various variables, P value < 0.05 was considered as statistically significant and < 0.01 as highly significant. SPSS version 24

3. Results

When the diabetic, siblings, control groups were studied for the fasting blood glucose, random blood glucose and HbA1C, siblings and control group showed normal and diabetic group showed higher levels of FBG, normal RBG levels in control and siblings and high level in diabetic patients, and normal HbA1C among control and high in siblings and diabetic patients. Diabetes group showed poor glycemic control than other two remaining groups (Table 1).

Table 1: FBG, RBG (mg/dl) and HbA1c among the study groups

Glycemic status	Diabetic (n=90)	Siblings (n=90)	Control (n=90)
Fasting blood glucose (FBG)	182	96	88
Random blood glucose (RBG)	268	156	142
Haemoglobin A1C (HbA1C) (%)	8.2	6.1	5.4

When siblings and control are tested for the oral glucose tolerance test (OGTT) in mg/dl for fasting and post-prandial, both showed normal OGTT levels. However, the glucose clearance rate is higher in the control as compared to the siblings (P≤0.05). Siblings showed non significantly higher fasting serum glucose levels as compared to control.

Table 2: Oral Glucose Tolerance Test (OGTT) in both siblings and control group (mg/dl)

Group	Serum fasting glucose (mg/dl)	Serum glucose (mg/dl) after 2-hr (PP)*	P-value
Siblings	99±0.83	134±0.46	0.05
Control	89±0.87	110±0.13	

*2-hr (PP): 2 hours post-prandial

After performing the above tests the blood sample is drawn by children and then tested for the cytokines such as IFN-γ, IL-2, IL-4, IL-10 which are determined among type-1 diabetes, siblings and control by ELISA method. The data are collected and tested by statistical method analysis of variance (ANOVA) test.

The tests have shown that IFN-γ levels were highly significant (P value-0.012) in type- 1 diabetes group, in comparison with both siblings and control groups. IL-2 was also significant (P = 0.037) in the diabetic group compared to both siblings and control groups. IL-4 mean levels were non-significant (P = 0.738) among all the study groups in spite it was higher in the control group (0.81936) than in both type 1 diabetics (0.74387), and siblings (0.77593). The IL-10 mean level shows a control group highly significant value (P = 0.030) in comparison to both type 1 diabetics and siblings.

Table 3: Cytokines levels IFN-gamma, IL-2, IL-4 and IL-10 (ng/ml) among the study groups

	IFN-γ	IL-2	IL-4	IL-10
Diabetic	0.94725	1.47049	0.74387	0.30399
Siblings	0.47949	0.91504	0.77593	0.36211
Control	0.84342	0.98960	0.81936	0.67602

The IFN-γ have shown that for type 1 diabetes and sibling groups, mean of differences of IFN-γ level was significant (P = 0.005), for type 1 diabetes and control group mean of difference in IFN-γ level was non-significant (P = 0.525), for siblings and control groups mean of difference of IFN-γ level was significant (P = 0.027). IL-2 showed that the type 1 diabetes and siblings group, mean of differences in IL-2 level was significant (P = 0.018), between type 1 diabetes and

siblings group the mean of difference in IL-2 level was significant (P = 0.040), between siblings and control group the mean of difference in IL-2 level was non-significant.

The results in case of IL-4 have showed that mean of difference have non-significant values between the study groups, type 1 diabetic group (P = 0.742), control group (P = 0.438), sibling group (P = 0.655). In case of control group the mean of difference in IL-4 was higher than both type 1 diabetic and control group.

Finally the results in case of IL-10 the mean of difference level in type 1 diabetes and control group were significant (P = 0.014), in siblings and control groups mean of difference level was significant (P = 0.037), in siblings and type 1 diabetic groups mean of difference level was non-significant.

4. Discussion

Type 1 diabetes is a most common chronic organ-specific autoimmune disease; occur due to a disorder of immunoregulation. Autoreactive T-cells specific for pancreatic islet cell constituent present normally, but are reserved by immunoregulatory mechanisms that eliminate and suppress the autoreactive T-cells. Islet cell autoreactive T-cells are supposed to develop clonally, become activated, and destroy cells when deletion and/or suppression of the autoreactive T-cells fails to occur normally.

As per the American diabetes association (ADA) the fasting (no caloric intake for at least 8 hr) blood glucose level is 126 mg/dl, post-prandial (2hr PP) is generally 200mg/dl, and HbA1C is around 6.5%. Cytokines are the low molecular weight extracellular proteins that serve as mediators of immune response. They play an important role in complex pathways which regulates the process of inflammation, and to conduct the response to lesion site they are necessary. In the pathogenesis of diabetes mellitus the low grade chronic inflammation and activation of the innate immune system have strong relation (Navarro and Mora, 2008).

There are several references which show that IFN-γ levels are lower in the normal individuals than the diabetes patients (Kukreja et al., 2002; Avanzini et al., 2005; Jessica et al., 2017). In our study also diabetic patients showed higher IFN-γ levels than siblings and control group. Our study is in accordance with previous reports (Avanzini et al., 2005: Kukreja et al., 2002; Jessica et al., 2017). Similarly, Yasar et al (2006) showed that the IL-2 levels are increased in the diabetic patients and compared to healthy individuals. IL-4 levels are very low in patients suffering from type 1 diabetes mellitus (Kukreja et al., 2002). Various references also show that the IL-10 levels are increased in patients suffering from type 1 diabetes if compared to control (Jessica et al., 2017). The study suggested that the increased in pro and anti inflammatory markers in the type 1 diabetes as compared to normal children.

5. Conclusion

In the present study, serum IL-2 levels significantly high among diabetic patients as compared to siblings and control individuals. However, IL-4 and IFN-γ levels in the diabetic group showed near control value. The mean levels of IFN-γ and IL-2 was significantly higher among diabetic children compared to their siblings and control group, while the mean levels of IL-4 were higher among children of control group than diabetic group and their siblings, although the difference was nonsignificant, and the mean levels of IL-10 was significantly higher among control children compared to diabetics and their siblings which may reflect a protective role for IL-4 against the development of diabetes type 1 and this needs to be confirmed by further studies

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