Investigation of Certain Immunological Markers in First Degree Relatives of Type 1 Diabetic Patients

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ABSTRACT:

BACKGROUND:

Detection of certain autoantibodies and other non-specific inflammatory markers were employed in to predict an ongoing process of developing diabetes in first degree relatives of T1D patients.

OBJECTIVE:

The main objective of this study was to evaluate the value of monitoring of selected specific and non specific serum markers in the presumptive prediction of Type 1 diabetes in first degree relatives of diabetic patients.

PATIENTS AND METHODS:

First degree relatives of diagnostically confirmed diabetic patients were used as a test group. Type 1 diabetic patients and non-relatives healthy control groups of both genders were used for comparison. Sera from all subjects were monitored for glutamic acid decarboxylase antibody, anti-insulin antibody, complement C3 and C4, C-reactive protein and fasting blood sugar and the standardization of the maximum and minimum limits of the studied markers level was plotted to reduce the overlapping in the markers' values between each pair of the studied groups.

RESULTS:

The frequency of seropositivity for glutamic acid decarboxylase antibody was 24.0% in first degree relatives group compared to 77.1% and 0% in patients and control groups respectively. For anti-insulin antibody and C-reactive protein, a very few members of the first degree relative group were positive compared to those in the patients group. The results of C3 revealed a higher than normal level in 44.0% of first degree relatives group, 65.7% in patients group and 0% in control group. In contrast to that, C4 showed a lower than normal level in 28.0% of first degree relatives group compared to 57.1% and 0% in patients and control groups respectively.

CONCLUSION:

Monitoring of glutamic acid decarboxylase antibody, C3 and C4, but not anti-insulin antibody and C-reactive protein levels may be used as markers for a possible developing T1D in first degree relatives that precede the elevation of fasting blood sugar in serum. a narrow scale border line in the quantitative serum values of these markers is helpful in the standardization of this prediction.

KEY WORDS: diabetes, relatives, autoantibodies, prediction, standardization.

INTRODUCTION:

Type 1 diabetes (T1D) is one of the greatest challenges in public health and one of the most frequent chronic diseases which can occur at any age but usually appears between infancy and the late 30s, most typically in childhood and adolescence [(1)]. It accounts for approximately 15% of diabetes population [(2)]. The incidence of T1D is ascending over the past few decades in USA, Finland and England (2) for unknown reasons, probably due to the ill-defined etiology of the disease.

In Iraq, the record for T1D in 1994 was 230 in every 100,000 population [(3)]. In 2007, the overall prevalence of diabetes in Iraq was 21.8 per 1000. Rates are greater in urban than rural areas (25.3 and 15.8 per 1000 respectively), and in the South/Centre than in Kurdistan (23.0 and 14.3 respectively). Prevalence of Type 1diabetes appears low as rates among 0-4 and 5-14 year olds of diabetes are negligible. Rates of diabetes increase markedly in the 30-49 age group, assumed to indicate the onset of Type 2 diabetes. Further increases in the rates are seen after age 50, with a prevalence rate of 143.8 per 1000 persons [(4)].

Over the past 20 years, evidences has accumulated that T1D (mainly Type 1 A) is an immune-mediated disease which lead to destruction of insulin-producing beta-cells in

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pancreatic islets of Langerhans [4]. Examinations of the pancreatic tissue from patients who have died shortly after being diagnosed with T1D, have revealed that there is a pronounced inflammatory infiltration by CD8+ and CD4+ cells, B lymphocytes, macrophages and natural killer cells, commonly referred to as insulitis [5]. However, the exact pathogenic process or the factors affecting the disease process are so far largely unknown, but it is generally accepted that both genetic predisposition and environmental factors are required for the initiation of the disease process leading ultimately to total β-cell destruction. This chronic prediabetic process may begin early in life, and it always starts months or even years before the presentation of clinical diabetes[6]. The burden of the disease, the inadequacy of treatment to prevent chronic complications and the risk of sever hypoglycemia justify the researches for preventive strategies of T1D.

On the genetic basis, a first degree relative is a family member who shares about fifty percent of their genes with a particular individual in a family including parents, offspring, and siblings [7]. The first-degree relatives of patients with T1D carry an eight- to tenfold higher risk for developing T1D than individuals from the general population; thus, this group was initially targeted for screening to identify individuals at risk for developing diabetes in many of the early studies [8]. An early diagnosis of the preclinical stage of T1D in first degree relatives of T1D patients was the main target of this study. Preclinical stages of T1D have different degrees of pancreatic β islet cells destruction that is in accordance with the stage; the earliest is the less degree of destruction. Treatment of people who are in an ongoing preclinical stage of T1D with immunotherapy (as anti-CD3 and IL-2) and chemotherapy (as rapamycin) before starting a replacement therapy is possible in such occasions and had some modest successes in a recent trial [9]. The rationale for immunological markers is to identify those at risk to whom such treatment might be given. The outcomes of this field are controversial, and this study is an attempt to explore some immunological parameters in first degree relatives of T1D patients as assuming or presumptive predictive markers for an ongoing diabetes preclinical process and to create standardization baseline for these parameters. Among many parameters, autoantibodies specific for islet antigens, such as glutamic acid decarboxylase (GAD65) and insulin have turned out to be useful markers for the risk of progression to overt T1D (10). However, it is not possible to predict precisely if an individual will develop T1D, or when the disease will clinically manifested. Identification of additional parameters such as complement components (C3 and C4) and C - reactive protein could be helpful which were also investigated in this study.

MATERIALS AND METHODS:

Selection of Study Groups

During the period from November/2009 to May/2010, 80 individuals, who were divided into three groups, were included in this cross sectional study. The first group (designated as group P) including 35 (19 males and 16 females) clinically diagnosed T1D patients with an age range of 5-24 years (mean of 16.54 ±5.05 years). All patients in this group were insulin dependent. The second group (designated as R group) including 25 (15 males and 10 females) first degree relative individuals of the T1D patients with an age range of 4-30 years (mean age of 16.6±8.17 years). All members of this group were clinically non-diabetics. The third group (designated as Control or C group) including 20 (11 males and 9 females) non-relatives of the T1D patients and clinically non-diabetics (healthy) individuals with an age range of 5-23 years (mean age of 13.0±5.42 years). Members of all groups were informed and instructed about the aims of the study and a verbal acceptance of the patients or their parents was obtained before sampling.

Clinical Data

According to a specified prepared case sheet, descriptive variables of the patients were recorded (obtained during collection of blood samples) including: name, age, sex, type of treatment (insulin, Personal Health Decisions or others), family history of diabetes (weather type 1, type 2 or both), duration and onset of the disease.

Laboratory Analysis

Anticubital venous blood (3 ml) was drawn from each subject of the three groups and sera were separated. Part of the separated sera was used immediately for enzymatic colorimetric method of Fasting Blood Sugar (FBS) analysis (supplied by Biomaghreb / Tunisia), whereas the remaining amount was stored at -20˚C until the following tests were performed:

- Anti-glutamic acid decarboxylase (GADA) ELISA test which is a quantitative test for the detection of circulating autoantibodies against GAD antigens (supplied by EUROIMMUN, Germany).
- Anti-Insulin (IAA) ELISA test which is a quantitative test for the detection of circulating autoantibodies against bovine, porcine and recombinant human insulin (supplied by ORGENTEC Diagnostika GmbH, Germany).
- C3 and C4 radio-immuno assay (RIA) which are quantitative tests for the determination of C3 and C4 concentration respectively (supplied by LTA s.r.l., Bussero/Milano - Italy).
- C-reactive protein – Latex (CRP) which is a qualitative slide agglutination test for the detection of CRP (supplied by SPINREACT, U.S.A.).

Statistical analysis

Statistical analysis was done using SPSS version 10 computer software (Statistical Package for Social Sciences). Contingency tables were conducted for studying the cause's correlation ship among the different responding in each group samples with any other related factors. In addition, a P-value was recorded within a causative cause's correlation ship to conclude the cases of a non significant cause's correlation ship that the three samples having the same responding towards the categorized related of studied variable. Stem-Leaf Plots were conducted to explain the statistics order of the marked respondents (i.e.arranged the results of responding within sort ascending in orderliness).

RESULTS:

Table 1 shows the sero-positivity of GADA and IAA in all study groups and their statistical analysis. For GADA, it was 77.1% in P group, compared to 24% and 0 5 for P and C groups respectively. By statistics, these results were with P value ranging from 0.000 (highly significant) between P X R, and PXC, to 0.019 (significant) between RXC. For IAA, seropositivity was 45.7%, 4%, and 0% for P, R, and C groups respectively, with similar statistical differences as that of GADA results.

The level of complement components C3 and C4 in all study groups are shown in Table 2. In patients group, the majority (65.7%) were with a higher than normal level of C3, compared to 44% and 0% with elevated C3 levels in relatives and control groups respectively. The statistical analysis of these results had revealed no significant difference (P value= 0.133) between P and R groups, highly significant difference (P value= 0.000) between P and C groups, and highly significant difference (P value=0.001) between R and C groups. On the contrary, the majority (57.1%) of patients group (P) had exhibit a lower than normal level of C4 compared to 28% and 0% of lower than normal level of C4 in R and C groups respectively (Table 2).

Table 3 shows the seropositivity of CRP in patients, relatives and controls groups. Positive CRP results were 22.9%, 4% and 0% in P, R, and C groups respectively with a significant differences (P value= 0.044 – 0.021). Concerning the distribution of positive GADA, IAA, CRP, C3 and C4 results in accordance with FBS results in the R group, 83.3% of positive GADA cases, 100% of positive IAA cases, 100% of positive CRP cases, 81.8% of higher than normal of C3cases, and 72.2% of lower than normal of C4 cases were normoglycaemic. The statistical analysis of these results had revealed a significant or a high significant difference (Table 4). Table 5 and figure 1 show steam-leaf (explore) plots for creating maximum standardized limits for the parameters that are heading towards elevation in the level.

Table 6 and figure 2 show steam-leaf (explore) plots for creating minimum standardized limits for the parameter that is heading towards decreasing in the level.

Table 1:Distribution of GADA and IAA Seropositivity in All Study Groups.

Marker	Result and statistics	Patients (P) n=35		Relatives (R) n=25		Control (C) n=20	
		Count	%	Count	%	Count	%
GADA	Positive	27	77.1	6	24.0	0	0.0
	Negative	8	22.9	19	76.0	20	100
	Total	35	100	25	100	20	100
	C.C	PXR= 0.466		PXC= 0.596		RXC= 0.331	
	P value	PXR= 0.000 (HS)		PXC= 0.000 (HS)		RXC= 0.019 (S)	
IAA	Positive	16	45.7	1	4.0	0	0
	Negative	19	54.3	24	96.0	20	100
	Total	35	100	25	100	20	100
	C.C	PXR = 0.415		PXC = 0.436		RXC = 0.134	
	P value	PXR = 0.000 (HS)		PXC = 0.000 (HS)		RXC = 0.366 (NS)	

C.C: cause's correlation, S: Sig. at P< 0.05; HS: Highly Sig. at P < 0.01

Table 2: C3 and C4 level in all study groups.

Marker	Result and statistics	Patients (P) n=35		Relatives (R) n=25		Control (C) n=20	
		Count	%	Count	%	Count	%
C3	Lower than normal	1	2.9	0	0	0	0
	Normal Level	11	31.4	14	56.0	20	100
	Higher than normal	23	65.7	11	44.0	0	0
	Total	35	100	25	100	20	100
	C.C	PXR = 0.251		PXC = 0.554		RXC = 0.453	
	P value	PXR = 0.133 (NS)		PXC = 0.000 (HS)		RXC = 0.001 (HS)	
C4	Lower than normal	20	57.1	7	28.0	0	0
	Normal Level	12	34.3	18	72.0	20	100
	Higher than normal	3	8.6	0	0	0	0
	Total	35	100	25	100	20	100
	C.C	PXR = 0.362		PXC = 0.540		RXC = 0.358	
	P value	PXR = 0.011 (S)		PXC = 0.000 (HS)		RXC = 0.010 (S)	

C.C: cause's correlation, S: Sig. at P< 0.05; HS: Highly Sig. at P < 0.01

Table 3: C - reactive protein seropositivity in all study groups.

Groups	Freq.'s Percentages	CRP results		Total	C.C P-value
		Positive	Negative		
Patient (P)	Count	8	27	35	C.C. PXR = 0.252 PXC = 0.298 RXC = 0.134 P-value PXR = 0.044 (S) PXC = 0.021 (S) RXC = 0.366 (NS)
	%	22.9%	77.1%	100.0%	
Relative (R)	Count	1	24	25	
	%	4.0%	96.0%	100.0%	
Control (C)	Count	0	20	20	
	%	0.0%	100.0%	100.0%	
Total	Count	9	71	80	
	%	11.3%	88.8%	100.0%	

C.C: cause's correlation, S: Sig. at P< 0.05; HS: Highly Sig. at P < 0.01

Table 4: Distribution of positive GADA, IAA, CRP, C3 and C4 in accordance with FBS results for the first degree relatives of T1D patients group.

Marker	Results	Normoglycaemia		Hyperglycaemia		Total		C.C	P value
		Count	%	Count	%	Count	%		
GADA	Positive	5	83.3	1	16.7	6	100	0.096	0.003 (S)
IAA	Positive	1	100	0	0	1	100	0.114	0.0001 (HS)
CRP	Positive	1	100	0	0	1	100	0.114	0.0001 (HS)
C3	Higher than normal	9	81.8	2	18.2	11	100	0.120	0.002 (S)
C4	Lower than normal	6	85.7	1	14.3	7	100	0.140	0.004 (S)

C.C: cause's correlation, S: Sig. at P< 0.05; HS: Highly Sig. at P < 0.01

Table 5: Estimations of Maximum Standardized Limits for FBS, GADA, IAA and C3 in study groups.

Study group	Marker	Maximum
Patients	Fasting Blood Sugar	512
	Glutamic Acid Decarboxylase Antibodies	325
	Insulin autoantibody	24
	Complement component (C 3)	340
Relative	Fasting Blood Sugar	152
	Glutamic Acid Decarboxylase Antibodies	11.1
	Insulin autoantibody	11.256
	Complement component (C 3)	258
Control - Healthy	Fasting Blood Sugar	109
	Glutamic Acid Decarboxylase Antibodies	4.4
	Insulin autoantibody	4.722
	Complement component (C 3)	156

Table 6: Estimation of Minimum Standardized Limit for C4 in study groups.

Groups	Minimum
Patients	8.7
Relative	12.3
Control - Healthy	35.1

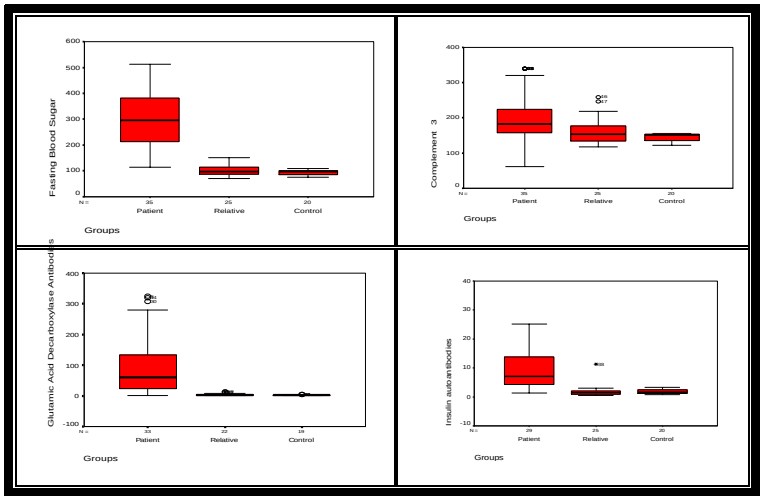

Figure 1: Stem-Leaf (Explore) Plots for Maximum Standardized Limits for FBS, GADA, IAA and C3 in study groups.

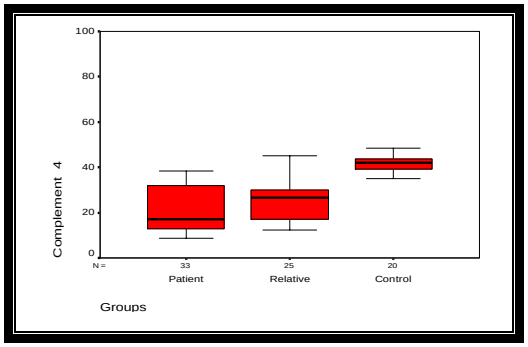

Figure 2: Stem-Leaf (Explore) Plot for Creating Minimum Standardized Limit of the Studied Parameter in Different Groups.

DISCUSSION:

Regarding first degree relatives (R) group of this study, the sero-positivity for GADA was 24% (Table 1). Similar result (22%) was reported by other study [11]. This study was partially a follow up for another previous one [12] in which the seropositivity for GADA was (8.4%) that indicates the progressive nature of the autoantibody stimulation which seems to be in consistency with the level of β-cell destruction[13]. It is clear from the natural history of T1D that the appearance of GADA in the individual's serum points out that these individuals actually in the second (or higher) stage of developing T1D. In other broad and prospective study, the actual predictive value for these autoantibodies for future diabetes was 28-66% within 3-5 years [14]. In comparison, a high GADA seropositivity (77.1%) was reported for the TID patients group in this study which verifies the suspected role played by these antibodies in the pathogenesis of the disease. These results indicate a good presumptive predictive value of this type of antibodies for a possible future development of T1D in the first degree relatives of T1D patients. This conclusion was confirmed by the statistical analysis as it is shown in Table 1. In the same Table, IAA in P, R, and C groups were 45.7%, 4.0% and 0% respectively. In spite of that IAA being the most specific type of autoantibodies for DM, its role in the pathogenesis is still ill-defined. The low seropositivity of IAA in the R group of the current study could be affected by the natural history of developing such autoantibody during the preclinical stages of T1D as well as the genetic constitution of the members of this group (which was not investigated in this study). For the above mentioned reasons, it seems that using IAA by its own is of no value in prediction of a future development of DM among first degree relatives of T1D patients. This result was in an agreement with another study, which detected IAA in 3.7% of relatives group [15]. The frequency of IAA in the patients group of this study, was in consistency with other studies[15,16], but it was not with other study conducted in Saudi Arabia [17] which showed that the prevalence of IAA was only 11% in T1D patients.

A higher than normal of C3 level in P group (65.7%) and R group (44%) compared to 0% in C group as it is shown in Table 2 confirms the involvement of complement-mediated mechanism in the pathogenesis of β-cell destruction in the preclinical and clinical phases of developing T1D as it was previously suggested [18]. It seems that estimation of C3 level in first degree relatives of diabetic people (in association with other predictive markers) is a good tool for prediction of future development of T1D. This concept is highly strengthened by the statistical analysis as it is shown in Table 2. On the contrary, the level of C4 was below than normal in 57% and 28% of P and R groups respectively (Table 2). This result was in an agreement with few other studies [19,20]. The deficiency of C4 can occur in many auto immune diseases [21] due to the fact that this protein is fundamental for the formation of C3-convertase in the classical pathway and for immune-complex dissociation. The statistical analysis of these results is highly supportive for the above mentioned concept as it is shown in the same table. Conclusively, measurement of C4 is of moderate value in the prediction of future development of T1D in people at risk.

Table 3 shows the sero-positivity of CRP in P, R and C groups which were 22.9%, 4% and 0% respectively. Statistically the difference between the P and R groups as well as between P and C groups was significant, but it was not between R and C groups. This result was in an accord with other studies [22,23] which reported that the level of CRP was elevated in type 1 diabetic patients. The result of an elevated CRP level in T1D patients is additional evidence that the disease is an immunoinflammatory disorder. On the other hand, the low sero-positivity of CRP in the relatives group in this study, in our opinion, is an indication of the delayed role of this inflammatory factor, if T1D is in a progress in the first place. This finding points out the low value of this factor in the prediction of T1D.

In Table 4, from 6 seropositive GADA individuals of the R group, 5 were with normal FBS level (83.3%, P value=0.003) indicating that using GADA as an early indicator in prediction of T1D for the first degree relatives of diabetic people is in preference of using FBS for such purpose. This is based on the natural history of preclinical developmental stages of T1D; GADA appears in serum before the hyperglycemic stage. Having both GADA positive and an elevated FBS level in this group was seen in one individual only which might prove the non coordination between these factors during the early stages of developing T1D. In the same table, one subject was positive for IAA and another was positive for CRP and both subjects were normoglycaemic. In spite of the highly significant statistical association between each of the immunological markers separately and the FBS, these results are fragile because of the single positive case for each of IAA and CRP which might happened coincidently. An independent association was found in one previous study between CRP and glucose in healthy men at risk of developing T2D as glucose by itself is a proinflammatory and can increase IL-6, TNF and IL-18 release in healthy subjects and person with impaired glucose tolerance [24].

From 11 higher than normal level C3 results, 9 (81.8%) were normoglycaemic and 2 (18.2%) were hyperglycaemic with a significant statistical analysis. Again, these results might point out that C3 level in the first degree relatives of T1D patients can be used as an earlier marker for the prediction of T1D compared to the measurement of FBS. This order might also base on the natural history of preclinical developmental stages of T1D; C3 level changes appear in serum before the hyperglycemic stage. Indeed, elevation of C3 level in association with elevated FBS (independently or dependently) in first degree relatives for T1D patients, to the best of our knowledge, was not documented in any other studies, but was documented in newly diagnosed T1D [25]. In the same Table, the lower than normal level of C4 in the serum of the first degree relatives of T1D patients and its association with the level of FBS had expressed a similar profile to that of the C3 and FBS association. Decreased level of C4 in the serum of first degree relatives of T1D patient was reported in other study [26].

Table 5 and figure 1 show steam-leaf (explore) plots for creating maximum standardized limits for the parameters that are heading towards the increase in the level. The maximum level of FBS in control, relatives, and patients were 109,152,512 mg/dl respectively; this indicates that the level of FBS was included in relatives group when it is above 109 mg/dl and it was included in patients when it is above 152 mg/dl.

The maximum level of GADA in control, relatives, and patients were 4.4, 11.1, 325 IU/L respectively; this indicates that the level of GADA was included in relatives group when it is above 4.4 IU/L and it was included in patients when it is above 11.1 IU/L. The same categorization was used to standardize the maximum limits for IAA and C3 and the minimum limits for C4 (Tables 5 and 6, and Figs. 1 and 2).

However, the above mentioned maximum and minimum standardized limits of the studied parameters represent a narrow scale values and not necessarily reflect the actual standardization values. A wider scale sample is needed to establish a more precise non-overlapping values in future studies.

CONCLUSION:

Glutamic acid decarboxylase autoantibodies, C3 and C4 levels (collectively or separately) may be used as an earlier marker than FBS for the prediction of developing of T1D in the first degree relatives of the diabetic patients. This is not true for IAA and CRP. Males appear to be more effected than females by T1D (1.2:1) irrespective of the age incidence and onset of the disease.

REFERENCES:

1. Weets I, Rooman R, Coeckelberghs M, and et al. The age at diagnosis of type 1 diabetes continues to decrease in Belgian boys but not in girls: a 15-year survey. Diab Metabol Res Rev, 2007; 23:637–43.
2. Kimpimaki T, Kulmala P, Savola K, and et al. Disease-associated autoantibodies as surrogate markers of type 1 diabetes in young children at increased genetic risk. Childhood Diabetes in Finland Study Group. J Clin Endocrinol Metab, 2000; 85(3):1126-32.
3. WHO. Prevalence rate of type 1 diabetes. (2006) [cited 2010 March 13]. Available from: http://www.who.int/en/.
4. Iraq Family Health Survey (IFHS) 2006/2007. Implementing agencies: Ministry of Health / Iraq Central Organization for Statistics & Information Technology Ministry of Health/Kurdistan Kurdistan Regional Statistics Office In collaboration with WHO/Iraq With the financial support by the European Commission
5. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. New England Journal of Medicine, 1994; 331:1428-36.
6. Sanna H . Characterization of Autoimmune Response in Preclinical Type 1 Diabetes. PhD [dissertation]. Faculty of Medicine: University of Tampere, 2005.
7. Lister Hill National Center for Biomedical Communications (2010). [cited 2010 July 10]. Available from: http://ghr.nlm.nih.gov/glossary=firstdegree relative.
8. Lisa K, Jerry P, Ake L. Autoantibodies and the disease process of Type 1 diabetes mellitus. In: Derek L, Simeon T and Jerrold O. Diabetes Mellitus: A Fundamental and Clinical Text. 3th ed. London: Grery HH, 2004, p. 506.
9. Staeva-Vieira T, Peakman M, Von Herrath M. Translational Mini-Review Series on Type 1 Diabetes: Immune-based therapeutic approaches for type 1 diabetes. Clinical Experimental Immunology, 2007; 148: 17–31.
10. Knip M. Disease associated autoimmunity and prevention of insulin dependent diabetes mellitus. Ann Med, 1997; 29:447-51.
11. Al-Hakbany M. Immunogenetics of type 1 diabetes in Saudi children. PhD [dissertation]. College of Medicine: King Saud University, 2009.
12. Hassan SM. The role of islet cell autoantibodies (GADA and IA-2A) in diabetes mellitus. [M.Sc thesis]. College of Medicine: Kufa University, 2008.
13. Knip M. Can we predict Type 1 diabetes in the general population?. Diabetes Care, 2002; 25:623-25.
14. Maclaren N, Lan M, Countant R, and et al. Insulin, GAD65, IA-2 &IA-2 beta predict immune mediated (type 1) diabetes in relatives. J Autoimmune, 1999; 12: 279-87.
15. Atkinson MA, Maclaren NK, Riley WJ, and et al. Are insulin autoantibodies markers for insulin-dependent diabetes mellitus?. Diabetes, 1986; 35:894-98.
16. Bingley PJ, Bonifacio E, Williams AJ, and et al. Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. Diabetes, 1997; 46:1701-10.
17. Lapolla A, Dalfra MG, Fedele D. Diabetes related autoimmunity in diabetes mellitus. Nutrition, Metabolism and Cardiovascular Diseases, 2009; 19:674-81.
18. Engstrom G, Hedblad B, Eriksson KF, and et al. Complement C3 Is a Risk Factor for the Development of Diabetes. Diabetes, 2005; 54:570-75.
19. Caraher EM, Conroy SJ, Newsholme P. Evidence for enhanced rates of complement activation in serum from patients with newly diagnosed insulin-dependent diabetes mellitus exposed to rat islet cells and complement-dependent induction of islet cell apoptosis, Journal of Endocrinology, 1999; 162:143-53.
20. Deschamps I. Life table analysis of the risk of type1 (insulin-dependent) diabetes mellitus in siblings according to islet cell antibodies and HLA markers. Diabetologia, 1992; 35:951-57.

21. Isaac L. Sistena complemento. In: Calich V, Vaz C, Immunologia. 1ed. Rio de Janeiro, Revinter, 2001, p 99.

22. Schulze MB, Rimm EB, Li T, and et al. C-reactive protein and incident cardiovascular events among men with diabetes. Diabetes Care, 2004; 27:889-94.

23. Kilpatrick ES, Keevil BG, Jagger C, and et al. Determinants of raised C-reactive protein concentration in type 1 diabetes. Q J Med, 2000; 93:231-236.

24. Niehoff AG and et al. C-reactive protein is independently associated with glucose but not with insulin resistance in healthy men. Diabetes care, 2007; 30:1627-1629.

25. Sundsmo JS, Papin RA, Wood L, and et al. Complement activation in type 1 human diabetes. Clin Immunol Immunopathol, 1985; 35:211-25.

26. Charlesworth J. A. , Timmermans V. , Golding J. , Campbell L. V., Peake P. W. , Pussell B. A., and et al. The complement system in Type 1 (insulin-dependent) diabetes, Diabetologia, 1987; 30: 372-9.