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# Prevalence of Diabetes-associated Antibodies and Impaired Insulin Response to Glucose in First Degree Relatives of Diabetic Patients

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Type 1 diabetes is a chronic autoimmune disease with a subclinical prodromal period characterized by the presence of circulating antibodies to various islet cell proteins. The main objective of this study was to estimate the prevalence of diabetes-associated autoantibodies in a group of 1st degree relatives, compared to healthy control subjects. Also, we tried to assess the insulin secretory capacity in subjects having multiple antibodies using First Phase Insulin Response (FPIR) to intravenous glucose. Eighty children and adolescents of the first degree relatives of diabetic patients attending the out patient clinic in the Diabetic Institute participated in our study. They were (34 boys and 46 girls, 50 siblings and 30 offspring of diabetic parents, aged 8-20 years with mean age 13.23+3.6). Twenty age and sex matched control subjects with negative family history of diabetes were enrolled from the child health clinic in the NRC. Sera of all subjects and controls were monitored for: islet Cell Antibodies (ICA), Anti-insulin Autoantibodies (IAA) and Glutamic Acid Decarboxylase antibodies (GAD) using ELISA technique and (IA-2) antibodies using radioligand binding assay. It was found that: according to the considered cut off point for positivity, 23 out of the 80 relatives (28.75%) showed positive ICA. 21 out of 80 relatives (26.25%) showed positive IAA. 17 out of 80 relatives (21.25%) showed positive GAD antibodies. 5/80 relatives (6.25%) showed positive IA-2 antibodies. As regard the control group only one subject tested positive for ICA and another one tested positive for IAA. None of the control group tested positive for GAD or IA-2 antibodies. On studying different combinations of positive antibodies, it was found that only two subjects of the study group (2.5%) had three positive antibodies, 10% had positive ICA and IAA, 3.75% had positive ICA and anti-GAD. The same percent of the study group had positive IAA and anti-GAD. Those subjects showing more than one positive antibody underwent IVGTT to determine FPIR to predict subjects at high risk for developing type 1 diabetes. One subject was found to be at risk and four subjects were found to be at high risk for developing type 1 diabetes.

**Key words:** Autoantibodies, Type 1 diabetes, 1st degree relatives, IVGTT-children

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## INTRODUCTION

Type 1 diabetes mellitus is one of the greatest challenges in public health and is one of the most frequent chronic diseases in the pediatric age (Krochiki *et al*., 2001). It can occur at any age but usually appears between infancy and the late 30s, most typically in childhood or adolescence (Auwera, 2003).

The incidence of type 1 diabetes has been rising over the past few decades in certain regions of the USA and some European countries, particularly Finland and England (Kulmala *et al*., 2000). In our country (Egypt), the prevalence rate among Egyptian preparatory school children was found to be 1.21 per 1000 (Ahmed, 1987). In 1990, the same rate was obtained in another epidemiological study among Egyptian school children (Abdel Dayem, 1990).

The burden of the disease, the inadequacy of treatment to prevent chronic complications and the risk of severe hypoglycemia justify the research of preventive strategies for type 1 diabetes (Thivolet *et al*., 2002).

Over the past 20 years, evidences have accumulated that type 1 diabetes is an immune-mediated disease, characterized by selective T cell mediated destruction of the pancreatic insulin-producing beta cells. Both genetic predisposition and environmental factors are required for the initiation of the disease process leading ultimately to total beta cell destruction. Throughout the long prediabetic period which starts months or even years before the presentation of clinical diabetes, autoantibodies specific for islet antigens such as Islet Cell Antibodies (ICA), Insulin Autoantibodies (IAA), Glutamic Acid Decarboxylase Autoantibodies (GADA) and the tyrosine phosphatase-like protein (IA-2) autoantibodies have turned out to be useful markers for the risk of progression to overt type 1 diabetes (Hoppu, 2005).

Risk assessment of type 1 diabetes in relatives was initially based on detection of circulating autoantibodies and evaluation of β-cell function by determination of the first-phase insulin response (FPIR) in the intravenous glucose tolerance test. In addition, the genetic susceptibility to type 1 diabetes, particularly that conferred by genes in the HLA class II region, has been more precisely defined and alleles conferring both susceptibility for and protection from the disease have been identified. This offers the possibility of combining immune, metabolic and genetic markers in strategies to identify family members at risk (Bingley *et al*., 2001).

The presence of ICA has for a long time formed the basis for predicting type 1 diabetes in first-degree relatives. Siblings of children with type 1 diabetes who are positive for ICA have a 40 to 50% cumulative risk of progressing to clinical disease within 10 years. (Bingley *et al*., 1994). The risk of type 1 diabetes has been reported to increase with the number of autoantibodies detected, rising from <10%, when ICA are detected alone to >80% when at least three antibodies are observed. It is evident that screening for all four essential antibodies (ICA, IAA, GADA and IA-2A) is more informative than ICA screening alone (Mrena *et al*., 2003).

The intravenous glucose tolerance test (IVGTT) is an efficient method for determining the degree of β-cell dysfunction and a reduced First-phase Insulin Response (FPIR) has been shown to be highly predictive of progression to type 1 diabetes in ICA-positive first-degree relatives and can be used to identify the subgroup of individuals with elevated levels of multiple antibodies (Krischer *et al*., 2003). Genetic testing is of limited value in family members in whom autoantibody levels are known. However, HLA class II typing may be useful in identifying infants for prospective follow-up for the appearance of islet autoantibodies or for recruitment into trials of interventions aimed at prevention of the initiation of autoimmunity (Bingley *et al*., 2001).

Increasing knowledge and intensive research on the pathogenesis of type 1 diabetes have produced hopes of finding an effective treatment to stop progressive β-cell damage in individuals en route to overt type 1 diabetes. Combinations of antibodies and FPIR seem to provide a promising tool for the identification of first-degree relatives at high risk of progression to the clinical disease. (Mrena *et al*., 1999).

The objectives of present study were to estimate the prevalence of diabetes associated autoantibodies (ICA, IAA, GADA and IA-2) in first degree relatives and trying to evaluate beta-cell function in subjects with multiple antibodies by determination of first phase insulin response (FPIR).

## MATERIALS AND METHODS

**Subjects:** First degree eighty children and adolescent relatives of type 1 diabetic patients attending the out patient clinic in the Diabetic Institute were invited to participate in the study. They were 34 boys and 46 girls; of whom 50 were unaffected siblings of type 1 diabetic children and 30 were unaffected offspring of diabetic parents. Their mean age was 13.23±3.6 years (the range, 8-20 years). The control subjects were recruited from the Child Health Clinic at the NRC. They were 20 children and adolescents, their ages and male to female ratio were in the same range as that of the first degree relatives. They had negative family history of diabetes or any other autoimmune disease. A written consent was obtained from the parents at the beginning of the study.

**Methods:** Thorough history and clinical examination, measuring weight and height and calculation of body mass index was done for each child recruited in the study. Fasting blood glucose was assessed for each subject to exclude any possibility of being diabetic.

Serum samples from all subjects and controls were taken starting from Jan. 2003 and each sample was divided into 4 tubes for the analysis of the autoantibodies. Then they were frozen (-20°C) till the time of analysis. The time of the first blood sample was recorded and then each subject was observed up to the end of May 2005 at intervals of 6 months for the development of overt diabetes. The diagnosis of overt diabetes was based on clinical symptoms and an increased random blood glucose concentration (>10 m mol $L^{-1}$). Elevated fasting (more than 6.7 m mol $L^{-1}$), or random blood glucose (>10 m mol $L^{-1}$) on 2 occasions in the absence of symptoms (Kulmala *et al*., 1998). The mean duration of follow up was 1.2 year.

Ten Subjects tested positive for more than 1 autoantibody were subjected to intravenous glucose tolerance test (IVGTT) for estimation of First Phase Insulin Response (FPIR) to glucose.

**Autoantibody assay:** The assay for autoantibodies was done in the laboratories of NRC.

**IA-2 assay:** Radioligand assay for the determination of autoantibodies to protein tyrosine phosphatase IA-2 in serum was done using kits from Biosource according to procedure described by Christie *et al.* (1997).

**ICA, IAA and GADA assay:** A qualitative ELISA test was used for the detection of circulating autoantibodies against Islet cell antigens (Islet test ICA), human insulin (Islet test-IAA) and GAD antigens (Islet test GAD) was used (Biomerica Inc;) according to the method described by Winter *et al.* (2002).

**IVGTT:** Subjects tested positive for more than 1 autoantibody were subjected to Intravenous Glucose Tolerance Test (IVGTT) for estimation of First Phase Insulin Response (FPIR) to glucose. Only 10 out of the 17 invited cases underwent IVGTT. The test was performed by administration of 0.5 g glucose $kg^{-1}$ body weight as a 25% glucose solution, up to a maximum of 35 g injected over 3 min (Vardi *et al*., 1991). The sum of insulin level at 1 and 3 minutes after the end of glucose infusion is determined (FPIR) (Chase *et al*., 2001). If the insulin sum is <48 mU $L^{-1}$ the case was considered 100% at high risk for type 1 diabetes within 4 years, while if it was 48 to 81 mU $L^{-1}$ it should be considered at risk only for type 1 diabetes (Bingley *et al*., 1992).

**Statistical methodology:** Analysis of data was done using SPSS (statistical program for social science) as follows:

- Description of quantitative variables as mean, SD and range
- Description of qualitative variables as frequency and%
- Chi-square test was used to compare qualitative variables
- Unpaired t-test was used to compare quantitative variables between two groups
- Cox regression analysis was performed to find the cumulative survival and the effect of different independent covariates which are considered as the independent predictors of DM among the 1st degree relatives included in the study using enter technique.

Sensitivity = Ability of the test to detect+ve cases
= True+ve/true+ve+false-ve

Specificity = Ability of the test to exclude negative cases
= True-ve/true-ve+false+ve

Positive predictive value = % of true+ve cases to all positive cases
= True+ve/true+ve+false+ve

Negative predictive value = % of true negative to all-ve cases
= True-ve/true-ve+false-ve

p value >0.05 insignificant
p<0.05 significant
p<0.01 highly significant

## RESULTS

**Descriptive data of the studied groups:** Eighty children and adolescents who are first degree relatives of patients with type 1 diabetes were included in our study. They were chosen randomly. They were 46 females (57.5%) and 34 males (42.5%). The mean age of the relatives was 13.19±3.63 years (range 8-20 years). Twenty control subjects who comprised 12 females (60%) and 8 males (40%) were included. Their mean age was 13.43±3.52 years (range 8-20 years). There was no significant statistical difference between 1st degree relatives and control group as regard the basic parameters included in this study except for height and BMI. There was highly statistically significant difference between both groups as regard mean level of ICA and IAA antibodies (p<0.01). Mean

level of GAD and IA-2 antibodies show statistically significant difference ($p<0.05$) (Table 1).

**Characteristics of diabetes-associated autoantibodies:** Testing for diabetes-associated autoantibodies (ICA, IAA, GAD and IA-2) in the unaffected 1st degree relatives reveled that: 33 (41.25%) had no antibodies, 30 (37.5%) had only one antibody (most frequently ICA or IAA), 15 (18.75%) had 2 antibodies (most frequently a combination of ICA and IAA and 2 (2.5%) had 3 antibodies specificities.

The cut off point for positivity for each autoantibody was calculated according to control mean level+2 SD.

The cut off point of each autoantibody and the distribution of positive results among the first degree relatives and the control group is shown in Table 2.

On studying different combinations of positive antibodies, it was found that only 2 relatives (2.5%) had 3 positive antibodies, 6 relatives (7.5%) had positive ICA and IAA, 3 relatives (3.8%) had positive ICA and GAD antibodies, 3 relatives had positive GAD and IAA, 2 relatives (2.5%) had ICA and IA-2 and one relative (1.3%) had positive GAD and IA-2 antibodies (Fig. 1).

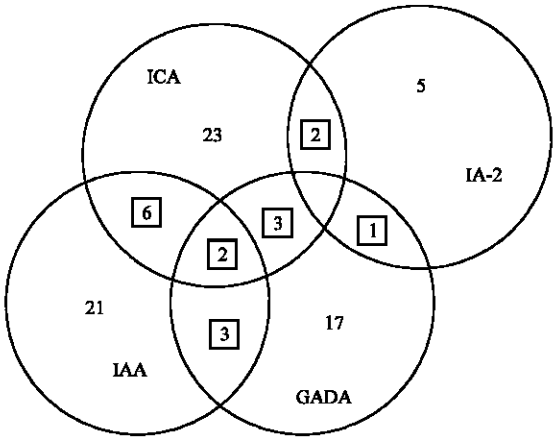


Fig. 1: Combinations of positive ICA, IAA, GAD and IA-2 antibodies in the 1st degree relatives

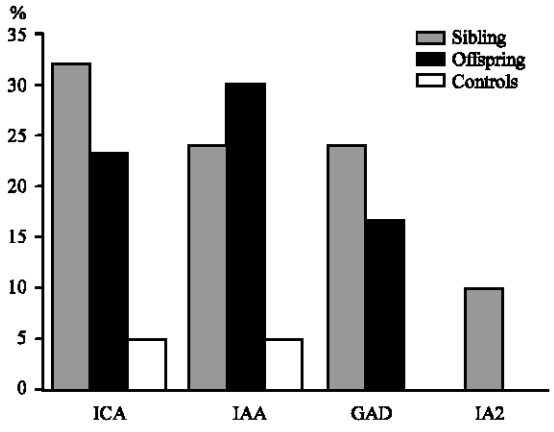


Fig. 2: The prevalence of positive antibodies among siblings, offspring and control groups

**Relation between diabetes associated autoantibodies and different risk factors**

**Relation to age:** The 1st degree relatives are classified into 2 groups: <10 year and >10 year of age. Using Chi square test, the relation between age and prevalence of autoantibody was examined. It seems that most of subjects who tested positive for different autoantibodies specificities were less than 10 year of age but on statistical analysis no significant difference could be detected between the two age groups ($p>0.05$).

**Relation to gender:** On studying the relation between antibodies positivity and gender of the 1st degree relatives, there was no statistically significant difference between males and females as regard positivity of autoantibodies by Chi square test $p>0.05$.

**Relation to proband:** Comparing the prevalence of positive antibodies among siblings, offspring and control groups revealed that, the prevalence of ICA, GAD and IA-2 antibodies is more common in siblings. When compared to the other two groups significant differences were shown using Chi square test ($p<0.05$). On the other hand no statistically significant difference was shown between the studied groups as regard IAA by the same test ($p>0.05$) (Fig. 2).

**Relation to Body Mass Index (BMI):** 1st degree relatives are classified into 2 groups as regard BMI: <25 (normal BMI) and >25 (over weight). There was no statistically significant difference between 1st degree relatives with normal BMI <25 and over weight >25 as regard positivity of autoantibodies titer.

To confirm the previous results, correlation between the prevalence of the autoantibodies versus age, BMI and FPG was examined using correlation coefficient test. No statistically significant correlation could be detected $p>0.05$.

**IVGTT:** Relatives who tested positive for more than 1 autoantibody under went IVGTT for estimation of FPIR to glucose. They were 10 out of 17 (Table 3).

The risk for developing DM is based on FPIR to glucose (Sum of insulin level at 1 and 3 min after infusion)

- HR : High Risk (if FPIR is <48 mU $L^{-1}$)
- R : Risk (if FPIR is 48-81 mU $L^{-1}$)
- NR : No Risk (if FPIR is above 81 mU $L^{-1}$)

Table 1: Descriptive data of 1st degree relatives and control groups

| Parameter | 1st degree relatives n = 80 Mean±SD | Control n = 20 Mean±SD | t-test | p-value |
|---|---|---|---|---|
| Age in years | 13.19±3.63 | 13.43±3.52 | 0.26 | >0.05 |
| Male/female ratio | 34/46 | 8/12 | | |
| Weight in kg | 46.70±14.39 | 47.45±12.20 | 0.21 | >0.05 |
| Height in cm | 144.07±14.68 | 153.25±13.28 | 2.55 | <0.05* |
| BMI | 21.89±3.84 | 19.77±2.28 | 2.36 | <0.05* |
| FPG mg $dL^{-1}$ | 96.59±12.59 | 101.35±6.96 | 1.63 | >0.05 |
| S. Blood pressure mmHg | 110.31±9.62 | 111.00±9.12 | 0.29 | >0.05 |
| D. Blood pressure mmHg | 70.13±9.48 | 73.5±6.71 | 1.50 | >0.05 |
| Antibodies to ICA (OD u.) | 1.01±0.57 | 0.62±0.28 | 2.95 | <0.01** |
| Antibodies to IAA (OD u.) | 1.03±0.52 | 0.65±0.29 | 3.19 | <0.01** |
| Antibodies to GAD (u $mL^{-1}$) | 6.16±11.26 | 1.30±0.76 | 2.00 | <0.05* |
| Antibodies to IA-2 (u $mL^{-1}$) | 3.41±12.54 | 0.2±0.52 | 2.28 | <0.05* |

*Significant test p<0.05, ** Highly significant p<0.01, Non significant p>0.05

Table 2: Frequency of autoantibodies among first degree relatives versus controls

| Parameter and cut off point | 1st degree relatives n = 80 No. (%) | Control n = 20 No. (%) | p-value |
|---|---|---|---|
| Antibodies to ICA (1.2 OD u.) | 23/80 (28.75) | 1/20 (5) | <0.05* |
| Antibodies to IAA (1.37 OD u.) | 21/80 (26.25) | 1/20 (5) | <0.05* |
| Antibodies to GAD (5 u $mL^{-1}$) | 17/80 (21.25) | 0/20 (0) | <0.01** |
| Antibodies to IA-2 (13 u $mL^{-1}$) | 5/80 (6.25) | 0/20 (0) | <0.01** |

*Significant test p<0.05, **Highly significant p<0.01

Table 3: Autoantibody profile and first phase insulin response to IVGTT in first degree relatives of type 1 diabetic patients and estimation of the risk for developing DM

| No. | IAA | ICA | GAD | Insulin at 1 min | Insulin at 3 min | Risk for DM |
|---|---|---|---|---|---|---|
| 1 | + | + | - | 4 | 8 | HR |
| 2 | + | + | - | 80 | 68 | NR |
| 3 | - | + | + | 9 | 10 | HR |
| 4 | + | + | + | 6 | 60 | R |
| 5 | + | + | - | 7 | 7 | HR |
| 6 | - | + | + | 90 | 68 | NR |
| 7 | - | + | + | 58 | 8 | R |
| 8 | - | + | + | 7 | 6 | HR |
| 9 | + | - | + | 80 | 60 | NR |
| 10 | + | + | - | 72 | 64 | NR |

Table 4: Sensitivity, specificity, PPV and NPV of different antibodies in relation to ICA

| Antibodies | IAA | GAD | IA2 |
|---|---|---|---|
| Sensitivity (%) | 30.40 | 51.70 | 20.00 |
| PPV(%) | 33.30 | 40.00 | 50.00 |
| Specificity (%) | 75.40 | 78.90 | 96.40 |
| NPV (%) | 72.80 | 71.40 | 73.30 |

As no one of the studied subjects developed diabetes during the short follow up period (14 months) restricted by the time limit of the project; we estimated the sensitivity and specificity of GAD, IAA and IA-2 antibodies taking ICA positive cases as a reference for true positive cases.

Table 4 shows that specificity of all studied autoantibodies is better than sensitivity. So, these autoantibodies are considered good negative predictors. IA-2 autoantibodies appear as the best specific marker (Specificity 96.4%) while GAD antibodies appear as the most sensitive marker for ICA positive cases (Sensitivity 51.7%) if compared with other markers.

Table 5: Relation between risk of DM and different risk factors among the studied 1st degree relatives (80) by cox regression analysis

| Independent variables | B | p-values | Odd's ratio (95%CI) |
|---|---|---|---|
| ICA | 2.35 | <0.05* | 13.0 (1.3-42.5) |
| GAD | 2.5 | <0.05* | 12.0 (2.6-35) |
| IAA | 2.1 | <0.05* | 7.5 (2-26) |
| IA-2 | 1.6 | >0.05 | 1.8 (1.2-9.8) |
| Age | 0.53 | >0.05 | 0.5 (0.2-5.9) |
| BMI | 0.89 | >0.05 | 1.1 (0.2-6.9) |
| Sex | 1.2 | >0.05 | 1.4 (0.5-10.2) |
| Relation to proband | 1.5 | >0.05 | 1.6 (0.3-12.6) |

*Significant test p<0.05

Therefore, testing for GAD autoantibodies was found to be more sensitive but less specific than testing for IA-2 autoantibodies to identify ICA positive subjects and vice versa testing for IA-2 was found to be highly specific but less sensitive to identify ICA positive subjects.

Table 4 shows that overall the studied antibodies are better negative than positive predictors because specificity is better than sensitivity for all, GAD had the best validity compared to the other two antibodies in reference to ICA.

**Relation between risk of developing DM and different risk factors:** Factors affecting the risk of developing DM (as estimated by FPIR) among first degree relatives, were assessed using Cox regression analysis (Table 5). Table 5 shows that the most important risk factors are the presence of ICA, GAD and IAA which are positively correlated to the risk of developing DM among 1st degree relatives. Other factors have no association with the risk of developing DM.

Table 5 shows that GAD, ICA and IAA antibodies are positively correlated with the risk of developing DM among the studied 1st degree relatives; GAD, IAA and

ICA are considered independent predictors of DM. Otherwise no significant correlation between other different risk factors and risk of DM was shown by Cox regression analysis.

## DISCUSSION

While most of subjects diagnosed with type 1 diabetes have no family history of the disease, there is an increased risk associated with siblings, parents and offspring of diabetic patients. This overall risk has been reported between 1 and 15% which can be compared to less than 1% for individuals without diabetic relatives (National Diabetes Data Group, 1995).

There are different approaches for the identification of individuals at risk for type 1 diabetes, genetic markers, autoimmune markers or metabolic markers. These alternatives may be combined in various ways to improve the prediction characteristics of the screening strategy (Knip, 1998). The aim of this study was to estimate the prevalence of diabetes-associated autoantibodies in a group of 1st degree relatives, compared to healthy control subjects. Also, we tried to assess the insulin secretory capacity in subjects having multiple antibodies using FPIR to intravenous glucose.

**Diabetes-associated autoantibodies:** Several autoantibodies are associated with autoimmune type 1 diabetes. Four autoantibodies have emerged as the most useful autoimmune markers of type 1 diabetes: ICA, IAA, GADA and IA-2. Detection of these autoantibodies in nondiabetic individuals indicates a significantly increased risk for the subsequent development of type 1 diabetes (Winter *et al*., 2002).

In this study testing for (ICA, IAA, GAD and IA-2) in the unaffected 1st degree relatives revealed that: 33 (41.25%) had no antibodies, 30 (37.5%) had only one antibody (most frequently ICA or IAA), 15 (18.75%) had 2 antibodies (most frequently a combination of ICA and IAA and 2 (2.5%) had 3 antibodies specificities.

Mrena *et al*. (1999) classified the unaffected siblings of diabetic patients into four stages of preclinical type 1 diabetes based on the number of disease-associated autoantibodies detected in the 1st sample. No prediabetic with no antibodies (87.2%); early prediabetics with one antibody specificity (6.5%), advanced with two antibodies (1.7%) and late prediabetics with at least three antibodies (4.6%). Follow up of these siblings proved that such a classification is very informative and is useful for assessing the risk of type 1 diabetes in healthy siblings (Mrena *et al*., 1999).

If we apply this classification on our results, the percentage of perdiabetics in each stage differs than that of Mrena's study. This may be due to small sample size and different techniques applied for antibody assay. However, such classification may become more clinically relevant if we follow our cases for a longer time till the development of diabetes.

The varying techniques used for antibodies assay in other different studies make it difficult to compare results directly.

In our study, a qualitative ELISA test was used for the detection of circulating autoantibodies against Islet cell antigens (Islet test ICA), human insulin (Islet test-IAA) and GAD antigens (Islet test GAD) (Biomerica Inc;). While radioligand assay was used for the determination of autoantibodies to protein tyrosine phosphatase IA-2 in serum using kits from Biosource.

In other studies: ICA were frequently determined by indirect immunofluorescence technique, while GAD, IA-2 and IAA were usually determined by radioligand assay (Kulmula *et al*., 1998, Kulmula *et al*., 2000; Thivolet *et al*., 2002).

According to the considered cut off point for positivity, 23 out of the 80 relatives (28.75%) showed positive ICA. 21 out of 80 relatives (26.25%) showed positive IAA. 17 out of 80 relatives (21.25%) showed positive GAD antibodies. 5/80 relatives (6.25%) showed positive IA-2 antibodies. As regard the control group only one subject tested positive for ICA and another one tested positive for IAA. None of the control group tested positive for GAD or IA-2 antibodies.

In the study of Kulmala *et al*. (1998) ICA were detected in (7.9%) of siblings, IAA were detected in (3.7%), GADA were detected in (7%) and IA-2 antibodies were detected in (5.3%) of siblings. On studying autoantibodies as markers of type 1 diabetes Kulmala *et al*., 2000 found that (9.4%) tested positive for ICA (4.4%) tested positive for IAA, (6.1%) tested positive for GADA and (5%) tested positive for IA-2.

While the results of a large French cohort of family members showed that (2%) were ICA positive, (0.8%) were IAA positive, (1.3%) were GADA positive and (0.7%) were IA-2 positive (Thivolet *et al*., 2002).

On the other hand, our results are nearly equivalent to the results of other studies using the same techniques for antibodies assay.

On screening 60 siblings of type 1 diabetic patients by combined autoantibodies, ICA were detected in (33.3%) and GADA were detected in (28.2%) of siblings (El-Habashi *et al*., 2002).

In another study assessing the prevalence of autoantibodies in siblings of patients with type 1 diabetes, Serum was analyzed for ICA by ELISA and GADA; IA-2 by radioimmuno assay: (19.4%) tested positive for ICA, (50%) tested positive for GADA and (12.5%) tested positive for IA-2 (Salah El_Din *et al*., 2002).

Moreover, the varying detection thresholds for positivity lead to wide variation in percentages of positive antibodies in different studies, even within the same study.

In Kulmala's study (1998), the percentage of ICA positive siblings was (7.9%) when the cut off point was considered at ≥2.5 Juvenile Foundation Units (JDFU), this percentage declined to (6.4%) when cut off point at ≥10 JDFU and reached (4.9%) when cut off point at ≥20 JDFU. Thus, raising the cut off increased the predictive power of autoantibodies, although the number of autoantibodies-positive individuals declined.

Nondiabetic individuals who express combinations of islet autoantibodies have a much higher risk for type 1 diabetes than individuals who express fewer types of islet autoantibodies (Knip *et al*., 1998).

From the Barbara Davis Center in Denver, Colo., it was found that the risk for type 1 diabetes in first degree relatives was 15% with one autoantibody, 44% with two autoantibodies and 100% with three autoantibodies (Verge *et al*., 1994).

Also, in the Milan, Italy, Family studies, the 6-year risk with no islet autoantibodies was 0% and it was 2.9% with only one islet autoantibody and it rose to 31.4% when two or more islet autoantibodies were present (Pastore *et al*., 1998).

The total number of types of islet autoantibodies is usually more important than the specific combination of positive islet autoantibodies. For example, in the Bart's Windsor, Bart's Oxford prospective family studies, the addition of any positive islet antibody to ICA positivity, raised the 15 years risk for type 1 diabetes from 47 to 66% (Gardner *et al*., 1999)

In our study only 2 relatives (2.5%) had 3 positive antibodies, 6 relatives (7.5%) had positive ICA and IAA, 3 relatives (3.8%) had positive ICA and GAD antibodies, 3 relatives had positive GAD and IAA, 2 relatives (2.5%) had ICA and IA-2 and one relative (1.3%) had positive GAD and IA-2 antibodies.

As no one of the studied subjects developed diabetes during the short follow up period (14 months) restricted by the time limit of the project; we can't estimate the precise risk of multiple autoantibodies detection, but this will be possible on longer follow up of those relatives. For the same reason-no one developed diabetes during follow up-the sensitivity and specificity of each autoantibody detected is estimated taking ICA positive cases as a reference for true positive cases. The results revealed that IA-2 autoantibodies appear as the best specific marker (Specificity 96.4%) while GAD antibodies appear as the most sensitive marker for ICA positive cases (Sensitivity 51.7%) if compared with other markers.

These results are in agreement with the results of Salah-El-Din *et al*. (2002) who found that the sensitivity of GADA to detect ICA positive subjects was 42.9% while specificity of IA-2 was 87.9%. Other studies reported that IA-2 testing had the highest specificity and also the highest predictive value out of the single antibodies tests (Gorus *et al*., 1997; Krip, 1998).

Thivolet *et al*. (2002) observed that combination of autoantibodies to GAD and IA-2 gave the best predictive value, since 29 out 30 relatives who developed diabetes, were tested positive for these 2 autoantibodies on an initial screening.

Another study for prediction of type 1 diabetes in siblings of children with diabetes stated that, all four islet antibody specificities are useful predictive markers for IDDM development. ICA having the highest sensitivity and IA-2 having the highest positive predictive value (Kulmala *et al*., 1998)

**Relation between diabetes associated autoantibodies and different risk factors:** The studied risk factors which may influence the prevalence and titer of diabetes associated antibodies are : age, gender, BMI, FPG and relation to the diabetic patient (whether sibling or offspring). It seems that the majority of subjects who tested positive for different autoantibodies specificities were less than 10 year of age. But this is not significant on statistical analysis ($p > 0.05$). Other risk factors had no influence on antibody positivity. No statistically significant correlation between gender, BMI and FPG versus different autoantibodies by r-test (correlation coefficient test $p > 0.05$).

These results are concordant with that of a study screening siblings of type 1 diabetic patients where age, gender, parental consanguinity and family history of diabetes were not related to ICA or anti-GAD status (El-Habashi *et al*., 2002).

On studying immunologic and genetic markers in an Argentine population, no correlation was found between antibodies prevalence and subject ages. IAA tended to be more prevalent among the population under 5 years and GADA was more frequent among those over 10 years of age, but these differences lacked statistical significance (Krochiki *et al*., 2001). On the other hand Bingley (1993) considered young age as an important factor enhancing the risk of progression to IDDM in ICA positive relatives.

Numerous reports proved that IAA was frequently detected among subjects under 5 years and its values tended to correlate negatively with increasing age (Verge *et al*., 1994; Feeney *et al*., 1997; Zimmet *et al*., 1994)

**Evaluation of insulin secreting capacity:** The first biochemical evidence of B-cell dysfunction can be detected by a loss of 1st phase insulin response to the administration of intravenous glucose. Usually, 1st phase insulin response is measured as the sum of the plasma insulin concentration at 1 plus 3 minutes after the acute glucose bolus injection (Chase *et al*., 2001)

The IVGTT is apparently more sensitive than the OGTT in indicating B-cell dysfunction. It is estimated that by the time the 1st phase insulin response is low, there has been a 50% decline in B-cell mass (Tarn *et al*., 1988).

In this study, relatives who tested positive for more than 1 autoantibody under went IVGTT for estimation of FPIR to glucose. The results reveled that: 4 subjects were recorded at high risk for developing type 1 diabetes within 4 years (FPIR is <48 mU $L^{-1}$) and one subject was recorded at risk (FPIR is 48-81 mU $L^{-1}$) and the rest of subjects were recorded at no risk (FPIR is above 81 mU $L^{-1}$) (Table 3).

Data analysis of a group of prospective studies, confirmed the importance of the loss of FPIR in enhancing the risk of progression to diabetes. Thus, FPIR <50 mU $L^{-1}$ gave 70% risk of progression within 5 years. The risk declined to 48% if FPIR was 50-100 mU $L^{-1}$ and it reached 17% if FPIR was >100 mU $L^{-1}$. However, this risk is modulated to a major extent by other markers of risk such as IAA or level of ICA (Bingley, 1994).

**Assessment of risk of developing type 1 diabetes:** The risk of progressing to type 1 diabetes is a product of many factors. In our study: age, sex, BMI, relation to proband and the presence of various disease-associated antibodies were assessed against the insulin secretory capacity which represent the impending risk of progression to type 1 diabetes. Cox-regression analysis revealed that the most important risk factors are the presence of ICA, GADA and IAA which are positively correlated to the risk of developing type 1 diabetes among 1st degree relatives ($p<0.05$). Other factors have no association with the risk of developing diabetes (Table 5).

The Islet Cell Antibody Register Users Study (ICARUS) predicting the different risk factors for progression to IDDM in ICA positive relatives, provided a rank order of importance for the markers: FPIR made the greatest contribution followed by the levels of IAA and ICA and then age. (Bingley *et al*., 1994). These results are to great extent in concordance with our results.

Many studies reported that, diabetes risk is highest in relatives with more than one islet autoantibody (Bingley *et al*., 1994; Kulmala *et al*., 1998; Kirscher *et al*., 2003).

Other studies defined the diabetes risk with high-titer islet cell antibodies (Bingley, 1993). Recent study for stratification of type 1 diabetes risk based on islet autoantibody characteristics showed strong association between risk and high titer IA-2 antibodies and IAA, IgG2, IgG3 and/or IgG4 subclass of IA2 and IAA.

## CONCLUSIONS

The combined analysis of the four diabetes-associated autoantibodies facilitates the estimation of type 1 diabetes risk in 1st degree relatives of diabetic patients. Estimation of FPIR is considered an effective tool for predicting the risk of progression to type 1 diabetes in the group with multiple positive autoantibodies. However, additional qualitative and quantitative tests and a longer period of follow up are required to improve the ability to predict diabetes.

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