Longitudinal Study for Prediction of Type 1 Diabetes in Siblings of Patients. An Initial Step for Prevention of Disease

Shereen Abdel Ghaffar, Mona H. Hafez, Fatma A. El-Mogi,[1] Asmaa Elsherei, Nermeen Salah, Faiza H. Abdel Hamid,[1] and Osama Khalafallah[1]

From the Departments of Pediatrics and Clinical Pathology,[1]Faculty of Medicine, Cairo University, Egypt

Abstract:

The aim of this study was identification of cut off points of positivity of different antibodies (Ab) against islet cell antigens (ICA, Anti GAD Ab, Anti IA2 Ab) in Egyptian children and adolescents who are sibs of patients with type 1 diabetes as well as determination of their insulin secretory capacity and identification of HLA-DQB1 alleles known to predispose to or protect against type 1 diabetes. The ultimate aim is to identify those at high-risk of the disease to enroll them in preventive trials.

This study was a longitudinal one that lasted for five years and included seventy-two sibs of type 1 diabetic patients recruited from the Diabetic Endocrine Metabolic Pediatric Unit (DEMPU) at Cairo University Children's Hospital. Thirty sex healthy subjects; age and sex matched with patients and with negative family history of autoimmune diseases were included as controls. Serum samples from all subjects and controls were analyzed for GAD_{65}, IA2 Ab using radioimmunoassay and ICA Ab using ELISA technique. Sibs who were positive for one or more Ab were further subjected to the assessment of first phase insulin response and HLA studies for detecting DQB1 alleles known to predispose or protect against type 1diabetes using SSP DNA-based technique.

The results showed that 36 sibs (50%) were GADAb positive, 10 sibs (13.9%) were IA2 Ab positive while 14 sibs (19.4%) were ICA positive with overlap. Mean FPIR in 41 sibs positive for one or multiple Ab was 41.407 mU/L $\pm$ 28.73 which was statistically significantly less than that in controls 79.414 mU/L $\pm$ 44.316 ($P<0.001$). Thirty-four sibs (83%) lied in the high-risk group defined by FPIR less than 5th percentile. HLA studies done in 32 sibs showed that 17/32 sibs (54.84%) had the predisposing alleles DQB1 (0201, 0202, 0302, 0303, 0401) while 7 sibs (22.28%) had protective alleles DQB1 (0301, 0601).

Conclusion: *Prevention of type 1diabetes will require reliable methods for early diagnosis of predisposition to the disease, using improved genetic and serological screening on a wide scale and identification of the primary antigenic target(s) for specific tolerance. Those at risk (multiple positive antibodies and reduced insulin secretory response) in absence of HLA protective alleles are to be enrolled in preventive trials.*

Abbreviations: Ab: antibody, GAD: glutamic acid decarboxylase, HLA: human leukocyte antigen, IA2: insulinoma associated protein2, ICA: islet cell antigen.

Introduction:

For successful prevention of type 1diabetes, it should be preceded by identification of individuals at increased risk for progression of disease who should be candidates for preventive intervention trials.[1] Autoantibodies are early detectable markers of an ongoing disease process and are used to diagnose prediabetes where glutamic acid decarboxylase (GAD), insulinoma associated protein (IA2) and islet cell antigen (ICA) represent the three major autoantigens. Among first-degree relatives of patients with type 1 diabetes, the risk for clinical disease can be graded from <5% in those with one or no antibodies to >90% in individuals who carry the HLA-DQB1* 02/0302 risk genotype and are positive for multiple autoantibodies.[2] The genes on the human leukocyte antigen (HLA) and insulin gene region are major genetic determinants for disease and the major histocompatibility complex namely HLA-DQ alleles still represent the strongest genetic risk. The highest risk was seen in those carrying the HLA DQ B1* 02/0302 genotype, on the other hand resistance to type 1 diabetes has been shown to be associated with DQ6, DQA*, 0102,DQB* 0602-0603 haplotype.[2,3]

Metabolic markers in the form of insulin response to an intravenous glucose load in the intravenous glucose tolerance test (IVGTT) is the most widely used measure of B-cell function used in the prediction of type1 diabetes. Presence of first phase insulin response (FPIR) below the first percentile of normal

control subjects (48mU/L) was found to be associated with an estimated risk of diabetes of 100% within 4 years.[4] Moreover, autoantibodies-positive individuals with low first-phase insulin response are highly predictive for rapid progression to the clinical disease.[2]

It has to be stressed that cut-off levels of positivity for different autoantibodies, as well as norms for insulin secretory capacity differ according to racial and ethnic factors with wide variations. Moreover, HLA DQB1 alleles known to predispose or protect against the development of type 1 diabetes also differ among populations of different ethnic origins.[2]

The aim of this study was identification of cut off points of positivity of different antibodies against islet cell antigens (ICA, Anti GAD Ab, Anti IA2 Ab) in Egyptian children and adolescents who are sibs of patients with type 1 diabetes as well as determination of their insulin secretory capacity and identification of HLA-DQB1 alleles known to be predispose to or protect against type 1 diabetes. Consequently, the trial of identification of sibs at high risk of development of type 1 diabetes depending on the presence of multiple autoantibodies, predisposing HLA-DQB1 alleles as well as reduced first phase insulin release were attempted with the ultimate goal of trial of prevention of type 1 diabetes in those sibs.

Subjects and Methods:

Study Population:

One hundred and eight subjects comprising:

- Seventy-two sibs of type 1 diabetic patients (derived from 41 families) recruited from those attending the Diabetic Endocrine Metabolic Pediatric Unit (DEMPU) at Cairo University Children's Hospital.
- Thirty sex healthy subjects, age and sex matched with patients and with negative family history of autoimmune diseases were included as controls.

Study Design:

A longitudinal study that lasted for five years, sibs were chosen consecutively from sibs of diabetic patients following at the Diabetic Endocrine Metabolic Pediatric Unit (DEMPU), Cairo University, while the controls were selected randomly from healthy relatives of patients coming with them to the hospital but had no positive family history (FH) of any autoimmune diseases. Informed consent was taken from all subjects.

Methods:

All subjects were subjected to:

- History taking including present history for signs and symptoms of diabetes, or autoimmune diseases, family history of autoimmune disease, including diabetes (type 1 or type 2), past history of viral infection, nutritional history: (whether subjects were fed breast-milk or cow's milk) and perinatal history of maternal illness during pregnancy.
- General examination for any signs of autoimmune diseases.
- Laboratory Investigations:

I. First Phase of the study

a) Fasting Blood Glucose

Fasting blood glucose was measured for all subjects and controls to exclude the possibility of being diabetics. Serum glucose was detected using automated autoanalyzer Cx5 (Beckman, USA).

b) Anti GAD, IA2 and ICA Autoantibodies

Serum samples from all subjects and controls were analyzed for GAD_{65}, IA2 and ICA autoantibodies. GAD and IA2 auto Ab were analyzed using radioligand assay with kits from MEDIPAN DIAGNOSTIC (Germany CENTAK). Islet cell autoantibodies were assayed using isletest-ICA Biomerica Inc; California USA, which is a qualitative ELIZA test for in vitro detection of circulating IgG antibodies against pancreatic islet cell antigens, calculation of data was done by recording the spectrophotometric readings (Optical density: OD) in absorbance units.

II. Second Phase of the study

a) First Phase Insulin Response (FPIR)

Sibs who were positive for one or more Ab as well as controls were further subjected to this assessment:

An intravenous glucose tolerance test (IVGTT) was performed by administration of 0.5gm glucose/kg BW as a 25% glucose solution, up to a maximum of 35 gm injected over 3 minutes, 3 blood samples were obtained one fasting, the 2nd after one min and the third after 3 minutes. Insulin level was measured in the 3 samples by solid phase radioimmune assay using kits from diagnostic product corporation (DPC) (West, 96th street Los Angeles CA, 90045-5597). The sum of insulin levels at 1 and 3 min after the end of glucose infusion was determined and was termed the FPIR. Normal range for insulin level [3-28.0 μIU/mL].[5]

b) HLA Studies

Sibs positive for one or more antibodies and who had undergone assessment of FPIR and accepted the analysis after informed consent were subjected to HLA studies searching for the presence of DQB1 alleles, which are related to genetic susceptibility for type 1diabetes. Biotest DQB SSP kit with low-resolution reading (Landsteinerstrasse.Germany) to perform DNA based HLA-DQB typing was used according to the technique described by Bunce et al.[6]

Statistical Analysis:

Quantitative data were expressed as mean ± SD while qualitative as percentages. Comparison between means of two different groups was done using student t test, P values were considered significant if ≤0. 05. Comparison between frequencies was done using Chi square test. Cut off values for antibodies positivity were determined using mean values of controls + 2SD.

Sensitivity and specificity were calculated for each of GAD antibodies and IA_2 Ab while considering ICA Ab as the gold standard for Ab positivity.

The positive likelihood ratio $\frac{\text{sensitivity}}{1-\text{specificity}}$ of each test (GAD, IA_2) that indicates the value for increasing certainty about positive diagnosis was calculated and compared in-between.

According to Polly and Bingley,[7] Mrena et al.,[8] Chase et al.,[9] with modification, subjects were classified as regards their FPIR into low risk group (>80-100mU/L), intermediate risk group (65-80 mU/L) and high risk group (<65-48mU/L) which is less than 5th percentile and (<48mU/L) which is less than first percentile.

Results:

The study included seventy two sibs of type 1 diabetic patients (derived from 41 families) as well as thirty six healthy subjects, age and sex matched with patients and with negative family history of autoimmune diseases including type 1 or type 2 diabetes as controls.

There was no statistically significant difference between the distribution of sex in sibs and controls, where in sibs 44 were females (61.1%) and 28 males (38.9%) while in controls 16 were females (44.4%) and 20 were males (55.6%) (P>0.05).

Mean age of siblings was 8.95 ± 8.684 yrs (range 1-19.67 yrs) while that of controls was 6.41 ± 3.57 yrs (range 1-20 yrs) with no statistically significant difference (P>0.05).

- In the first phase of the study:

Autoantibodies against islet cells, GAD and IA2 were assayed in 72 sibs of patients with type 1diabetes and compared to 36 healthy controls.

Table I shows mean levels of the antibodies assayed in 72 sibs and 36 controls. The mean levels were significantly decreased in sibs than in controls (p<0.05 for each antibody). Fig 1,2 and 3 show different cut off levels of positivity of each Ab according to mean + 2 SD of levels of controls.

Table I: The levels of antibodies to GAD, IA2 and ICA among the studied samples

Type of antibody	Control group Mean ±SD	Group of siblings Mean± SD	P value
Antibodies to GAD (µg/ml)	0.217 ± 0.312	0.663 ± 0.584	<0.01
Antibodies to IA2 (µg/ml)	0.658 ± 0.279	0.724 ± 0.415	<0.05
Antibodies to ICA (OD µ)	0.307 ± 0.099	0.403 ± 0.147	<0.01

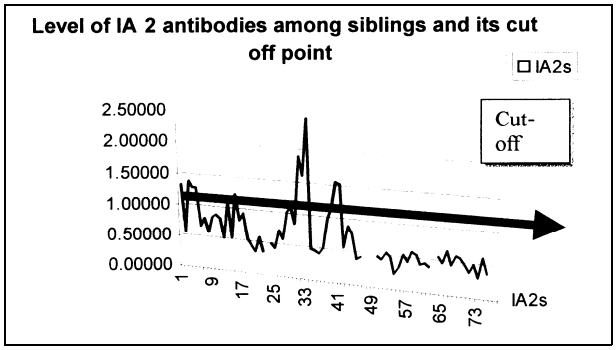

Fig.1: Cut-off point of positivity of IA2 antibodies = 1.217 µg/ml

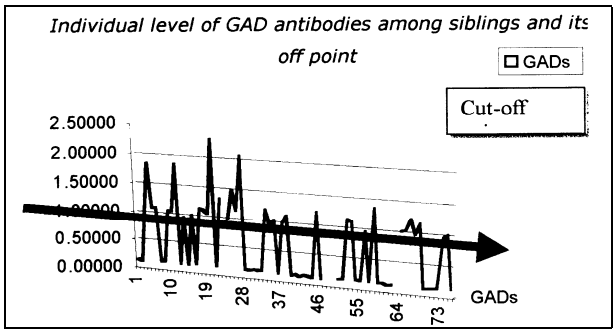

Fig.2: Cut-off point of GAD antibodies = 0.841 µg/ml

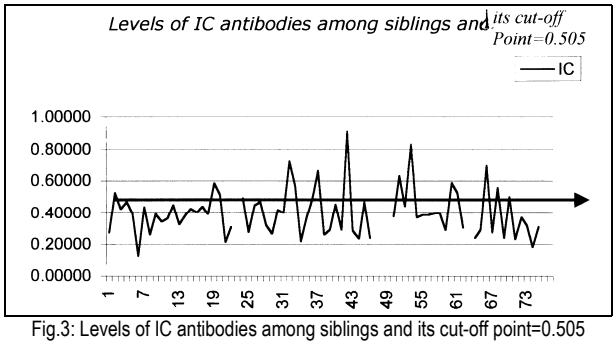

Fig.3: Levels of IC antibodies among siblings and its cut-off point=0.505

Distribution of sibs sample according to the cut off point of GAD, IA2 and IC antibodies positivity was as follows: 36 sibs (50%) were GADAb positive, 10 sibs (13.9%) were IA2 positive while 14 sibs (19.4%) were ICA positive with overlap of multiple Ab positivity.

When ICA positivity was considered as the gold standard test, sensitivity of GAD antibodies was 42.9% (positive GAD and positive ICA) and its specificity was 48.3% (negative GAD and negative ICA). On the other hand, sensitivity of IA2 antibodies was 21.4% (positive IA2 and positive ICA) and its

specificity was 87.9% (negative IA2 and negative ICA).

- In the second phase of the study:

Forty-one sibs who had positive one (n=30) or two (n =11) autoantibodies were included for further testing for metabolic markers (FPIR) (flow chart: Fig. 4).

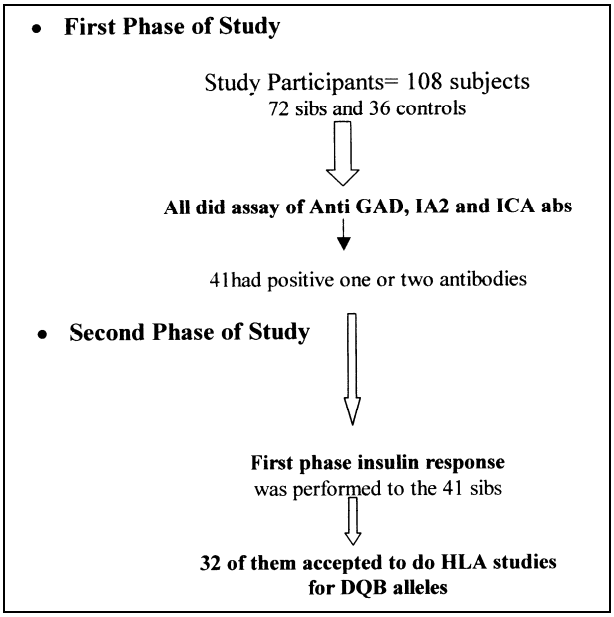

Fig.4: Flow chart of the longitudinal study

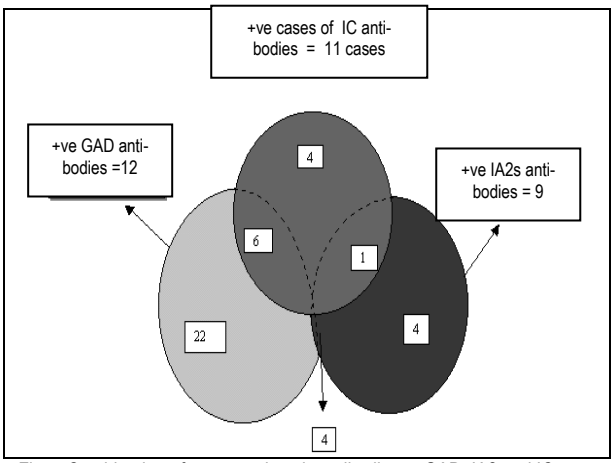

Fig.5: Combination of +ve cytoplasmic antibodies to GAD, IA2 and IC
From this figure it is apparent that six sibs had both IC and GAD antibodies positive, one case had both IC and IA2 antibodies positive, while four cases have both IA2 and GAD antibodies positive.

Fig 5 shows different combinations of positive Ab: six sibs had both IC and GAD antibodies, four sibs had both IA2 and GAD antibodies, four sibs had only IA2Ab positive, 22 sibs had only GAD Ab positive and 4 sibs had only ICA positive.

Among the 41 sibs positive for one or two Ab in the second phase of study: 30 (73.1%) were exclusively breast fed for (4-8m), 2 sibs (4.9%) for 3 m and one sib (2.4%) for 2 m. As regards past history of viral infection: 5 sibs (12.3%) had measles, 4 sibs (9.8%) had measles and chicken pox, and one sib (2.5%) had mumps. No one had prenatal history of maternal viral illness during pregnancy or any evidence of autoimmune disease other than type 1 diabetes.

As regards consanguinity, only 10 sibs (24.6%), their parents were consanguineous. Positive family history of diabetes mellitus in sibs was found to be as follows: 34 sibs (82.8%) had one member with Type1Diabetes and 7 sibs (17.2%) had multiplex family history of Type1Diabetes. On the other hand, 18 sibs (43.6%) had one member and 6 sibs had (14.6%) a multiplex family history for Type 2 Diabetes.

First phase insulin response

It was performed for all sibs positive for one or more antibodies (n=41) and controls (n=36), Mean FPIR in sibs was 41.407 mU/L ± 28.73, which was significantly decreased than the mean in controls, the latter was 79.414 mU/L ± 44.316 (P<0.001).

Table II. Grading of decreased levels of First Phase Insulin Release (FPIR) among the studied sample of siblings

Grading of decreased levels of FPIR	Number (n=41)	Frequency
I- Low risk group		
* Preserved FPIR (> 100 mU/L)	1	2.4%
* Non-preserved FPIR (> 80-100 mU/L)	3	7.3%
II- Intermediate risk group		
* Border line FPIR (80–65 mU/L)	3	7.3%
III- High risk group		
* FPIR (<65-48 mU/L) (less than 5th percentile)	7	17.1%
* FPIR (< 48 (mU/L) (less than 1st percentile)	27	65.9%
Total	41	100%

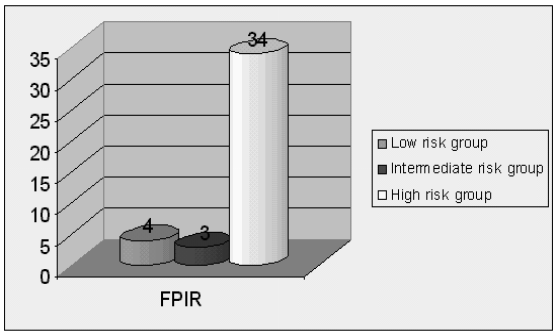

Fig.6: Grading of FPIR levels amongt siblings

Table II and Fig 6 show frequency of different grades of decreased levels of the first phase insulin release (FPIR) among sibs, the majority of sibs n=34/41 (83%), lied in the high risk group who had FPIR less than 5th percentile (< 65 mU/L).

Sibs with positive IC antibodies had mean FPIR 45.677mU/L ± 23.293 which was not significantly statistically different from mean FPIR of those with negative IC antibodies (41.067 mU/L ± 30.937 (P>0.05).

Similarly, mean FPIR among sibs with positive IC antibodies (32.889 mU/L ± 17.85) was not statistically different significantly from FPIR of sibs who had both

positive GAD antibodies and IA2 antibodies (33.02 mU/L ± 30.28) (P>0.05).

Moreover, there was no statistically significant difference between mean levels of FPIR in sibs with one positive antibody (42.28 mU/L ± 30.14) and those who had two positive antibodies (39.26 mU/L ±34.13) (P> 0.05).

Similarly, there was no statistically significant difference in the grading of FPIR between groups with either positive one or two Abs (P>0.05).

HLA studies

Only thirty-two of the 41 sibs accepted to perform HLA studies for determination of DQB1 alleles that could be related to genetic susceptibility or protection against type 1 diabetes.

Table III. Frequency of DQB1 alleles among 32 siblings

DQB1 alleles	Number (total No.=32)	Frequency %
0201 *	14	45.16
0202 *	11	35.48
0304 *	11	35.48
0501 *	10	32.26
0601 *	7	22.58
0203 *	6	19.35
0301 *	6	19.35
0302 *	5	16.13
0401 *	3	9.68
0402 *	3	9.68
0504 *	2	6.45
0303 *	1	3.23

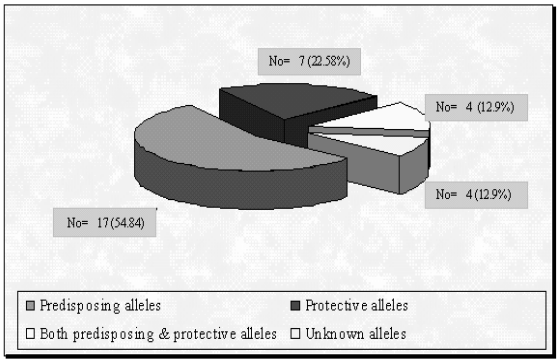

Fig.7: Frequency distribution of DQB1 alleles among studied siblings

Predisposing alleles : 0201 .0303 ,0302 ,0202
Protective alleles: ,0301 .0601
Unknown alleles: 0203 .0501,0504 ,0402 ,0401 ,0304

Table III shows the frequency of DQB1 alleles among sibs. Fig. (7) shows that 17/32 sibs (54.84%) had the predisposing alleles DQB1(0201, 0202, 0302, 0303, 0401) while 7 sibs (22.28%) had protective alleles DQB1(0301, 0601).

There was no statistically significant difference in the prevalence of predisposing or protective DQB1 alleles between those who had either positive one or two antibodies (P >0.05)

Similarly, there was no statistically significant difference in mean FPIR between sibs with predisposing or protective DQB1 alleles (p>0.05).

Discussion:

There are different approaches for identification of individuals at risk for development of type 1 diabetes, during the asymptomatic preclinical period which may last for years during which progressive beta cell destruction occur. These approaches are based on the presence of positive family history of type 1 diabetes, genetic, autoimmune or metabolic markers. These alternatives may also be combined in various ways to improve the predictive characteristics of the screening strategy.[10]

Autoantibodies to various beta cell antigens have proved to be an early marker of ongoing-B-cell destruction and are widely used to assess the risk of future manifestations of clinical disease in first-degree relatives of patients with type 1 diabetes.[11,12]

Prospective studies in subjects with and without family history of type 1 diabetes have conclusively demonstrated that the risk for type 1 diabetes is strongly correlated with the number of positive antibodies, the 5-10 years risk for type 1 diabetes varied from 0-1% in individuals with only one positive antibody to 62-100% in subjects who were positive for three or more antibodies.[4,13]

In the first phase of this study, cut off limit for positivity for GADAb was 0.841 unit/ml, for IA-2Ab was 1.217 unit/ml, while that of ICA positivity was 0.505 OD units.

There had been wide variations in the cut-off points of positivity of each of the assessed antibodies mentioned in different studies carried on populations of different ethnic and racial origins. Kulmala et al.,[14] in Finland, defined the cut off limit for GAD positivity as 6.6 relative units (RU) and for IA2 Ab 0.43 KU representing the 99th centile for both Abs in a series of 372 healthy control children and this definition conferred a disease sensitivity of 79% and a disease specificity of 97% for GAD and sensitivity of 62% and specificity 97% for IA2 Ab. On the other hand, Bingley et al.,[15] in Italy, determined a cut off limit of 2.74 for GADAb and 1.4U for IA2, which represented the 97.5th centile of control subjects for both Ab. This conferred a disease sensitivity of 82% and a specificity of 100% for GAD and 71% disease sensitivity and 97% specificity for IA2 Ab.

As for ICA, Kulmala et al.[14] determined a detection limit by immunofluorescence as 2.5 JDFU, which conferred a sensitivity of 100% and a specificity of 98% while Strebelow et al.[16] found that a cut off point of 40 JDFU favored rapid progression of type 1diabetes.

The different cut off limits between this study and other studies and in-between other studies could be explained by different methodological techniques as

well as racial and ethnic factors. Therefore, levels of Abs should be always compared to the same population.

ICA is the most definitive indication of autoimmunity as has been previously confirmed, however, since the usefulness of ICA for routine screening of susceptible individuals of the general population is limited by the cumbersome nature of the ICA assay and problems with the consistency of the test, several studies have analyzed the value of other antibody markers for prediction of type 1 diabetes. Many studies have confirmed that screening for antibodies to IA2 in combination with GAD Ab may represent a powerful strategy of routine screening to identify subjects at increased risk for TIDM with sensitivity similar to ICA and with improved specificity.[14,17] This matches well with the results of this study.

In this study, the sensitivity of GAD to discover ICA-positive subjects was 42.9% while its specificity was 48.3%. On the other hand, in a similar fashion, sensitivity of IA2 Ab was 21.4%, while it specificity was 87.9%. Therefore, testing for GAD Ab was found to be more sensitive but less specific than testing for IA2Ab to identify ICA-positive subjects and vice versa. These characters offer an advantage of combining the assay of both antibodies together, one with high sensitivity and the other with high specificity to reach a good predictive value for type 1diabetes.

In 2nd phase of study, there was high positive family history of type 1 diabetes mellitus in sibs. However, positive family history for type 1diabetes has a low sensitivity for type 1diabetes at the general population level and does not provide a clinically useful tool alone.[1] Although a positive history of type 1diabetes is a risk factor for the disease, about 90% of cases have no affected first degree relative.[17]

Similarly, the prevalence of type 2diabetes in family members of sibs under study was relatively high. This has been previously confirmed by Quatraro et al.,[18] who found that the patients with TIDM have an increased family history of T2DM; in their study, 112 patients were affected by type 1diabetes: 54 of them were related with at least one subject suffering from type 2diabetes. Similarly, Kajio et al.[19] observed that 23% (12 of 52) of the parents of TIDM patients had T2DM.

The presence of positive family history of type 2 diabetes was found to increase blood pressure and decrease insulin sensitivity in type 1 diabetic patients. Thus, such patients should be treated more aggressively in terms of both cardiovascular risk factors and glucose control.[20] On the other hand, it was found that family history of T2D, but not T1D, may delay the age at onset of TID.[21]

In this study, 41 sibs with positive one or two antibodies as well as 36 controls were subjected to determination of first phase insulin response by intravenous glucose test, mean levels of FPIR was significantly reduced in sibs than in controls. A reduced first phase insulin response to intravenous glucose is perceived as a sign of far advanced deterioration of beta-cell function during the development of type 1 diabetes mellitus.[13]

Mrena et al.[22] designed a classification system for staging of preclinical type 1 diabetes in siblings of affected children, based on a combination of the initial number of antibodies and the first phase insulin response (FPIR) to I.V. glucose: no prediabetes (no antibodies), early (one positive antibody, normal FPIR), advanced (two or more positive antibodies, normal FPIR), and late prediabetes (at least one positive antibody, reduced FPIR).

In this work, according to Srikanta et al.,[23] the majority of sibs, n=34 (83%) lied in the high-risk group who had FPIR less than 5th percentile (< 65 mU/L) while 27 sibs (65.9%) had FPIR less than the 1st percentile (< 48mU/L).

The presence of FPIR below the first percentile of normal control subjects (48mu/L) was found to be associated with an estimated risk of diabetes of 100% within 4 years. Moreover, the risk of type 1diabetes was highest in family members with FPIR <50mu/L (85%) within 5 years, 48% in those with FPIR 50-100mu/L and 17% with FPIR >100mu/L.[7]

Contrary to these results, none of the siblings developed diabetes after a follow up of 5 years since the start of this study. An explanation to this finding was proposed previously by Keskinen et al.,[13] who reported that very low FPIR is common shortly after seroconversion to autoantibody- positivity in young children with HLA-DQB1 associated genetic risk for type 1 diabetes. This suggested that in young children, the autoimmune-mediated destruction of beta cells may occur early in the course of prediabetes; the observation that a number of children have remained non-diabetic for a relatively long-time period despite very low FPIRs, (similar to our study), may raise the issue of metabolic compensation mechanisms (changes in insulin sensitivity) that could alter disease progression. Moreover, the high positive family history of type 2 diabetes in sibs in this study may delay the age at onset of type 1diabetes as has been previously mentioned by Zalloua et al.[21]

In this study, it was found that the mean level of FPIR didn't differ significantly according to the presence or absence of IC antibodies. Similarly, there was no statistically significant difference between mean level of FPIR in sibs in the group with positive ICA or those with positive GAD and IA2 antibodies. This is

expected as combined GAD and IA2Ab testing was reported to be equivalent to ICA testing as regards sensitivity and specificity.[14] Moreover, in this study the group of siblings with negative ICA had positive GAD and or IA2Ab, this could explain why ICA positive or ICA negative Ab groups showed comparably low FPIR; all of them show ongoing autoimmune B-cell destruction.

Contrary to our results, Chase et al.[9] reported that first-phase insulin response was attenuated early especially in children with high ICA titers and children positive for multiple autoantibodies. This has been also confirmed by Veijola et al.,[24] who mentioned that despite the fact that the mechanisms of decline in the functional secretory capacity of beta cells remain speculative, ICA titres could to some extent be used as indicator of the stage of impairment of insulin-secretory capacity of the beta cells.

On the other hand, Carel et al.[25] reported that ICA-negative siblings of diabetic children could have abnormally low FPIR, suggesting an intrinsic, non immune mediated defect of insulin secretion in these children, however, as in that study only ICA was measured, children with low FPIR could have had other diabetes associated autoantibodies as is the case with our study.

Moreover, in this study, there was no statistically significant difference between mean levels of FPIR in sibs with one or two positive antibodies. This can be explained by that most sibs, n=34 (83%) lied in the high-risk group (<5th percentile). However, none of the sibs developed T1DM. Greenbaum et al.[26] mentioned that the extent to which FPIR reflected the rate of beta-cell destruction and predicted the time of onset of type 1diabetes was a matter of controversy. Moreover, Lorini and Vanelli [27] found that reference ranges of FPIR in healthy young children were poorly defined and it increased with age, especially during puberty when insulin sensitivity was decreased.

Therefore, the sensitivity, specificity and predictive value of FPIR in diabetes prediction and the time scale of the FPIR loss before the onset of clinical type 1diabetes have remained poorly defined. Longitudinal studies with long follow up times, large cohorts of study subjects and frequently repeated tests are obviously needed to evaluate the usefulness of FPIR in diabetes prediction as has been mentioned by Keskinen et al.[13]

In this work, the most frequent DQBI alleles among the siblings were 0201, 0202, 0304, 0501 and less frequent alleles were 0601, 0203, 0301, 0302, 0401, 0402, 0504 and 0303.

Similarly, HLA class II typing was performed by Gaber et al.,[28] for 50 IDDM patients and 50 healthy control subjects by a restriction fragment length polymorphism (RFLP) techniques. The analysis of position 57 of the DQB1 molecule was conducted by polymerase chain reaction and specific sequence oligonucleotide hybridization. The frequency of DRB1*0301-DRB3 *0201-DQA1*0501-DQB1*0201 haplotype was 43.9% in IDDM Egyptian patients and 7.1% in control subjects (P<0.00001), reflecting the increased prevalence of DQA1*0501 susceptibility allele coding arginine at position 52 and DQB1 0201 susceptibility allele non coding aspartic acid at position 57. DQB1 *0601 and 0603 alleles, both carrying Asp at position 57 of the β-chain, and DQA1 *0203, encoding a non-Arg52 α-chain were significantly decreased among the IDDM patients. The presence of four susceptibility residues (two DQA1 Arg52+ and two DQB1 Asp 57-) conferred the highest relative risk at 20.2, on the other hand, homozygous genotypes for DQA1 non-Arg52 and DQB1Asp57 were found only in the control group.[28]

In this study, it was found that 17 sibs had the predisposing DQB1 alleles (0201, 0202 0302, 0303, 0401) with frequency of 54.84%, 7 sibs had the protective DQB1 alleles (0301, 0601) with frequency of 22.58% and 4 sibs had both protective and predisposing alleles with frequency of 12.90%.

Bingley et al.[1] found that the presence of predisposing or protective alleles can genetically modify the risk of development of type 1diabetes. The major histocompatibility complex namely HLA-DQ alleles, still represent the strongest genetic risk factor. The analysis of HLA DQ alleles may be important to discriminate between subjects at high or intermediate risk from antibody positive individuals carrying protective haplotypes as Seissler et al.,[4] had previously mentioned.

Greenbaum et al.[29] mentioned that the presence of human leukocyte antigen (HLA) haplotype DQA1* 0201, DQB1* 0602 was associated with protection from type 1 diabetes. Similarly, Kulmala[2] reported that the resistance to type 1 diabetes has been shown to be associated with DQ6 (DQA1* 0102, DQB*, 0301 and 0602-03 haplotype, and that it may be important to identify those relatives carrying DQB1* 0602 and are ICA positive to avoid their enrolment into prevention trials.

On the other hand, the highest risk was seen in those carrying the HLADQB1* 02/0302 genotype.[2,3] Similarly, Keskinen et al.[13] detected that children with the 02*/0302 were categorized into high genetic risk (risk of diabetes before the age of 15 years about 8%), and those with 0302/X genotype (X not 02, 0301, 0602 or 0603) into the group with moderate genetic risk (Risk 2.3%).

Mrena et al.[22] mentioned that autoantibodies alone were more sensitive in the prediction of future

diabetes in siblings than when combined with genetic susceptibility. Genetic susceptibility played a role in whether the initial prediabetic stage progressed (Progression in 29% of the high risk siblings compared with 6.6% of the siblings with DQB1 genotypes conferring decreased risk; $p<0.001$) and whether overt type 1 DM became manifest or not. They concluded that genetic susceptibility has an impact on both the initiation and progression of the autoimmune process leading to clinical diabetes in siblings of affected children. Moreover, the progression in sibling of TIDM patients seemed to be characteristic of younger age, higher number of detectable antibodies, higher autoantibody levels and had reduced FPIR.

In this study, the mean level of FPIR according to the presence of predisposing or protective DQB1 alleles was statistically insignificantly different ($P>0.05$). Similarly, Veijola et al.[24] found that the association between the HLA-DQB1 genotypes and FPIR has been controversial, among ICA-positive siblings of children recently diagnosed with type 1 diabetes. This has been confirmed in another study, which stated that there was no association between a diminished FPIR and high-risk HLA-DQB1 genotype in a group of ICA positive school children.[14]

To conclude, prevention of type 1diabetes will require reliable methods for early diagnosis of predisposition to the disease, using improved genetic and serological screening on a wide scale and identification of the primary antigenic target(s) for specific tolerance. The aim of the prediction of high-risk subjects is their enrolment in prevention trails where, numerous international studies are currently underway to develop therapies for the preservation of islet cell function and the prevention of type 1diabetes.

The route towards effective prevention of type 1diabetes will hardly be a well-paved high way, but rather a path lined by both success and disappointment, and one can only hope a significant decrease in the number of children progressing to clinical type 1diabetes.

References:

1. Bingley PJ, Bonifacio E, Zeigler AG, Schatz DA, Atkinson MA, Eisenbarth GS. Imunology of Diabetes Society: Proposed guidelines on screening for risk of type 1 diabetes. Diabetes Care 2001; 24(2): 398.
2. Kulmala P. Stability of autoantibodies and their relation to genetic and metabolic markers of type 1 diabetes in initially unaffected school children. Paediatr Drugs 2003; 5(4): 211-21.
3. Kimpimaki T, Kupila A, Hamalainen AM, Kukko M, Kulmala P, Savola K, Simell T, Knip M. The first signs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: The finish type 1 diabetes prediction and prevention study. .J Clin Endocrinol Metab 2000; 86(10): 4782-8.
4. Seissler J, Hatziagelaki E, Scherbaum WA. Modern concept for the prediction of type 1 diabetes. Exp Clin Endocrinol Diabetes 2001; 109 Suppl. 25: 304-16.
5. Yalow R, Rauman WR, Kifkin H. Plasma insulin in health and disease. Diabetes mellitus, Theory and Practice. New York. Experta Medica 1983; 119-50.
6. Bunce M, O'nierll CM, Krausa P, Morris PJ. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP).Tissue antigens 1995; 46: 355-67.
7. Polly J, Bingley. For the ICARUS group. Interaction, of age, islet cell antibodies insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA positive relatives. Diabetes 1996; 45:1724.
8. Mrena S, Savola K, Kulmala P, Akerblom HK, Knip M. Staging of preclinical type 1 diabetes in siblings of affected children. Pediatrics 1999; 104:924-930.
9. Chase HP, Cuthbertson DD, Dolan LM, Kaufman F, Krischer JP, Schatz White DA, Wilson DM: The Dpt-1 Study Group. First phase insulin release during the intravenous glucose tolerance test is a risk factor for type 1 diabetes. J Pediatr 2001; 138(2): 244-9.
10. Gale EA. Intervening before the onset of type 1 Diabetes: Baseline data from the European Nicotinamide Diabetes Intervention Trial (ENDIT). Diabetologia 2003; 46(3): 339-46.
11. Winter WE, Harris N, Schatz D. Type 1 diabetes islet autoantibody markers. Diabetes-Technol-Ther 2002; 4(6): 817-39.
12. Alemzadeh R, Wyatt DT. Diabetes Mellitus in children. In: Behrman RE, Kligman R.M., Jenson HB(eds.) Textbook of Pediatrics: 7th Edition. W.B. Saunders, Pennsylvania, 2004: chapter 583.
13. Keskinen P, Korhonen S, Kupila A, Knip M. First-phase insulin response in young healthy children at genetic and immunological risk for type 1diabetes. Diabetologia 2003; 45(12): 1639-48.
14. Kulmala P, Rahko J, Savola K, Vahasalo P, Veijola R, Sjoroos M, Rennanen A, Ilonen J, Knip M. Stability of autoantibodies and their relation to genetic and metabolic markers of type 1 diabetes in initially unaffected school children. Diabetologia 2000; 43(4): 457-64.
15. Bingley PJ, Bonifacio E., Williams AJ; Genoveses Boltazzo GF, Gale EA. Prediction of IDDM in the

general population strategies, based on combination of autoantibody markers. Diabetes 1997; 46: 1701-1710.
16. Strebelow M.; Schlosser M.; Ziegler B.; Ryasanowski I.; Ziegler M.C. Type 1 diabetes risk study of a general population: Frequencies and interactions of the four major type 1 diabetes-associated autoantibodies studied in 9419 school children. Diabetologia 1999; 42(6): 661-70.
17. Kulmala P, Savola K, Petersen JS, Karjalinea J, Lopponen T, Dyrberg T, Knip M. Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes. A population-based study. The childhood diabetes in Finland Study Group. J Clin Invest 1998; 101: 327-336.
18. Quatraro A, Consoli G, Magno M, Caretta F, Ceriello A, Giugliano D. Analysis of diabetic family connection in subjects with insulin-dependent diabetes mellitus (IDDM). Diabet Metab 1990; 16(5): 449-52.
19. Kajio H, Kobayashi T, Nakanishi K, Okubo M, Tsukada T. Relationship between insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus; beta cell function; islet cell antibody, and haptoglobin in parents of IDDM patients. Metabolism 1995; 44(7): 869-75.
20. Damci T, Osar Z, Ilkova H. Higher blood pressure in normal albuminuric type 1 diabetic patients with a familial history of type 2 diabetes. Diabetes Metab 2002; 28(5): 417-20.
21. Zalloua PA, Shbaklo H, Halaby G, Terwedow H, Xu X, Azar ST. Type-2 diabetes family delays the onset of type-1 diabetes. Clin. Endocrinol Metab 2002; 87(7): 3192-6.
22. Mrena S, Savola K, Akerblom HK, Knip M. Natural course of preclinical type 1 diabetes in siblings of affected children. Acta Paediatr 2003; 92(12): 1403-10.
23. Srikanta S, Canda PP, Gleason RF, Jackson RA, Soaldner JS, Eisenbarth GS. Pre-type 1 diabetes, linear loss of beta cell response to intravenous glucose. Diabetes 1984; 33:717-20.
24. Veijola R, Vahasalo P, Tuomilehto-Wolf E. Human leukocyte antigen identity and DQ risk alleles in autoantibodies-positive siblings of children with IDDM are associated with reduced early insulin response. Diabetes 1995; 44: 1021-8.
25. Carel JC, Boitard C, Bougneres PF. Decreased insulin response to glucose in islet cell antibody-negative siblings of type 1 diabetic children. J Clin Invest 1993; 92: 509-13.
26. Greenbaum CJ, Sears KL, Kaha SE, Palmer JP. Relationship of β-cell function and autoantibodies to progression and non progression of subclinical type 1 diabetes. Diabetes 1999; 48:170-5.
27. Lorini R, Vanelli M. Prediabetes Study Group of Italian Society for Pediatric Endocrinology and Diabetology (SIEDP): Normal values of first-phase insulin response to intravenous glucose in healthy Italian children and adolescents. Diabetologia 1996; 39: 370-1.
28. Gaber SA, Mazaola G, Berrino M, Canale L, Coronaglia M, Ghali I, Sergio EF, Amoroso A. Human leukocyte antigen class II polymorphisms and genetic susceptibility of IDDM in Egyptian children. Diabetes Care 1994; 17(11): 1341-4.
29. Greenbaum CJ, Schatz DA, Cuthbertson D, Zeidler A, Eisenbarth GS, Krischer JP. Islet cell antibody-positive relatives with human leukocytes antigen DQA1 *0102, DQB1 *0602. Identification by the diabetes prevention trial type 1. J Clin Endocrinol Metab 2000; 85(3): 1255-60.