Abstract
Type 1 diabetes is most often associated with auto antibodies (AAbs) against beta cell antigens and high levels of HLA mediated genetic susceptibility. The purpose of this study was to identify at risk siblings of type 1 diabetic patients at an early stage by screening for HLA–DQB1 alleles, which carry the high-risk genotype (DQB1*0201 / 0302) and its associations with measured islet cell antibody (ICA). Twenty-five siblings of subjects with type 1 diabetes aged 4 – 13 yr, median age (8.4 ± 1.02) were studied. They were screened for ICA as well as HLA-DQB1 (0201 and 0302) alleles.

Results of the study showed that among the 25 siblings, 20% tested positive for ICA. HLA-DQB1 was detected in 16% of siblings. Allele DQB1 0201 presented higher frequency (48%) than DQB1 0302 (36%). Association of DQB1 0201 and / or 0302 with ICA positive cases was detected in 20% of cases, while frequency of DQB1 0201 and / or 0302 in ICA negative cases was detected in 48% of them.

In conclusion: - Our results showed that among siblings of type 1 diabetes HLA-DQB1 alleles associated with highest genetic susceptibility i.e. DQB1 0201 and / or 0302 were detected with high significance. Also HLA-DQB1 0201 is more frequently associated with ICA. Initial screening by high risk DQB1 *0201/0302 with subsequent autoantibody testing is shown to be useful in the assessment of diabetes risk among siblings of subjects with type 1 diabetes.

Introduction
Type 1 diabetes is most often associated with auto antibodies (AAbs) against "beta" cell antigens as AAbs to glutamic acid decarboxylase (GADA), insulin (IAA) as well as the heterogeneous islet cell cytoplasmic antibodies (ICA).

Although the complex associations and linkage of HLA antigens with type 1 diabetes are not yet fully understood, HLA–DQB1 and DQA1–DQB alleles provide the strongest genetic contribution to the disease (Robinson etal, 1989) accounting for approximately 50% of the genetic risk in type 1 diabetes (Todd and Farral 1996).

In Caucasian, DQB1 0302 and DQB10201 and their linked DR specificities DR4 and DR3 provide disease susceptibility (Michelson et al,1990), where’s dominant protection is conveyed by DQB1 0602 linked to DR2 (Reijonen et al, 1991). The relationship between HLA markers and the occurrence of AAbs has been previously examined in recent onset patients of type 1 diabetes indicating positive correlation (Vande walle et al,1997) in recent years, several studies have been conducted to identify subjects at risk of developing type 1 diabetes by screening for ICA as well as by human leukocyte antigen (HLA) typing.

Aim of the work
To identify at risk siblings of type 1 diabetic patients at an early stage, by screening for HLA–DQB1 alleles which carry the high-risk genotype (DQB1 0201 and 0302) and its associations with measured islet cell antibody (ICA).
Subjects and methods

This study was carried out on twenty-five healthy siblings of type 1-diabetes. Fourteen females and 11 males. They aged 4-13 years, mean age was (8.4±1.02).

Samples of serum and blood were collected for immunological and HLA allele typing test, respectively. HbA1c was also measured.

Each family provided informed consent before samples were collected.

Methodology of HLA typing

Whole blood on EDTA was obtained from each subject. DNA was extracted from whole blood using DNA extraction kit (Manufactured by QIAGEN, USA) according to recommendation.

HLA DQB1 typing for both of the studied alleles was carried on using polymerase chain reaction (PCR) kit using sequence specific primaries multiplex PCR (all set SSP) manufactured by (DYNL BIOTECH Ltd. UK.). PCR cycling parameters were as followed: A denaturation step at 96 degrees centigrade (°C) for two min., 10 cycles of denaturation at 96 °C for 15 sec., annealing and extension at 96 °C for 60 sec. Twenty cycles of denaturation at 96 °C, for 10 sec. annealing at 61 °C. for 50 sec., and finally extension at 72 °C. for 30 sec. (Ilonen et al, 2000).

Immunological studies

ICA was determined by indirect immunofluorescence (IIF), (Reijonen et al 1994).

The ICA assay was controlled externally by an international proficiency test, (Immunology Diabetes Workshop, University of Florida USA, 1993).

The DNA locus of HLADQB1 genotype was typed using the amino acid sequencer of DNA (Sequence Specific Oligonucleotide) (SSO) probes 6. (Adojaan, et al 1996)

Statistical methods

Mean values, ranges and standard deviations were obtained for normally distributed values. Percentage of results were also calculated

Results:

The results are shown in tables 1, 2, and 3

Table (1) mean value ± SD of age / year – FBS/mg/dl and HbA1c for all studied cases(25).

<table>
<thead>
<tr>
<th>Age / year</th>
<th>FBs/mg/dl</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>65-101mg/dl</td>
<td>4.2-6.3</td>
</tr>
<tr>
<td>mean</td>
<td>(84.8±5.07)</td>
<td>(6.26±1.58)</td>
</tr>
</tbody>
</table>

Table (2) percentage of –ve and +ve HLADQB1 0201, 0302 and ICA in all studied cases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>-ve</th>
<th>+ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLADQB1 0201</td>
<td>52%</td>
<td>48%</td>
</tr>
<tr>
<td>HLA-DQB1 0302</td>
<td>64%</td>
<td>36%</td>
</tr>
<tr>
<td>HLADQB1 0201 and 0302</td>
<td>84%</td>
<td>16%</td>
</tr>
<tr>
<td>ICA</td>
<td>80%</td>
<td>20%</td>
</tr>
</tbody>
</table>
Association of Islet Cell Antibody

Table (3) Frequency of Association of HLADQB1 0201 and 0302 with positive and negative ICA.

<table>
<thead>
<tr>
<th>ICA (-ve)</th>
<th>HLADQB1 0201</th>
<th>HLADQB1 0302</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA (+ve)</td>
<td>7 (28%)</td>
<td>5 (20%)</td>
<td>12</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>3 (12%)</td>
<td>2 (8%)</td>
<td>5</td>
<td>20%</td>
</tr>
</tbody>
</table>

Discussion:

Type 1 diabetes is an autoimmune disease caused by destruction of pancreatic insulin secreting B-Cells, a process that may occur sub clinically years before the onset of clinical symptoms. The risk of sibling is 15 fold higher than is the population prevalence, but the etiology of the disorder is complex and probably involves multiple genetic and environmental factors (Charles et al, 1998).

Among the general population, the presence of islet autoantibodies carries increased risk (Schatz et al, 1993). Thus it would appear that islet autoantibodies are universal markers of the destruction of insulin producing islet cells that ultimately results in type 1 diabetes.

In the present study, ICA were used as primary screening tool for beta – cell autoimmunity in siblings with increased genetic risk of type 1 diabetes. This decision was based on the observation that ICA were more sensitive and specific than GADA or IAA, with more than 84% of children with newly diagnosed type 1 diabetes testing positive (Savila et al,1998 and Kimpim etal, 2002).

In the current study, ICA were detected in 20% of studied cases. Kulmala et al,(2001) reported that the prevalence of ICA had been observed to be 3-4% among Finnish school children, while Krischer et al (2003) found 82% positive cases of ICA. Further follow up and analysis of actual progression to diabetes may revert some of our negative ICA cases to positive one. Bonifacio et al, (1999) had shown that ICA titer was directly related to risk of type 1 diabetes. Also for trails in which screening is necessary to identify a population with sufficient diabetes risk, ICA would augment the population identified by GADA and ICA 512 AA (or vise versa).

Type 1 diabetes has strong class II HLA association, with linkage to the DQA and B genes and is influenced by the DRB genes. The risk presented by the DR4 allele is primarily attributable to an association in a haplotype with HLADQB1 *0302. Susceptibility associated with HLA-DR3 may be determined directly by HLADQB1 *0201 (Cavan etal, 1992).

In the current study, high risk HLADQB1 0201 allele represented higher frequency in siblings of type 1 diabetes (48%) than HLADQB1. 0302 allele (36%). Analysis for both HLADQB1 allele was found in 16% of cases. These results are in agreement with Hagopain (1995) and Bonifacio et al (1995).

High proportion of this genetic marker indicate that those siblings carry the susceptibility for developing type 1 and that their predicted risk of diabetes is greater with more than one high risk allele.

In a study, investigating genetic and humoral markers for prediction type 1 diabetes in siblings has reported that among the genetic markers DQB1 *0201/0302 was associated with the highest positive predictive value of 22% (Schlosser et al, 2002).

In our study, the frequency of HLADQB1 *0201 and / or 0302 in ICA negative cases was detected in 48% of cases. This is in accordance with results of Schlosser et al (2002), who found 46% association.

On the other hand HLADQB1 *0201 and / or 0302 was detected in 20% of our ICA positive cases. Kimpini et al, (2002)
found the proportion of children who tested positive for ICA was significantly higher among those with the high risk genotype (5.1%).

Schlosser et al (2002) found HLA heterozygostiy to occur in 4.5% with single AAb.

ICA was associated with 0201 in 8% of cases and was associated with both alleles in 12% of cases. Subjects with positive AAbs revealed an increased frequency of diabetes when associated with HLA-DQB1 alleles 0201 or 0302 (P=0.001 and 0.006) respectively (Kimpim etal, 2002).

Vandewalle et al, (1997) in their study on recent onset diabetic patients as well as their siblings found positive correlation between the occurrence of diabetes associated AAbs and high risk HLA markers. These results might be of importance for population screening strategies based on detection of islet autoantibodies in associations with high risk HLA genotypes. Furthermore they indicate significant increased risk for subsequent development of type 1 diabetes.

In conclusion: - our results showed that among siblings of type 1 diabetes HLA-DQB1 alleles associated with highest genetic susceptibility i.e. DQB1 0201 and / or 0302 were seen with high significance. Also HLA-DQB1 0201 is more frequently associated with ICA. Initial screening by high risk DQB1 *0201/0302 with subsequent autoantibody testing is shown to be useful in the assessment of diabetic risk among siblings of subjects with type 1 diabetes.

References


Association of Islet Cell Antibody……..


مصاحبة الأجسام المضادة لخلايا جزر لانجرهانز مع الجزيء الجيني DQB1 0302

، 2001 في إشقاء مرضى السكر من النوع الأول

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السكر من النوع الأول هو أكثر الالتواعب مصحوبة بظهور الأجسام المضادة ضد خلايا البيتزا (خلايا بيتزا في جزر لانجرهانز) وأيضًا مستويات عالية من الجزيئات الجينية القابلة لظهور

المرض

الهدف من هذا البحث هو تحديد مدى قابلية تعرض إشقاء مرضى السكر من النوع الأول لهذا المرض وهذا عن طريق قياس الأجسام المضادة لخلايا بيتزا وأيضًا عن طريق التنبيه الجيني لهذا المرض ومدى مصاحبة الأجسام المضادة لخلايا بيتزا وهذا عن طريق قياس الأجسام المضادة لخلايا بيتزا في المرضى. وقد تم اجراء هذا البحث على 25 فردًا من إشقاء مرضى السكر من النوع الأول يتراوح اعمارهم ما بين 4 إلى 13 سنة بمتوسط عمر 8.4±0.9. وقد تم قياس الأجسام المضادة لخلايا بيتزا في جميع الأفراد وأيضًا تم تتبع التحليل الجيني للحمض الأميني في الكروموسوم المسنول عن ظهور هذا المرض (0302).

HLA-DQB

وكانت النتائج الآتى

20% من الحالات كانت إجابة للاجسام المضادة لخلايا بيتزا HLA-DQB1 0201

16% من الحالات كانت إجابة لتحليل الجيني 1 HLA-DQB1 0110

48% من الحالات كانت إجابة لتحليل الجيني المضادة لخلايا بيتزا HLA-DQB1 0201

36% من الحالات كانت إجابة لتحليل الجيني المضادة لخلايا بيتزا HLA-DQB1 0201

وألات هذه النتائج ذات دلالة إحصائية قوية لصصاح الأجسام المضادة لخلايا بيتزا مع التحليل الجيني 0201. التي تزيد من احتمالات ظهور اعراضه ومحاولة ايجاد العلاقة بين موجود وذان أن DQB1 0201 ، DQB1 0201 فقط أكثر من 0201

الجسم المضادة لخلايا بيتزا أكثر مصاحبة للجزيء الجيني 0201 HLA-DQB1 0201 فقط أكثر من 0201

والخالية من هذه النتائج وجد أنه بخصوص إشقاء مرضى السكر من النوع الأول وجد عندما قابلية وراثية عالية لظهور مرض السكر وذلك في حالة وجود الجزيء الجيني 0201 مع الأجسام المضادة لخلايا بيتزا واقل منها احتمال في حالة إيجابية DQB1 0201 مع الأجسام المضادة لخلايا بيتزا ويزيد احتمالات ظهور المرض في حالة إيجابية الجينيين الجينيين 0201.

0201 مصاحب للاجسام المضادة لخلايا بيتزا ويkład ينعدد احتمال الأصابة بالمرض في حالة سلبية الجزيء الجيني 0201 0201. ومن هذا يمكننا التنبؤ المبتكر لحذف مرض السكر من النوع الأول في أشقاء مرضى السكر من هذا النوع الأول وذلك قبل ظهور المرض. وذلك يمكن أن يفيد في محاولة تاجيل ظهور المرض أو التدخل المبكر للعلاج لمنع المضاعفات.