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Human Leukocytes Antigens in Insulin Dependent Diabetes Mellitus (IDDM) Type I

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Abstract: This study consists of 72 diabetic patients of type I and 52 diabetic patients of type II besides 35 normal persons of matched age and sex. There are some alleles more frequent in IDDM group than in control. In patients, they are DQA1 *0301/2, *0102, *0101 DQBl * 0201, * 0302, * 0602-3, * 030I-DRB1*0101, *0301, *04, *1101, While in normal control, they are DQA1*0101, *0102, *0l03, *0201, '0501 DQB1*030l, *0602-3, *020l, *0601 DRB1 *1101 *1501/2/3. Three times high positive percent were observed regarding rubella 1gM of IDDM as compared to NIDDM while it was zero percent with respect to control.

Key words: Leukocytes · IDDM

INTRODUCTION

The earliest prediction of type I diabetes is one based upon the identification of its genetic basis. If the genes responsible for the disease are known it might eventually be possible to predict the disease in the prenatal phase [1].

There is an association between specific human leukocytic antigens (HLA) and predisposition to type I diabetes [2].

The HLA system constitutes part of the major histocompatibility complex (MHC) located on the short arm of the sixth chromosome, the gene products of MHC include not only the HLA but also C2 and C4 components of the complement system (class III genes) and other proteins as the adrenal enzyme hydroxylase. The region of the MHC is divided into loci designated as A, B, C and D the alleles at the A, B and C loci are described as class I genes and encode for signal-chain glycoproteins expressed as surface antigenes on all nucleated cells. The HLA-D region encodes for class II antigens which are polymorphic dimers each containing an A chain and B-chain, these antigenes may be expressed on a variety of cells particularly in response to various stimuli Within the D region of the HLA complex at least three loci DR, DP and DQ have been identified at each of which several alleles have been identified [3].

Neither the mechanisms of the HLA association nor the mode of inheritance of IDDM associated haplotypes are understood. Numerous studies have failed to identify a simple mode of inheritance of IDDM [4].

There is evidence suggesting that HLA class II alleles are not only associated with but also genetically linked to IDDM [5].

A strong association of IDDM with the absence of one particular amino acid (aspartic acid) at position 57 on the DQB chain has been found 171.

Modes of inheritance have been suggested as autosomal recessive with two alleles, one normal and one diabetogenic and more complex modes as two or more diabetogenic alleles in linkage disequilibrium with DR3 and DR4 respectively or multiple loci [6, 7].

The higher risk represented by absence of Aspartic acid at position 57 of beta chain. The haplotypes DR / DQW2, DR1 / DQW5, DR2 / DQW1, DR6 / DQW6 possess different amino acids either alanine or valine or serine at position 57 of the DQ beta gene [8, 9].

The findings have also confirmed in an animal model of IDDM where at position 57 aspartic acid is replaced by serine.

Approximately 12% of islet cell autoantibodies (ICA) positive relatives of patient with type I diabetes express this allele the majority of DQB1 O6O2 + ICA + express an ICA subtype absorbed glutamic acid decarboxylase (GAD). This means that HLADQB 1 0602 associated ICA might represent a pattern of ICA which confers lower risk for progression to diabetes [10, 11].

On the other hand the frequencies of the highest risk genotypes. DR3 /DR4 or DQBI 0201 /0302 Buzzetti *et al.* [12] were significantly increased in autoantibody positive relatives compared with auto- antibody —ve relatives [13].

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So HLA encoded susceptibility to disease relevant autoantibody production and IDDM are concordant with the susceptibility alleles but discordant for the protective DQB 1 0602 allele. Within families the risks are not equally distributed between siblings and depend on the number of HLA haplotypes shared with the proband, HLA haploidentical siblings have a similar risk to the general population for developing IDDM while HLA identical siblings have an approximately 100 times higher risk [14].

DR4 positive affected fathers have shown to transmit this allels more frequently to their offspring diabetic more than the affected mothers [151. This distorted mode of inheritance may explain the lower risk of IDDM in the offspring of diabetic mother than do offspring of diabetic fathers [16].

Although a number of autoantibodies to several auto-antigenes have been detected in sera of individuals in their pre-diabetic phase, islet cell antibodies were the first autoantibody used in the prediction of type I diabetes [17, 18].

ICA (Islet cell autoantibodies) are present in 80% new onset diabetic patients and in 5% of their relatives. The prevalence of these autoantibodies among prediabetic relatives vary in several studies but it has been estimated that approximately 70% have an ICA titer greater than 10 units [19, 20].

ICA direct at beta cell surface antigen, islet cell surface antibodies (ICSA) have been observed in patients with newly detected type I diabetes. A cytotoxic effect on beta cells is generally demonstrabic with ICSA but not with cytoplasmic ICA Skyler *et al.* [21, 22] and Muir *et al.* [3]. ICA have been shown to represent a heterogenous family of autoantibodies. Insulin is a 5 1 amino acids disulfide linked hetei-odjmer both A and B chains contribute to form the epitope of anti-insulin auto-antibodies [23].

The presence of these auto-antibodies is secondary to B-cell destruction process during which insulin is released and processed by antigen presenting cells [24].

Viral Infection Consideration: Viral infection may play a role in the etiology of diabetes by triggering autoimmune mediated destruction of beta cells in generally predisposed individuals. The mechanism of such viral triggering may involve the systemic release of various cytokines, including interferon. The cytokines induce the expression of specific antigen (e.g. the 64 KD, glutamic acid decarboxylase) on the surface of beta cells, thus exposing some of these cells to immune destruction, in addition homology has been observed a relation between amino acid sequences in the coxsachie virus and glutamic acid decarboxylase expressed in islet cells so antibodies induced by and directed at the coxsachie virus may therefore induce islet cell destruction [25].

The major histocompatibility complex class II alleles, HLA-DQ and HLA-DR are the chief genetic elements of human type I diabetes [21]. Thus the aim of this study is to illustrate the more frequent alleles of HLA in IDDM and the role of Rubella infections.

MATERIALS AND METHODS

The study consisted of 72 diabetic patients of type I (IDDM) and 52 diabetic patients of type II (NIDDM) who were taken from Elmataria Hospital and Sphinx Hospital beside 35 normal controls of matched age and sex. The blood sample were taken at fasting, the blood sample withdrawn with sterile disposable syringes. In a Wassermann test tubes the blood mixed with heparin and/or fluoride sodium salts. The following parameters were measured.

Rubella Antigens 1gM & lgG: About 10-20% of children with congenital rubella, particularly those who carry high risk HLA alleles, develop autoimmune type I diabetes [26].

The ELISA has been shown to be a sensitive and reliable procedure for detection of antibodies to Rubella. Rubelia antigens are fixed to the interior surface of microwells patient's serum is added and any antibody presents to Rubella antigens will bind to these antigens. The microwells are washed to remove unbounded serum proteins. Anti bodies conjugated with Horseradish peroxidase enzyme and directed against human 1gM or IgG are added and will in turn bind to any human 1gM or IgG prersent. The microwells are washed to remove unbound conjugate and then chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolysed to a coloured end product. The colour intensity is proportional to the amount to antibodies present in the patient's serum (Equipyr, Company code 0150-0150, 1999) [27].

HLA Class II: The test is based on three major processes: PCR target amplification, hybridization of the amplified products to an array of immobilized sequence specific oligonucleotide probes and detection of the propound amplified product by colour formation [28].

The statistical analysis of data by using t-test, Critical vales of pearson's correlation coefficient and chi square test.

RESULTS

Numbers and percentage of highly positive, positive and negative cases infected by Rubella virus in IDDM, NIDDM and control groups expressed as Rubella 1gM are shown in Table (1). The percentage of patients who was infected by rubella virus in the group of IDDM and possessed an antibodies to the virus were 68.1% (high positive), 6.9% (positive) and 25% negative respectively. While in group NIDDM it was found that about 21.15% (high positive), 5.77% (positive) and 73.8% (negative) respectively regarding control 0% (high positive), 17.14% (positive) and 82.68% (negative).

Mean values of rubella IgG of the IDDM, NIDDM and control groups are shown in Table (2) as indicated from the table that the mean value of rubella lgG of IDDM group is markedly more than the corresponding values of the two other groups.

Frequencies of HLA-DQA1 alleles of type I diabetes mellitus and normal control are shown in Table (3). The obtained result reveled that the most frequent alleles regarding patients are, the DQA1 *0301/2, *0102, *0101 While in normal control the more frequent are DQA1*0101, *0l02, *0103, *0201, *0501. Frequencies of HLA-DQB 1 alleles of type I diabetes mellitus and normal control are shown in Table (4). It illustrates that the most frequent alleles in patients and control are, DQB1 * 0201, * 0302, * 0602-3, * 0301, DQB l*0301, *0602-3, *02O1, *0601 respectively. Frequencies of HLA-DRB 1 alleles of type I diabetes mellitus and normal control are shown in Table (5). It shows that the most frequent alleles, in patients and control are DRB 1*010l, *0301, *04, *1101, DRB1*1 101, *1501/2/3 respectively.

DISCUSSION

The genetic susceptibility is strongly associated with HLA-DQ and DR on chromosome 6, but genetic factors on other chromosomes such as the insulin gene on chromosome 11 and cytotoxic T-lymphocyte antigene gene on chromosome 2 may modulate disease risk. So, 60% of genetic susceptibility to type 1 is conferred by HLA [29- 31].

The polymorphism of HLA class II are simply in a linkage disequilibrium with the true susceptibility genes maped within or nearby the HLA complex locus. Also a particular three dimensional structure of HLA class II molecules determined by amino acid substitution at position 52 of HLA -DQ α chain and /or position 57 of HLA -DQ β chain has something to do with the presentation of pancreatic autoantigens by antigene presenting cells and subsequent recognition of them by CD4+ T-helper lymphocytes [32].

Although recent studies have concluded that DQA 1 -DQB 1 haplotypes are the primary markers of susceptibility for type 1 diabetes, their effect can be modified by DRBI. Studies investigating this issue have examined differences in DRB 1*4 alleles among DQA 1*0301 -DQB 1*0302 positive cases and controls. In Caucasians, the DRB1*040l DQA 10301 -DQB 1*0302 haplotype has been shown to be increased infrequency among type I diabetes compared to controls. However, in combination with DQA1*0301 DQB1*0301, DRB1 *0401 was negatively associated with the disease. Thus it is unlikely that DRB1*0401 confers an independent risk for type I diabetes. Studies of the DRB 1*0404 DQA 1*0301 -DQB 1*0302 haplotype have yielded conflicting result. DRB 1 *o405-DQA 1*0301 - DQB 1*0302 appears to be more common among type 1 diabetic cases compared to controls in groups such as Mexican Americans [26, 31].

Just recently, one group managed to obtain T-cell lines from peripheral blood of several type 1 diabetic patients that are specific to the insulin B9- 23 peptide, restricted to the susceptibility allele DQA1*0301/B1*0302, the structure of this particular peptide HLA-DQ8 complex has been determined by crystallography and it will certainly provide futher insight into the pathways through which HLA-DQ molecules determine the susceppribility or resistance to type 1 diabetes [32]. It was found a positive association between HLA-DRB 1*01 and DRB 103 alleles and IDDM, the frequency of HLA-DQA1*0101, DQB1*0201 and *0302 was high in patients. Also the frequency of the DRBI *0301/ DQA1 *0501/DQB 1 *020 /1; DRB 1 *0405/DQA1 *0301/DQB1 *0302 and DRB1 *0405/DQA1 0301 /DQB1 *0401 haplotypes were higher in comparison to controls [33].

In the present study there are some alleles more frequent in IDDM group than in control group. In patients, the DQA1 *0301/2, *0102, *0l0l..DQB1*0201, *0302, *0602-3, *0301/DRB1 *0101, *030l, *04, *l10l. While in normal control, DQA1*0101, *0102, *0103, 0201 *0501 -DQB1*0301, *0602..3 *0201.

Type I diabetes is believed to result from an infectious or toxic environmental insult to the pancreatic beta cells in genetically predisposed persons. Environmental factors that have been associated with altered pancreatic islet cell function include viruses (mumps, rubella, coxackie virus) and other destructive cytotoxin [34].

Table l: Numbers and percentage of highly positive, positive and negative cases infected with Rubella virus in IDDM, NIDDM and controls that estimated as Rubella 1gM

	IDDM group				NIDDM group				Control group		
	N	High+ve	+ve	-ve	N	High+ve	+ve	-ve	N	High+ve	+ve	-ve
Rubella IgM	72	49.72	5/72	18/72	52	11/52	3/52	38/52	35	0.35	6/35	29/35
Percentage		68.1%	6.9%	25%		21.15%	5.77%	73.8%		0%	17.14%	82.86%

Table 2: Mean values of Rubella IgG in insulin dependent group, non insulin dependent group as compared to control group

Studied parameters	IDDM groupN=72	NIDDM group N=52	Control groupN=35
Rubella IgG (I.U.)	24.967±0.133	13.483±0.084	11.543±0.095

Table 3: Different gene frequency of DQAl in control group and IDDM group

DQAl Alleles	Control group N=20	IDDM group N=50
0101	0.1818	0.1709
0102	0.1545	0.2025
0103	0.1364	0.0633
0104	0.1182	0.0696
0201	0.1273	0.0759
0301.2	0.1091	0.2405
04010	0.0364	0
0501	0.1364	0.1772

Table 4: Different gene frequency of DQBl in control group and IDDM group

DQBl Alleles	Control group N=20	IDDM group N=50
0201	0.1261	0.2687
0301	0.2432	0.1269
0302	0.0901	0.2164
0303	0.0541	0.0373
0500	0.0721	0.0672
0601	0.1171	0.0896
0602-3	0.2072	0.1418
0604-5	0.0721	0.0522
0401/2	0.018	0

Table 5: Different gene frequency of DRBl in control group and IDDM group

DRBl Alleles	Control group N=20	IDDM group N=50.
0101	0.0673	0.1714
0102	0	0
151/2/3	0.101	0.0714
1601/2	0.0721	0.0429
0301	0.0817	0.2857
04	0.0385	0.1571
070.2	0.0529	0.1429
0803.4	0.0096	0
0901	0.0337	0
1001	0.0481	0.0571
1101	0.2308	0.1286
1202	0.024	0
1302/3/5	0.0673	0
1401/2	0.0625	0.0429

Viruses have been implicated in the etiology of type 1 diabetes for the past several decades, they are thought to act as initiator, accelerator or precipitators of the disease and may function by direct or indirect mechanisms. Viruses may attack and destroy the beta cells of the pancreas and directly cause diabetes, with or without autoimmunity [35].

An adequate antioxidant therapy may represent a strategy to protect pancreatic beta cells against destruction during the development of autoimmune diabetes Oral antidiabetic compounds such as sulfonylureas (gliclazide) and thiazolidindiones can have a potential antioxidant activity. Metformin treatment can reduce s level of methylglyoxal and so, inhibit AGEs formation. Improvement of glycemic control seems to be a beneficial factor to decrease oxidative stress in diabetes [36].

In the correlation of different parameters in type I (1DDM) group, It's found that, viruses have long been considered a major environmental factor to cause type I diabetes. So, congenital rubella is the only infection clearly associated with development of type I diabetes [37].

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