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Research article

HLA Genotyping in Patients with Multiple Sclerosis and Insulin-Dependent Diabetic Patients: Descriptive Study in A Multiplex Libyan Families

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Abstract
Multiple Sclerosis (MS) is an autoimmune, chronic inflammatory demyelinating central nervous system (CNS) insult initially, and a neurodegenerative disorder eventually. This paper is a descriptive cross-sectional study that involved two Libyan families, with a total number of 8 and 13 members, across two and three generations, respectively. The first family included 2 MS-affected members, and there were 4 MS patients among the second family members. As well as, 3 out of those 6 MS-affected individuals are Insulin-Dependent Diabetic Patients (IDDM). Every MS patient of both families was genotyped for HLA. The HLA-DRB1, DRQB1, and DQA1 were explored. HLA haplotypes were observed in each MS patient, taking into consideration the IDDM comorbidity status. The 3 individuals with MS and type I DM are carriers of the HLA-DRB1*0402-DQB1*0302 pattern. While the patients affected with MS only are carriers of the pattern: HLA- HLA-DRB1*1501-DQB1*0302. In conclusion, this paper brings to our attention that the common HLA genotypes among Libyan MS patients with or without IDDM comorbidity might be the same HLA genotypes among the European MS patients.
Keywords. Multiple Sclerosis, Human Leukocyte Antigens, Major Histocompatibility Complexes, Diabetes Mellitus.

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating central nervous system (CNS) insult initially, and a neurodegenerative disorder eventually. It is an unpredictable and incurable disabling disease, mainly affecting young adults. To date, Genome-wide association studies (GWAS) have identified almost 236 independent genetic variants associated with increased MS susceptibility, including 32 independent variants located in the human leucocyte antigen (HLA) region on chromosome 6. The strongest MS associations are found in the HLA region, in particular, HLA DRB1*15:01 being the most prominent of them, conferring an approximately three-fold increase in the risk of MS [1,2]. Being a complex, multifactorial, polygenic disorder, MS is thought to involve gene-gene and gene-environment interactions. Some of the major environmental contributors to MS is the previous childhood infection with Epstein-Barr Virus (EBV) that has been recently proved to increase the risk of MS in genetically-susceptible individuals by 32-fold. Additionally, several lifestyle factors are associated with elevated MS risk, such as vitamin D deficiency, low sun exposure, unhealthy diet, smoking, and obesity [3].

The genetic and environmental contributions of the MS etiology have been supported a long time ago by the occurrence of familial aggregations, as the family members share the same genetic composition and similar environmental influencing factors as well [4]. During the last decade, its incidence and prevalence have been increasing among Libyan families with multiple affected family members, enhancing the idea that the answer lies within our genes [5]. However, HLA genes play a crucial role in immune functions, and they are well known to be associated with a wide variety of autoimmune and complex diseases in general, certain HLA haplotypes are considered to be the major heritable factors involved in developing IDDM disease; as HLA-DRB1, -DQA1 and -DQB1 [6]. Moreover, lifestyle and environmental factors such as obesity and unhealthy diet are well-established risk factors that increase the possibility of comorbidities with other autoimmune disorders among patients with IDDM [7].

Methods

Study Participants

This paper is a descriptive cross-sectional study that involved two Libyan families, with a total number of 8 and 13 members, across two and three generations, respectively. The first family (MSF1), included 8 members of two generations. It included 2 MS-affected family members: the father on the first generation and one of his sons on the second generation, with an existing diagnosis of Clinically Definitive MS (CDMS). In addition to MS, both patients have an alternative autoimmune disease, which is type I Diabetes Mellitus (IDDM) (Figure 1-a). The father is also a heavy smoker, obese, and has hypertension. While the son has a history of previous EBV infection during childhood (additional environmental risk factors). Although the father had been diagnosed with MS at the age of 43 years, his son had an early onset of disease, as he had been diagnosed with MS at the age of 12 years. The second family was denoted as (MSF2); it was composed of 13 members across three generations. It included 4 MS patients: the mother on the first generation, a son, and two daughters on the second generation with an existing diagnosis of CDMS. Additionally, the

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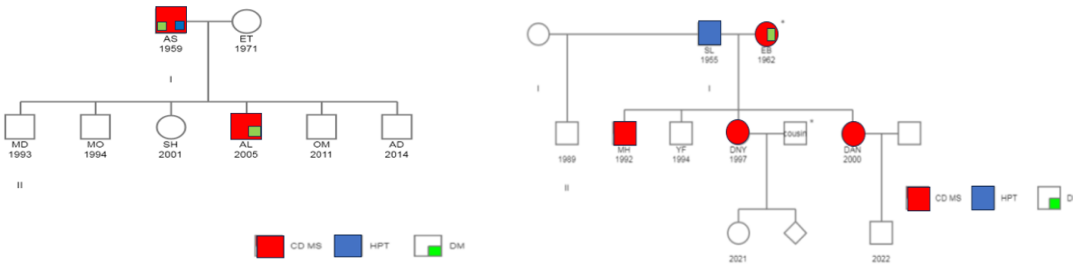
mother has type I diabetes mellitus (IDDM) (Figure 1-b); And all of her offspring had a history of a childhood EBV infection, including the currently MS-unaffected son. The mother was diagnosed with MS at the age of 42 years, while her children had an early onset of the disease; as her son was presented with CDMS at the age of 18 years; and his sisters had CDMS at 17 and 15 years of age.



(**a**) (**b**)

Figure 1. The pedigree of the two participating families: (a) The pedigree of the first MS family (MSF1). It is composed of two generations, the MS-affected patients are a child and his father, both of whom have type I DM. (b) The pedigree of the second MS family (MSF2). It is composed of three generations, the MS-affected patients are three siblings and their mother, who has type I DM concomitantly. MS-affected males (red square), MS-affected females (red circles). CD MS: clinically definitive Multiple Sclerosis (red), HPT: Hypertension (blue), DM: Diabetes Mellitus (green)

HLA genotyping

Every MS patient of both families was genotyped for HLA. The genomic DNA was extracted from white blood cells (WBCs) of the peripheral venous blood. The blood samples were drawn in EID private Clinic–Tripoli/Libya in October 2018, then the samples were sent abroad for HLA genotyping (Bioscientia Labs-Germany). The HLA-DRB1, DRQB1, and DQA1 were explored. HLA haplotypes were observed in each MS patient, taking into consideration the IDDM comorbidity status.

Results

This study included 6 MS patients, collectively, 3 males and 3 females. Three of the MS patients have type I DM (1 female and two males). The individuals with MS and type I DM (3/6) are carriers of the HLA-DRB1*0402-DQB1*0302 pattern. While the patients affected with MS only are carriers of the pattern: HLA-HLA-DRB1*1501-DQB1*0302. Table 1 shows the clinical characteristics and the associated HLA haplotype for every MS patient among the studied families. The mean age of the patients at MS disease onset was 29.1 years, ranging from 12 years to 49 years. As the diagnosis of MS had been made according to the McDonald criteria [8]. All of the patients had positive MRI Brain and Cervicodorsal spine, with positive oligoclonal bands (OCB) in cerebrospinal fluid (CSF) analysis. Also, a delayed P100 response on the visual evoked potential (VEP) study. Additionally, for the EDSS score, all of the patients showed some sort of functional disability ranging from minimal concealed disability (EDSS score= 0.5 to ≤ 2.5), to reveled partial dependence (EDSS= ≥ 3.5 to ≤ 6), according to the disease clinical phenotype severity being either mild or severe, and the years of disease duration. Only 3 MS patients had type I Diabetes Mellitus.

Table 1. Clinical characteristics and associated HLA haplotype of the studied MS patients.

MS Patient	Family No./ Sex	MS Onset Age	Clinical Phenotype	Phenotype Severity	Clinical Symptoms	EDSS Score	IDDM Comorbidity	HLA haplotype
1	F1/ M	43 Y	SPMS	Severe	Motor+optic	6	IDDM	DRB1*0402-DQB1*0302
2	F1/ M	12 Y	RRMS	Severe	Cerebellar+optic+brain stem	2.5	IDDM	DRB1*0402-DQB1*0302
3	F2/ F	49 Y	RRMS	Mild	Sensory	1	IDDM	DRB1*0402-DQB1*0302
4	F2/ M	18 Y	RRMS	Severe	Motor +cerebellar	2.5	-	DRB1*1501-DQB1*0302
5	F2/ F	21 Y	RRMS	Mild	Sensory+optic	1	-	DRB1*1501-DQB1*0302
6	F2/ F	17 Y	RRMS	Mild	sensory	1	-	DRB1*1501-DQB1*0302

F/No. Family number, M male, F female, RRMS relapsing remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis, PRMS progressive relapsing multiple sclerosis, IDDM insulin-dependent diabetes mellitus, EDSS Expanded Disability Status Scale, HLA human leucocyte antigen.

Discussion

Multiple Sclerosis was proven to be a complex, multifactorial, autoimmune disorder, long time ago. As well as IDDM has been considered to be a hereditary disease involving a wide variety of genetic and environmental factors. Both of them had a strong association with autoimmunity, with common potential genetic risk factors that are involved in autoimmune diseases causation and association [9,10]. The HLA genes are among the most important genetic factors that have been proven to be correlated to MS and IDDM,

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concomitantly; through several familial studies, and there was a great deal of research that supports and demonstrates the shared etiology between MS and IDDM [11,12]. As HLA DRB1*15 is the most frequent HLA allele that is associated with MS [13]. Moreover, HLA-DR2 is associated with increased susceptibility to MS in Caucasian multiplex families [12,13]. Whereas, IDDM increased susceptibility is associated with DRB1*04 more frequently [6,15]. Additionally, the DRB1*0401-DQB1*0302 haplotype was shown to be associated with IDDM predisposition [16].

After analyzing the previously reported HLA alleles of Class II (DPB1, DQB1, DRB1) for 14 different Western European countries that were established on the Global HLA allele estimation report done in October 2020 [17]; There were 2 (DQB1, DRB1) out of the 58 HLA class II alleles occurred in the MS patients from those countries, that were the same as we explored in our Libyan families, in agreement with the proposed association of specific alleles as predisposing risk factors for autoimmunity and another protective effect of their absence [18]. Further research should be done involving a larger sample of Libyan MS multiplex Families, with and without another autoimmune disease comorbidity, to explore and estimate the HLA allele frequencies and their risk and protective effects. In conclusion, this paper brings to our attention that the common HLA genotypes among Libyan MS patients with or without IDDM comorbidity might be the same HLA genotypes among the European patients who carry specific HLA alleles that are associated with MS pathogenicity and autoimmune diseases comorbidity.

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Conflicts of Interest
The authors declare no conflicts of interest.

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التصلب المتعدد هو إصابة مناعية ذاتية مزمنة التهابية مزيلة للميالين للجهاز العصبي المركزي في البداية، واضطراب تنكسي عصبي في النهاية. هذه الورقة هي دراسة وصفية مقطعية شملت عائلتين ليبيتين، بإجمالي عدد أفراد يبلغ 8 و13 فردًا، على مدى جيلين وثلاثة أجيال، على التوالي. ضمت العائلة الأولى فردين مصابين بالتصلب المتعدد، وكان هناك 4 مرضى بالتصلب المتعدد بين أفراد العائلة الثانية. بالإضافة إلى ذلك، فإن 3 من هؤلاء الأفراد الستة المصابين بالتصلب المتعدد هم مرضى السكري المعتمدون على الأنسولين. تم تحديد النمط الجيني لكل مريض بالتصلب المتعدد من كلتا العائلتين من أجل مستضدات الكريات البيضاء البشرية .(HLA) تم استكشاف HLA-DRB1 و DRQB1و .DQA1لوحظت أنماط HLA في كل مريض بالتصلب المتعدد، مع الأخذ في الاعتبار حالة الأمراض المصاحبة لمرض السكري المعتمد على الأنسولين. الأفراد الثلاثة المصابون بالتصلب المتعدد ومرض الســـكري من النوع الأول هم حاملون للنمط .HLA-DRB1*0402-DQB1*0302 في حين أن مرضى التصـــلب المتعدد فقط يحملون النمط .HLA- DRB1*1501-DQB1*0302 ختامًا، تلفت هذه الورقة انتباهنا إلى أن الأنماط الجينية الشائعة لـ HLA بين مرضى التصلب المتعدد الليبيين، سواءً كانوا مصابين بمرض السكري المزمن أو غير مصابين به، قد تكون هي نفسها بين مرضى التصلب المتعدد الأوروبيين.

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