



## Detection of Human Leukocyte Antigen and Celiac Disease Auto Antibodies in serum of Patients with Multiple Sclerosis

Rana S. Aboud<sup>1\*</sup>, May K. Ismael<sup>1</sup>, and Haider J. Mohammed<sup>2</sup>

<sup>1</sup> Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

<sup>2</sup> Department of Biology, College of Science, AL-Mustansiriyah University, Baghdad, Iraq.

### Abstract

To determine the important pathogenic role of celiac disease in triggering several autoimmune disease, thirty patients with Multiple Sclerosis of ages (22-55) years have been investigated and compared with 25 healthy individuals. All the studied groups were carried out to measure anti-tissue transglutaminase antibodies IgA IgG by ELISA test, anti-reticulin antibodies IgA and IgG, and anti-endomysial antibodies IgA and IgG by IFAT. There was a significant elevation in the concentration of anti-tissue transglutaminase antibodies IgA and IgG compared to control groups ( $P \leq 0.05$ ), there was 4(13.33%) positive results for anti-reticulin antibodies IgA and IgG, 3(10%) positive results for anti-endomysial antibodies IgA and IgG. There were 4 positive results (13.33%) for HLA-DQ8 by using HLA-DQ8 Real-Time PCR test. These results indicated that patients with celiac disease play an important role in pathogenesis of Multiple Sclerosis.

**Keywords:** Multiple sclerosis, celiac disease, auto antibodies.

## التحري عن مستضدات التوافق النسيجي والاضداد الذاتية لحساسية الحنطة في مصول المرضى المصابين بتصلب الاعصاب المتعدد

رنا سعدي عبود<sup>1\*</sup>، مي خليل اسماعيل<sup>1</sup>، حيدر جاسم محمد<sup>2</sup>

<sup>1</sup> قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

<sup>2</sup> قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.

### الخلاصة

غرض تعيين الدور الامراضي المهم لمرض حساسية الحنطة في قدح العديد من امراض المناعة الذاتية، تم التحري عن (30) شخص مريض يعانون من تصلب الاعصاب المتعدد باعمار تتراوح من (22-55) سنة وتمت المقارنة مع (25) شخص سليم. خضعت جميع عينات الدراسة لقياس مستوى اعداد anti-tissue transglutaminase Ab الصنف (IgA, IgG) باستخدام تقنية الامتزاز المناعي المرتبط بالانزيم، وقياس مستوى اعداد anti-reticulin Ab الصنف (IgA, IgG) و anti-endomysial Ab الصنف (IgA, IgG) باستخدام تقنية التالىق المناعي غيرالمباشر. اظهرت نتائج الدراسة ارتفاعا معنويا ( $P \leq 0.05$ ) في تركيز اعداد anti-tissue transglutaminase Ab الصنف (IgA, IgG) مقارنة بمجاميع السيطرة، كما لوحظ ان هنالك اربعة نتائج موجبة (13.33%) بالنسبة لاضداد anti-reticulin Ab (IgA, IgG) وثلاثة نتائج موجبة 10% بالنسبة لاضداد anti-endomysial Ab (IgA, IgG)، كما اظهرت نتائج الدراسة ان هنالك اربعة نتائج موجبة (13.33%) بالنسبة لـ HLA-DQ8 باستخدام تقنية HLA-DQ8 Real time PCR.

\*E-mail: dranasadi@yahoo.com

تشير نتائج الدراسة الحالية بان مرض حساسية الحنطة يلعب دورا مهما في قدح مرض تصلب الاعصاب المتعدد.

## Introduction

Multiple Sclerosis (MS) is a chronic disease of unknown etiology, characterized by the presence of disseminated demyelinating lesions in the central nervous system (CNS), and associated with autoimmunity [1]. MS patients have other auto immune diseases that mimic MS symptoms and play a certain role in the pathogenesis like intrinsic factor antibodies [2]. The prevalence of MS is approximately 60 cases per 100000 and the incidence is 3 cases per 100000 per year [3], but these statistics vary considerable between countries; in Iraq, MS is not rare; its demographic and clinical data, in general, similar to those reported in Caucasian populations, and there was some evidence for North-South gradient and a possible increasing incidence characterized by an increase in female preponderance during the last 2 decades. [4]. While its etiology is unknown, MS is clearly immune mediated disease influenced by genetic and environmental factors [2].

Celiac disease (CD) is a T cell-mediated, tissue-specific autoimmune disease that is activated in a subset of genetically susceptible people following dietary exposure to proline and glutamine-rich proteins found in certain cereal grains [5]. The discoveries in the 1970s of strong associations between various diseases and certain human leukocyte antigen (HLA) factors were a revolution within genetic epidemiology in the last century by demonstrating for the first time how genetic markers can help unravel the genetics of disorders with complex genetic backgrounds. HLA controls immune response genes and HLA associations indicate the involvement of autoimmunity [6]. The genetic basis for gluten intolerance is located in the region of chromosome 6 coding for HLA class-II [7]. CD is closely associated with genes that code HLA-II antigens, mainly of DQ2 and DQ8 classes. Previously, it was considered to be a rare childhood disorder, but is actually considered frequent condition, present at any age, which may have multiple complications [8].

Numerous neurological conditions, including epilepsy, sensory ataxia, and neuropathy, have a reported association with established CD. Associations between AGA positivist (as distinct from CD) and cerebella ataxia have also been reported, with speculation that the ataxia is gluten induced [9]. Previous researchers have investigated the role of a gluten free diet in the treatment of multiple sclerosis (MS) found no benefits [10]. MS and CD are considered T-cell-mediated autoimmune disease, and the involvement of Th1 cell in their pathogenesis has been suggested. Some studies found the interaction between MS and CD related inflammatory processes may result in an amplification of Th-1 immune response. Anti gliadin antibodies are also found in the cerebrospinal fluid, they might be responsible for headache and white-matter abnormalities as cerebellar ataxia [11]. The aim of the present study was to detect the prevalence of some auto antibodies of celiac disease in patients with Multiple Sclerosis.

## Materials and methods

The study included (30) patients suffering from well-established and clinically definite Multiple sclerosis aged (25-55) years. They were previously diagnosed and checked up for demyelinating disorders within the department of neurology at the medical city teaching hospital of Baghdad; A (25) healthy blood donors aged years, mean age (25-47) were taken as controls from spouses or friend and from volunteers in the University of Baghdad, biology department; they were excluded if they had a known autoimmune disease. Blood samples (5 ml) were collected by disposable syringe into gel tubes and stand at room temperature until the coagulant was form. Then the samples are centrifuged at 3000 rpm for 5 minutes. Serum samples were dispensed on a seven Ependroff tubes. All samples were stored at (-20C) until carried out to immunological examinations.

## Serological markers:

Tissue transglutaminase (tTG) anti-IgA, IgG antibodies levels quantification was achieved with the aid of commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (Euroimmun) Germany; while anti-reticulin antibody and anti-endomysial antibodies IgA, IgG were measured by immunofluorescence test IFAT (Euroimmun) Germany, according to the leaflet of kits [12].

**Genetic markers:**

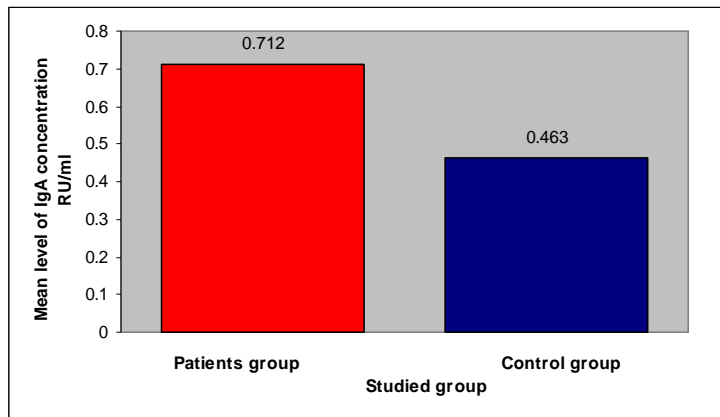
Whole blood samples were tested to assess genetic susceptibility to celiac disease; the HLA-DQ8 was assayed by using a Real time- polymerase chain reaction (Real time-PCR) with a commercially available kit (Bioneer, Germany).

**Statistical analysis:**

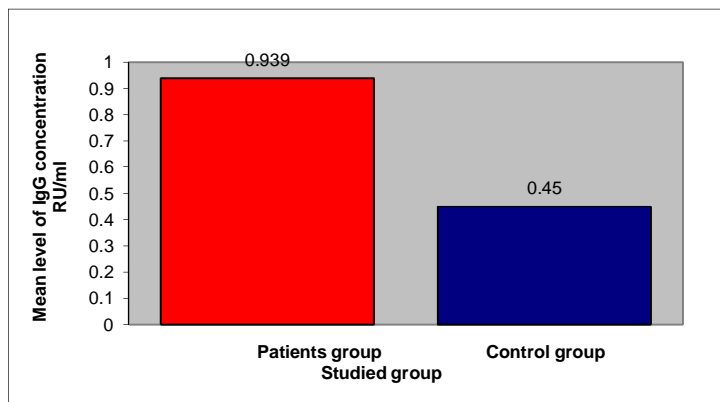
The statistical analysis used included Student t-test and Pearson chi-square test ( $\chi^2$ ). The Statistical Package for Social Science V.13 (SPSS) was used. A p-value  $<0.05$  was considered statistically significant [13].

**Results and discussion**

The results of the present study showed that there was a significant elevation in the concentration of anti-tissue transglutaminase antibodies IgA(0.712+0.550) compared to the control groups (0.463+0.207 ) $P\leq 0.05$ ; and in the level of anti-tissue transglutaminase antibodies IgG(0.938+0.274) compared to the control groups(0.450+0.232 ) . $P\leq 0.05$  as shown in figure 1 and 2 respectively.

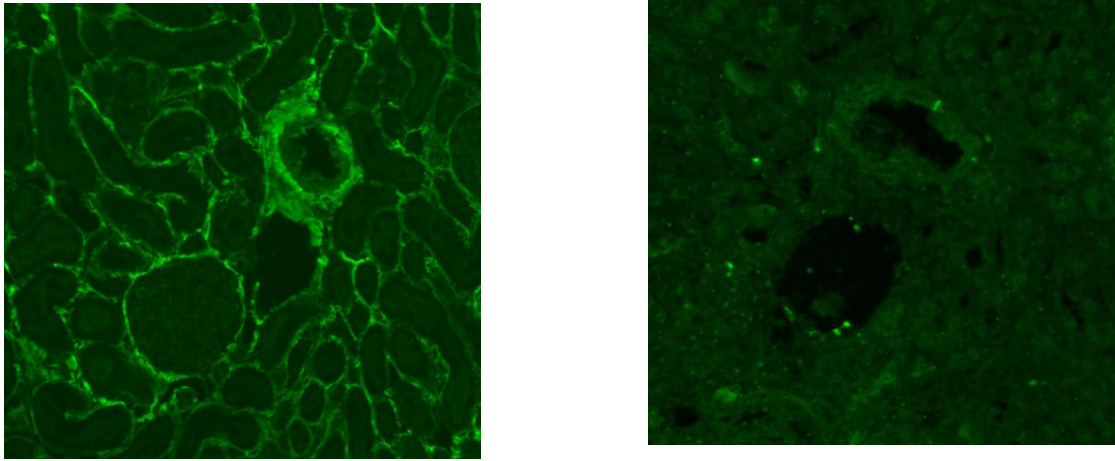


**Figure 1-** Mean level of anti- tissue transglutaminase IgA in the sera of patient with Multiple Sclerosis and control groups.

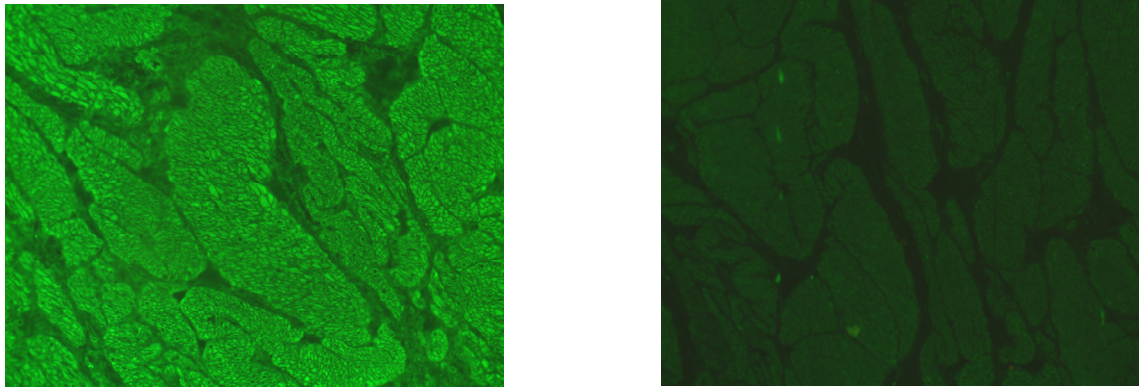


**Figure 2-** Mean level of anti- tissue transglutaminase IgG in the sera of patient with Multiple Sclerosis and control groups

Whereas, there were positive results 4(13.33%)for anti-reticulin antibodies IgA, IgG and 3 (10%)positive results for anti-endomysial antibodies IgA, IgG by IFAT test in the serum of patients with multiple sclerlosis compared to the control groups, as shown in figure 3a,3b and 4a,4b.

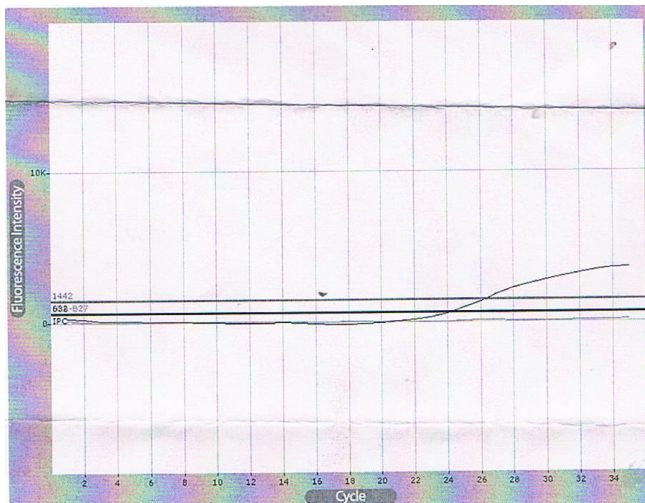


**Figure 3a-** Positive anti-reticulin Ab IgA, IgG and **3b-** Negative anti-reticulin Ab IgA and IgG by IFAT test for serum of patient with MS.



**Figure 4a-** Positive anti-endomysial Ab IgA, IgG and **4b-** Negative anti-endomysial Ab by IFAT test for serum of patients with MS .

On the other hand, there were 4 positive results (13.33%) for HLA-DQ8 by using HLA-DQ8 Real-Time PCR test in the serum of patients with multiple sclerosis compared to control groups, as shown in figure 5.



**Figure 5-** HLA-DQ8 by using HLA-DQ8 Real-Time PCR for serum of patients with MS

Several studies have found a high prevalence of celiac disease among MS patients, (11.1%) based mainly on the presence of villous atrophy in the duodena l biopsy; this is between 5-10 times higher than the frequency found in general population [14]. One study showed that one patient (2%) had strongly positive IgA anti-TtG and antiendomysial antibodies [15]. Another study revealed seroprevalence of anti-tissue transglutaminase antibodies IgA in 7(10%) MS patients [1]. Some reports showed certain neurological symptoms that respond to a gluten free diet , especially if it is started in the first few months after their appearance [16]. In 1996, it was reported that 57% of patients with cryptogenic neurological disorders were antigliadin antibodies seropositive (IgG or IgA or both) compared to 5% of patients with known neurological diseases and 12% of normal control subjects, Further, 70% of these seropositive cases were human leukocyte antigen (HLA) DQ2 [1]. CD provides a good model for HLA-associated diseases, and insight into the mechanism of this disease may well shed light on oral tolerance in humans. The primary HLA association in the majority of CD patients is with DQ2 and in the minority of patients with DQ8. Gluten-reactive T cells can be isolated from small intestinal biopsies of celiac patients but not of non-celiac controls. DQ2 or DQ8, but not other HLA molecules carried by patients, are the predominant restriction elements for these T cells [17].The specific role of serological gluten markers in the pathogenesis of multiple sclerosis remains uncertain.

Some data deserve to be considered because firstly, they reinforce previously reported findings of an excess of autoimmune pathology in first degree relatives of MS patients, and further suggest the hypothesis that a common genetic susceptibility for autoimmunity co-exists with additional disease-specific factors (non-MHC self-antigen, environmental factors), Secondly, they point to a role for DR4 in autoimmune predisposition, and support the hypothesis of a preferential association of MS with type-1 diabetic mellitus , at least in Southern Europe. Thirdly, they show that MS is not necessarily associated with a particular DR phenotype [18,19] . Initial studies of healthy normal subjects (HLA-DQ2 and other DQ genotypes) showed no consistent IFN responses when peripheral blood mononuclear cells (PBMCs) were stimulated with gliadin peptides, similarly, showed no responses in patients with celiac disease either before they started a gluten-free diet, or while they were maintained on a gluten-free die [20] . It is now evident that the link between CD and neurological disorders results, in part, from common genetic background, most importantly, the HLA region on chromosome 6, and other markers. In addition to genetic predisposition, immunologic factors probably also play a role, one way that this may occur is by antibody or T-cell cross-reactivity, a mechanism that is suspected of triggering the immune response in some autoimmune diseases. Alternatively, it may result from the involvement of additional auto antigens through epitope spreading [21,22 ].HLA molecular typing for Celiac disease is, therefore, a genetic test with a negative predictive value. Nevertheless, it is an important tool able to discriminate individuals genetically susceptible to CD, especially in at risk groups such as first-degree relatives (parents, siblings and offspring) of patients and in presence of autoimmune conditions (type 1 diabetes, thyroiditis, multiple sclerosis) or specific genetic disorders (Down, Turner or Williams syndromes).[23] . The HLA genes, located in the major histocompatibility (MHC) region on chromosome 6 play a role in multiple autoimmune disorders, like celiac disease (CD), type 1 diabetes (T1D), rheumatoid arthritis, multiple sclerosis, psoriasis and others [24]. The prevalence of DQ2 in the general population is 20-40%, it reaches 90% among celiac patients in which a series of patients with presumed irritable bowel syndrome and predominant diarrhea were screened for possible CD [25]. Another study detected a significant association between a genetic polymorphism in the IL23 receptor gene and CD and Harris et al[28], found higher production of IL-23 after stimulation of human monocytes derived from CD patients with peptic fragments of wheat gliadin and very recently, gluten-specific IL-17A-producing cells have been found in the duodenum of CD patients[26,27] . CD is also associated with several neurological conditions at a significantly higher frequency more than in the general population, as can be observed in patients with migraine Cerebellar ataxia associated with gluten is the syndrome most commonly associated with CD, especially in adult patients; many immuno pathogenic mechanisms and different antibodies associated with gluten have been described which are capable of crossing the blood-brain barrier and deposit at the level of the Purkinje cells, where they produce a marked inflammatory response followed by neuronal generation and cerebellar atrophy an isoenzyme of tissue transglutaminase has been recently described, specifically the subtype 6, which is

present in the cerebellum of patients with CD-associated ataxia, and its positivity would be useful in explaining the pathogenesis of this process[28,29].

#### .Conclusion:

These results indicated that patients with celiac disease play an important role in pathogenesis of Multiple Sclerosis

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