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Anti-axonal and anti-neuronal autoantibodies in Iraqi patients with multiple sclerosis

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ABSTRACT

Multiple sclerosis is an autoimmune disease of unknown cause in which an immunologic reaction to myelin proteins of the central nervous system is triggered by one or more exogenous agents in a genetically susceptible individual. the association of Multiple sclerosis with autoantibodies to axonal and neuronal components, myelin-associated glycoprotein (MAG) and antinuclear antibody (ANA). 54 Iraqi patients with multiple sclerosis were included in this study. 35 apparently healthy individuals were enrolled in this study as control group. Immunofluorescence technique was used. elevated serum antibodies to axonal and neuronal nuclear antigens with a low level of autoantibodies to MAG. Conclusion: Anti-axon and ANNA auto-antibodies were elevated suggest that production of these antibodies may reflect ongoing neurodegeneration & disease progression. The differences between the response to MAG and PCA-1, anti-axon and ANNA auto-antibodies suggest some degree of selectivity in the humeral response.

Keywords: multiple sclerosis; anti-axonal; anti-neuronal; myelin-associated glycoprotein (MAG); antinuclear antibody (ANA); Immunofluorescence assay.

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INTRODUCTION

Multiple sclerosis (MS), also called disseminated sclerosis, is an inflammatory demyelinating disorder of the central nervous system (CNS) and the most common cause of neurologic disability in young adults [1]. MS is autoimmune disease in which an immunologic response to myelin proteins of the CNS is triggered by one or more exogenous agents in a genetically susceptible individual [2].The disease is characterized by regions of demyelination of varying size and age scattered throughout the white matter of the CNS, especially in the cerebrum, brainstem, optic nerves, and spinal cord [3].Clinically, it is characterized by recurrent or chronic progressive neurologic dysfunction [4]. Typically, it presents in young adults between 20 and 40 years with a peak at 30 and with a female to male ratio of 2:1[5].

The incidence and prevalence of MS varies geographically [6]. High frequency areas of the world include all of Europe (including Russia), southern Canada, northern United States, New Zealand, and southeast Australia [7]. In many of these areas the prevalence is more than 1:1000; the highest reported rate of 3:1000 is in the Orkney and Shetland Islands. In the United States, the prevalence is 1:1000 [8]. In most of Asia, Africa, India, china and Japan the prevalence rate is much lower (5:100000) [2]. The disease is rare in tropical areas and the prevalence increased proportionally to the distance from the equator [9]. However, it is not rare in Iraq [10] the disease affects females more than males (2:1) and this ratio is even higher (may reach up to 3:1) if it started before the age of 15 or after 50 years [11]. The disease occurs in all major racial groups but is most common in whites, less common in blacks [4]. All high prevalent areas for MS have predominantly white populations [11].

The cause of MS remains unknown [2]. Evidence suggests that an environmental agent operates in a genetically susceptible individual. The most likely causative agent is viral; a range of viruses may be involved [12].

Certain HLA types, especially class II determinants, particularly HLA DR2 [13], HLA DRB1 and HLA DR15 have association [14, 15]. Family clustering is present, where the first-degree relatives of the patients have a 2% to 5% increased risk of developing MS and the risk is greater for siblings than for parents [16].Twin studies show that the concordance rate is much higher in monozygotic twins (25-30%) than dizygotic twins (2-5%) [17].

Although many viruses have been associated with MS [18]. No specific evidence linking viruses directly to the development of the disease has been reported. Increasing attention has centered on the Epstein Barr Virus (EBV), which causes infectious mononucleosis, as a possible cause or trigger of the disease [19]. Other viruses, such as hepatitis B virus (HBV),Varicella zoster virus (VZV),influenza A virus, Human herpes virus type 6 (HHV-6), and measles virus are also implicated as the possible cause of multiple sclerosis[20]. No virus has definitely been shown to be the environmental trigger at this time [2].

An association between latitude and the risk of MS where the risk increasing from south to north [7]. The disease is most common in northern hemisphere and, interestingly, in the United States [16]. Migration studies show that Persons migrating from a high to low-risk area after the age of puberty carry their former high risk with them, while those that migrate during childhood seem to have the risk associated with the new area to which they migrated [6].

This study was done to shed some light on the immunologic detection of anti-myelin (anti-MAG), anti-neuron (ANNA, PCA-1) and ANA auto-antibodies by IIFA in sera of MS patients. And to investigate whether progression in MS is associated with antibodies to axonal and neuronal components and compared this with antibodies to MAG and ANA, also to investigate whether serum anti-axon and anti-neuron antibodies can used as biological markers for neurodegeneration and/or disease progression in MS. In addition to determination of the common clinical presentation of multiple sclerosis in Iraqi patients.

PATIENTS AND METHODS

This prospective study included 54 patients (31 RRMS “relapsing remitting MS”, 17 SPMS “secondary progressive”, 6 PPMS “primary progressive MS”) were diagnosed as multiple sclerosis. The gender of the patients were 20 males and 34 females and their age range from 18 to 53 years. Those patients recruited from the multiple sclerosis units in Baghdad teaching hospital in medical city, Baghdad during the period from the

April to the end of June 2009. The diagnosis in each case was established by clinical history and examination, radiologic finding, and laboratory investigations.

Thirty five apparently healthy individuals (12 males and 23 females) were enrolled in this study as control group, their age range from 17 to 43 years. Those individuals were recruited from donors of the central blood bank in medical city complex.

Base line data about patients were obtained from the history and clinical examination; these include name, age, sex, residence, presentation, family history, past history, and other information which arrange in questioner and fulfilled for each patient. After receiving approval of human ethics committee, Informed consent was obtained from each individual involved in this study.

Materials:

- Immunofluorescence assay kit for detection of IgG auto-antibodies against myelin sheaths and myelin associated glycoprotein (anti-MAG) in serum. (BINDING SITE COM-UK).
- Immunofluorescence assay kit for detection of IgG auto-antibodies against neurons (ANNA & PCA-1) in serum. (BINDING SITE COM-UK).
- Immunofluorescence assay kit for detection of IgG auto-antibodies against cell nuclei (ANA) in serum. (BINDING SITE COM-UK).

Blood sample collection and preparation: five milliliters (5 ml) venous blood were obtained from each subject included in the study and placed in a sterile plain tube, then centrifuged and the serum was separated and stored at -20 Ċ.

Immunofluorescence test for detection of IgG auto-antibodies against myelin sheaths (anti-MAG) and against nerve axon, against neurons (anti-Hu, anti-Ri & anti-Yo), and against cell nuclei (ANA) in serum was performed in the immunology department of teaching laboratories, Baghdad medical city, following the procedure protocol included within the kit packing as issued from the manufacturer company.

Statistical Package for Social Sciences (SSPS) was used for data entry and analysis. Results were expressed in simple statistical terms such as means, percentages, and standard deviations. Chi square test was used for testing the significance of association between two discrete variables. Finding with P value less than 0.05 was considered

RESULTS

This prospective study includes 54 (60.8%) patients with different patterns of multiple sclerosis, RRMS 31(34.8%), SPMS 17(19.2%) and PPMS 6 (6.7%), all attended the MS unit in Baghdad teaching hospital in medical city complex. Also 35 (39.3%) healthy individual served as control, all are free of clinically evident neurologic disease.

The age groups of the patients with multiple sclerosis enrolled in this study were ranged between (10 and 60) years with a mean age (35.377 + 9.007) years. The peak age of patients in this study was between (21-40) years, no significant correlation with age group was founds (P value was 0.7913).

Patient group comprised 34 (63%) females and 20 (37%) males having multiple sclerosis. There was female preponderance with female: male ratio = 1.9:1. With non-significance correlation (P value is 0.7913).

Among 54 MS patients there were only 9 (16.7%) having positive family history of multiple sclerosis in the first degree relatives while 45 (83.3%) were not have. This considered not significant (P value is 0.1730). There were 25 (46%) were having some psychological stress (6 months to one year) prior to onset of the disease. Among them 20 (59%) were females and 5 (25%) were males. (P value is 0.01608), which considered to be significant.

The initial presentation in the MS patients where the motor weakness in upper or lower extremities were presented in 38 patients (70.3%), followed by paresthesia in 35 patients (65%), blurred vision in 34

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patients (63%), unsteady gait in 22 patients (41%) and urine incontinence in 18(33.4%). Other uncommon symptoms was constipation (9.2%), Stool incontinence (1.8%) and impotence (1.8%).

Table (1) demonstrate the results of IFA test for IgG anti-myelin sheath auto-antibodies of all study groups, in which the sero-positive results were 2(11.7%) in SPMS and 1(16.7%) in PPMS while in RRMS and controls were zero. Table (1) also demonstrate the results of IFA test for IgG anti-axon auto-antibodies of all study groups, in which the sero-positive results were 15(88.2%) in SPMS, 12(38.7%) in RRMS and 4(66.7%) in PPMS while in control were 6 (17.1%).

Table (1): Distribution of IgG anti-myelin and anti-axon auto-antibodies in all study groups.

Study group	**Anti-myelin sheath Abs** (anti-MAG)*				**Anti-axon Abs** (non-specific)**				Total	
	Positive		Negative		Positive		Negative			
	N=	%	N=	%	N=	%	N=	%	N=	%
RRMS	0	0	31	100	12	38.7	19	61.3	31	100
SPMS	2	11.7	15	88.3	15	88.2	2	11.8	17	100
PPMS	1	16.7	5	84.3	4	66.7	2	33.3	6	100
Control	0	0	35	100	4	11.4	31	88.6	35	100
Total	3	3.4	86	96.6	35	39.3	54	60.7	89	100

***P value is 0.4679, considered not significant. ****P value is 0.0001, considered very significant.**

Table (2) demonstrate the results of IFA test for IgG ANNA auto-antibodies of all study groups, in which the sero-positive results were 7 (22.5%) in RRMS, 10 (58.8%) in SPMS and 5 (83.4%) in PPMS. While in control were 3(8.6%). Table (2) also demonstrate the results of IFA test for IgG PCA-1 auto-antibodies of all study groups, in which the sero-positive results were 3 (9.7%) in RRMS, 4 (23.5%) in SPMS and 1 (16.7%) in PPMS. While in control were 2 (5.7%).

Table (2): Distribution of IgG anti-neuron auto-antibodies in study groups.

Study group	**ANNA I & II** (anti-Hu & anti-Ri)*				**PCA-1** (anti-Yo)**				Total	
	Positive		Negative		Positive		Negative			
	N=	%	N=	%	N=	%	N=	%	N=	%
RRMS	7	22.5	24	77.5	3	9.7	28	90.3	31	100
SPMS	10	58.8	7	41.2	4	23.5	13	76.5	17	100
PPMS	5	83.4	1	16.6	1	16.7	5	83.3	6	100
Control	3	8.6	32	91.4	2	5.7	33	94.3	35	100
Total	25	28.1	64	71.9	10	1.1	79	98.9	89	100

***P value is 0.0002801, considered highly significant. **P value is 0.27257, considered not significant.**

Table (3) demonstrate the results of IFA test for IgG ANA auto-antibodies of all study groups, in which the sero-positive result was 2 (6.5%) in RRMS, 2 (11.7%) in SPMS and 1(16.7%) in PPMS. While in control were 2 (7.8%).

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Table (3): Distribution of IgG ANA in all study groups.

Study group	ANA				Total	
	Positive		Negative			
	N=	%	N=	%	N=	%
RRMS	2	6.5	29	93.5	31	100
SPMS	2	11.7	15	88.3	17	100
PPMS	1	16.7	5	84.3	6	100
Control	2	5.7	33	94.3	35	100
Total	7	7.8	82	92.2	89	100

P value is 0.7274, consider not significant.

DISCUSSION

In this study, we used an indirect Immunofluorescence (IIFA) method to determine the prevalence of anti-myelin (anti-MAG), anti-axonal, anti-neuronal (ANNA & PCA-1) and ANA in patients with multiple sclerosis and healthy controls (HC).

The number and percentage of female patients with multiple sclerosis was higher than those in male 1.8:1 comprised 63%. This ratio not significant but agree with Iraqi study (Muna, 1999) [21]. This is expected since most autoimmune disease are well documented to be aggravated in female more than male and this is because women mount more robust immune responses because the estrogen hormone shown to have an ability to alter immune response by altering the pattern of gene expression (Rogers, Levine et al. 2012) [22].

The age of the patients ranged between 10-60 years (mean= 35.377 + 9.007) and the peak of incidence was between 21-40 years, these were compatible with Iraqi studies, (Muna, 1999) [21] and (Ayad ,2001) [23], also compatible with other study (Cardenas-Roldan, Rojas-Villarraga et al. 2013) [24].

There is no significant correlation between multiple sclerosis and family history; only nine (16.7%) patients gave positive family history of multiple sclerosis while the rest were negative. This study agree with other study (Cardenas-Roldan, Rojas-Villarraga et al. 2013) [24].

The history of psychological trauma, 6 months to 1 year before the onset of the disease was more pronounced in females (59%) than in males (25%). These results were compatible with Mohr (2004) [25] and Ayad (2001) [23]. This point to probability that females might be more susceptible to development of MS in relation to psychological stress than males. In spite of small No. of samples, this significant finding can be explained on the basis of hormonal differences between males and females.

In our study the sequence of the presenting symptoms in patients was different from other studies. In our patients the motor weakness was the first (70.3%) followed by paresthesia (65%), blurred vision (63%) and unsteady gait (41%) while in Muna (1999) [21], bowel and bladder symptoms was the third. In Ayad (2001) [23], the sensory symptoms was the third. In all studies the motor weakness heads the list. The differences in sequence of frequency of presenting symptoms in our patients from other studies can explained by the fact that our number of patients is not large enough to demonstrate the various distributions of symptoms.

We established an IIFA to screen sera of MS 54 patients (31 RRMS, 17 SPMS, 6 PPMS) and 35 HC for serum IgG autoantibodies to myelin sheath (anti-MAG) and to neuronal antigens (anti-axon, ANNA & anti-PCA-1). 16.7% 0f PPMS and 11.7% 0f SPMS were seropositive for anti-MAG antibodies while all RRMS patients and HC were seronegative. Nobile-Orazio (1985) [26] was found nearly similar results by using enzyme linked immune-sorbent assay (ELISA) while a study by (Pihan, Decaux et al. 2012) [27] a higher result were obtained, 20.4% of PPMS, 17.2% of SRMS and 3.1% of RRMS were seropositive for anti-MAG antibodies by Radioimmunoassay (RIA) which is a very sensitive method. The results suggest that there is a low level of humoral immunity to MAG in MS patients that can only be detected by the most sensitive methods as in (Pihan, Decaux et al. 2012) [27] study. This feeble immune response to MAG may be secondary to the demyelinating process, but could play a role in the progression of the disease.

In screening for anti-axonal antibodies (non-specific), the sero-positivity was 88.2% in SPMS, 66.7% in PPMS, 38.7% in RRMS and 11.4% in HC. MS patients had a significantly higher antibody response than HC

(sero-positivity were 57.4% in MS and 11.4% in HC), and within MS population, we found significantly higher responses in SPMS(88.2%) in comparison to PPMS (66.7%) and a tendency for higher antibody responses when compared to patients with RRMS (38.7%). These results were agree with Silber (2002)[28] but disagree with Ehling (2004)[29] which found higher antibody response in PPMS (93.6%)than SPMS(77.9%) , this difference may be due to use of Elisa test which is more sensitive.

In this study, the frequency of serum IgG ANNA was greater in the PPMS (83.4%) and SPMS (58.8%) groups compared with the RRMS(22.5%) and HC(8.6%) , while in screening for PCA-1 the frequency were low in all study groups (23.5% in SPMS, 16.7% in PPMS, 9.7% in RRMS and 5.7% in HC). These results were agree with Rawes (2002)[30] and Silber(2002)[28]. The sero-positivity of ANA in PPMS were 16.7% and in SPMS were 11.7%, this means the positive ANNA in PPMS and SPMS were true anti-neuronal nuclear antibodies and not ANA.

Using IIFA, this study found elevated serum antibodies to axonal antigens and neuronal nuclear antigens (anti-axon & ANNA) in subjects with progressive MS. It is feasible that increased amount of axonal proteins are released with axonal degeneration and that may behave as antigens. The elevated level of anti-axon antibodies may be a consequence of the ongoing axonal damage. Potentially increasing the level of antigens present and thereby contributing to the immune response to these axonal proteins. Elevated antibodies in progressive MS suggest a rise in antibody related to ongoing axonal destruction. The possibility that anti-axon antibodies may play a more direct pathogenic role in progressive MS should be considered.

A pathogenic role for antibodies to intracellular antigens (neuronal nuclear antigens) in progressive disease has been sought but not convincingly proven. High level of antibodies to neuronal components are present in progressive MS, yet it has not been possible to demonstrate their contribution to tissue damage.

It is notable that the antibody responses to myelin associated glycoprotein and Purkinje cytoplasm antigen-1, other proteins that may be expected to be present with tissue destruction, was considerably weaker. This suggests some degree of selectivity in the humeral response.

CONCLUSION

1. Low level of auto-antibodies to MAG in Iraqi patients with MS comprising 5.6% ; that can only be detected by the most sensitive methods, such as RIA or Elisa.
2. Anti-axon and ANNA auto-antibodies were elevated in progressive MS suggest that production of these antibodies may reflect ongoing neurodegeneration.
3. It is also possible that these antibodies reactive with axonal and neuronal components may play a potential pathogenic role in disease progression.
4. The differences between the response to MAG and PCA-1as well as anti-axon and ANNA auto-antibodies suggest some degree of selectivity in the humeral response.
5. Our finding support the use of serum anti-axon and ANNA as a marker for axonal destruction.

Conflicts of interest

The authors of this study receives research support from Baghdad College of Medicine with an equipment loan from the Immunology unit in Teaching Laboratories / Baghdad Medical City complex. Some authors also serve as a lecturer in University of Baghdad.

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