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Clinical Characteristics of Anti-Myelin Oligodendrocyte Glycoprotein Antibody among Aquaporin -4 Negative Neuromyelitis Optica Spectrum Disorders in Egyptian Patients

Thesis

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List of Contents

Title	Page No.
List of Tables	i
List of Figures	ii
List of abbreviations	3
Introduction	6
Aim of the Work	8
Review of Literature	
Neuromyelitis Optica Spectrum Disorders	9
Aquporin-4	33
Anti-myelin Oligodendrocytic Glycoprotein (MOG) Antibody	48
Subjects and Methods	56
Results	62
Case Presentation	69
Discussion	72
Summary and Conclusion	82
References	84
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Comparison between symptomatic episodes between anti-AQP-4-Ab positive NMOSD patients and anti-MOG-Ab positive patients	52
Table (2):	Description of demographic data, clinical characteristics and anti-MOG-Ab results of NMOSD patients.	62
Table (3):	Descriptive statistics of disease modifying drug taken by the NMOSD patients in-between attacks.	65
Table (4):	Comparison between the anti-MOG-Ab seropositive and seronegative patients regarding their EDSS	66
Table (5):	Comparison between the anti-MOG-Ab seropositive and negative patients and clinically studied parameters	68

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Immunopathogenesis of neuromyelitis optica spectrum disorders	11
Figure (2):	Mammalian AQPs expression pattern among tissues	35
Figure (3):	Anti-MOG-antibodies when bind to MOG on surface lamellae of myelin sheath eliciting an immune response and releasing IL-6	49
Figure (4):	Bar chart shows risk factor distribution among the included patients.	63
Figure (5):	Bar chart shows the number of relapses in the 13 relapsed patients included in the study.	63
Figure (6):	Comparison between the anti-MOG-Ab seropositive and negative patients regarding the EDSS	67

List of Abbreviations

Abb.	Full term
Abs	Antibodies
ADEM	Acute disseminated encephalomyelitis
ANA	Anti-nuclear antibody
APC	Antigen presenting cell
AQP	Aquaporin
ARR	Annualized relapse rate
BAFF	B- cell activating factor
BBB	Blood brain barrier
CBA	Cell based assay
CCL	Chemokine ligand
CCR	Chemokine receptor
CD	Cluster of differentiation
CHO	Chinese hamster ovary
CNS	Central nervous system
CO2	Carbon dioxide
CRMP5	Collapsing response-mediator protein-5
CSF	Cerebrospinal fluid
CV	Coefficient of variation
DMD	Disease modifying drug
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
EDSS	Expanded disability status scale
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
Fc	Fragment crystallizable
FIPA	Fluorescence-based immune precipitation assay
FITC	Fluorescein isothiocyanate
FL	Full length
GFAP	Glial fibrillary acidic protein
HEK	Human embryonic kidney
HLA	Human leukocyte antigen
ICAM	Intercellular adhesion molecule
ICC	Immunocytochemistry

ICCF	Fluoroimmunocytochemistry
IFN	Interferon
Ig	Immunoglobulin
IHC	Immunohistochemistry
IHC-C	Conventional immunohistochemistry
IHC-F	Fluoro-immunohistochemistry
IIF	Indirect immunofluorescence
IL	Interleukin
IVIG	Intravenous immunoglobulin
kDa	Kilo Dalton
LETM	Longitudinally extensive transverse myelitis
MAC	Membrane attack complex
MBP	Major basic protein
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NF	Neurofilament
NH3	Ammonia
NK	Natural killer
NMDA	N-methyl D-aspartate
NMDAR	N-methyl-D-Aspartate receptor
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorder
OAPs	Orthogonal array of particles
OSMS	Optic spinal multiple sclerosis
PBS	Phosphate buffered saline
PD1	Programmed cell death protein-1
PLEX	Plasma exchange
PLP	Proteolipid
PTPN22	Protein tyrosine phosphate non-receptor type 22
RIPA	Radio-immuno-precipitation assay
RNA	Ribonucleic acid
Rpm	Revolutions per minute
SL	Short length
SLE	Systemic lupus erythematosus
S100B	S100 calcium binding protein B
SPSS	Statistical Package for Special Sciences

SSA	Sjogren syndrome A
SSB	Sjogren syndrome B
TGF-β	Transforming growth factor beta
Th	T-helper
VCAM	Vascular cell adhesion molecule
VGEF	Vascular endothelial growth factor
WB	Western blot

Title

Clinical Characteristics of Anti-Myelin Oligodendrocyte Glycoprotein Antibody among Aquaporin -4 Negative Neuromyelitis Optica Spectrum Disorders in Egyptian Patients

Running title: Anti-MOG Antibodies in NMOSD

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ABSTRACT:

Background: Neuromyelitis optica spectrum disorder (NMOSD) is a central nervous system inflammatory disease that was once thought to be a form of multiple sclerosis (MS); it now has independent clinical, pathological, and immunological characteristics because of the discovery of anti-aquaporin-4 (anti-AQP4) and anti-myelin oligodendrocyte glycoprotein (anti-MOG) antibodies. **Objectives:** The goal of our research was to determine the prevalence of anti-MOG antibodies in anti-AQP4 seronegative NMOSD Egyptian patients and link their presence with NMOSD clinical characteristics and disease-induced disability. **Methods:** This cross-sectional study included 40 anti-AQP-4 antibody negative NMOSD patients, 6 children and 34 adults. They were screened for anti-MOG antibodies by the indirect immunofluorescence technique. **Results:** Of all included NMOSD patients, only 7.5% (n=3) were positive for anti-MOG antibodies and had significantly higher disability scores compared to seronegative patients (p=0.021). The presence of anti-MOG antibodies was not significantly associated with patients' age (p=0.696), gender (p=0.232), type (p=0.488) or frequency of relapse (p=0.488), family history of consanguinity (p=0.211), family history of autoimmune disease (p=0.608), nor with smoking (p=0.608). **Conclusions:** Anti-MOG antibody seropositivity in anti-AQP4 negative NMOSD patients could be used as a primary indicator of disease-related impairment in the future.

Keywords:

Anti-Myelin Oligodendrocyte Glycoprotein; Aquaporin-4; Disability; Neuromyelitis optica spectrum disorder

1. INTRODUCTION:

Neuromyelitis optica spectrum disorder (NMOSD) is a rare autoimmune demyelinating disorder of the central nervous system (CNS) with a prevalence that rarely exceeds 5 per 100,000 [1]. The disease causes disabling episodes of optic neuritis and transverse myelitis [2] associated with astrocyte death, axonal loss, perivascular lymphocytic infiltration, and vascular proliferation [3]. It is characterized by longitudinally extensive spinal cord lesions (>3 vertebral segments) and the absence of oligoclonal IgG bands (in about 70-85% of cases) [4]. Because of the discovery of anti-aquaporin-4 (anti-AQP4) and anti-myelin oligodendrocyte (anti-MOG) antibodies in serum of NMOSD patients, it is now considered as a distinct clinical entity from multiple sclerosis (MS) [5].

AQP4 is the most abundant water channel in the mammalian CNS; it is highly expressed in the membrane of the astrocytic end-feet. Anti-AQP4 antibodies are pathogenic and primarily mediate a humoral immune neuroinflammatory response [6], leading to high complement activation [7]. Despite the development of highly sensitive and specific assays for anti-AQP-4 antibodies, up to 40% of NMOSD patients do not have these antibodies at initial presentation and during the course of the disease [8].

MOG glycoprotein is a member of the immunoglobulin superfamily; it makes up about 0.05% of total myelin proteins [2]. It is expressed on the outer lamella of the myelin sheath but not expressed nor on the thymus nor on peripheral organs, making it more likely to be immunogenic than other CNS myelin proteins [9]. Anti-MOG antibodies trigger both encephalitogenic T-cell response and antibody-mediated humoral demyelinating response in a synergistic way [10].

Thus, anti-AQP-4 associated NMOSD is an astrocytopathy, while anti–MOG–associated inflammatory demyelinating diseases represent an oligodendropathy [8].

Clinical, biological, and immunological features of NMOSD appear to differ in patients with positive anti-MOG antibodies compared to patients with positive anti-AQP-4 antibodies [2]. In this context, we designed this study in order to discover the prevalence of anti-MOG antibodies among anti-AQP4 seronegative NMOSD Egyptian patients and to establish a link between their existence and NMOSD clinical features and disease-induced impairment.

2. METHODOLOGY:

2.1.Study design and subjects:

This cross-sectional study included 40 NMOSD patients, 6 children (age: <18 years old) and 34 adults (age: ≥18 years old), of them, 35% (n=14) were males and 65% (n=26) were females, diagnosed by clinical picture and radiological findings according to the 2015 international consensus diagnostic criteria of NMOSD [11]. They were recruited from the outpatient clinic of the Neurology Department, Ain Shams University Hospitals, Cairo, Egypt. All included patients were seronegative for antiAQP4 antibody. MS patients and those who received corticosteroids within a month before the study were excluded. This study was carried out after the approval of the ethical committee of Ain Shams University, Faculty of Medicine (FMASU M5176/2019). Before taking part in this study, all subjects signed a written informed consent form. All collected data were kept private and confidential and were solely utilized for the study's purposes.

2.2.Clinical assessment:

The neurologist examined the disability status of all participants using The expanded disability status scale (EDSS), which offers a total score on a scale ranging from 0 to 10 in 0.5-unit increments, with higher scores representing higher levels of disability. People with a high degree

of ambulatory ability are classified as levels 1.0 to 4.5, whereas those with a loss of ambulatory capacity are classified as levels 5.0 to 9.5 [12].

2.3.Sample collection and anti-MOG antibody analysis:

Laboratory work was conducted in the Clinical Pathology Department, Ain Shams University Hospitals, Cairo, Egypt. From each participant, 3 ml venous blood was collected by aseptic venipuncture into a serum separation vacutainer tube. Blood samples were allowed to clot completely then were centrifuged at 3000xg for 10 minutes. Separated sera were collected and stored in the freezer (-80◦C) until analysis. Anti-MOG IgG antibody detection was done using indirect immunofluorescence slides (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany, order no.: FB 1156-1005-50) according to manufacturer's instructions. The slides were assessed with a fluorescence microscope (Olympus CX33 Biological Microscope, Tokyo, Japan) using a 40 magnification power lens where positive reactions produced a flat, smooth to coarse-granular fluorescence of the cell with an accent of the cell membrane. The area of the cell nucleus was only slightly stained. **Figure 1**

2.4.Statistical analysis:

The data were coded and analyzed using Statistical Package for Special Sciences (SPSS) software computer program version "V. 23.0" (IBM Corp., USA, 2015). Description of quantitative nonparametric data was carried out by using median, and IQR and quantitative parametric data were carried out by mean ± SD. Description of qualitative data was presented as numbers and percentages. In comparison, the Mann-Whitney test was used for quantitative data, while the Chi-square test was used to compare qualitative data. Significance level was set at *p*-value < 0.05.