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Clinicopathological Features of Pakistani Patients with Anti Aquaporin-4 (Aqp-4) Antibody Positive Neuromyelitis Optica Spectrum Disorder (NMOSD): A Comparison with Local, Regional and International Studies

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ABSTRACT

Objective: To characterize clinicopathological characteristics of patients with anti AQP-4 antibody positive Neuromyelitis Optica Spectrum Disorder in local population in order to facilitate clinicians in timely diagnosis and treatment of this rare debilitating illness.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jun 2021 to Dec 2022.
Methodology: The study included 369 individuals presenting at Armed Forces Institute of Pathology for serum anti AQP-4 antibody testing by indirect immunofluorescence. Commercial Anti Aquaporin-4 Indirect Immunofluorescence Test Slides along with control serum (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) were used and immune-fluorescence was observed using immunofluorescent microscope BA-310.
Results: Out of 369 patients who were tested for Anti Aquaporin-4 antibody, 168(45.5%) were females and 201(54.5%) were males. Total 9(2.43%) patients tested positive including 5(55.5%) females and 4(44.4%) males (p=0.947). Most prevalent core clinical characteristics among patients with Neuromyelitis Optica Spectrum Disorder were optic neuritis 7(77.8%), acute myelitis 7(77.8%) and area postrema syndrome 6(66.6%). Common presenting core clinical characteristics were area postrema syndrome 4(44.4%) and acute myelitis 4(44.4%). Average diagnostic delay in our study was 2.7±3.2 years.
Conclusion: By keeping high index of suspicion for presenting core clinical characteristics (area postrema syndrome and acute myelitis) clinicians may be able to diagnose and treat this rare disease timely hence decrease average diagnostic delay and prevent associated morbidity and mortality.

Keywords: Acute Myelitis, Anti Aquaporin-4 Antibody, Neuromyelitis Optica Spectrum Disorder.

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INTRODUCTION

Autoimmune demyelinating disorders are a heterogeneous group of diseases, which occur as an acute or chronic inflammatory process.[1] Neuro-Myelitis Optica Spectrum Disorder (NMOSD) is an antibody associated autoimmune demyelinating disease of the central nervous system (CNS) with predisposition for optic nerves and spinal cord.[2] It predominantly affects women and in majority cases is characterized by serum IgG antibodies against aquaporin 4 (AQP-4) which are of immense importance in the accurate diagnosis and treatment.[2,3]

Pathologically NMOSD is an astrocytopathic disorder rather than demyelinating disease and has a prevalence of ~0.5–4 per 100,000 and may be up to 10 per 100,000 in certain races.[4] The prevalence of pathogenic anti AQP-4 antibodies was reported in 0.7-4.5% of pediatric population presenting with acquired demyelinating syndrome (ADS).[5] Anti AQP-4 antibody seropositivity is highly specific for NMOSD and have high female-male ratio (9:1).[6,7]

Diagnosis of NMOSD is often challenging in clinical practice despite phenotypical and serological manifestations of the disease; because vast number of disorders with autoimmune, infectious, vascular and neoplastic etiologies may mimic with NMOSD. Patients with NMOSD may have few clinical manifestations especially in their initial disease stages; test results for AQP-4 antibody can also be influenced by many factors such as test methods, serologic result, disease stage or types of management; some patients with NMOSD do not show positivity for anti AQP-4 antibody and test results for the AQP-4 antibody may not be timely available for the acute management of

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patients[8] of anti AQP-4 antibody negative NMOSD or when serologic testing is not available.[9] If not timely diagnosed and remained untreated, upto 50% of patients with NMOSD will be wheelchair bound and blind; a third may die within 5 years of their presentation.[10] NMOSD is scarcely described in local Pakistani literature and very few studies have been done, which forms the rationale for our study.

METHODOLOGY

The cross-sectional study was carried out at Department of Immunology Armed Forces Institute of Pathology, Rawalpindi Punjab Pakistan, from June 2021 to December 2022, after obtaining approval from the Institutional Ethical Review Board (IRB/21/1590). Sample size was calculated using Open Epi sample size calculator (online) by keeping following population prevalence of NMOSD 4%.[4] Subjects were recruited using non-probability consecutive sampling, after obtaining written informed consent.

Inclusion Criteria: Patients of either gender, aged 01 month to 85 years presenting at AFIP for serum anti AQP-4 antibody testing were included.

Exclusion Criteria: Patient with hemolysed, lipaemic or icteric serum samples were excluded.

The study included 369 patients presenting at AFIP for serum anti AQP-4 antibody testing by indirect immunofluorescence. We applied 30µl of diluted serum of each patient [1/10 dilution in Phosphate Buffer Saline (PBS)-Tween] on wells of Commercial Anti AQP-4 Indirect Immunofluorescence Test Slides along with positive and negative control serum (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) and then slides were incubated for 30 minutes at room temperature (18-25°C). After incubation slides were rinsed with PBS-Tween and kept in it for 5 minutes. After removing from PBS-Tween 25 µl FITC labelled secondary antibody was applied on wells of slides and again incubated for 30 minutes at room temperature. After incubation slides were again rinsed with PBS-Tween and then kept in it for 5 minutes. Up to 10 µl of mounting medium was applied onto the cover glass for each reaction well. Then slides were removed from PBS-Tween, back and all four sides were dried with paper towel and then slides was applied onto the prepared cover glass. Fluorescence was observed using immunofluorescent microscope BA-310 and observations were noted in predesigned proforma (Figure).

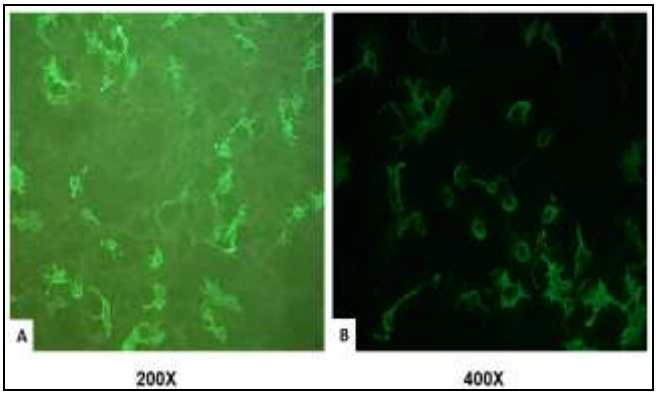

Figure: Anti AQP-4 Antibody on Indirect Immunofluorescence

Qualitative variables including gender, positivity of anti AQP-4 antibody and intensity were expressed as frequency and percentages and quantitative variables, such as age, were expressed as Mean±SD. Data was analyzed using Statistics Package for Social Sciences (SPSS) version 23.0 and Chi-square test was used to compare positivity and negativity in male and female patients. The *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Table-I: Results of Immunological Investigations (n=9)

Parameters	n(%)
Age (Mean±SD)	40.2±2.3 years
Gender (Male/Female)	4/5 (1:1.25)
Result for Anti AQP-4 Antibody	
Time of testing (n=9)	During Attack
Specimen (n=9)	Serum
Result (n= 9)	Positive
Intensity (n= 9)	Weak positive 2, Positive 5, Strong positive 2
Repeat testing (n=3)	Positive
Result of Immunological tests	
Anti MOG Antibody (n = 8)	Negative
Oligoclonal Band / CSF IgG index (n=3)	Normal
Anti-Nuclear Antibodies (n = 6)	Negative
Anti Neutrophil Cytoplasmic Antibodies (=6)	Negative
RA factor (n=3)	Negative
Anti CCP Antibodies (n=3)	Negative
Anti dsDNA Antibodies (n=3)	Negative
Anti TTG Antibodies (n=3)	Negative
Anti Thyroid Microsomal Antibodies (n=2)	Negative
Anti-ENA Antibodies (n=3)	Negative (n=2), Positive SSA (n=1)
Anti Cardiolipin IgG / IgM Antibodies (n=2)	Negative
Anti Neuronal Antibodies (n=1)	Negative

Out of 369 patients tested for anti AQP-4 antibody 168(45.5%) were females and 201(54.5%) were males. Total 9(2.43%) patients tested positive for

anti AQP-4 antibody including 5(55.5 %) females and 4(44.4%) males (female to male ratio, p = 0.947). Mean age of diagnosis was 40.2±2.3 years (range 27-65), however mean age of onset of sign and symptoms was 37±2.0 years (range 24-64). All patients at time of diagnosis had two or more core clinical manifestations and most prevalent characteristics among patients with NMOSD were optic neuritis 7(77.8%), acute myelitis 7(77.8%) and area postrema syndrome 6(66.6%). Common presenting core clinical characteristics among patients with NMOSD were area postrema syndrome 4(44.4%) or acute myelitis 4(44.4%). Average diagnostic delay noted in our study was 2.7 years (range 6 months -7 years). Among the nine antibody-proven NMOSD patients, five had + intensity, two had weak positivity (WP) and two had ++ intensity. In most patients (66.6%) with NMOSD, MRI findings were typical. Disease course appears to show all antibody proven NMOSD patients relapsing.

DISCUSSION

Anti AQP-4 antibodies are serological markers for NMOSD. According to our study mean age of diagnosis of NMOSD was 40.2±2.3 years (Range 27-65) and mean age of presentation was 37±2.0 years (range 24-64) which are in coherence with findings by Mealy *et al.* according to them mean age of onset of disease was 39.2 years, however Sherani *et al.* in a Pakistan-based study showed mean age of presentation as 31.93±8.97 years.[12,13] Our findings showed that 5(55.5%) females and 4(44.4%) males were tested positive for anti AQP-4 antibody, whereas study conducted by Sherani *et al*. showed 61.25% female and 38.5% male diagnosed with antibody-proven NMOSD.[12] Present study showed female to male ratio 1.25:1 where as Edgar *et al*. in Ecuador-based study showed that NMOSD was more prevalent in women with female/male ratio 4.4:1.[14]

Table-II: Clinicopathological Characteristics of Patients (n=9)

Parameters	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Age (Years)	29	35	28	51	42	27	35	65	47
Gender	Female	Male	Male	Male	Female	Female	Female	Male	Female
Onset	28 years & 6 months	34 years & 6 months	24 years	50 years	35 years	25 years	27 years	64 years	46 years & 6 months
Diagnostic delay	6 months	6 months	4 years	1 years	7 years	2 years	8 years	1 year	6 months
Presentation	Area Postrema Syndrome	Area Postrema Syndrome	Acute Myelitis	Acute Myelitis	Symptoma-tic cerebral syndrome	Area Postrema Syndrome	Acute Myelitis	Area Postrema Syndrome	Acute Myelitis
Core Clinical Characteristic									
Optic Neuritis	Absent	Present	Present	Present	Absent	Present	Present	Present	Present
Acute Myelitis	Present	Present	Present	Present	Absent	Absent	Present	Present	Present
Area Postrema Syndrome	Present	Present	Absent	Absent	Present	Present	Present	Present	Absent
Acute Brainstem Syndrome	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Symptomatic cerebral syndrome	Absent	Present	Absent	Absent	Present	Absent	Present	Present	Absent
Acute Diencephalic Syndrome	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent
Total Characteristic	3	4	2	2	2	2	5	5	2
MRI Findings	Typical	Typical	Typical	-	-	Unremark-able	-	Unremark-able	Typical
Course of disease	Relapsing	Relapsing	Relapsing	Relapsing	Relapsing	Relapsing	Relapsing	Relapsing	Relapsing
Autoimmune diseases	Yes	No	No	Not clear	No	No	No	No	No
Comorbids	No	No	No	Hyper-tension	No	No	No	No	No
Vaccination Status									
EPI	Vaccinated	Vaccinated	Vacci-nated	Vacci-nated	Vaccinated	Vacci-nated	Vacci-nated	Vaccinated	Vacci-nated
Covid	Vaccinated	Vaccinated	Vacci-nated	Vacci-nated	Vaccinated	Vacci-nated	Vacci-nated	Vaccinated	Unvacci-nated
Flu	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated	Unvacci-nated
Current Status	Symp-tomatic	Asymp-tomatic	Symp-tomatic	Asymp-tomatic	Symp-tomatic	Asymp-tomatic	Symp-tomatic	Symp-tomatic	Symp-tomatic

One study noted that the mean delay in diagnosis or use of a preventive treatment was 3.6 years in a cohort of 182 individuals with NMOSD, while present study showed average diagnostic delay of 2.7 years (range 6 months -7 years).[13] Most common presenting core clinical characteristic in present study was area postrema syndrome present 4(44.4%) and acute myelitis in 4(44.4%) patients respectively which was in coherence with findings by Akhmetgaleeva *et al*.[15] According to the present study most of patients at diagnosis have 2 or more core clinical characteristics which is consistent with study conducted by Sherani *et al*. according to them 63% patients have 2 or more core clinical characteristics.[12]

All of our NMOSD patients showed relapsing course with is consistent with findings of Ashtari *et al*. they reported an increase in prevalence women and a relapsing course in NMOSD patients.[16] Carnero *et al*. also showed disease onset age range as (32.6–45.7) which is in agreement with present study.[17] A study by Bano *et al*. showed longitudinally extensive transverse myelitis as most common finding in NMOSD which was present in 93.8% patients, our study showed similar findings in which acute myelitis was present in 7(77.8%) of antibody proven NMOSD patients.[18]

CONCLUSION

High index of suspicion should be kept for NMOSD in patients presenting with area postrema syndrome (hiccups, nausea, vomiting) and acute myelitis (lower back pain, numbness and weakness in arms and legs, bladder dysfunction). By keeping low threshold level for presenting core clinical characteristics clinicians may be able to diagnose and treat this rare disease timely, hence decrease average diagnostic delay and prevent associated morbidity and mortality.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MZA & MOR: Data acquisition, data analysis, critical review, approval of the final version to be published.

DA & MH: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AT & MAH: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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