

Aquaporin-4 Immunoglobulin G testing in 36 consecutive Jamaican patients with inflammatory central nervous system demyelinating disease

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Abstract

Epidemiological studies of neuromyelitis optica (NMO) in Jamaica are lacking. Here we reviewed the clinical records of 700 patients undergoing neurological evaluation at the Kingston Public Hospital, the largest tertiary institution in Jamaica over a 4 month period. We investigated the diagnostic utility of Aquaporin-4 ImmunoglobulinG (AQP4-IgG) testing in 36 consecutive patients with a diagnosis of an inflammatory demyelinating disorder (IDD) of the central nervous system (CNS). Patients were classified into 3 categories: i) NMO, n=10; ii) multiple sclerosis (MS), n=14 and iii) unclassified IDD (n=12). All sera were tested for AQP-IgG status by cell binding assay (Euroimmun). No MS cases were positive. Ninety per cent of NMO cases were positive. Four of 12 patients with unclassified IDD tested positive for AQP4-IgG. AQP4-IgG seropositivity was associated with a lower socioeconomic status, higher EDSS (P=0.04) and lower pulmonary function than the seronegative cases (P=0.007). Aquaporin-4 autoimmunity may account for a significant proportion of Jamaican CNS IDDs.

Introduction

Neuromyelitis optica (NMO) is much less common than multiple sclerosis (MS) with a worldwide prevalence of 1-2 per 100,000.¹ In the Caribbean basin, where existing data is sparse, the prevalence of NMO has been studied in the African-descent population of Martinique and Cuba,²⁻⁴ where the prevalence was reported to be 4.2 and 0.52 per 100,000 respectively. Although some information exists on Afro-Caribbean MS in the United Kingdom,⁵

similar studies of central nervous system (CNS) demyelinating conditions in the English-speaking Caribbean are lacking. Jamaica, a multi-ethnic predominantly Afro-Caribbean middle income developing country is the third most populous anglophone country in the Americas. In this study, we investigated the diagnostic utility of Aquaporin-4 ImmunoglobulinG (AQP4-IgG) testing in Jamaican patients with inflammatory demyelinating disease (IDD). We also compared demographic (including socioeconomic status), clinical and outcome data (Expanded Disability Status Scale, pulmonary function) stratified according to disease group and serostatus.

Materials and Methods

The study was institutional review board approved. Thirty six consecutive patients with IDD were identified among 700 patients undergoing evaluation at the Kingston Public Hospital (KPH) neurology clinic over a 4 month period and classified into 3 categories: i) NMO [n=10; Wingerchuk 1999 criteria (9 relapsing/1 monophasic)]; ii) MS [n=14; McDonald 2005 criteria (11 relapsing remitting, 1 secondary and 2 primary progressive)]; iii) *unclassified* where patients failed to fulfill either MS or NMO criteria and distinction from MS and NMO was not possible resulting in unclear treatment plan (n=12).^{6,7} These *unclassified* cases included: recurrent optic neuritis (n=7), longitudinally extensive transverse myelitis (LETM, n=2), single TM with optic disc pallor (visual acuity/evoked potentials normal), multifocal presentation (n=1), brainstem CIS with multifocal lesions on MRI (n=1). All patients were Jamaican nationals. All were seronegative for human immunodeficiency virus and human T cell lymphotropic virus type-1.

AQP4-IgG assay

All serum samples, stored in -70°C freezer, were tested at Mayo Clinic's Neuroimmunology Laboratory, Rochester, MN. M1 and M23 transfected HEK-293 cells provided in kit form by EUROIMMUN (Luebeck Germany) were used to detect AQP4-specific IgG. Patient sera (1:10) or control sera were incubated for 30 minutes at room temperature and washed once for 5 minutes with PBS containing Tween-20. Cells were then incubated with fluorescein-labeled goat anti-human-IgG for 30 minutes at 22°C, washed again with PBS/Tween, then coverslipped. Specific IgG binding was confirmed by comparison of binding to non-transfected HEK293 cells.⁸

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Conflict of interests: Dr. Pittock is a named inventor on patents (publication #US-2010-0226860-A1 and #US-2010-0092478-A1) that relate to functional AQP4/NMO-IgG assays and NMO-IgG as a cancer marker; receives research support from Alexion Pharmaceuticals, Inc., the Guthy Jackson Charitable Foundation, and the NIH. The other authors declare no conflict of interests.

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Outcome measurements

Disability was measured using Kurtzke Expanded Disability Status Scale (EDSS).⁹ FEV1 and FVC were measured using a handheld turbine spirometer (MicroSpirometer Cat No: MS01 Micromedical Limited P.O. Box 6 Rochester, Kent, ME1 2AZ, UK).

Statistical analysis

Normally distributed continuous variables were compared by t-test otherwise Mann-Whitney or Wilcoxon signed ranks test were used. Comparisons of the three categories of patients (MS, NMO and Unclassified) and continuous variables were further analyzed using ANOVA. Relationships between categorical variables were examined by Chi-squared tests. Linear regression analyses were performed with FVC or EDSS as outcome variables and presence of NMO-IgG and transverse myelitis (both coded as present or absent) and duration of illness as independent variables. Data were

analyzed using SPSS (version 12 for Windows).

Results

Table 1 shows the demographic and clinical characteristics of the 3 groups of patients. Nine of 10 NMO patients were seropositive for AQP4-IgG. Seven (70%) had optic neuritis as their onset attack and 3 were blind at last follow up. The mean interval between optic neuritis and transverse myelitis was 10.3 months. Despite a much shorter duration of disease (2.3 vs 11.7 years) the NMO patients had a higher mean EDSS (6.5 vs 4.9) compared to MS patients.⁹ None of the MS patients tested positive. Of the 12 unclassified patients, 4 tested positive; 2 had recurrent optic neuritis, 1 had LETM and 1 had TM with optic disc pallor.

Using Jamaica's high Gini Index, a standard economic measure of income inequality,¹⁰ we found that NMO patients tended to come from lower socioeconomic households. The monthly household income for 70% of NMO patients was less than 25,000 Jamaican Dollars (USD 300). In contrast to NMO, the monthly household income of 70% of MS patients was greater than 25,000 Jamaican dollars (USD 300).

AQP4-IgG and lung function

Despite a shorter disease duration and similar body mass indices, AQP4-IgG seropositive patients had worse pulmonary function than seronegative IDD cases [FEV1, sitting value (mean 1.75L; SD 0.61 vs mean 2.33L; SD 0.56), P=0.007].

Discussion

Jamaica, a multi-ethnic predominantly Afro-Caribbean middle income developing country is the third most populous Anglophone country in the Americas. This study expands the geographic lens and ethnic profile of populations typically depicted in the literature on CNS inflammatory diseases. It is noteworthy that during the study period, a similar number of relapsing NMO and relapsing remitting MS cases were encountered suggesting that in Jamaica both disorders may occur with similar frequency. This is in sharp contrast to western MS where MS outnumbers NMO by about 100 fold. In addition the 33% AQP4-IgG seropositivity in the unclassified IDD group adds further support for a disproportionate representation of AQP4 autoimmunity in Afro-Caribbean IDDs. Our NMO and MS cohorts were demographically similar to other published studies. In our study all NMO patients were Afro-Caribbean. In Martinique, NMO cases appar-

ently predominated in mixed blacks, whereas in Cuba it was equally prevalent in the white, black and mixed populations.^{2,3} The duration of illness for MS was much longer than for NMO in our study. A multitude of factors may account for this including the high mortality rate associated with untreated NMO, socioeconomic factors, selection bias and, recently, increasing recognition of NMO spectrum disorders.

This study is the first to investigate socioeconomic status in patients with neuromyelitis optica. It utilizes Jamaica's high Gini Index, a standard economic measure of income inequality.¹⁰ NMO patients tended to come from lower socioeconomic households. Though our findings are preliminary and based only on small numbers they have potentially important pathophysiologic implications. Research from the French West Indies has suggested that parasitic eradication through effects on immune system tolerance may predispose to MS.² Conversely, patients from a lower socioeconomic status are more prone to parasitic infestations.^{11,12} Further research investigating the potential link between NMO and parasitic infection may shed light on the etiology of NMO.

Afro-Caribbean NMO patients in this study rapidly developed disability (mean EDSS 6.5 over median disease duration of only 2.3

years) which is similar to the Cuba/French WI experience.⁴ Comparison to French and Italian studies supports the argument that Afro-Caribbean NMO has a worse prognosis than Caucasian NMO.¹²⁻¹⁴ We also found that the time interval between onset of optic neuritis and the development of transverse myelitis or the reverse appears short compared with other NMO ethnic groups. This difference may be genetically based though limited availability and range of treatment options and delays in both diagnosis and treatment may be important variables.

Patients with IDD seropositive for AQP4-IgG had worse pulmonary function. Given the more severe phenotype of NMO in Jamaican patients and limited intensive care resources, close attention to preservation of lung function by earlier diagnosis and initiation of attack prevention therapies will be important.

None of the patients in this study were seropositive for HTLV-1 despite its high prevalence in Jamaica.¹⁵ Delgado *et al.* have described a case of a patient with transverse myelitis who was seropositive for NMO-IgG antibody and HTLV-1 and postulated that the immunopathogenesis of NMO may be linked to HTLV-1 exposure.¹⁶ Our findings do not support this hypothesis. In addition, none of the NMO cases had a coexisting autoimmune disease despite the high population prevalence of

Table 1. Clinical and demographic characteristics of 36 consecutive Jamaican inflammatory demyelinating disorder patients subgrouped according to diagnosis.

	NMO	MS	Unclassified
Age, mean (SD)	35.1 (13.4)	43.1 (8.6)	36.5 (12.6)
Age at onset, mean (SD)	33.0 (13.1)	30.6 (8.7)	33.8 (11.3)
Relapses, mean (SD)	1.6 (1.1)	2.6 (2.7)	0.67 (0.78)
Duration of disease, years (SD)	2.3 (1.2)	11.7 (6.3)*	2.8 (2.7)
Males (%)	2 (20.0)	3 (21.4)	2 (16.7)
Ethnicity, blacks (%)	10 (100.0)	8 (57.1)	11 (91.7)
First clinical presentation (%)			
Impaired vision	7 (70.0)	5 (35.7)	6 (50.0)
Other presentation	3 (30.0)	9 (64.3)	6 (50.0)
Monthly household income, Jamaican currency (%)			
<10,000	3 (30.0)	1 (7.1)	2 (16.7)
10,000-25,000	4 (40.0)	2 (14.3)	2 (16.7)
25,000-50,000	1 (10.0)	2 (14.3)	2 (16.7)
50,000-100,000	1 (10.0)	1 (7.1)	4 (33.3)
>100,000	1 (10.0)	8 (57.1)	
Education (%)			
Primary	2 (20.0)	1 (7.1)	1 (8.3)
Secondary	6 (60.0)	5 (35.7)	5 (41.7)
Tertiary	2 (20.0)	8 (57.1)	6 (50.0)
Clinical course (%)			
Remitting - Relapsing	9 (90.0)	11 (78.6)	5 (41.7)
Primary progressive	0 (0.0)	2 (14.3)	1 (8.3)
Single events	1 (10.0)	1 (7.1)	6 (50.0)
Expanded Disability Status Scale	6.5 (2.5)	4.9 (2.2)	3.2 (2.6)**

NMO, neuromyelitis optica; MS, multiple sclerosis; SD, standard deviation. *Comparison of NMO and MS, P<0.05; **comparison of NMO and unclassified demyelinating disease, P<0.05. All were seronegative for human immunodeficiency virus and human T cell lymphotropic virus type-1.

systemic lupus erythematosus and other autoimmune diseases in Jamaica.¹⁷

Conclusions

This small clinic based series of Jamaican IDD's highlights the need for population-based studies of demyelinating conditions, especially NMO and NMO spectrum disorders, in Jamaica, the wider Caribbean, as well as other parts of the world, to better understand the contribution of factors (including ethnic and environmental) to the risk of developing these conditions. The high prevalence (36%) of AQP4-IgG seropositivity among the whole group and the high seropositivity rate among unclassified IDD's warrants emphasis and supports the argument that aquaporin-4 autoimmunity may account for a significant proportion of Jamaican CNS inflammatory demyelinating diseases. This is in sharp contrast to western MS where MS outnumbers NMO by about 100 fold.

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