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Anti-John Cunningham Virus antibody prevalence in multiple sclerosis patients in Turkey

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ABSTRACT

Exposure to John Cunningham Virus (JCV) has been associated with Progressive Multifocal Leukoencephalopathy (PML) in Multiple Sclerosis (MS) patients under specific treatments. In order to assess the identifiable risks of Turkish relapsing-remitting multiple sclerosis (RRMS) patients, the seroprevalence rate of JCV antibodies was investigated and compared with demographic and therapy related factors. Serum samples were collected from 308 RRMS patients at 24 centers, and tested by STRATIFY JCV assay for the presence of anti-JCV antibodies. Also, demographic, disease and treatment data were collected at the same time and were used for analysis of associations with seropositivity. The prevalence of anti-JCV antibodies was observed to be 68.2%, which is higher than most countries. There was a trend in terms of higher seroprevalence of antibodies in older patients, and male patients. There was no association with previous natalizumab use, but there was a significantly higher seroprevalence in patients who used immunosuppressant therapies ($p<0.05$). JCV prevalence rate is observed to be high in Turkish MS patients, therefore it would be beneficial to use this marker as a risk stratification tool in clinical decision making.

Keywords:
John Cunningham Virus
Multiple Sclerosis
Natalizumab
Turkey

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1. Introduction

In recent years, there has been an increase in the number of pharmaceuticals used in treatment of Multiple Sclerosis (MS), made available by new high technology developments. Although these new treatments are becoming more and more effective in the way we manage this debilitating disease, they may come with a burden. MS disease effects over one million people around the world, and it is mediated by the patient's own immune response targeting the central nervous system (Steinman, 2012). It is not surprising that when the immune systems of MS patients are modified in order to treat the disease, opportunistic infections kept under control by immunity may start to cause unwanted side effects. Weighing the benefits and risks of any given treatment in daily clinical practice and using all the available tools to determine which is the most benefit for the patients is crucial. One of the most efficient therapies for MS, the monoclonal antibody natalizumab, has been associated with Progressive Multifocal Leukoencephalopathy (PML), a rare but potentially fatal disease (Clifford et al., 2010; Hellwig and Gold, 2011; Vermersch et al., 2011). Typically, PML is caused by opportunistic John Cunningham Virus (JCV), in patients with immunocomprimising diseases, or under immunosuppressive/ immunomodulatory therapies (Major, 2010). JCV exposure among different populations was reported to be 33%-91%, in various geographies and using different methods (Sorensen et al., 2012).

JCV infection is asymptomatic in healthy people; the virus needs to gain pathogenicity and some additional factors play role in development of PML (Sunyaev et al., 2009). Data from multinational studies show approximately 50-69.5% prevalence in various populations, by testing JCV specific antibodies from serum samples by specifically developed assays, optimized to a false negative rate of 2.5-2.7% (Gorelik et al., 2010; Bozic et al., 2011; Bozic et al., 2012, ECTRIMS). As a guide for clinical decision making, risk stratification algorithms are developed, for which the JCV antibody assay plays a central role. In patients exposed to JCV, prior immunusuppressant treatments and duration of natalizumab treatment are shown to effect the risk of development of PML (Sorensen et al., 2012). The estimated incidence of PML is still quite low in patients who have been exposed to JCV and immunosuppressants during the first two years of natalizumab treatment: 1.6/1,000 (Bloomgren et al., 2012). The mortality rate in natalizumab treatment related PML is 22% (Foley et al., 2011). Thus, it is recommended that patients who are currently being treated or candidates for natalizumab treatment are tested for JCV antibody status and therapeutic decision is made considering the likely benefit/ risk. Serum antibody testing was recently optimized using a 2-step ELISA (Gorelik et al., 2010).

Since there have been many reports with different seroprevalence rates from various countries, we have started a programme in Turkey to identify patients who will benefit more from a highly effective therapy, in order to give the clinician a more informed decision making during everyday practice. As the first step, we tested 308 relapsing-remitting multiple sclerosis (RRMS) patients for anti-JCV antibodies, from various geographical regions, in order to determine the seroprevalence in Turkey.

2. Experimental procedure

Study patients and sample collection

This observational study was approved by the Ethical Committee and Ministry of Health before patient enrollment. For the collection of patient samples and demographic data, 24 study centers were included in the study. These centers represent the most experienced clinical care and high patient rate MS clinics from various geographical regions of Turkey. Serum samples were collected from 308 adult male and female RRMS patients, over 10 months. Patients with a diagnosis of RRMS according to McDonald criteria, admitted to the clinics for routine exams and using various therapies were included. Written consents were taken from patients, in accordance with the Declaration of Helsinki and Turkish laws and legislations on clinical studies.

All serum samples were tested for anti-JCV antibody positivity for a single time point. Serum samples were prepared from peripheral blood according to protocol (Focus Diagnostics, Cypress, CA) and tested by 2-step ELISA method specially developed for JCV antibody detection.

Demographic data and disease history

Each patient was coded in order to protect privacy. At the time of blood withdrawal, demographic data and disease characteristics were collected from the patients; gender, age, time when first symptoms started and time of diagnosis. Treatment history was recorded in detail including previous immunomodulatory and immunosuppressant treatments, natalizumab treatments as well as the duration of each treatment. Clinical disease characteristics included number of relapses in last 2 years, Expanded Disability Status Scale (EDSS) scores, magnetic resonance imaging (MRI) status of gadolinium-enhanced lesions, and presence of any other diseases.

Anti-JCV antibody determination

After collection, serum samples were stored in -20°C until they were shipped to Focus Diagnostics, Cypress, CA, USA. Samples were tested by STRATIFY JCV™ , a 2- step ELISA validated for JCV antibody detection. Serum antibody status was dichotomous, being positive or negative for each patient.

Data analysis

Primary goal of this study was to determine the prevalence of anti-JCV antibodies in Turkish population. Additionally, clinical data like disease duration, relapse rates, EDSS score, treatment and MRI lesion status are defined in detail in this cohort.

The demographic data and the anti-JCV antibody prevalence were described using descriptive statistics (minimum, maximum, mean, median and standard deviation values for the continuous variables, and frequency and percentage values for the categorical variables). For specific data like disease and treatment history, some patient data were missing. No substitution was done for the missing values, these were excluded from the statistical analysis. Anti-JCV antibody prevalence was defined as the percentage of patients with positive anti-JCV antibodies. Annualized relapse rate was defined by dividing the number of relapses in last two years by 2.

Demographics (age and gender), disease and treatment history were analyzed for association to seropositivity. For the analysis of association with age, patients were separated into several age groups and the p-value across age categories was determined using Fisher's Exact Test because of the small sample size. For the analysis of association of anti-JCV antibody with gender, immunosuppressant use, and natalizumab use, Pearson Chi-Square Test was used. Results at $p<0.05$ level were considered statistically significant.

Study data were analyzed by an independent statistician. Statistical analyses were performed using IBM SPSS 20 software.

3. Results

Demographic information was available from 307 patients, summarized in Table 1. Mean age of the patients that were enrolled in the study was 32.93 (±9.05) years. Among 307 patients, 223 (72.6%) were female. Mean total duration of MS disease was 8.8 (±5.68) years from the start of first symptoms. From the diagnosis of RRMS, a mean of 7.4 (±4.92) years had passed.

Disease characteristics are listed in Table 2. Relapse history in the 2 years prior to sample collection were available from 304 patients. Most of the patients had 2 or more relapses, with a mean annualized relapse rate of 1.2 (±0.90). EDSS scores were available at the time of sample collection from 296 patients. Median EDSS was 3 and most of the patients had EDSS scores between 2 and 3. Only EDSS scores of 10.5% patients were above 5. According to the MRI results in the past 1 year, 175 of 278 patients (62.9%) had gadolinium-enhanced lesions.

Table 1. Demographic data and background disease characteristics

	(N=308)
Age (yrs.)	
n	307
Mean (SD)	32.93 (9.05)
Median	32.0
Min, max	16.0, 63.0
Gender	
Male	84/307 (27.4%)
Female	223/307 (72.6%)
MS disease duration (yrs.)	
n	303
Mean (SD)	8.8 (5.68)
Median	7.7
Min, max	0.2, 36.0
MS diagnostic duration (yrs.)	
n	307
Mean (SD)	7.4 (4.92)
Median	6.5
Min, Max	0.0, 25.0

Table 2. Summary of relapses, EDSS, and contrast lesion status

Relapses in last 2 years	
0	41/304 (13.5%)
1	56/304 (18.4%)
2	83/304 (27.3%)
3	49/304 (16.1%)
4	37/304 (12.2%)
5	16/304 (5.3%)
6	14/304 (4.6%)
>= 7	8/304 (2.6%)
Annualized relapse rate	
n	304
Mean (SD)	1.2 (0.90)
Median	1.0
Min, max	0.0, 4.5
Last EDSS	
n	296
Mean (SD)	3.05 (1.62)
Median	3.0
Min, Max	0.0, 7.5
Last EDSS	
>=0 and <=1	47/296 (15.9%)
>1 and <=2	55/296 (18.6%)
>2 and <=3	71/296 (24.0%)
>3 and <=4	55/296 (18.6%)
>4 and <=5	37/296 (12.5%)
>5 and <=6	21/296 (7.1%)
>6	10/296 (3.4%)
Contrast lesion in last 1 year	
Yes	175/278 (62.9%)
No	103/278 (37.1%)

Prior treatments are listed in Table 3. A total of 99/307 (32.2%) patients were using/had used natalizumab, and 47/307 (15.3%) patients were exposed to immunosuppressant treatment previously with nine receiving mitoxantrone. Almost all patients had received one of four Disease Modifying Treatments (DMTs): Interferon beta-1a sc, interferon beta-1a im, interferon beta-1b, glatiramer acetate. Most received only one type of DMT (56.2%), while 33.0% switched to a second DMT. Mean durations of treatment were 15.35, 8.63, 15.55 and 9.95 months for IF1a sc, IF1a im, IF1b, GA, respectively.

The number of the patients who tested positive for anti-JCV antibodies was 210 of 308 patients (68.2%). For the analysis of association of anti-JCV antibody positivity with age, the patients were separated into age groups (<30, 30-39, 40-49, 50-59, ≥60) (Fig. 1). When the patients under 30 years old and above 50 years old were compared, there was a higher seropositivity rate in the older group (64.7% and 86.7%, respectively). However, when all age groups were compared, no significant difference was shown (p=0.218). There was a significant difference between women and men

Table 3. Treatment history

Prior Tysabri Use	
Yes	99/307 (32.2%)
No	208/307 (67.8%)
Prior IS Use	
Yes	47/307 (15.3%)
No	260/307 (84.7%)
Prior DMT use	
0	11/306 (3.6%)
1	172/306 (56.2%)
2	101/306 (33.0%)
3	21/306 (6.9%)
>= 4	1/306 (0.3%)
IF1 a sc	
n	304
Mean duration of use (months) (SD)	15.35 (27.84)
Min, max	0.0, 168
IF1 a im	
n	305
Mean duration of use (months) (SD)	8.63 (19.97)
Min, max	0.0, 144
IF1b	
n	299
Mean duration of use (months) (SD)	15.55 (29.55)
Min, max	0.0, 180
GA	
n	299
Mean duration of use (months) (SD)	9.95 (18.80)
Min, max	0.0, 108

(64.1% vs 78.6%, respectively p<0.05) (Fig. 2). Looking at the effects of treatment on anti-JCV antibody prevalence, there was no significant difference between the patients who have been treated by natalizumab and the patients who have never used natalizumab, p=0.155 (Fig. 3). Patients who were exposed to immunosuppressant therapies had a seropositivity of 83.0%, significantly different from patients who did not use immunosuppressants, 65.4% (p<0.05) (Fig. 4).

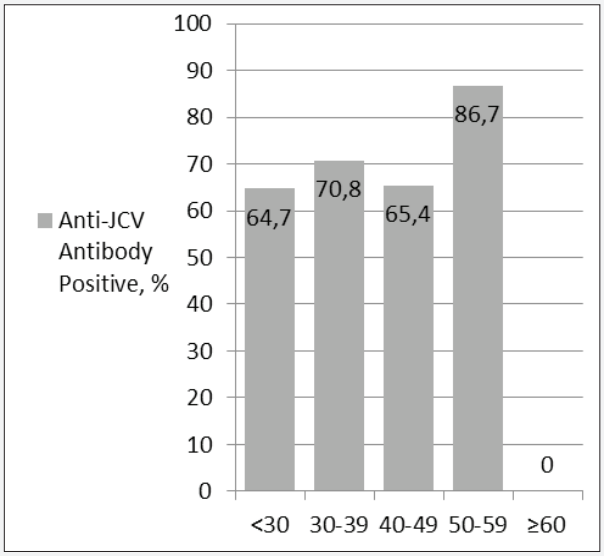

Fig. 1. Seroprevalance among the age groups. p value across age categories was determined by Fisher's Exact Test (p=0.218).

4. Discussion

There has been a growing interest in JCV epidemiology since the emergence of PML cases associated with the use of monoclonal antibodies for the treatment of MS. Various national and multinational studies have reported the prevalence of JCV antibodies to be around 50-60%, assessed by STRATIFY JCV assay (Bozic et al., 2011; Bozic et al., 2012; Trampe et al., 2012). By determining the serostatus of patients, it is possible to make more informed decisions about treatment options, minimising risks and giving the patients best possible care. Natalizumab is a powerful MS treatment, especially in patients with highly active disease, and it is warranted to assess JCV status of patients using or thinking of using natalizumab (Polman et al., 2006; Rudick et al., 2006; Sorensen et al., 2012). This study revealed the seroprevalence of anti-JCV antibodies in Turkey. RRMS patients came from various geographical regions of Turkey, without any selection of demographics or disease/treatment history. Analysis of JCV antibodies in serum samples with the STRATIFY JCV assay resulted in 68.2% prevalence rate in Turkish patients. This represents one of the highest reported rate. To date, the highest seropositivity was reported for Portugal (69.5%) (Bozic et al., 2012). JCV is a ubiquitous virus usually transmitted in childhood, but the main route of transmission is not clearly defined (Matos et al., 2012).

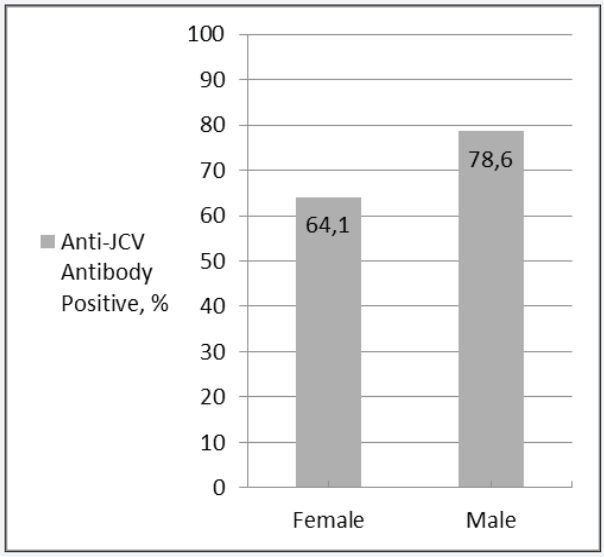

Fig. 2. John Cunningham Virus antibody positivity is higher in men than in women (p<0.05).

It may be speculated that the social mode of interaction, close family relations and cultural habits are factors affecting the prevalence. Also, yet undiscovered genetic factors might play a role in susceptibility to infection.

Evaluation of demographic factors in the present patient population showed age, gender and disease histories to be similar to other studies conducted in various countries (mean patient age 32.93± 9.05, 72.6% women, mean disease duration 7.4±4.92 years, median EDSS 3.0). Thus, this group reflects general MS population in terms of disease characteristics. In previous reports, older age was correlated with an increase in seropositivity, maybe as a result of more exposure in years (Bozic et al., 2011; Outteryck et al., 2012; Trampe et al., 2012). In the present study, seropositivity was higher in the

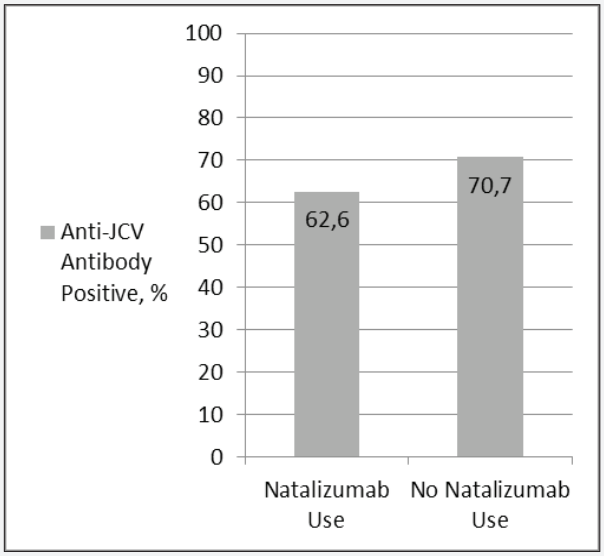

Fig. 3. Prevalence between the patients exposed to natalizumab (p=0.155).

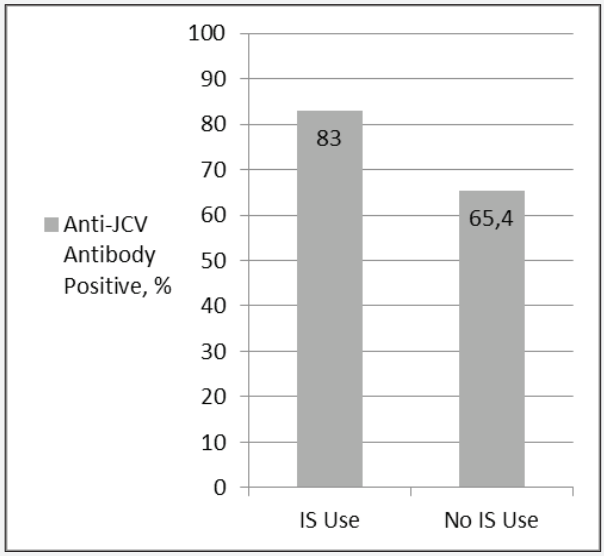

Fig. 4. Prevalence of anti-JCV antibodies is higher in the patients who have used immunosuppressant treatments (p<0.05).

older group; however the 40-49 age group does not reflect the expected rate of positivity, so there was no statistically significant difference among groups. Seropositivity was higher in males, as previously reported although the reason behind this remains unknown (Gorelik et al., 2010; Outteryck et al., 2012; Trampe et al., 2012). Exposure to natalizumab was not found to be correlated with seropositivity, as reported in the earlier studies (Bozic et al., 2011; Trampe et al., 2012).

However, there was a significant correlation between prior immunosuppressant use and JCV antibody positivity (83.0% in users, 65.4% in non-users). In previous studies, no significant correlation was found, although there was a a tendency towards more positivity in immunosuppressant users groups in some cohorts (Bozic et al., 2011; Olsson T. ECTRIMS 2011, P338; Trampe et al., 2012; Outteryck et al., 2012). Our findings may be spurious and may have resulted because of the overall high seropositivity rate while the number of patients using immunosuppressants was low (15.3%).

Given the fact that JCV-antibody seropositivity is high in our population, this study was a major step forward in risk stratification for daily clinical practice in Turkey. Although a majority of the patients may test positive for this marker, this does not necessarily mean that they will develop PML if they are treated with natalizumab and immunosuppressants. The benefits and risks of treatment need to be assessed in such cases. Risk assessments for various conditions have been determined quite clearly and guidelines are available for clinicians to aid in decision making (O' Connor, 2012; Sorensen et al., 2012). It should be kept in mind that the estimated false negative rate of the test is 2.5-2.7% (Gorelik et al., 2010; Bozic et al., 2011). If a patient is negative for the anti-JCV antibody, relative PML risk is estimated to be equal or less than 1 in 10,000 (Bloomgren et al., 2012; Sorensen et al., 2012). It is recommended that these patients are tested periodically as about 5-10% are shown to have a serostatus change to positivity over time (Trampe et al., 2012). In positive patients, estimated incidence differs according to treatment history. It is still 0.56/1000, in positive patients who have not used immunosuppressants and had up to 2 years of natalizumab treatment (Bloomgren et al., 2012). After 2 years of treatment, it becomes 4.6/1000 (Bloomgren et al., 2012). If the patient has used immunosuppressants and up to 2 years of natalizumab treatment, estimated incidence of PML is 1.6/1000 and 11.1/1000 after 2 years. This information can be used for risk assessment in clinical decision making. Some patients with very active disease who benefit from natalizumab treatment may prefer to continue after 2 years, accepting the risks, if they are fully aware of them. The risks and benefits of treatment according to patient status should be discussed. It should also be kept in mind that not much is known about long-term use associated with PML risks. Nevertheless, patients suffering from debilitating effects of MS can still be offered potent therapies following recommendations published for early recognition and treatment of PML (Bloomgren et al., 2012; O' Connor, 2012; Sorensen et al., 2012).

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