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ORIGINAL ARTICLE

# Prevalence of JC polyomavirus among rheumatoid arthritis and systemic lupus erythematosus patients and its correlation with vitamin D levels

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## ABSTRACT

**Background and Objectives:** Vitamin D deficiency in viral infection associated with autoimmune diseases is well documented. This study assessed the prevalence of JC virus in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and its correlation with vitamin D level.

**Materials and Methods:** Serum and urine samples were collected from 50 patients with RA and SLE. DNA was extracted and subjected to PCR test. Positive PCR products were sequenced, phylogenetic tree was constructed to determine the JC virus genotype. The patient's vitamin D level was evaluated.

**Results:** Of 50 patients, 19 (38%) were diagnosed as RA, and 31 (62%) were identified as SLE. JC virus DNA was detected in 17 (34%) patients' urine samples including 5 (26.3%) RA and 12 (38.7%) SLE cases. JC virus DNA was detected 2 (4%) in patients' serum samples (one RA. and one SLE). JC virus genotype 3A was dominant. Interestingly, the SLE patients with positive JC virus showed lowered vitamin D compared to patients with negative JC virus ($P<0.005$).

**Conclusion:** Given the high rate of JC virus, DNA detection and susceptibility of patients for PML development, it is recommended that detection of JC virus DNA and vitamin D level should be implemented for patients with RA/SLE prior to treatment.

**Keywords:** JC virus; Arthritis; Rheumatoid; Lupus erythematosus; Polymerase chain reaction

## INTRODUCTION

JC virus or JC Polyomavirus ( JCPyV) is generally acquired at an early age, mostly between 10 to 14, through the fecal-oral and respiratory routes, which results in a persistent infection that remains dormant in various parts of the body including kidneys, peripheral blood lymphocytes (PBL), tonsils, kidney, liver, lungs, lymph nodes, spleen, bone marrow, urine upper and lower gastrointestinal tract, and CD34+ lymphocytes in healthy individuals (1, 2). JCV excretes through urine and stool, disseminated and affects a significant portion of 50% to 80% of individuals worldwide (2, 3). However, in individuals with

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compromised immune systems, the B lymphocytes harboring JC virus have the ability to infiltrate the central nervous system, leading to the viral attack on astrocytes and oligodendrocytes and subsequent development of PML (2, 4). JC virus belongs to the Polyomaviridae family and is a double-stranded DNA virus. Through the analysis of VP1 sequences, seven distinct genotypes of JC virus have been identified (5). In this region of Iran, JC virus genotype 3A is the most prevalent (6). The primary mechanism underlying the pathogenesis of PML development due to JC virus is the occurrence of rearrangement of the non-coding control region (NCCR) of the virus (7-10).

Rheumatoid arthritis (RA) is a systemic inflammatory disorder that predominantly impacts the joints, potentially resulting in deformities and functional constraints in the absence of proper treatment, leading to elevated disability rates and decreased survival among affected individuals. The disease is typified by inflammation, synovial hyperplasia, the production of autoantibodies (such as rheumatoid factor (RF) and anti-citrullinated protein), cartilage destruction, bone deformities, and systemic engagement affecting the cardiovascular, pulmonary, neurological, and endocrine systems (11, 12). Systemic lupus erythematosus (SLE) represents an autoimmune condition characterized by significant chronic features, affecting various organ systems, with a course marked by periods of remission and relapse (13). While the precise cause remains unidentified, the disease's development is acknowledged to involve a complex interplay of genetic, environmental, hormonal, and immunological factors (13). Vitamin D deficiency is widely acknowledged as a significant global issue in public health. Numerous epidemiological studies have demonstrated that inadequate levels of vitamin D can impact a variety of diseases (14-16). Furthermore, Vitamin D plays a role in stimulating the production of human β-defensins (HBDs), which in turn can assist in establishing an antiviral state against viral infections (17). The antiviral effects of Vitamin D are mediated through both direct and indirect mechanisms. The indirect modulation of immune cell migration to the infection site by HBDs is coupled with potential direct antiviral actions such as disruption of viral membranes, interference with viral glycoproteins, inhibition of virus replication, and suppression of virus receptors (18). This has been observed in various viruses including HIV-1 (19-21), vaccinia (22), adenovirus (23), Rhinovirus (RV) (24), and influenza virus (25). Vitamin D deficiency has also been demonstrated to have a correlation with an elevated CD4/CD8 ratio (26). Inadequate levels of vitamin D could potentially diminish the capacity of the immune system to generate activated T lymphocytes, specifically CD8+ T cells, which have the capability to combat virally infected B cells, thereby compromising the management of viral infections like EBV (27). Vitamin D induces antiviral activity of cathelicidin against HSV-1/2 (28, 29), influenza, (30) rhinovirus (RV) (31), and HCV (32). Vitamin D can contribute to decreased replication of hepatitis B virus (33).

The majority of therapeutic options for rheumatologic disease are immunosuppressive agents including monoclonal antibodies, Methotrexate, Glucocorticoids or TNF inhibitors such as infliximab (34-36). Using immunosuppres- sive treatment can be considered as a risk factor for JC virus activation and PML development (34-36). Therefore, this study was aimed to evaluate the pres- ence of JC virus in urine and serum of patients with RA and SLE in Ahvaz city, Iran.

## MATERIALS AND METHODS

In this cross-sectional study, a consecutive 50 patients, comprising individuals with RA and SLE, who were referred to Golestan Hospital in Ahvaz, Iran, during the year 2021, were recruited. After obtaining written ethical consent, sampling was conducted. All study protocols were approved by the ethical committee of Ahvaz Jundishapur University of Medical Sciences, with the ethical committee code IR.AJUMS.REC.1400.062. The patient’s urine samples were also collected in a completely clean, sterile screw-top container then decanted into sterile capped tubes and stored at -70°C. A volume of 5mL of peripheral blood was collected from each patient for serum separation, and stored at -70°C. The inclusion criteria were patients with RA or SLE confirmed by a rheumatologist. The exclusion criteria were patients who did not receive treatment and cancer patients under chemotherapy. Addition- ally, certain clinical laboratory data, including the erythrocyte sedimentation rate (ESR), the vitamin D levels deficiency considered as <20 ng/ml (37), anti-double-stranded deoxyribonucleic acid antibodies (dsDNA), and rheumatoid factor (RF), were extracted from the patients' files. The treatment protocols were

also documented from the patients' files.

**Molecular testing.** For the extraction of DNA from the serum and urine samples, a viral nucleic acid extraction kit (Favorgen, Taiwan) was utilized in accordance with the manufacturer's brochure. The confirmation of the quality of the DNA samples that were extracted was achieved through the use of spectrophotometry at 260/280 nm.

**JC virus detection and molecular evaluation.** The DNA extracted from serum and urine samples were used as a template for detecting and determining the JC virus genotype using conventional PCR. A set of specific JC virus VP1 primers, consisted of a forward primer "ACAGTGTGGCCAGAATTCCACTAC" and a reverse primer "TAAAGCCTCCCCCCCAACAGAAA" were employed (38). The PCR reaction mixture containing 20 pmol of each forward and reverse primers, 12 µL of master mix Yekta Tajhiz (Tehran, Iran), 400 ng of the extracted sample, and PCR grade water to bring the total volume to 25 µL. All the reactions were achieved along with the negative (distilled water) and positive controls were subjected to PegLab thermal cycler (Germany) with the following thermal program, the initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for second, and extension at 72°C for 50 seconds. A final extension step was performed at 72°C for 10 minutes. The amplification products were visualized using 2% agarose gel electrophoresis. The expected size of the PCR prod- uct was 215 base pairs (38).

**Sequencing and phylogenic analyses.** Consequently, a total of five (three urine and two serum samples) PCR products from the VP1 were selected and subjected to sequencing in both the forward and reverse directions. This sequencing process was conducted using the Applied Biosystem 3500 instrument, ABI Scientific USA. The nucleotide sequencing results of the partial "VP1" region of the isolates JC virus genome was aligned utilizing the NCBI JC virus database. The JC virus VP1 sequences from urine and serum patients were also aligned with the consensus sequence JC virus VP1 using SnapGene software (version 3.2.1). In order to determine the JC virus genotyping, a phylogenetic tree was constructed for partial VP1 region of the isolates utilizing the Maximum Likelihood method under the Kimura 2-parameter distance model with 1000 bootstrap replicates. The MEGA software version 6 was employed to implement these methods.

**Statistical evaluation.** The chi-square test and Mann-Whitney U test were used for evaluation of variables. All statistical evaluation preformed in SPSS version 22 (IBM, SPSS). The $P<0.05$ was considered as significant results for each test.

## RESULTS

**Demographical and patients' data.** By evaluation of 50 included patients, it was demonstrated that, the mean age of included patients was 50.3 ± 14.4 and only 4 (8%) were male and 46 (92%) were female. From 50 included patients 19 were RA (38%) and 31 SLE (62%). More information about other features is provided in Table 1. The ESR rate was statistically significant higher in SLE group compare with RA patients (P=0.04).

**JC virus status and molecular study.** The PCR test can detect JC virus in 17 (34%) of urine samples [for RA, one male and four female, for SLE, 12 female and 0 male]. The distribution of vitamin D level among the patients was 9.8 ng/ml to 39.9 ng/ml. All other variables for JC virus positive urine samples are illustrated in Table 2. 2 (4%) of serum samples were found to be positive for JC virus. Of the JC virus positive serum samples were one RA and one SLE patients, both the patients were female.

The JC virus molecular evaluation showed that out of 5 sequences samples, 4 were cluster with genotype 3A including 2 urine (OR778408, OR778409) and 2 serum samples, (OR778410, OR778411) and only one urine sample was cluster with genotype 1A (OR778407). The Maximum likelihood phylogenetic tree represents high similarity form evaluated samples with other Iranian strains (Fig. 1).

## DISCUSSION

As mentioned earlier RA and SLE are two important rheumatological diseases which are distributed all around the word. In fact, the RA is a prototype

**Table 1.** The demographical and laboratory data of included RA and SLE patients

| Variables/Disease | RA | SLE | P-value |
|---|---|---|---|
| | 19 (38%) | 31 (62%) | |
| Mean age ± SD | 54.1 ± 13.3 | 48 ± 14.8 | 0.08 |
| Gender | Male 3 (15.8%) | 1 (3.2%) | 0.1 |
| | Female 16 (84.2%) | 30 (96.8%) | |
| dsDNA Positive* | - | 6 (19.4%) | - |
| FANA** | 3 (15.7%) + | 8 (25.8%) ++ | |
| RF Positive | 2 (10.5%) | - | - |
| Vitamin D | 20.8 ± 9 | 25 ± 10.2 | 0.1 |
| ESR | 29.95 ± 19.48 | 17.55 ± 17.22 | 0.04*** |

*dsDNA evaluated by ELISA, **FANA (Fluorescent antinuclear antibody test) evaluated by IF (Immunofluorescence) in Hep-2 cells, Vitamin D value ng/mL, ESR rate mm/hr, *** represents statistically significant difference in Mann-Whitney U test. + Positive FANA in RA patients with non-specific patterns, ++ Positive FANA in SLE patients with SLE-specific pattern

**Table 2.** Evaluated variables for patients with JC virus-positive urine samples

| Variables/Disease | RA | | | SLE | | |
|---|---|---|---|---|---|---|
| | JC virus Positive | JC virus Negative | P-value | JC virus Positive | JC virus Negative | P-value |
| Number (%) | 5 (26.3%) | 14 (73.7%) | - | 12 (38.7%) | 19 (61.3%) | 0.36 |
| Mean age ± SD | 54.8 ± 15.4 | 53.9 ± 13.1 | 0.8 | 50.25 ± 12.46 | 46.63 ± 16.29 | 0.4 |
| Gender Male | 1 (20%) | 2 (14.3%) | 0.7 | 0 | 1 (5.3%) | 0.3 |
| Female | 4 (80%) | 12 (85.7%) | | 12 | 18 (94.7%) | |
| Treatment drugs | | | | | | |
| PSL | 1 (20%) | 1 (7.1%) | | 0 | 3 (15.8%) | |
| PSL+NSAID | 1 (20%) | 9 (64.3%) | | 0 | 2 (10.5%) | |
| PSL+HCQ | 3 (60%) | 0 | | 4 (33.3%) | 5 (26.3%) | |
| PSL+MTX | 0 | 2 (14.3%) | - | 2 (16.7%) | 0 | - |
| PSL+CLC | 0 | 0 | | 1 (8.3%) | 1 (5.3%) | |
| PSL+HCQ+NSAID | 0 | 1 (7.1%) | | 3 (25%) | 0 | |
| PSL+HCQ+MTX | 0 | 1 (7.1%) | | 1 (8.3%) | 0 | |
| Vitamin D | 18.6 ± 8.8 | 21.7 ± 9.2 | 0.5 | 17.67 ± 3.7 | 29.6 ± 10.3 | 0.005** |
| ESR | 26.6 ± 19.6 | 31.1 ± 20 | 0.8 | 22.5 ± 12.6 | 14.4 ± 19.2 | 0.01** |

HCQ: hydroxychloroquine, PSL: prednisolone, NSAID: nonsteroidal anti-inflammatory drug, MTX: Methotrexate, CLC: colchicine, Vitamin D value ng/mL, ESR value mm/hr, ** represents statistically significant difference in Mann-Whitney U test.

for rheumatologic joint diseases and SLE is a prototype for rheumatologic systemic diseases (39). The nature of all rheumatological diseases is associated with flare and latent episodes (40). This phenomenon makes clinicians try to overcome this flare episode or try to prevent flares with immunosuppressive treatments. These treatments and the disease pathophysiology lead to disease and treatment-related comorbidities (41). This immunosuppressive condition could be a start for JC virus activation and PML development in SLE (41, 42) or RA patients (43, 44). Some of this immunosuppressive treatments are approved for RA such as Rituximab (RTX) that deplete $CD^{20+}$ B cells, and can increase the risk of PML development in RA patients (45). There are some reports of increased risk of PML development in RA patients with Methotrexate and Infliximab (46). In this regard, we tried to evaluates the JC virus status in some of the referred RA and SLE patients in Ahvaz city, Iran. Results of the current study indicates

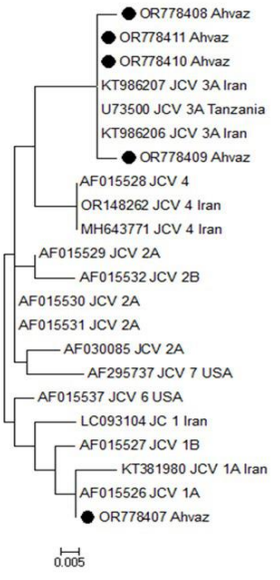


**Fig 1.** Phylogenetic tree using the Maximum likelihood method was constructed to compare partial sequences of the VP1 region of JC virus isolates from patients' urine with rheumatoid diseases in Ahvaz city with different JC virus genotypes. The corresponding accession numbers were retrieved from GenBank, and the genotypes were isolated from various regions around the world. The constructed phylogenetic tree demonstrated the clustering of four JC virus isolates (accession numbers OR778408-OR778411) from Ahvaz city, among them 4 marked with black circles, with genotype 3A from Iran (KT986207), Tanzania (U73500) and one marked with black circle (OR778407) isolate from Ahvaz with genotype 1A from USA (AF015526). The accuracy of the phylogenetic tree was evaluated by conducting 1000 bootstrap replicates. The scale bar = 0.005.

that, the mean age of included patients was 50.3 ± 14.4 and only 4 (8%) were male and 46 (92%) were female. From 50 included patients 19 were RA (38%) and 31 SLE (62%). The PCR test can detect JC virus in 2 (4%) of evaluated serum samples and 17 (34%) of urine samples. The JC virus positive serum samples were one RA and one SLE patients; urine samples were positive in 5 (26.3%) of RA and 12 (38.7%) of SLE. The JC virus molecular evaluation represents that the majority of evaluated samples were associated with genotype 3A and only one was associated with genotype 1 of the JC virus. Patients treated by only prednisolone (PSL) and, PSL plus nonsteroidal anti-inflammatory drug (NSAID) showed less frequency for JC virus in Urine sample in comparison with other combination of treatments.

In a conducted study by Rodio et al. (47) the evaluation of rheumatological disease for JC virus during anti TNF-α treatment indicated that, 16 (47%) and 4 (11%) had positive JC virus results in urine and serum, respectively. Furthermore, Verheyen and colleagues (48) observed JC virus positive results in 26 (32%) of urine and 2 (2.5%) of serum samples in RA patients. The urine positive PCR result was associated with JC virus increased antibody titer in RA patients in Verheyen's study as well. Other studies reported similar results, for instance JC virus prevalence in Italy from rheumatologic disease during treatment was 22.6% in urine samples (49). By considering this, it seems that our results are confirming previous studies and minor differences could be due to differences in methods of detection, study setting and geographical region (50).

The genotype of the JC virus in different populations is associated with geographical location. The JC virus genotype 2 seems to be more prevalent in Comar et al. study in Italy (49). Meanwhile, Hu et al. (51) reported the dominancy of JC virus genotype 6 in China. Karimi et al. (50) introduced genotype 3 as major genotype in Iran. Higher frequency of JC virus type 3 in Ahvaz and Isfahan, Iran was reported in other previous studies (52, 53). It seems our current study results confirmed previous studies and JC virus type 3 is the major type in Iran.

In instances where patients exhibit severe phase and flare symptoms while being unaware of carrying the JC virus, clinicians may opt to administer Rituximab, a monoclonal antibody against CD20 on B cells. Rituximab functions by reducing the population of B cells (45). Alternatively, treatment involving natalizumab, a monoclonal antibody directed at alpha-4 integrin, can impede the migration of various T cells, such as CD8 T cells, within the central nervous system (54). Similarly, the utilization of infliximab has also been associated with the development of progressive multifocal leukoencephalopathy (PML) (55, 56). The level of vitamin D in RA patients positive JC virus and JC virus negative was not significant (P=0.8), while the level of vitamin D in SLE patients positive JC virus and JC virus negative was found to be significant (P=0.005). The rate of ESR in RA patients positive JC virus and JC virus negative was not significant (P=0.8), while the rate of ESR in SLE patients positive JC virus and JC virus negative was found to be significant (P=0.01). This finding could be justified by the systemic nature of SLE in com-

parison with RA as a joint associated disease (38).

The significant point of this study reveals the absence of JC virus among the patients with SLE with sufficient vitamin D level above the cutoff value > 20 ng/mL than those SLE patients positive JC virus who had vitamin D level lower than cutoff value <20 ng/mL ($p<0.005$). This finding highlights the important effect of vitamin D in controlling JC virus however, further comprehensive studies are required. The effect of vitamin D was found to impede replication of some viruses and bacteria which cause infection including HIV-1 and Mycobacterium tuberculosis infection (57), HCV (58), Influenza virus (59) and hepatitis B infection (60) via expression of cathelicidin LL-37. Vitamin D could help to reduce the severity of the COVID-19 pandemic as well (61). Vitamin D exhibits immunomodulatory, anti-inflammatory, antioxidant, and anti-fibrotic properties that possess the capacity to impact the progression and prognosis of immune-mediated disorders (62). The insufficiency of vitamin D leads to the advancement of autoimmune disorders such as multiple sclerosis and autoimmune hepatitis (63).

Furthermore, it needs to be mentioned that our current study faced different limitations, including limited number of patients in each group, limitation for evaluation of healthy control group, limitation in evaluation of JC virus, other genomic regions such as NCCR, and limitation of evaluation of patients in different phases of SLE or RA.

## CONCLUSION

The JC virus genotype 3A is the major genotype dominant in evaluated patients. Given the high rate of JC virus DNA detection and susceptibility of patients for PML development, it is recommended that detection of JC virus DNA and vitamin D level should be implemented for patients with RA/SLE prior to treatment. Monitoring of RA, SLE patients harboring JC virus during immunosuppressive treatment is critical. This finding highlights the necessity of future investigations for JC virus status, genetic variations and load in rheumatological disease.

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