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Original Article

Lymphotropic polyomavirus and Merkel cell polyomavirus in patients infected with HIV or hepatitis B or C virus

Bahman Abedi Kiasari [a], Amir Hossein Alipour [a,b], Negar Hemmati [c], Mohammad Gholamnezhad [d,**,1], Fatemeh Hoda Fallah [e,*,1]

[a] Microbiology and Immunology Group, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[b] Gene Therapy Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[c] Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[d] Clinical Research Development Unit, Yasuj University of Medical Sciences, Yasuj, Iran
[e] Allergy and Clinical Immunology Department, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: LPV and MCV emerge as recent additions to the Polyomaviridae family, capable of inducing important infections. Studies have suggested the presence of LPV in human populations, with potential involvement in central nervous system (CNS) diseases. Additionally, MCV, closely related to LPV, has been implicated in Merkel cell carcinoma (MCC). This study aimed to explore the prevalence of LPV and MCV in individuals with compromised immunity due to chronic viral infections.

Methods: 340 specimens, including HIV PCR-positive, HBV PCR-positive, HCV PCR-positive, and HIV/HBV/HCV negative sera, underwent screening via PCR technique to identify LPV and MCV genomes. Subsequently, sequencing was employed to validate the viral identity.

Results: Out of all specimens, MCV DNA was detected in 8.52 % of participants, with a significantly higher prevalence in HIV-positive individuals (26.4 %). LPV was detected in only one HIV-positive patient. No co-detection of MCV and LPV was observed. Phylogenetic analysis confirmed the genetic similarity of the detected MCV strains to known references, while the LPV sequence showed 99 % identity to the published sequences of LPV-K38.

Conclusion: This research provides insights into the prevalence of LPV and MCV in individuals with chronic viral infections. The study highlights the potential association between MCV and immunocompromised states, emphasizing the need for comprehensive investigations to understand the epidemiology, transmission routes, and clinical implications of these polyomaviruses in human populations.

1. Introduction

Evidence indirectly suggests the potential for human infection with the lymphotropic polyomavirus (LPV) from African green monkeys, as demonstrated by the reactivity of human sera with the virus [1]. Early serological investigations disclosed a 30 % seropositivity in human sera through various assays [2]. Viscidi and Clayman identified LPV-reactive antibodies in a significant portion of human sera (15 %) using a VLP-based immunoassay [3]. Competitive inhibition assays ruled out cross-reactivity with BKV or JCV antibodies [4], leading to the suggestion that LPV reactivity in human sera may arise from cross-reacting antibodies induced by unidentified LPV-related polyomaviruses.

The discovery of Merkel cell polyomavirus (MCV) as a closely related virus to LPV in human Merkel cell carcinoma (MCC) by Feng et al. [5] implies a potential role for this virus in MCC carcinogenesis. Their findings indicated viral genome integration into tumor cell DNA, suggesting viral infection preceding the clonal expansion of tumor cells [5].

Numerous studies, including those in Australia (1.3 %) [6], Finland (2.1 %) [7], Sweden (4.3 %) [8] and the UK (3.27 %) [9], have reported

* Corresponding author. Allergy and Clinical Immunology Department, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran.
** Corresponding author. Clinical Research Development Unit, Yasuj University of Medical Sciences, Yasuj, Iran.
E-mail addresses: mo.gholamnezhad@yahoo.com (M. Gholamnezhad), hodafallahfatemeh@gmail.com (F. Hoda Fallah).
[1] Fatemeh Hoda Fallah and Mohammad Gholamnezhad contributed equally to this work.

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the detection of MCV in respiratory specimens. This suggests the respiratory tract as a potential route for MCV transmission. Consequently, the reactivity of human sera with LPV in previous studies may be attributed to cross-reacting antibodies induced by MCV. However, a seroprevalence study with a competition assay indicated that MCV and LPV are distinct antigenically [4]. They revealed persistent LPV infection (15 % seropositivity) in humans throughout their lives [4]. Delbue et al. validated current seroepidemiological evidence by detecting LPV genome in human peripheral blood using PCR [10]. The LPV control region was successfully amplified from DNA extracts, suggesting potential involvement in central nervous system (CNS) leukoencephalopathies, particularly in patients with progressive multifocal leukoencephalopathy)PML (and JC virus-negative leukoencephalopathy [10].

Hence, this study aims to detect the occurrence of LPV or MCV among individuals with chronic viral infections and potentially weakened immune systems. To achieve this objective, we investigated HIV-infected patients, as well as those infected with hepatitis B or C virus (HBV or HCV), alongside a control group comprising individuals negative for HIV/HBV/HCV.

2. Materials and methods

2.1. Study populations and samples

This study involved the screening of four distinct groups, totaling 340 serum samples, which were collected between January 2023 and September 2023 at the Clinical Virology Laboratory. The samples were obtained from patients attending various departments, including the Outpatient Department (OPD), Inpatient Department (IPD), and the HIV clinic, ensuring a diverse representation of patient populations. The samples included 53 HIV PCR-positive sera, 98 HBV PCR-positive sera, 49 HCV PCR-positive sera, and 100 HIV/HBV/HCV-negative sera from a control group. All participants were informed about the study, and written consent was obtained before sample collection. The patients' age, gender, and ethnicity were recorded. The median age of the study participants was 37 years (mean 31 years; range 17–73 years), with a male-to-female ratio of 1.19:1 (185 males and 155 females).

All specimens were promptly transported to the laboratory and stored at −70 °C until further analysis. To maintain patient confidentiality, samples were anonymized, with all identifiers removed and specimens renumbered before inclusion in this research.

2.2. Molecular analysis

Specimens were extracted using the QIAamp® MinElute® Virus Spin kit (Qiagen) following the manufacturer's instructions. For the detection of polyomaviruses KI, WU, BK, JC, LPV, and MCV, in-house DNA PCRs were employed, as previously outlined [9,11,12]. PCR examinations were conducted using two sets of primers for MCV (LT3F/LT3R; VP1F/VP1R) and two sets for LPV (LPR1/LPR2; LPV1/LPV2) (Table 1). Positive sample extracts from the first PCR, targeting the LT region of the genome, underwent a second PCR analysis using primers focusing on an alternative genome region (VP1). The virus loads of HIV, HBV, and HCV were determined using quantitative reverse transcription–PCR (qRT-PCR) with commercially available diagnostic kits.

Table 1
Details of primers used in the PCR assays.

Primer name	Sequences (5′-3′)	Target gene	References
LT3F	TTTGCCAGCTTACAGTGTGG	LT-Ag	Feng et al., 2008
LT3R	TGGATCTAGGCCCTGATTTTT	(MCV)	[5]
VP1F	TTTGCCAGCTTACAGTGTGG	VP1	Feng et al., 2008
VP1R	TGGATCTAGGCCCTGATTTTT	(MCV)	[5]
LPR1	GCAACTAGACCGCAGAACAGTTG	LT-Ag	Lednicky et al.,
LPR2	CTCAGGGCAGCTTACCTAATGAG	(LPV)	2002 [13]
LPV1	AGGGCCTGATGCTATTACCC	VP1	Delbue et al.,
LPV2	CCCACATCAAAATGGTGTCA	(LPV)	2008 [10]

2.3. DNA sequencing

Sequencing was carried out using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and the ABI 3100 Genetic Analyzer (Applied Biosystems), following established protocols [12].

3. Results

3.1. Characteristics of study participants

The LPV/MCV assay examined 340 specimens from patients, with a median age of 37 years (mean 31 years; range 17–73 years), and a male-to-female ratio of 1.19:1 (185:155). Specimens were categorized into four groups: Group I comprised 53 serum samples from HIV-1 positive patients (n = 53), Group II included 98 serum samples from HBV positive cases (n = 98), Group III had 49 serum samples from HCV positive cases (n = 49), and Group IV consisted of 100 serum samples from HIV/HBV/HCV negative cases (n = 100).

3.2. Prevalence of MCV and LPV and clinical observations

PCR analysis, using LT3F/LT3R primers for MCV and LPV1/LPV2 primers for LPV, was performed on serum samples (n = 340) from HIV, HBV, or HCV-infected patients, and a control group negative for HIV/HBV/HCV. The negative and positive controls yielded the expected results. Positive samples underwent a second PCR analysis using primers targeting an alternative region of the virus genome (VP1), followed by bidirectional sequencing of the LT and VP1 assay amplification products. MCV DNA was detected in 8.52 % of all participants. Among HIV-1 positive patients, 26.4 % (14/53) were positive for MCV, significantly higher than HIV-1-negative patients (5/100, 5 %, $P < 0.01$, Chi-square test). Additionally, 6.1 % and 8.1 % of HBV-positive and HCV-positive samples, respectively, tested positive for MCV (Fig. 1). Mean and median HIV, HBV, and HCV loads for MCV-positive and MCV-negative patients in the respective groups are presented in Table 2. LPV screening in 340 specimens revealed only one LPV-positive patient in the HIV-1 positive group, with no LPV detection in HCV-positive, HBV-positive, and the control group specimens. Co-detection of MCV and LPV was not observed in any of the patient groups (Fig. 1).

Phylogenetic analysis using MEGA 4.0 software and the neighbor-joining algorithm showed that all MCV strains, based on the LTag gene sequences, clustered with MCV reference strains (EU375803; EU375804) with 99 % DNA sequence homology (Fig. 2). LPV sequences demonstrated 99 % identity to published sequences of LPV-K38 in GenBank (Fig. 3).

4. Discussion

In this study, we explored the presence of LPV and MCV in serum samples from four patient groups: those positive for HIV, HBV, HCV, and a control group negative for all three viruses. Our findings revealed a significant detection rate of MCV in HIV-positive patients compared to other groups, with MCV DNA present in 26.4 % of HIV-positive samples. In contrast, LPV was detected in only one HIV-positive patient, suggesting a lower prevalence or different dynamics of LPV infection in this study.

The detection of MCV in HIV-positive individuals is in line with previous research that suggests a possible association between MCV and immunosuppressed states [9,14,15]. The higher MCV positivity observed in HIV-positive patients, particularly those not on antiretroviral therapy and many of whom had progressed to AIDS, could indicate that immunosuppression may play a role in facilitating MCV reactivation. However, since our study focused on HIV-positive patients,

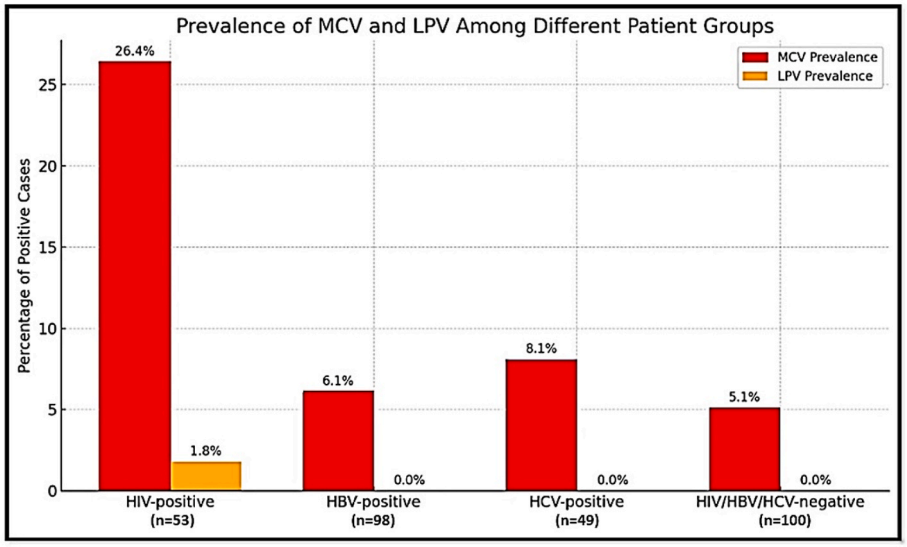

Fig. 1. Prevalence of MCV and LPV among different patient groups. The graph shows the percentage of positive cases in four patient categories: HIV-positive (n = 53), HBV-positive (n = 98), HCV-positive (n = 49), and HIV/HBV/HCV-negative (n = 100). MCV prevalence is significantly higher in HIV-positive patients (26.4 %), with lower rates observed in HBV-positive (6.1 %), HCV-positive (8.1 %), and HIV/HBV/HCV-negative individuals (5.1 %). LPV prevalence is observed only in the HIV-positive group (1.8 %). Importantly, no simultaneous infection of both MCV and LPV was detected in any of the samples.

Table 2
Mean and Median HIV/HBV/HCV Load in MCV positive and negative patients.

	Mean Load (copies/Ml)			Median Load (copies/Ml)		
	HIV +	HBV+	HCV+	HIV+	HBV+	HCV+
MCV Positive Patients	31,900	17,442	1,312,100	27,200	13,645	1,708,000
MCV Negative Patients	141,000	14,592	3,001,100	64,500	12.698	1,171,000

further research is necessary to determine whether similar associations exist in other immunosuppressive conditions [16–18]. For instance, Silling et al. reported a rise in the prevalence of cutaneous MCV among those with HIV [17]. In another study, MCV DNA was detected in 59.0 % of forehead swabs from non-lesional skin of 210 HIV-infected men [18]. Furthermore, individuals with poorly managed HIV exhibited notably higher MCV DNA loads compared to those with well-controlled HIV [18].

While MCV has primarily been associated with MCC, its detection in various non-cancerous tissues and blood raises the possibility of a broader lymphotropic nature [5,19–22]. Our findings, showing MCV DNA in serum samples from HIV, HBV, and HCV-positive patients, suggest that MCV might disseminate through the bloodstream and could potentially reactivate under conditions of immunosuppression. Although the design of our study focuses on the association between MCV and HIV, rather than immunosuppression per se, these results prompt further investigation into the clinical implications of MCV viremia and its potential role in disease progression among immunocompromised individuals. Additionally, the detection of MCV in blood samples supports the theory of its hematogenous spread, which may explain its presence in diverse tissues and its involvement in conditions beyond MCC [19].

On the other hand, the singular detection of LPV in an HIV-positive patient, with sequences matching known LPV strains, highlights the rarity of LPV detection in this study. Previous reports have suggested LPV presence in the human population and its possible involvement in CNS leukoencephalopathies, particularly in HIV-positive individuals [23]. Moreover, Italian researchers reported the amplification of LPV genome from three DNA samples: one extracted from PBMCs obtained from a PML patient and two from PBMCs collected from leukoencephalopathy patients negative for the JC virus [10]. The potential for LPV to infect B lymphocytes and disseminate through PBMCs underscores the need for further research into its transmission routes and prevalence.

The detection of LPV only in one HIV-positive patient might suggest a sporadic presence or reactivation under specific conditions of immunosuppression. Given the polyomavirus family's known behaviors, it is plausible that LPV might reactivate in immunocompromised states, similar to other polyomaviruses like JC virus, which is known to cause PML in such populations.

Our study emphasizes the necessity for comprehensive molecular and serological investigations to understand the epidemiology and clinical significance of LPV and MCV infections, especially in immunocompromised populations. Future research should focus on larger-scale studies with standardized methodologies to elucidate the interactions between these viruses and other pathogens, and their impact on health outcomes. Understanding the transmission dynamics and persistence of LPV and MCV in the human population will be crucial for developing effective diagnostic and therapeutic strategies.

In conclusion, our research contributes to the growing body of knowledge on the prevalence of LPV and MCV in individuals with chronic viral diseases. The significant detection of MCV in HIV-positive patients and the isolated LPV case highlight the importance of these viruses in immunocompromised states. Further investigation is warranted to fully understand their role and to develop appropriate interventions to mitigate their impact on vulnerable populations.

Limitations: While our study provides valuable insights into the prevalence of MCV and LPV among individuals with chronic viral infections, it is not without limitations. First, the study sample size, particularly in the subgroups of HIV, HBV, and HCV-infected individuals, is relatively small. This may limit the generalizability of our findings to broader populations. Larger studies are needed to validate these results and to better understand the true prevalence of LPV and MCV in different patient groups.

Second, the cross-sectional nature of this study does not allow for conclusions about causality or the temporal relationship between viral infections and immune status. Longitudinal studies would be necessary to determine whether LPV or MCV infections precede or are a consequence of immune suppression in these patients.

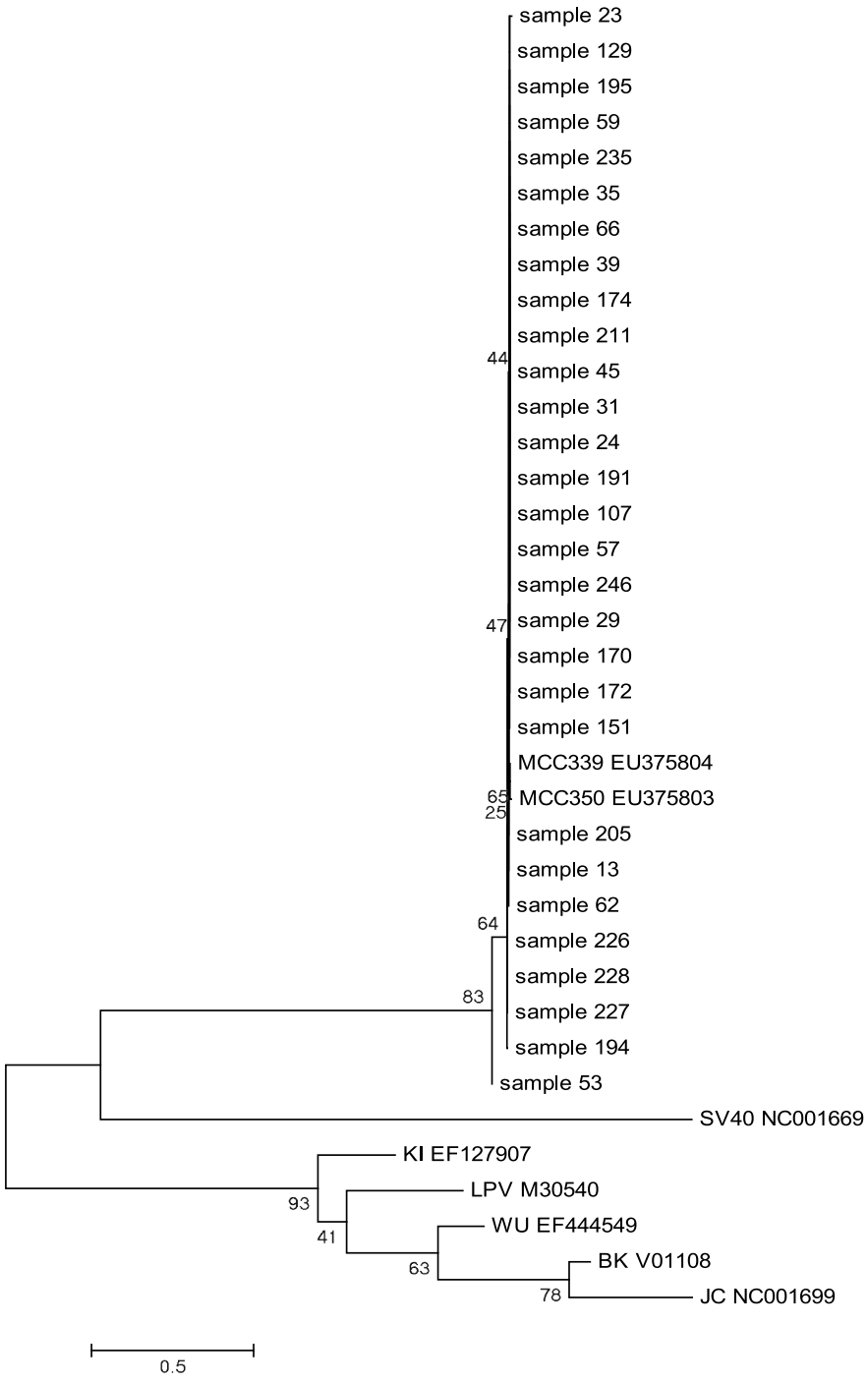

Fig. 2. Phylogenetic analysis of nucleotide sequences of MCV large T antigen (LT) partial genes from positive sera. The tree was built with the MEGA 4.0. Software by using neighbor-joining algorithm; bootstrap values were determined by 1000 replicates; the percentage bootstrap values are shown at nodes. The scale indicates 0.5 substitutions per base pair indicated horizontal distance. The analysis includes MCV references sequences from GenBank including MCC339, EU375804 and MCC350, EU375803 and representative BK, JC, KI and WU, LPV, SV40 sequences from GenBank.

Third, the use of serum samples alone might not fully capture the presence of these viruses in other body compartments where they might be more prevalent, such as in the skin for MCV or in the central nervous system for LPV. The lack of data from other tissues limits our understanding of the full spectrum of viral dissemination and pathogenesis.

Lastly, while we employed rigorous molecular methods to detect these viruses, the possibility of false negatives cannot be entirely ruled out, especially given the low viral loads typically associated with LPV. More sensitive assays or alternative sample types, such as cerebrospinal fluid for LPV or skin swabs for MCV, could potentially yield higher detection rates and more comprehensive insights.

Future research should aim to address these limitations by incorporating larger, more diverse cohorts, longitudinal follow-up, and multi-tissue sampling to provide a more complete picture of the role of MCV and LPV in immunocompromised individuals.

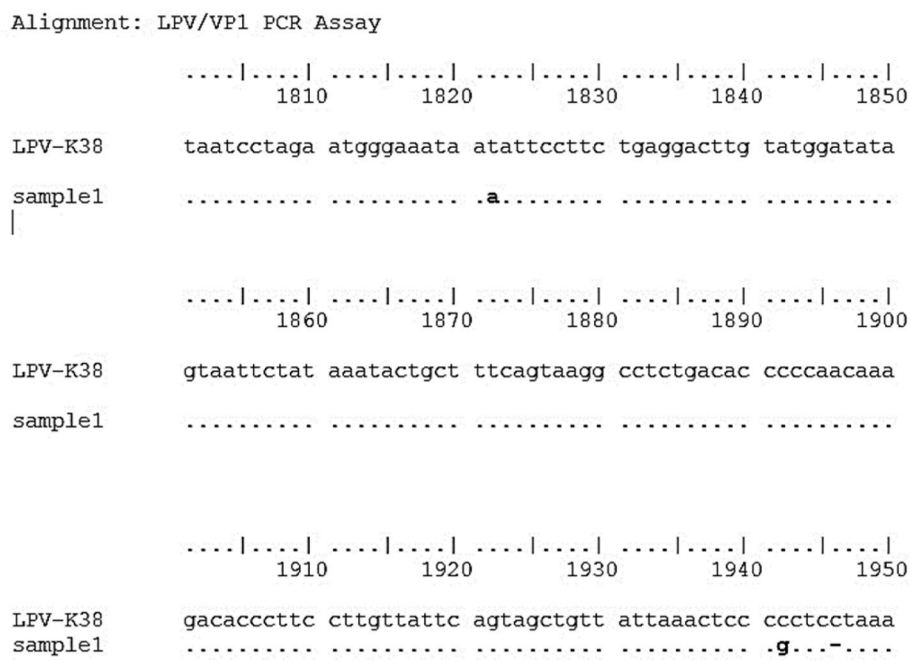

Fig. 3. Nucleotide sequences of the partial VP1 antigen of LPV isolates compared to positive control LPV- K38. Each sample is identified at the left. Dots indicate identity and dashes indicate a deletion compared to the sequence of positive control, which is given on the top line.

Ethical statement

The final manuscript's content has been reviewed and approved by all of the authors.

Data availability

The original data underpinning the findings and conclusions presented in this article can be provided by the authors upon a reasonable request.

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CRediT authorship contribution statement

Bahman Abedi Kiasari: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Amir Hossein Alipour:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Negar Hemmati:** Writing – review & editing, Methodology, Formal analysis. **Mohammad Gholamnezhad:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Fatemeh Hoda Fallah:** Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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