Breif Original Article | Iran J Med Microbiol. 2025; 19(4): 278-282

Category: Medical Virology | Peer Reviewed | Open Access

10.30699/ijmm.19.4.278

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Varicella Zoster Virus as a Potential Risk Factor in Stroke: A Pilot Case-Control PCR-Based Investigation

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ABSTRACT

Background and Aim: Viral contributions to cerebrovascular disease are increasingly recognized. Varicella zoster virus (VZV) can cause a variety of pathologies in humans, including stroke, which increases the risk of developing the condition. As part of a broader pilot project on neurotropic herpesviruses (VZV/CMV/HSV), we aimed to evaluate the prevalence of VZV DNA in stroke patients compared with demographically matched controls and to estimate the strength of association.

Materials and Methods: In this pilot case–control study, whole blood sample was obtained from 28 consecutive adult stroke patients admitted to ICUs in northern Iran and 28 demographically matched controls without stroke. DNA was extracted and quality-checked by spectrophotometry. VZV DNA was detected by conventional PCR using virus-specific primers. Beta-globin was used as internal control. For the statistical comparisons chi-square test was used.

Results & Conclusion: VZV DNA was detected in 7/28 (25.0%) stroke cases and 0/28 (0%) controls (χ^2 P=0.005). The analysis based on the chi-square test showed a significant association (P=0.005) between the frequency of VZV and stroke with 95% confidence. This pilot study suggests an association between VZV DNA presence and stroke occurrence. Given the limited sample size and cross-sectional design, causality cannot be inferred and reactivation remains a plausible explanation. Larger, time-resolved studies incorporating serology and mechanistic biomarkers are warranted.

Keywords: Neurotropic Virus, PCR, Stroke, Varicella Zoster Virus, VZV DNA

Received: 2025/06/24; **Accepted**: 2025/09/06; **Published Online:** 2025/10/10;

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1. Introduction

Stroke is a leading cause of death and disability globally. Beyond traditional risk factors, infections including neurotropic herpesviruses have been linked to vascular events. Population-level studies have suggested that viral infections may contribute to stroke risk, though unclear mechanisms (1). VZV establishes lifelong latency with capacity for reactivation and vasculopathy (2). Building on our regional work on herpesviruses and stroke, we report here the VZV arm of a broader pilot study that screened three neurotropic herpesviruses (VZV/CMV/HSV) in stroke

patients. This study was designed to determine the prevalence of VZV DNA in peripheral blood of stroke patients compared with demographically matched controls and to estimate the strength of association in this pilot dataset.

2. Materials and Methods

2.1 Study design and setting

This pilot case–control study was conducted in 2016 across ICU wards of hospitals in northern Iran. Blood samples were collected from 28 consecutive adult stroke patients (≥18 years) with neurologist-confirmed acute stroke (ischemic or hemorrhagic) based on clinical evaluation and neuroimaging. Additionally, 28 blood samples were collected from individuals in the control group, consisting of adults without current or prior stroke/transient ischemic attack (TIA), frequency-matched to cases by age (±5 years) and sex; recruited from hospital staff and community volunteers. Controls had no acute infection symptoms at the time of sampling.

Inclusion criteria were age ≥18 and ability to provide consent or proxy consent, and exclusion criteria were prior antiviral therapy within 14 days, current chemotherapy or high-dose steroids at sampling time, known primary immunodeficiency, and inability to provide a blood sample. Whole blood was collected at ICU admission for stroke onset. Control samples were collected at a single visit during the same calendar period. Stroke patients were confirmed by a neurologist, and questionnaires and samples were collected with the patients' consent and in accordance with medical ethics.

2.2 Sample collection and DNA extraction

Blood samples were collected during the acute phase of stroke. For the patients admitted directly with a new stroke/TIA diagnosis, sampling was performed within several hours to one day after ICU admission. For patients already hospitalized at the time of investigator arrival (e.g., referrals from other centers), sampling was conducted during the first 1–3 days of hospitalization. Peripheral blood was taken into sodium citrate tubes and transported to the Genetics Research Laboratory (Tonekabon Branch) at 4°C. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen) per manufacturer's instructions. DNA yield and purity were assessed by spectrophotometry (A260/A280).

2.3 PCR assays

Human beta-globin fragment (122 bp) was co-amplified in each sample as an internal control for extraction and amplification quality.. VZV detection was performed using virus-specific primers targeting a 267 bp region of the VZV genome. Specific primers were synthesized by TAG Copenhagen, Denmark. The primers sequences are provided in Table 1 with source citations. For detection of VZV genome in the samples, PCR was conducted using specific primers **(3)**. Each reaction was carried out in a total volume of 20 µL. Thermal cycling conditions were as follows: 95°C for 5 min; 40 cycles of 94°C for 45 s, 54.5°C for 40 s, and 72°C for 35 s; followed by a final extension at 72°C for 10 min. Amplicons were resolved on 1.5% agarose gel (100 bp ladder), stained with ethidium bromide, and visualized by UV transillumination. For the quality assurance and contamination control each PCR run included: (i) no-template control (NTC); (ii) extraction blank; and (iii) a confirmed VZV-positive control DNA. All positive clinical samples were re-amplified in duplicate from the original extract for confirmation. Pre- and post-amplification areas were physically separated with unidirectional workflow and aerosol-resistant tips.

2.4 Statistical Analysis

Proportions were compared using the chi-square test to determine whether there was a significant relationship between the prevalence of VZV in stroke patients and stroke incidence. Analyses were performed in SPSS v17 and verified by manual calculations.

Table 1. Primer sequences used for VZV and beta-globin amplification

Primer	Primer sequence 5'→ 3	PCR product (bp)
Betaglobin-F	5- TCC AAC ATC AAC ATC TTG GT-3	122
Betaglobin-R	5- TCC CCC AAA TTC TAA GCA GA- 3	
VZV-F	5-ATGTCCGTACAACATCAACT-3	267
VZV-R	5- CGATTTTCCAAGAGACGC-3	

3. Results & Discussion

3.1 Participants

Fifty-six individuals were included (28 cases, 28 controls). Demographic characteristics are summarized in Table 2.

3.2 Assay performance

Beta-globin (122 bp) amplified in all samples confirmed DNA quality. All NTCs and extraction blanks were negative. The positive control was amplified as expected (Figure 1).

3.3 VZV detection

VZV DNA was detected in 7 of 28 stroke patients (25.0%), whereas none of the 28 control subjects tested positive (0%). The difference in detection rates between two groups was statistically significant (χ^2 test, P=0.005) (Figure 2).

Data are presented as number of participants and percentage within each group (n/N, %). Age groups were categorized as <40, 40–60, and >60 years. Sex, history of cardiovascular disease, hypertension, diabetes mellitus, and family history of stroke were reported as counts and percentages. Percentages were calculated relative to the total number of participants in each group (stroke or control).

3.4 Discussion

In this pilot case–control study, VZV DNA was more frequent among stroke patients than controls. These findings align with epidemiologic and mechanistic evidence implicating VZV reactivation in cerebrovascular events **(4)**, and mechanistic studies have shown potential pathways for VZV-induced vasculopathy **(5, 6)**. Importantly, our cross-sectional blood-based design cannot establish causality; detection may reflect reactivation accompanying critical illness rather than a direct cause of stroke. As a pilot study with a small sample size, our findings are exploratory and should be interpreted with caution **(7)**.

If validated, incorporating targeted viral diagnostics and risk stratification particularly in older or immunocompromised patients may be informative. However, proposals regarding zoster vaccination as a stroke-prevention strategy should be framed cautiously, recognizing that current evidence for direct stroke prevention is suggestive but not definitive and arises largely from observational data **(8, 9)**.

This analysis represents the VZV component of a broader pilot screening of three neurotropic herpesviruses (VZV/CMV/HSV) in regional stroke cohorts; our earlier local work detected CMV DNA in stroke patients from the same area **(10)**. Integrated multi-virus analyses with larger samples are underway, in line with recent evidence that cumulative herpesvirus infection burden may contribute to stroke risk **(11, 12)**. Larger, time-resolved studies incorporating standardized sampling windows, VZV serology, and viral load quantification, CSF testing when clinically indicated, and vascular biomarker panels are warranted. Prospective cohorts should assess outcomes and interactions with vaccination status.

Table 2. Baseline characteristics of stroke patients and control subjects.

Variable		Stroke (n=28)	Control (n=28)
Age (years)	<40	1 (3.57)	1 (3.57)
	40–60	6 (21.43)	20 (71.43)
	>60	21 (75)	7 (25)
Sex	Male	13 (46.43)	13 (46.43)
	Female	15 (53.57)	15 (53.57)
History of cardiovascular disease	Yes	8 (28.6)	3 (10.71)
	No	20 (71.4)	25 (89.29)
History of stroke in family	Yes	5 (17.86)	0 (0)
	No	23 (82.14)	28 (100)
Hypertension	Yes	9 (32.14)	11 (39.29)
	No	19 (67.86)	17 (60.71)
Diabetes mellitus	Yes	9 (32.14)	1 (3.57)
	No	19 (67.86)	27 (96.43)

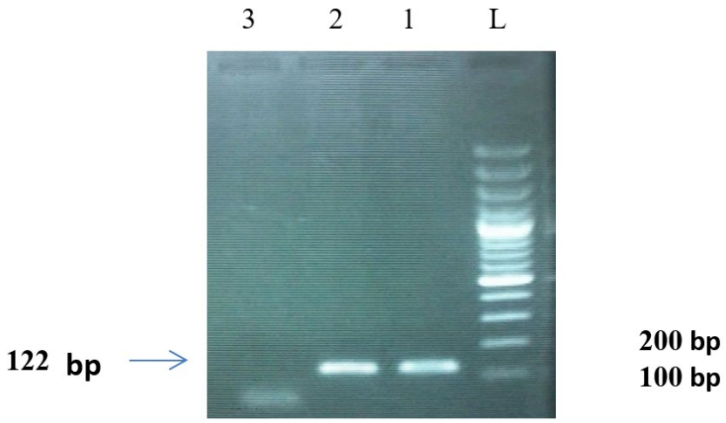

Figure 1. Electrophoresis of representative PCR products targeting beta-globin gene on 1.5% agarose gel. Lane L: 100–1000 bp DNA ladder; lanes 1–2: DNA from patient samples; lane 3: negative control **(Prepared by Authors, 2025)**.

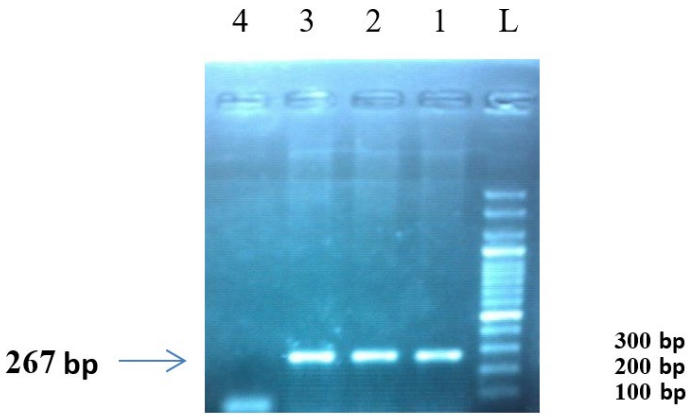

Figure 2. PCR products for VZV detection on 1.5% agarose gel. Lane L: 100–1000 bp DNA ladder; lane 1: positive control; lanes 2–3: VZV-positive patient samples; lane 4: negative control **(Prepared by Authors, 2025)**.

4. Conclusion

In this pilot case–control study from northern Iran, VZV DNA was detected in a subset of stroke patients but not in matched controls, supporting an association between VZV and stroke. Confirmation in larger cohorts with mechanistic assessments is required.

5. Declarations

5.1 Acknowledgment

The authors thank the staff of the participating hospitals for their assistance with sample collection and the laboratory team for technical support.

5.2 Ethical Considerations

This study was conducted using human blood samples collected under the ethical approval of the primary research protocol (Ethics Code: IR.SBMU.RIGLD.REC.1395.97), which covered sample collection, handling, and storage. No separate ethics approval was required for this secondary analysis, as confirmed by the institutional review board. Verbal informed consent was obtained from all participants prior to sample collection. All procedures adhered to institutional ethical standards and to the principles of the 1964 Helsinki Declaration and its later amendments. Participant confidentiality was strictly maintained throughout the study.

5.3 Authors' Contributions

FM performed laboratory experiments, collected samples, analyzed the data, and drafted the manuscript. MG designed the study, provided methodological supervision, and critically revised the manuscript. Both authors read and approved the final version of the manuscript.

5.4 Conflict of Interests

The authors declare no competing interests.

5.5 Financial Support and Sponsorship

This research received no external funding.

5.6 Using Artificial Intelligence Tools (AI Tools)

The authors declare that no artificial intelligence tools were used for data analysis, image generation, or manuscript writing. All parts of the study and manuscript were prepared manually by the authors.

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