Serum microRNA Levels including miR-125a-5p, miR-125b-5p, and miR-433-5p as Biomarkers for Differentiating Acute Vertigo between Cerebellar or Brainstem Infarction and Peripheral Vertigo (Interim Analysis)

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Abstract

Background: Differentiating central vertigo caused by posterior circulation stroke from peripheral vertigo remains diagnostically challenging. Early ischemic lesions may be missed on neuroimaging, and bedside examination can be limited in patients with severe symptoms. Circulating microRNAs (miRNAs) have emerged as potential biomarkers of cerebrovascular injury. This study aimed to validate the diagnostic performance of serum miR-125a-5p, miR-125b-5p, and miR-433-5p in distinguishing central from peripheral vertigo, while extending the age range to improve generalizability in clinical practice.

Methods: This single-center prospective cohort study enrolled adults presenting with acute vertigo within 72 hours of symptom onset. Central vertigo was diagnosed by neurologists using clinical and neuroimaging criteria, while peripheral vertigo was diagnosed by otolaryngologists or by the absence of stroke on MRI. Acute-phase venous blood samples were collected, and serum miRNA levels were quantified using absolute RT-qPCR. Group comparisons were performed using appropriate statistical tests, with $p<0.05$ considered significant. This is an interim analysis.

Results: A total of 48 patients were enrolled (central=27; peripheral=21). Serum miRNA levels did not differ significantly between groups. Median levels of miR-125a-5p (278.27 vs. 324.78, p=0.8111) and miR-125b-5p (570.43 vs. 519.60, p=0.4734) were comparable between central and peripheral vertigo. Median miR-433-5p levels were higher in the central group (51.81 vs. 28.97), though not statistically significant (p=0.4117).

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Conclusion: Although no significant differences were observed, miR-433-5p showed a trend toward higher levels in central vertigo. Interpretation is limited by reduced statistical power due to incomplete sample size, and continued recruitment is required to determine the diagnostic performance of these miRNAs.

Keywords : Acute vertigo, Posterior circulation stroke, Central vertigo, Peripheral vertigo, Micro RNA, Biomarkers, miR-433-5p, miR-125a-5p, miR 125b-5p

Introduction

Acute ischemic stroke is a leading cause of death and disability in Thailand and worldwide[1,2]. Moreover, the incidence of ischemic stroke among young adults is rising globally, especially in developing countries, including Thailand. In recent years, the prevalence of stroke among patients under 40 years of age in Thailand has increased[3,4]. Vertigo is one of the common presentations of posterior circulation stroke, and differentiating central vertigo due to cerebellar or brainstem infarction from peripheral vertigo remains a major clinical challenge. Clinical assessment is often limited by severe vertiginous symptoms. Neuroimaging also has limitations, as computed tomography (CT) has low sensitivity for posterior fossa infarction[5], and early diffusion-weighted magnetic resonance imaging (MRI) performed within 48 hours of symptom onset may miss acute infarction[6]. Blood-based biomarkers have therefore been studied as potential tools to improve diagnostic accuracy in patients presenting with acute vertigo. MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene regulation and have gained attention as stable and disease-specific circulating biomarkers among stroke patients[7-9]. In our previous studies[10], we identified miR-125a-5p, miR-125b-5p, and miR-433-5p as being significantly elevated in patients with acute vertigo due to posterior circulation stroke compared with those with peripheral vertigo.

This study aims to validate the diagnostic performance of these miRNAs for distinguishing central from peripheral vertigo and to extend the age range of enrolled patients to better reflect real-world clinical practice and increase clinical applicability.

Materials and Methods

1. Study design and study population

This was a single-center prospective cohort study conducted between December 2024 and November 2025. The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No. 0476/67). Initial screening was performed by emergency medicine physicians, neurologists, or otolaryngologists who first encountered the patients. Inclusion criteria were patients aged ≥18 years who presented with acute vertigo at King Chulalongkorn Memorial Hospital within 72 hours of symptom onset. Exclusion criteria included altered consciousness; body temperature >38 °C; white blood cell count >15,000 cells/μL; serum creatinine >2 mg/dL; liver enzyme levels >3-fold the upper limit of normal or the presence of cirrhosis; a known history of active malignancy or autoimmune disease; and concurrent intracranial pathology other than acute ischemic stroke. Informed consent was obtained in the hospital after patient encounter in the emergency department, outpatient clinic, or inpatient ward in cases of hospitalization.

2. Diagnosis of central and peripheral vertigo

The diagnosis of central vertigo due to acute ischemic stroke (cerebellar or brainstem infarction) was made by a neurologist based on clinical evidence of an episode of neurological dysfunction caused by focal cerebral or cerebellar infarction and was confirmed by imaging evidence of infarction in a defined vascular distribution, demonstrated by restricted diffusion on diffusion-weighted MRI or by a lesion detected on CT scan. The diagnosis of peripheral vertigo was confirmed either by a board-certified otolaryngologist according to established diagnostic criteria and classified into benign paroxysmal positional vertigo (BPPV)[11], Ménière's disease[12], or vestibular neuritis, or by the absence of evidence of acute ischemic stroke on brain MRI.

3. Specimen collection and processing

Venous blood samples were obtained during the acute phase and at a 3-month follow-up visit. A total of 8 mL of venous blood was collected into blood collection tubes containing clot activator. After collection, the blood samples were left to clot completely at room temperature for 30–60 minutes and then centrifuged at 1,000 g for 15 minutes at 4°C. The serum supernatant was transferred to a new tube and centrifuged again at 2,500 g for 15 minutes at 4°C to remove residual debris. Serum samples were stored at −80°C until analysis.

4. miRNAs Extraction and quantification

MicroRNAs were isolated from samples using a commercially available miRNA isolation kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) according to the manufacturer's protocol. Purified RNA was stored at −70°C in RNase-free water. Quantification of serum miRNA was performed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Absolute miRNA expression levels were determined using standard curves generated from serial dilutions of known miRNA concentrations. Quantification of miRNA levels was performed by laboratory staff blinded to clinical presentation and neuroimaging findings.

5. Sample size calculation and enrollment

The sample size was calculated based on our previous study[10] using the area under the receiver operating characteristic curve (AUC). Among the three candidate miRNAs, the lowest AUC value (0.71) was used for estimation. With an alpha level of 0.05, 80% power, and an assumed case proportion of 0.5, a total of 100 participants were required. Allowing for an estimated 10% loss to follow-up at 3 months, the final target sample size was set at 110 participants (55 with central vertigo and 55 with peripheral vertigo). Sample size calculation was performed using RStudio.

At the time of this interim analysis, a total of 48 patients with acute vertigo had been enrolled (central = 27, peripheral = 21). Of these, 35 patients reached the 3-month follow-up window. Six patients were lost to follow-up, leaving 29 patients who completed the 3-month evaluation (central = 15, peripheral = 14) (Figure 1). As the number of patients with available 3-month follow-up data remains limited, these data were not included in the current analysis and will be evaluated once additional follow-up data are obtained.

6. Statistical analysis

The distribution of the data was determined using the Kolmogorov–Smirnov test. Continuous variables were presented as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables were presented as frequencies and percentages. Comparisons

between the central and peripheral vertigo groups were performed using the unpaired t-test or Mann–Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. Biomarker levels between groups were compared using the Mann–Whitney U test, while within-group comparisons between acute and 3-month follow-up samples were analyzed using the Wilcoxon signed-rank test. All statistical analyses were performed using STATA version 19.5 (Stata Corp LLC, College Station, TX, USA). A two-sided p-value < 0.05 was considered statistically significant.

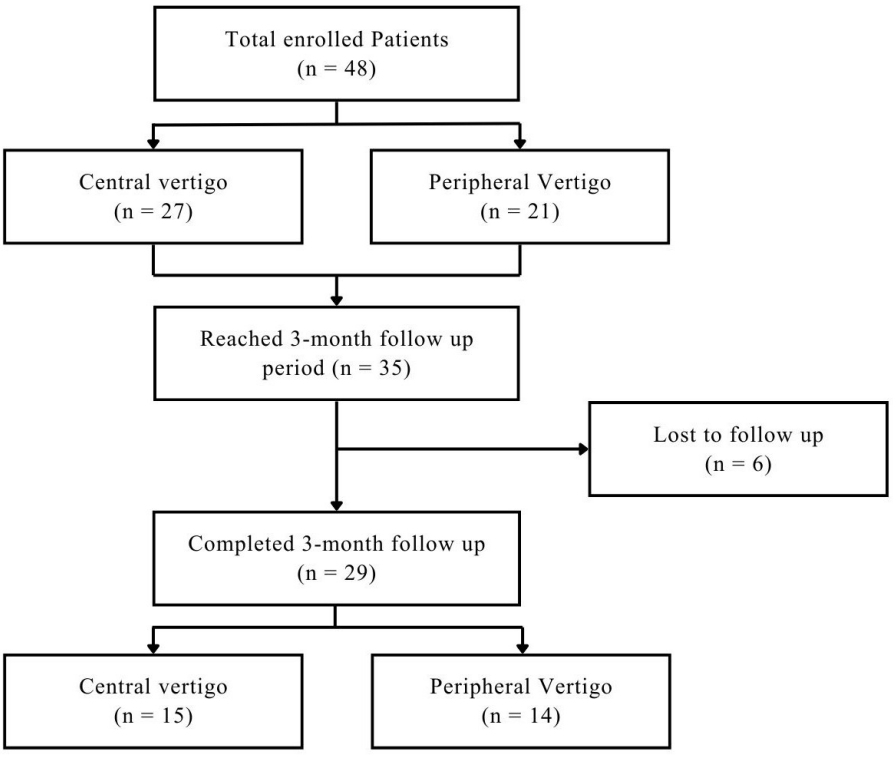

Figure 1: Patient enrollment

Results

1. Patient characteristics

A total of 48 patients presenting with acute vertigo were included in the study, consisting of 27 patients with central vertigo and 21 patients with peripheral vertigo. Patients with posterior circulation stroke included cerebellar infarction (37%), brainstem infarction (41%), and combined cerebellar and brainstem infarction (22%). In the peripheral vertigo group, there were patients with BPPV (38%), vestibular neuritis (19%), and MRI-negative other peripheral vertigo (42%). The baseline characteristics are summarized in Table 1.

The mean age was comparable between the central and peripheral vertigo groups (66.26 ± 12.58 vs. 65.38 ± 13.84 years, p = 0.819). The proportion of female patients did not differ significantly between groups (55.6% vs. 57.1%, p = 0.912). Patients with central vertigo had a significantly longer onset-to-blood collection time compared with those with peripheral vertigo (41.87 ± 16.90 vs. 25.03 ± 17.55 hours, p = 0.0018) (Table 1).

Most vascular risk factors were more prevalent in the central vertigo group, including hypertension (88.9% vs. 66.7%), diabetes mellitus (40.7% vs. 23.8%), and dyslipidemia (92.6% vs. 80.9%), although these differences did not reach statistical significance. Smoking history, past smoking, and alcohol intake were observed only in the central vertigo group. Regarding vital signs, systolic and diastolic blood pressures were significantly higher in patients with central vertigo (159 vs. 151 mmHg, p = 0.0345; and 90 vs. 83 mmHg, p = 0.0009, respectively). Atrial fibrillation was more frequently observed among central vertigo patients (14.8% vs. 4.8%), although the difference was not statistically significant. Laboratory parameters were similar between groups, except that patients with central vertigo had significantly higher fasting blood glucose (114 vs. 93 mg/dL, p = 0.0221) (Table1).

2. Clinical presentation

Neuro-otologic findings are shown in Table 2. A positive head impulse test (HIT) was predominantly observed in patients with peripheral vertigo (38.1% vs. 3.7%), with a statistically significant difference between groups (p = 0.001). Various patterns of nystagmus were observed in both groups. Bilateral direction-changing and vertical nystagmus were more frequent in the central vertigo group, whereas unidirectional nystagmus was more common in the peripheral vertigo group. However, none of the nystagmus subtypes reached statistical significance. There was also a significant difference in functional disability at presentation, with patients in the central vertigo group having higher modified Rankin Scale (mRS) scores compared with those in the peripheral vertigo group (p = 0.010).

Table 1 Baseline characteristics, laboratory parameters

	Central vertigo (n=27)	Peripheral vertigo (n=21)	P-value
Age, year, mean (SD)	66.26 (12.58)	65.38 (13.84)	0.819
Female% (n)	55.6% (15)	57.1% (12)	0.912
Onset to blood collection (hrs), mean(sd)	41.87 (16.90)	25.03 (17.55)	0.0018*
Stroke risk factors, % (n)			
Hypertension	88.9% (24)	66.7% (14)	0.081
Diabetes mellitus	40.7% (11)	23.8% (5)	0.217
Dyslipidemia	92.6% (25)	80.9% (17)	0.383
Ischemic heart disease	8% (2)	4.8% (1)	1.000
Smoking	11.1% (3)	0	0.246
Past smoking	14.8% (4)	0	0.121
Alcohol	3.7% (1)	0	1.000
Past drinking	3.7% (1)	0	1.000
Systolic BP, mmHg, median (IQR)	159 (30)	151 (22)	0.034*
Diastolic BP, mmHg, median (IQR)	90 (14)	83 (12)	0.001*
Pulse rate, bpm, median (IQR)	80 (20)	74 (14)	0.243
Atrial fibrillation % (n)	14.8% (4)	4.8% (1)	0.369
Laboratory parameters			
Hemoglobin, g/dL, med (IQR)	13.6 (3.5)	13.1 (1.4)	0.212
WBC count, $*10^3$/μL, mean (SD)	9.14 (2.51)	6.87 (1.81)	0.001*
Platelets, $*10^3$/μ, med (IQR)	247 (88)	222 (70)	0.164
Cr, mg/dL, med (IQR)	0.74 (0.22)	0.77 (0.26)	0.43
Fasting blood sugar, mg/dL, med (IQR)	114 (42)	93 (24)	0.022
HbA1c, %, med (IQR)	5.8 (2.4)	5.75 (0.9)	0.508

*P < 0.05

Table 2 Clinical presentation

Clinical presentation, % (n)	Central vertigo (n=27)	Peripheral vertigo (n=21)	P-value
Carotid bruit	0	0	-
Murmur	0	0	-
Irregular heartbeat	3.7% (1)	4.8% (1)	1.000
Positive HIT			0.001*
- Not presence	96.3% (26)	61.9% (13)	
- Presence (left)	0	28.6% (6)	
- Presence (right)	3.7% (1)	9.52% (2)	
Nystagmus	48.12% (13)	42.9% (9)	0.715
- Bilateral direction changing	22.2% (6)	9.5% (2)	0.437
- Vertical	7.4% (2)	0	0.497
- Torsional	14.8% (4)	14.3% (3)	1.000
- Unidirectional horizontal	25.9% (7)	33.3% (7)	0.575
Skew deviation	0	0	-
mRS			0.010*
- 1	18.5% (5)	61.9% (13)	
- 2	18.5% (5)	14.3% (3)	
- 3	29.6% (8)	19.0% (4)	
- 4	33.3% (9)	4.8% (1)	
- 5	0	0	

HIT head impulse test

*P < 0.05

3. Serum biomarker expression level

At this point of interim analysis, serum miRNA expression levels measured during the acute phase did not show significant differences between central and peripheral vertigo. Median miR-125a-5p levels were similar between groups (278.27 [IQR 350.10] vs. 324.78 [IQR 288.90], p = 0.8111). Similarly, miR-125b-5p showed no significant group difference (570.43 [IQR 974.10] vs. 519.60 [IQR 1293.55], p = 0.4734). Median miR-433-5p levels were slightly higher in the central group compared to the peripheral group (51.81 [IQR 57.63] vs. 28.97 [IQR 51.18]), but the difference did not reach statistical significance (p = 0.4117).

Table 3 Biomarker level during acute phase in patient with central and peripheral vertigo

miRNA level [median (IQR)]	Central vertigo (n=27)	Peripheral vertigo (n=21)	P-value
miR-125a-5p	278.27 (350.1)	324.78 (288.9)	0.8111
miR-125b-5p	570.43 (974.1)	519.6 (1293.55)	0.4734
miR-433-5p	51.81 (57.63)	28.97 (51.18)	0.4117

Discussion

In this interim analysis, serum miRNA levels measured during the acute phase did not show statistically significant differences between central and peripheral vertigo. However, miR-433-5p tended to be higher in patients with central vertigo, suggesting a possible biological marker that may become clearer once more patients are included. Since the study has not yet reached its calculated sample size, the current results may be affected by limited statistical power and should be interpreted with caution. Technical factors may also contribute to the lack of statistical significance at this stage. Mei et al. [13] showed that miRNA quantification using RT-qPCR requires optimized and sensitive assays, as small differences in miRNA levels can be difficult to detect without proper methodological control. This supports the idea that early non-significant findings do not necessarily rule out potential diagnostic value, especially when the sample size is still small.

From a biological perspective, the trend observed in miR-433-5p may relate to vascular and endothelial changes that occur in posterior circulation stroke. Reijerkerk et al.[14] demonstrated that specific miRNAs can regulate endothelial barrier function during inflammation, affecting blood–brain barrier integrity. Their findings support the broader concept that circulating miRNAs may reflect cerebrovascular or inflammatory processes, which are relevant in central vertigo caused by ischemia.

Conclusion

Overall, the preliminary trend in miR-433-5p is noteworthy, but the current data are insufficient to draw definitive conclusions. Continued patient recruitment will be important to complete the planned sample size and to determine whether miR-433-5p or other miRNAs can help distinguish central from peripheral vertigo. Further analyses with a full dataset will also allow assessment of temporal changes, diagnostic performance, and multivariable associations.

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