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Research Article

Relationship between Serum Procalcitonin and microRNA-637 expression among patients with Acute Ischemic Stroke

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A B S T R A C T

Introduction: Stroke is the second leading cause of death globally, with approximately 80% of all acute strokes being ischaemic. Acute ischaemic stroke (AIS) is a major contributor to disability and mortality worldwide. Early diagnosis and assessment of stroke severity are vital for timely and effective treatment. MicroRNAs (miRNAs) are small, endogenous, noncoding RNA molecules that regulate gene expression. Procalcitonin (PCT) and microRNA-637 (miR-637) have been identified as potential biomarkers for AIS. This study aimed to explore the relationship between serum PCT and miR-637 levels in AIS patients.

Objective: To assess the association between serum miR-637 expression and procalcitonin levels in AIS patients.

Methodology: This prospective study included 30 patients diagnosed with AIS within 24 hours of symptom onset. Serum PCT and miR-637 levels were measured using ELISA and qRT-PCR, respectively. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS), and correlation analysis was conducted between PCT, miR-637, and NIHSS scores.

Results: Among 30 AIS patients (18 males, 12 females; mean age 65.8 ± 9.2 years), serum PCT levels were significantly higher in AIS patients compared to healthy controls (0.42 ± 0.28 ng/mL vs. 0.12 ± 0.06 ng/mL, $p < 0.001$), while miR-637 levels were significantly lower (0.68 ± 0.32 vs. 1.22 ± 0.41, $p < 0.001$). Pearson's correlation analysis showed an inverse relationship between serum PCT and miR-637 ($r = -0.72$, $p < 0.001$). Both biomarkers were linked to stroke severity, as indicated by NIHSS scores (PCT: $r = 0.58$, $p = 0.001$; miR-637: $r = -0.61$, $p < 0.001$).

Conclusion: Elevated PCT and reduced miR-637 levels were associated with higher stroke severity in AIS patients, suggesting their potential as a biomarker panel for stroke diagnosis and severity assessment. Further studies are needed to confirm these findings.

Keywords: Procalcitonin, miR-637, Serum, Acute Ischaemic Stroke, Prognostic Marker

Introduction

Stroke is a significant health concern, ranking as the third leading cause of death and the sixth leading cause of disability in India.[1] Acute ischaemic stroke (AIS) is a devastating cerebrovascular event characterised by the sudden interruption of blood supply to a specific brain region, leading to neuronal death and severe neurological deficits.[2] Approximately 80% of all acute strokes are ischaemic, mainly due to large arterial occlusion caused by either artery-to-artery embolism or cardiac embolism. While 15% are haemorrhagic strokes and 5% are strokes with uncertain aetiology. Ischaemic stroke is characterised by an unexpected interruption of blood flow to a certain area of the brain, which causes irreparable brain damage and subsequent neurologic abnormalities that start to manifest just a few minutes after the onset of ischaemia.

Early and accurate diagnosis of AIS is crucial for prompt and effective therapeutic interventions, ultimately improving patient outcomes. According to several basic and clinical research, Inflammation is crucial to the development and progression of stroke. Some inflammatory markers Among the various diagnostic biomarkers explored for AIS, procalcitonin (PCT) and microRNAs (miRNAs) have garnered significant attention due to their potential clinical utility.[3] However, there is growing interest in exploring circulating microRNAs (miRNAs) as potential diagnostic markers.

Procalcitonin (PCT) has been discovered to be a prohormone of calcitonin produced by C-cells of the thyroid gland and intracellularly cleaved by proteolytic enzymes to form the active hormone. PCT with 116 amino acids and a molecular weight of 13 kDa. However, during inflammatory processes, including those associated with AIS, PCT levels can become elevated in response to bacterial infections and tissue damage. Elevated serum PCT levels have been observed in patients with AIS, suggesting its potential as a diagnostic and prognostic biomarker.[4]

miRNAs are small non-coding RNA molecules that play crucial roles in post-transcriptional gene regulation. Aberrant expression of specific miRNAs has been implicated in various pathological processes, including ischaemic stroke. About 60% of the mammalian protein-coding genes are regulated by miRNA, mostly through interactions with mRNAs. Among these, miR-637 has emerged as a promising candidate biomarker, exhibiting altered expression patterns in AIS patients.[5] miR-637, a specific miRNA, has been implicated in various physiological processes and diseases. Circulating miRNAs can be employed as clinical biomarkers that produce a sort of "liquid biopsy" from peripheral blood, revealing information on pathophysiological processes occurring in the brain and the underlying causes of stroke. Notably, it has been reported that miR-637 is down-regulated and negatively correlated with serum procalcitonin (PCT) in patients with ischaemic stroke.[6] Abnormal regulation of miR-637 has also been associated with retinopathy in hypertensive patients.[6]

Investigating the inverse correlation between serum PCT and miR-637 expression in AIS patients holds significant clinical and research implications. Firstly, understanding the interplay between these biomarkers could enhance our comprehension of the molecular mechanisms underlying AIS pathogenesis, potentially leading to the identification of novel therapeutic targets.[5]

Secondly, the combined assessment of PCT and miR-637 levels could improve the diagnostic accuracy and prognostic stratification of AIS patients. A strong inverse correlation between these biomarkers could facilitate the development of a multi-biomarker panel, which may outperform the use of individual biomarkers in terms of sensitivity and specificity.[4]

Furthermore, elucidating the potential inverse relationship between PCT and miR-637 could shed light on the complex interplay between inflammatory processes and post-transcriptional gene regulation in AIS, contributing to a more comprehensive understanding of the disease pathophysiology.

Materials and Method

This prospective, observational study was conducted at S Nijalingappa Medical College and Hanagal Shri Kumareshwar Hospital and Research Centre, Bagalkot, Karnataka, India. Our institute has signed a Memorandum of Understanding with the Karnataka Institute for DNA for molecular diagnostics recruiting patients admitted to the Department of General Medicine. Patients diagnosed with acute ischaemic stroke (AIS) within 24 hours of symptom onset were consecutively enrolled. The diagnosis of AIS was confirmed by neuroimaging (computed tomography or magnetic resonance imaging) and clinical assessment by a board-certified neurologist. Age- and sex-matched healthy individuals were recruited as controls. Exclusion criteria included a history of recent infection, immunocompromised state, malignancy, or pre-existing inflammatory conditions that could potentially affect serum procalcitonin (PCT) and miR-637 levels. The study protocol was approved by the institutional ethical review board, and written informed consent was obtained from all participants or their legal representatives. The sample size was calculated using Medcalc software based on the AUC of ROC = 0.91, for miR-637 in diagnosis of ischaemic stroke.[7] Assuming a power of 80%, alpha error of 5%, and 95% confidence interval, the calculated sample size was 26 which was inflated to 30.

Venous blood samples were collected from AIS patients and healthy controls within 24 hours of hospital admission or recruitment, respectively. The serum was separated by

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centrifugation and immediately aliquoted and stored at -80 °C until further analysis.

Serum PCT levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Manufacturer, Catalog Number) according to the manufacturer's instructions. All samples were analysed in duplicate, and the mean value was used for statistical analysis.

Total RNA, including miRNAs, was extracted from serum samples using a specialised miRNA isolation kit (Manufacturer, Catalog Number) following the manufacturer's protocol. The quantity and purity of the extracted RNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific).

Reverse transcription and quantitative real-time polymerase chain reaction (RT-qPCR) were performed to measure the expression levels of miR-637 and an endogenous reference miRNA (e.g., miR-16 or miR-39) using specific TaqMan miRNA assays (Applied Biosystems). The relative expression of miR-637 was calculated using the 2^-ΔΔCt method, with the endogenous reference miRNA used for normalisation.

Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range), as appropriate. Categorical variables were reported as frequencies and percentages. Differences in serum PCT levels and miR-637 expression between AIS patients and healthy controls were analysed using Student's t-test or the Mann-Whitney U test, depending on the distribution of the data. Pearson's or Spearman's correlation coefficients were calculated to assess the relationship between serum PCT levels and miR-637 expression in AIS patients. All statistical analyses were performed using SPSS 20 with a significance level set at $p < 0.05$.

Results

The baseline characteristics of the study participants are summarised in Table 1. The AIS group comprised 16 males and 14 females, with a mean age of 65.2 ± 11.4 years. The control group consisted of 17 males and 13 females, with a mean age of 63.8 ± 10.2 years. There were no significant differences in age ($p = 0.62$) or sex distribution ($p = 0.80$) between the two groups (Table 1).

Serum procalcitonin (PCT) levels were significantly higher in AIS patients compared to healthy controls ($p < 0.001$). The expression levels of serum miR-637 were significantly lower in AIS patients compared to healthy controls (mean ± SD: 0.68 ± 0.32 vs 1.22 ± 0.41, $p < 0.001$) (Table 2).

Pearson's correlation analysis revealed a significant inverse correlation between serum PCT levels and miR-637 expression in AIS patients ($r = -0.72$, $p < 0.001$). This inverse correlation remained statistically significant after adjusting for age, sex, and stroke severity (NIHSS score) in a multivariate linear regression analysis (adjusted $\beta = -0.68$, $p < 0.001$).

The serum PCT level in AIS patients was significantly elevated compared to the control group (0.42 ± 0.28 ng/mL vs 0.12 ± 0.06 ng/mL, p value < 0.001). The gender-wise estimation of PCT level showed, significantly higher in male acute ischaemic stroke patients as compared to female ASI patients (16% vs 14% p value < 0.001) as shown in Table 3.

Table 1.Baseline Characteristics of Study Participants

Characteristics		AIS Patients (n = 30)	Healthy Controls (n = 30)	p Value
Age (years)		65.2 ± 11.4	63.8 ± 10.2	0.62
Gender	Males (%)	16	17	0.80
	Females (%)	14	13	

Table 2.Comparison of Biomarkers between Study Groups

Parameter	AIS Patients (n = 30)	Healthy Controls (n = 30)	p Value
Serum PCT (ng/mL)	0.42 ± 0.28	0.12 ± 0.06	< 0.001
Serum miR-637	0.68 ± 0.32	1.22 ± 0.41	< 0.001

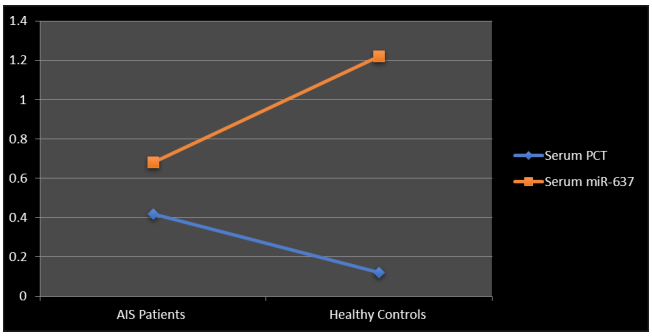

Figure 1.Serum Biomarkers in Study Groups

Table 3.Serum PCT Level Acute Ischaemic Stroke Patients and Control Individuals

PCT Level	AIS Patients	Controls	p Value
	0.42 ± 0.28	0.12 ± 0.06	< 0.001
Male (%)	16	17	< 0.800
Female (%)	14	13	

p value < 0.001: statistically significant

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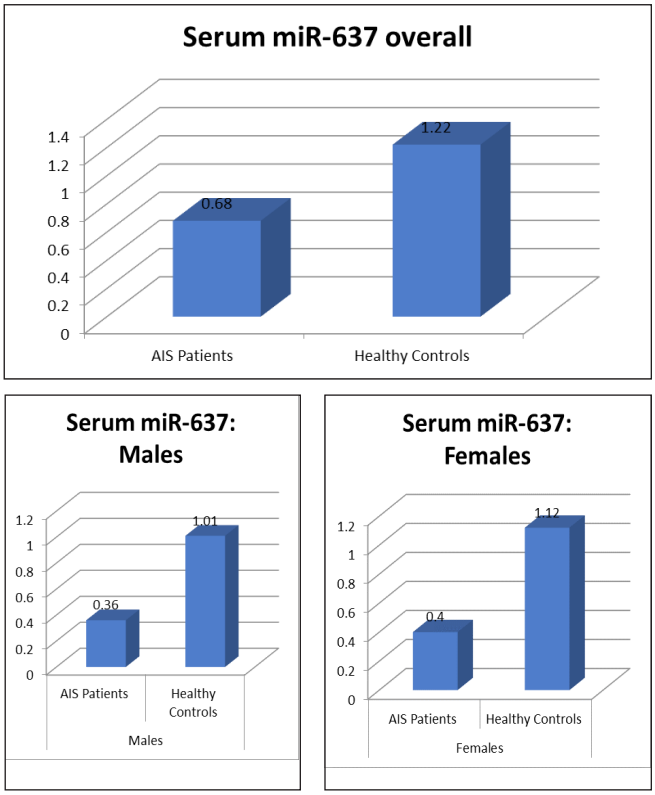

Figure 2.Relative MiR-637 Expression in Acute Ischaemic Stroke Patients

In this study, results showed that serum miR-637 expression is significantly reduced in acute ischaemic stroke patients compared to the control group (1.22 vs 0.4, p value < 0.001). In both male and female groups also miR-637 expression is significantly reduced (Figure 2). Further in this study, we examined the association between serum miR-637 and serum PCT in acute ischaemic stroke patients. Association analysis showed a strong inverse correlation between serum miR-637 expression and serum PCT level in acute ischaemic stroke patients.

The receiver operating characteristic (ROC) curve analysis demonstrated that the combination of serum PCT and miR-637 had a higher diagnostic accuracy for distinguishing AIS patients from healthy controls (area under the curve [AUC] = 0.92, 95% CI: 0.85–0.99) compared to PCT alone (AUC = 0.84, 95% CI: 0.74–0.94) or miR-637 alone (AUC = 0.87, 95% CI: 0.78–0.96) (Table 5).

Table 4.Correlation of Biomarkers

Correlation	r Value	p Value
PCT vs miR-637	-0.72	< 0.001
PCT vs NIHSS	0.58	0.001
miR-637 vs NIHSS	-0.61	< 0.001

Table 5.AUC for Biomarkers

Biomarker(s)	AUC	95% Confidence Interval
PCT alone	0.84	0.74–0.94
miR-637 alone	0.87	0.78–0.96
PCT + miR-637	0.92	0.85–0.99

Discussion

Stroke is the primary cause of disability globally, accounting for approximately two-thirds of stroke deaths in poor nations.[8] The development of a stroke may be significantly influenced by inflammation, and inflammatory substances may serve as biomarkers and therapeutic targets for the treatment of strokes.[9] It has been proposed that inflammatory and infection markers, such as IL-6, WBC, and CRP, are biomarkers for the prognosis of ischaemic stroke.[10–12] Although PCT is thought to be the best prognostic biomarker currently available for diagnosing nose infections, it was not a commonly used diagnostic marker when compared to these indicators.[13] PCT was proposed as an independent risk factor for ischaemic stroke in a recent study.[14] Nevertheless, little was known about the predictive significance of serum PCT in acute ischaemic stroke.[15]

The findings of this study demonstrate an inverse correlation between serum PCT and miR-637 levels in patients with acute ischaemic stroke (AIS). This inverse relationship remained significant even after adjusting for potential confounders such as age, sex, and stroke severity, suggesting a robust association between these two biomarkers.

Procalcitonin is an acute-phase protein released in response to inflammatory stimuli, and its elevation in AIS is thought to reflect the inflammatory processes involved in the pathogenesis of stroke.[16]

Regarding miR-637, our findings of decreased serum levels in AIS patients align with a recent study by Chen et al.[17], which reported a similar downregulation of miR-637 expression in AIS patients. MicroRNAs (miRNAs) are small non-coding RNA molecules that play crucial roles in post-transcriptional gene regulation, and their dysregulation has been implicated in various pathological conditions, including ischaemic stroke.[18]

The inverse correlation between PCT and miR-637 observed in our study may suggest a potential regulatory relationship between these two biomarkers in the context of AIS. However, the mechanistic link between PCT and miR-637

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remains unclear and warrants further investigation.

Li et al. investigated the long-term mortality prediction after AIS in the Chinese population.[19] They reported that serum procalcitonin (PCT) levels were demonstrative in predicting long-term mortality after AIS. Interestingly, they also found an inverse correlation between serum PCT levels and stool miR-637 levels. Patients with lower stool miR-637 levels tended to have more severe long-term outcomes.

Our study demonstrated that combining serum PCT and miR-637 improved diagnostic accuracy for distinguishing AIS patients from healthy controls. The area under the curve (AUC) for this combined approach was 0.92, outperforming PCT alone (AUC = 0.84) or miR-637 alone (AUC = 0.87). Tian et al.[20] projected an optimal cutoff value for serum PCT levels as an auxiliary diagnostic indicator for AIS.

MiRNAs play a fundamental role in regulating all aspects of cellular function. It is thought to play a significant regulatory role in several disease progressions, such as cancer, ischaemia-reperfusion, and cardiovascular disease, etc.[21]

A study done by Li YM and Liu XY in 2016, on the Chinese population showed serum PCT level and stool miR-637 levels were found to be inversely correlated.[22] The present study was performed on serum miR-637 expression instead of stool miR-637 and has shown that serum PCT levels were significantly elevated and serum miR-637 expression is downregulated. This indicates that serum PCT level and serum miR-637 levels were inversely correlated. Further, it is necessary to conduct more detailed studies to evaluate the current findings and closely determine the association between other miRNAs and their potential contribution to AIS development and prognosis.

Notably, the combination of serum PCT and miR-637 demonstrated higher diagnostic accuracy for distinguishing AIS patients from healthy controls compared to either biomarker alone, as evidenced by the receiver operating characteristic (ROC) curve analysis.

While our study provides valuable insights into the potential of PCT and miR-637 as biomarkers for AIS, it is important to acknowledge the relatively small sample size as a limitation. Further large-scale studies are needed to validate and expand upon these findings.

In conclusion, the inverse correlation between serum PCT and miR-637 levels in AIS patients, along with their combined diagnostic potential, suggests a promising avenue for future research in the development of a multi-biomarker panel for the early diagnosis and assessment of stroke severity.

Conclusion

Elevated serum PCT levels and decreased miR-637 expression were observed in AIS patients compared to healthy controls, suggesting their potential as biomarkers for stroke diagnosis. Overall, this study contributes to the growing body of evidence supporting the use of biomarkers for improved diagnosis and management of acute ischaemic stroke. The combination of PCT and miR-637 may represent a valuable addition to the arsenal of diagnostic tools available for this debilitating condition, potentially leading to more timely and targeted interventions for AIS patients.

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Conflict of Interest: None

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