RESEARCH PAPER

Serum Tnf-A and Nitric Oxide Levels In Relation To Infarct Volume Following Acute Ischemic Stroke: A Cross-Sectional Study

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ABSTRACT

Background: Inflammation and oxidative stress contribute substantially to secondary brain injury following acute ischemic stroke. Tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) are key mediators of these processes; however, their relationships with the extent of cerebral infarction remain incompletely understood. This study aimed to investigate the relationships between serum TNF-α and NO levels and infarct volume in patients with acute ischemic stroke.

Methods: This cross-sectional study included 53 patients with acute ischemic stroke. Serum TNF-α and NO levels were measured using enzyme-linked immunosorbent assay (ELISA), and infarct volume was quantified from non-contrast brain computed tomography using the Broderick method. Data normality was assessed using the Kolmogorov–Smirnov test, and correlations between serum biomarker levels and infarct volume were analyzed using Spearman's rank correlation test. A p value <0.05 was considered statistically significant.

Results: The mean serum TNF-α level was 4.74 ± 3.33 pg/mL, the mean serum NO level was 271.29 ± 331.93 µmol/L, and the mean infarct volume was 53.95 ± 136.44 cm³. Serum TNF-α showed a very weak negative correlation with infarct volume ($r = -0.064$, $p = 0.647$), with no statistically significant relationship. Serum NO showed a weak positive correlation with infarct volume ($r = 0.268$, $p = 0.052$), although this relationship did not reach statistical significance.

Conclusion: Serum TNF-α was not significantly correlated with infarct volume in acute ischemic stroke, while serum NO demonstrated a weak positive correlation that approached but did not reach statistical significance. These findings suggest that single measurements of TNF-α and NO may not adequately reflect the extent of cerebral infarction and support further longitudinal investigation using serial biomarker assessments.

Keywords: acute ischemic stroke; infarct volume; nitric oxide; oxidative stress; tumor necrosis factor-alpha

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INTRODUCTION

Acute ischemic stroke remains a major cause of mortality and long-term disability worldwide and results from an abrupt reduction in cerebral blood flow that initiates a complex cascade of cellular and molecular events [1]. Beyond the primary ischemic insult, infarct progression is influenced by excitotoxicity, oxidative stress, neuroinflammation, endothelial dysfunction, and disruption of the blood–brain barrier [2]. These processes contribute to the conversion of potentially salvageable ischemic tissue into irreversible infarction [3]. Infarct volume therefore represents an important structural indicator of the extent of cerebral tissue injury and may provide an objective measure for investigating biological responses occurring during acute ischemic stroke [4].

Neuroinflammation is a central component of secondary brain injury following cerebral ischemia [5]. Ischemic tissue injury activates microglia, astrocytes, endothelial cells, and infiltrating immune cells, resulting in the release of proinflammatory mediators, including tumor necrosis factor-alpha (TNF-α) [6]. TNF-α can modulate leukocyte recruitment, blood–brain barrier permeability, neuronal survival, and inflammatory signaling through its receptors, TNFR1 and TNFR2 [7]. Although increased circulating TNF-α levels have been reported during the acute phase of ischemic stroke, its relationship with the extent of

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structural brain injury remains uncertain [8]. The biological effects of TNF-α are complex and may vary according to receptor signaling, temporal phase after ischemia, and the magnitude of the systemic and local inflammatory response [9].

Nitric oxide (NO) represents another important mediator in the pathophysiology of cerebral ischemia, with effects that depend on its cellular source and the phase of ischemic injury [10]. Endothelial nitric oxide synthase (eNOS)-derived NO contributes to vasodilation and maintenance of cerebral perfusion, whereas excessive NO production through neuronal and inducible nitric oxide synthase (nNOS and iNOS) may promote oxidative and nitrosative stress [11]. In particular, high NO production can interact with superoxide to generate peroxynitrite, contributing to mitochondrial dysfunction, lipid and protein oxidation, blood–brain barrier disruption, and neuronal injury [12]. TNF-α may further interact with this pathway through inflammatory signaling that promotes iNOS expression, suggesting that inflammation and NO-related oxidative stress represent interconnected mechanisms that may influence infarct progression after acute cerebral ischemia [13].

Despite the established roles of TNF-α and NO in ischemic brain injury, clinical evidence regarding their relationship with infarct volume remains limited and inconsistent, particularly when both biomarkers are evaluated simultaneously [14,15]. Moreover, evidence from Southeast Asian populations remains scarce. Evaluating these circulating biomarkers alongside quantitative neuroimaging may provide further insight into the relationship between systemic inflammatory and oxidative responses and the extent of cerebral tissue injury [16,17]. Therefore, this study aimed to investigate the relationships of serum TNF-α and NO levels with infarct volume in patients with acute ischemic stroke.

MATERIALS AND METHODS

Study Design and Participants

This analytical observational study employed a cross-sectional design and included patients with acute ischemic stroke admitted to Dr. Wahidin Sudirohusodo General Hospital and affiliated hospitals in Makassar, Indonesia. Participants were recruited consecutively during the study period until the required sample size was achieved. A total of 53 patients who fulfilled the eligibility criteria were included in the final analysis.

Eligible participants were patients diagnosed with acute ischemic stroke who met the predefined inclusion criteria. Patients with conditions that could substantially influence systemic inflammatory or oxidative responses were excluded according to the study protocol. All participants underwent clinical evaluation, serum biomarker measurement, and non-contrast brain computed tomography (CT) for infarct volume assessment. Serum TNF-α and nitric oxide (NO) levels were subsequently analyzed in relation to infarct volume.

Clinical and Laboratory Assessment

Clinical and demographic data were collected at the time of hospital admission, including age, sex, and vascular risk factors such as hypertension, diabetes mellitus, heart disease, and smoking history. All participants underwent clinical evaluation and non-contrast brain computed tomography (CT) to confirm acute ischemic stroke and assess the ischemic lesion. Venous blood samples were collected during the acute phase for measurement of serum tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) levels.

Serum TNF-α and NO concentrations were measured using enzyme-linked immunosorbent assay (ELISA). TNF-α levels were expressed in pg/mL, while NO was assessed through its stable metabolites, nitrite and nitrate, and expressed in μmol/L. Infarct volume was assessed using non-contrast brain CT images and calculated manually using the Broderick method. Measurements were performed by an experienced radiologist who was blinded to serum TNF-α and NO levels and the participants' clinical data to minimize assessment bias.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as means and standard deviations, while categorical variables were presented as frequencies and percentages. Data normality was assessed using the Kolmogorov–Smirnov test. Spearman's rank correlation test was used to evaluate the correlations between serum TNF-α and nitric oxide (NO) levels and infarct volume. A two-sided p value <0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Hasanuddin (approval No. 686/UN4.6.4.5.31/PP36/2026). All participants received information regarding the study objectives, procedures, and potential risks before enrollment. Written informed consent was obtained from all participants prior to their participation in the study.

RESULTS

Participant Demographics and Clinical Profile

A total of 53 patients with acute ischemic stroke who fulfilled the eligibility criteria were included in the analysis. Baseline characteristics comprised sex, age, and major vascular risk factors, including hypertension, diabetes mellitus, heart disease, smoking, and other vascular risk factors. The demographic and clinical characteristics of the study participants are summarized in Table 1.

Table 1. *Demographic and Clinical Characteristics of the Study Participants*

Characteristics	Category	n	%
Sex	Male	30	56.6
	Female	23	43.4
Age	25–44 years	3	5.7
	45–59 years	27	50.9
	>60 years	23	43.4
Hypertension	Yes	31	58.5
	No	22	41.5
Diabetes mellitus	Yes	9	17.0
	No	44	83.0
Heart disease	Yes	5	9.4
	No	48	90.6
Smoking	Yes	14	26.4
	No	39	73.6
Other vascular risk factors	Yes	7	13.2
	No	46	86.8

Source: *Primary data*

Male participants accounted for 56.6% of the study population, while 43.4% were female. Most participants were aged 45–59 years (50.9%), followed by those aged >60 years (43.4%). Hypertension was the most prevalent vascular risk factor, affecting 58.5% of participants, followed by smoking (26.4%), diabetes mellitus (17.0%), other vascular risk factors (13.2%), and heart disease (9.4%).

Descriptive Profile of Serum Biomarkers and Infarct Volume

Serum TNF-α and nitric oxide (NO) levels were measured in all participants, while infarct volume was quantified from non-contrast brain CT using the Broderick method. The descriptive values of the two serum biomarkers and infarct volume are presented in Table 2.

Table 2. *Serum TNF-α, Nitric Oxide, and Infarct Volume in Patients With Acute Ischemic Stroke*

Variable	Mean	SD
TNF-α (pg/mL)	4.74	3.33
Nitric oxide (NO) (µmol/L)	271.29	331.93
Infarct volume (cm^3)	53.95	136.44

Source: *Primary data*

The mean serum TNF-α level was 4.74 ± 3.33 pg/mL, whereas the mean serum NO level was 271.29 ± 331.93 µmol/L. The mean infarct volume measured using the Broderick method was 53.95 ± 136.44 cm^3. The relatively large standard deviations, particularly for serum NO and infarct volume, indicate substantial variability among participants.

Distribution of Serum TNF-α and Nitric Oxide Levels Across Infarct Volumes

To characterize the individual distribution of serum biomarkers across infarct volumes, TNF-α and nitric oxide (NO) levels were arranged in descending order and plotted alongside the corresponding infarct volume for each participant. Individual-level data are provided in Supplementary Tables S1 and S2. This descriptive visualization was performed to illustrate interindividual variability before evaluating the relationships between biomarker levels and infarct volume using correlation analysis.

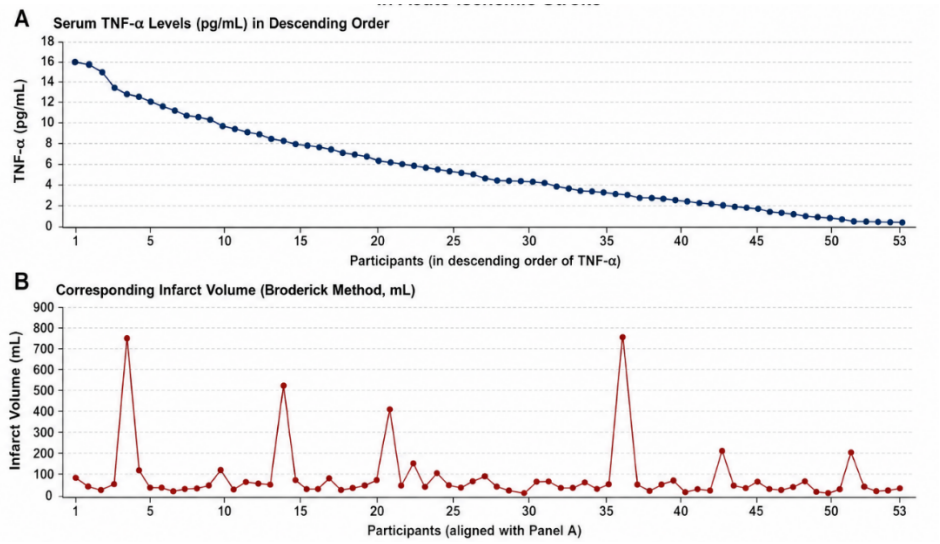

Figure 1. Distribution of Serum TNF-α Levels and Corresponding Infarct Volumes in Acute Ischemic Stroke

Serum TNF-α levels varied substantially across participants, with corresponding infarct volumes showing marked heterogeneity throughout the range of TNF-α concentrations. Larger infarct volumes were observed at several different TNF-α levels, and no consistent parallel pattern between decreasing TNF-α concentrations and infarct volume was visually apparent.

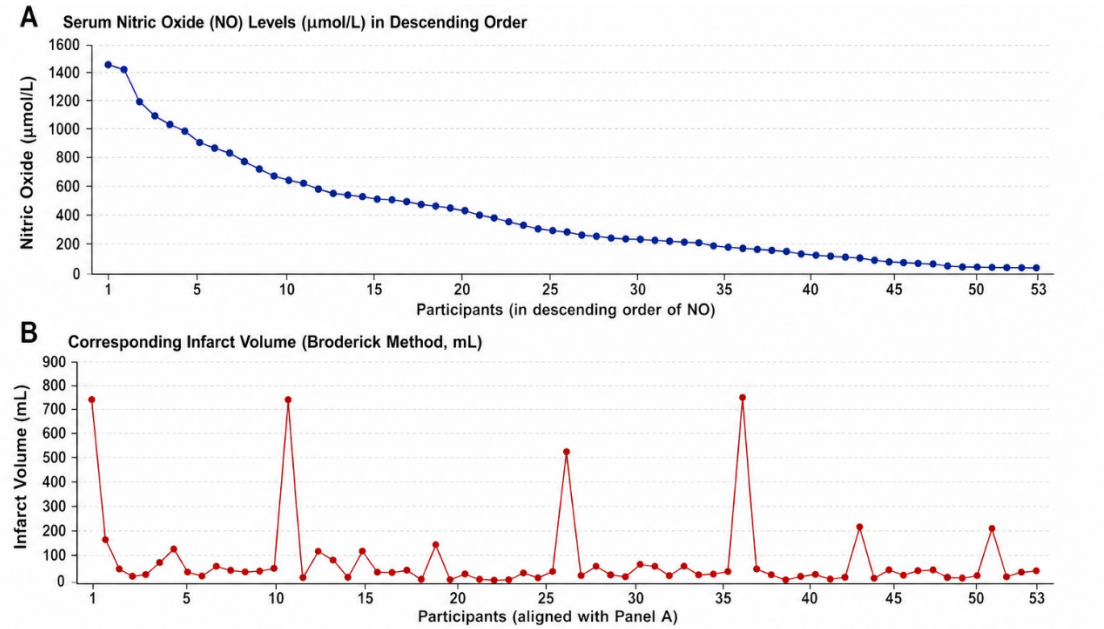

Figure 2. Distribution of Serum Nitric Oxide Levels and Corresponding Infarct Volumes in Acute Ischemic Stroke

Serum NO levels also demonstrated considerable interindividual variability. Corresponding infarct volumes varied across the full range of NO concentrations, with relatively large infarcts occurring at both higher and intermediate NO levels. Although some larger infarct volumes were observed among participants with higher NO concentrations, the descriptive distribution did not demonstrate a uniform pattern across all participants.

Correlation Between Serum TNF-α and Infarct Volume

The relationship between serum TNF-α levels and infarct volume was evaluated using Spearman's rank correlation analysis. This analysis was performed to determine the direction and strength of the association between circulating TNF-α concentrations and the extent of cerebral infarction in patients with acute ischemic stroke.

Table 3. Correlation Between Serum TNF-α Levels and Infarct Volume

Variables	*n*	Spearman's *r*	*p* value
Serum TNF-α vs. infarct volume	53	−0.064	0.647

** Spearman's rank correlation test; statistical significance was defined as $p < 0.05$.*

Spearman's correlation analysis demonstrated a very weak negative correlation between serum TNF-α levels and infarct volume ($r = -0.064$). This association was not statistically significant ($p = 0.647$), indicating that serum TNF-α levels were not significantly correlated with infarct volume in the study population. The relationship between individual serum TNF-α concentrations and infarct volumes is further illustrated in Figure 3 using a scatter plot.

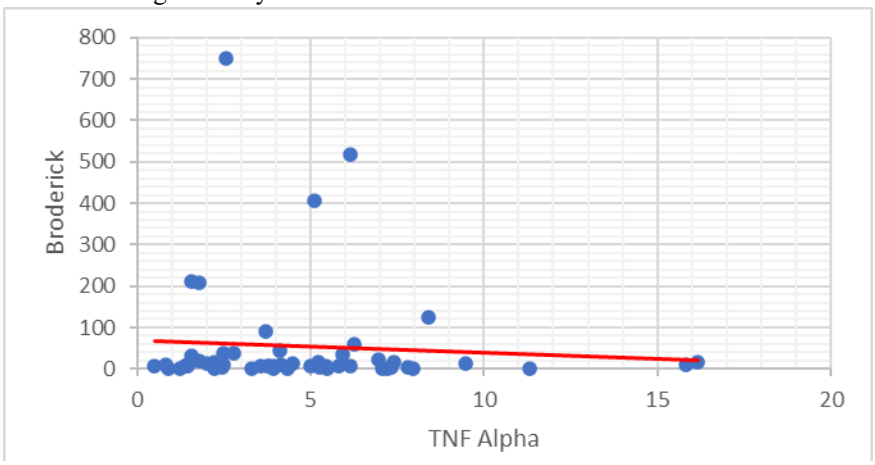

Figure 3. *Correlation Between Serum TNF-α Levels and Infarct Volume in Acute Ischemic Stroke*

The scatter plot demonstrates a broad dispersion of infarct volumes across serum TNF-α concentrations, without a discernible monotonic pattern. Several participants with relatively large infarct volumes had low-to-intermediate TNF-α concentrations, whereas higher TNF-α levels were generally accompanied by comparatively smaller infarct volumes. This visual distribution is consistent with the negligible correlation coefficient observed in the Spearman analysis ($r = -0.064$; $p = 0.647$).

Correlation Between Serum Nitric Oxide and Infarct Volume

The relationship between serum nitric oxide (NO) levels and infarct volume was evaluated using Spearman's rank correlation analysis. This analysis was performed to determine the direction and strength of the association between circulating NO concentrations and the extent of cerebral infarction in patients with acute ischemic stroke.

Table 4. Correlation Between Serum Nitric Oxide Levels and Infarct Volume

Variables	*n*	Spearman's *r*	*p* value
Serum nitric oxide vs. infarct volume	53	0.268	0.052

**Spearman's rank correlation test; statistical significance was defined as $p < 0.05$.*

Spearman's correlation analysis demonstrated a weak positive correlation between serum NO levels and infarct volume (r = 0.268). However, this association did not reach statistical significance (p = 0.052), indicating that serum NO levels were not significantly correlated with infarct volume in the study population. The direction of the correlation nevertheless suggested a tendency toward larger infarct volumes with increasing serum NO levels. The relationship between individual serum NO concentrations and infarct volumes is further illustrated in Figure 4 using a scatter plot.

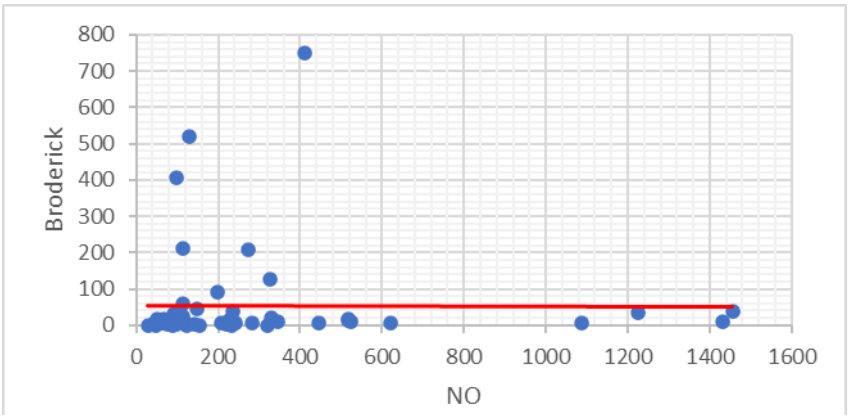

Figure 4. Correlation Between Serum Nitric Oxide Levels and Infarct Volume in Acute Ischemic Stroke

The scatter plot demonstrates substantial dispersion of infarct volumes across the range of serum NO concentrations. A slight upward tendency is visually apparent, consistent with the weak positive Spearman correlation ($r = 0.268$); however, considerable interindividual variability remains and the association

did not reach the predefined threshold for statistical significance ($p = 0.052$).

DISCUSSION

This study evaluated serum TNF-α and nitric oxide (NO) levels in relation to infarct volume among patients with acute ischemic stroke [18,19]. The principal findings were that serum TNF-α showed a very weak negative correlation with infarct volume that was not statistically significant, whereas serum NO demonstrated a weak positive correlation that approached, but did not reach, statistical significance. These findings suggest that circulating inflammatory and nitrosative stress markers do not necessarily parallel the extent of structural cerebral injury in a simple linear manner [20,21]. They also emphasize the biological complexity of the ischemic cascade, in which inflammatory and oxidative responses evolve dynamically and may be influenced by multiple systemic and temporal factors [22].

The absence of a significant relationship between serum TNF-α and infarct volume may reflect the pleiotropic and temporally dependent actions of TNF-α after cerebral ischemia [23]. TNF-α acts through TNFR1 and TNFR2, which may mediate partly opposing biological effects [24]. TNFR1 is more closely associated with pro-inflammatory, cytotoxic, and apoptotic signaling, whereas TNFR2 may contribute to immune regulation, tissue repair, angiogenesis, and neuroplasticity [25]. Consequently, a single peripheral TNF-α measurement may represent the combined activity of several biological pathways rather than directly reflecting the amount of irreversibly injured brain tissue [26-27].

The lack of a clear TNF-α–infarct volume relationship is also consistent with the temporal heterogeneity of inflammatory signaling after stroke [28]. TNF-α-related pathways change substantially during the first several days after ischemia, with TNFR1-associated inflammatory activity occurring early and TNFR2-related reparative signaling becoming more prominent thereafter [29]. Because biomarker concentrations are therefore highly dependent on the interval between stroke onset and blood sampling, measurements obtained at different biological phases may weaken an otherwise time-dependent relationship with infarct size. In the present study, serum TNF-α therefore appears to reflect the complexity of the systemic inflammatory response more than the magnitude of structural brain injury alone [8,30].

In contrast, serum NO demonstrated a weak positive relationship with infarct volume, with a correlation coefficient of 0.268 and a p value of 0.052. Although this finding did not meet the predefined threshold for statistical significance, the direction of the relationship is biologically plausible. The effects of NO during ischemic stroke depend strongly on its enzymatic source [10,31]. NO generated by endothelial nitric oxide synthase (eNOS) may exert protective vascular effects by promoting vasodilation, maintaining cerebral perfusion, and reducing leukocyte and platelet adhesion [18,32]. Conversely, activation of neuronal nitric oxide synthase (nNOS) and particularly inducible nitric oxide synthase (iNOS) can produce greater amounts of NO that contribute to excitotoxicity and nitrosative stress [33].

Excessive NO production may become particularly detrimental when NO reacts with superoxide to form peroxynitrite [34]. This highly reactive species can induce lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, blood–brain barrier disruption, and neuronal death [35]. Larger ischemic lesions may also provoke a more extensive inflammatory response and greater iNOS expression in microglia, astrocytes, macrophages, and vascular endothelial cells, potentially increasing circulating NO metabolites [32,36]. Such mechanisms provide a plausible explanation for the positive direction of the association observed in this study, despite its lack of statistical significance [37].

The simultaneous evaluation of TNF-α and NO is particularly relevant because inflammation and nitrosative stress are biologically interconnected rather than independent processes [38]. Ischemic tissue injury activates inflammatory cells and increases TNF-α release; subsequent NF-κB activation can upregulate iNOS expression and increase NO production [30,36]. In turn, reactive nitrogen species such as peroxynitrite may further activate inflammatory pathways and amplify secondary tissue injury [11]. This bidirectional interaction creates a positive feedback loop linking neuroinflammation, oxidative/nitrosative stress, blood–brain barrier dysfunction, and progression of ischemic injury [39]. The differing correlations observed for TNF-α and NO may therefore indicate that circulating biomarkers capture distinct components and phases of this interconnected biological response [9,36,40].

From a clinical perspective, the present findings do not support the use of serum TNF-α or NO alone as independent surrogates for infarct volume in acute ischemic stroke. The absence of a significant TNF-α correlation and the borderline, weak association for NO indicate that a single peripheral biomarker is unlikely to adequately characterize the extent of cerebral tissue damage [41]. A multimarker approach combining inflammatory mediators, oxidative or nitrosative stress markers, and markers of neuronal or glial injury may provide a more comprehensive biological assessment than any individual marker alone [42].

Several limitations should be considered when interpreting these findings. First, the cross-sectional design allowed biomarker assessment at only one time point and therefore could not characterize the dynamic temporal profiles of TNF-α and NO after stroke. Second, the relatively modest sample size may have limited statistical power, particularly for the weak NO–infarct volume relationship that approached statistical

significance. Third, variation in the interval between stroke onset and blood sampling may have resulted in biomarker measurements representing different biological phases among participants. Fourth, serum measurements may not fully reflect inflammatory and oxidative processes occurring locally within ischemic brain tissue.

Despite these limitations, this study has several strengths. Both an inflammatory biomarker and a mediator of oxidative/nitrosative stress were evaluated simultaneously, allowing two interconnected components of ischemic pathophysiology to be examined within the same population [41]. Infarct volume was quantified using a CT-based Broderick method, providing an objective structural measure of cerebral injury. In addition, this study represents one of the early investigations in an Indonesian acute ischemic stroke population to assess TNF-α and NO simultaneously in relation to infarct volume [43]. Future studies should employ larger longitudinal cohorts, serial biomarker measurements at standardized intervals after stroke onset, and more specific evaluation of TNFR1/TNFR2 signaling and eNOS, nNOS, and iNOS activity to better define the temporal and mechanistic relationships between inflammation, nitrosative stress, and infarct progression.

CONCLUSION

Serum TNF-α levels were not significantly correlated with infarct volume in patients with acute ischemic stroke, whereas serum nitric oxide (NO) levels demonstrated a weak positive correlation that did not reach statistical significance. These findings indicate that neither biomarker, when measured individually at a single time point, adequately reflects the extent of cerebral infarction. The observed tendency for higher NO levels to accompany larger infarct volumes warrants further investigation in larger longitudinal studies using serial biomarker measurements and more detailed assessment of inflammatory and nitric oxide–related pathways.

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CONFLICT OF INTEREST

The authors declare that this research was conducted without any conflicts of interest affecting its objectivity.

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Supplementary Table S1. Individual Serum TNF-α Levels and Corresponding Infarct Volumes

Participant rank	Serum TNF-α (pg/mL)	Infarct volume (cm^3)
1	16.17	16.90
2	15.82	11.40
3	11.32	0.29
4	9.48	12.70
5	8.41	126.08
6	7.96	1.61
7	7.79	5.30
8	7.40	16.84
9	7.34	2.73
10	7.23	0.46
11	7.06	0.71
12	6.95	22.57
13	6.27	61.06
14	6.16	518.50
15	6.16	7.84
16	5.94	34.52
17	5.83	5.70
18	5.49	0.83
19	5.44	5.46
20	5.27	4.69
21	5.21	16.77
22	5.10	407.26
23	4.99	6.90

24	4.49	12.04
25	4.32	1.19
26	4.16	9.40
27	4.10	44.01
28	3.93	7.74
29	3.93	0.42
30	3.77	7.80
31	3.77	6.30
32	3.71	89.54
33	3.55	7.30
34	3.32	2.08
35	2.77	38.55
36	2.55	749.54
37	2.49	36.80
38	2.49	9.50
39	2.44	5.30
40	2.22	14.80
41	2.22	0.66
42	2.00	13.20
43	1.78	207.01
44	1.78	20.68
45	1.56	211.15
46	1.56	32.09
47	1.50	13.06
48	1.45	7.98
49	1.39	6.12
50	1.23	0.82
51	0.90	1.37
52	0.84	8.82
53	0.51	6.80

Supplementary Table S2. Individual Serum Nitric Oxide Levels and Corresponding Infarct Volumes

Participant rank	Serum nitric oxide (µmol/L)	Infarct volume (cm^3)
1	1456.65	36.80
2	1430.29	8.82
3	1224.21	34.52
4	1086.34	4.69
5	622.42	5.30
6	522.59	9.40
7	519.24	16.84
8	518.13	13.20
9	444.69	6.90
10	411.74	749.54
11	346.99	11.40
12	331.02	20.68
13	325.45	126.08
14	319.91	0.71
15	282.19	7.74
16	272.61	207.01
17	243.73	7.80
18	235.46	38.55
19	231.36	0.46
20	226.48	13.06
21	219.22	2.08
22	206.51	7.84
23	198.70	89.54
24	153.20	0.66

25	148.93	44.01
26	141.88	2.73
27	129.46	518.50
28	123.37	0.82
29	116.03	9.50
30	115.37	22.57
31	114.05	211.15
32	112.73	61.06
33	112.08	14.80
34	103.00	5.70
35	102.36	6.12
36	99.17	1.19
37	98.54	407.26
38	93.49	32.09
39	89.75	6.30
40	87.89	0.42
41	79.95	7.98
42	76.94	1.37
43	73.96	12.70
44	72.77	12.04
45	66.89	16.90
46	66.31	5.30
47	57.14	6.80
48	52.64	16.77
49	48.22	0.83
50	47.67	7.30
51	46.03	5.46
52	43.85	1.61
53	28.57	0.29