Short Communication

Iranian Journal of Immunology

https://iji.sums.ac.ir

# Increased Serum Levels of IL-1β after Ischemic Stroke are Inversely Associated with Vitamin D

Mahnaz Bayat[1], Niloufar Razavi Moosavi[1], Najmeh Karimi[1, 2], Moosa Rahimi[3], Afshin Borhani-Haghighi[1, 4, 5*]

[1]Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; [2]Department of Neurology, Shiraz University of Medical Sciences, Shiraz, Iran; [3]Laboratory of Basic Sciences, Mohammad Rasul Allah Research Tower, Shiraz University of Medical Sciences, Shiraz, Iran; [4]Department of Neurology, John Hunter Hospital, University of Newcastle, Newcastle, Australia; [5]Hunter Medical Research Institute and the University of Newcastle, Newcastle, Australia

## ABSTRACT

**Background :**The initial inflammatory reaction starts following occlusion in ischemic stroke (IS). Interleukin-1β (IL-1β) is a pro-inflammatory cytokine with a crucial role in the pathogenesis of neurodegenerative disorders.

**Objective:** To investigate the levels of IL-1β and vitamin D (VitD) in patients with IS compared with controls and their correlation.

**Methods:** The serum level of 25-OH VitD and IL-1β was assessed in 102 IS patients (0-24 h after stroke) and 102 controls with an enzyme-linked immunosorbent assay (ELISA) kit.

**Results:** We found a significant increase in IL-1β (80.14±6.8 *vs.* 60.32±4.1 pg/ml, $p<0.05$) and a decrease in VitD level (24.3±1.4 vs. 29.9±1.5 ng/ml, $p<0.01$) in the IS patients compared with the controls. There was a significantly positive correlation between the National Institutes of Health Stroke Scale (NIHSS) and IL-1β according to both the Spearman correlation ($r=0.35$, $p=0.0003$) and the linear regression (beta=0.255, $p=0.014$). Also, a significant negative association between VitD and NIHSS was detected by the Spearman correlation ($r=-0.41$, $p<0.0001$) and the linear regression (beta=-0.381, $p=0.000$). Moreover, we found a significant negative correlation ($r=-0.26$, $p=0.006$) between the serum levels of VitD and IL-1β in the patient group.

**Conclusion:** Ischemic stroke correlates positively with IL-1β and negatively with VitD levels. The speculated role of VitD deficiency in the evolution and severity of stroke may be justified by its role in the modification of inflammation.

**Keywords:** Inflammation; Interleukin-1β; Ischemic Stroke; Vitamin D

*Corresponding author:
Afshin Borhani Haghighi, Clinical Neurology Research Center, Shiraz University of Medical Sciences, P. O. Box: 71936-35899, Shiraz, Iran
**Email:** neuro.ab@gmail.com

*Cite this article as:*
Bayat M, Razavi Moosavi N, Karimi N, Rahimi M, Borhani-Haghighi A. Increased Serum Levels of IL-1β after Ischemic Stroke are Inversely Associated with Vitamin D. *Iran J Immunol.* 2023; 20(3):359-367, doi: 10.22034/iji.2023.96384.2432.

Received: 2022-08-10
Revised: 2022-11-29
Accepted: 2022-11-30

## INTRODUCTION

Stroke with a high mortality rate and permanent disability incidence has attracted extensive clinical and experimental research attention. Current therapeutic options for patients with ischemic stroke (IS) are limited to the tissue plasminogen activator (tPA) and endovascular mechanical thrombectomy, which have their own limitations (1).

Meanwhile, inflammation plays a critical role in IS pathogenesis, and the suppression of inflammation may improve the functional outcomes (2). The damaged brain cells can produce soluble glycoproteins called cytokines. The high pro-inflammatory to anti-inflammatory cytokines ratio is associated with stroke damage in patients (3).The family of interleukin-1 (IL-1) has eleven isoforms, and IL-1β has shown a key role in the pathogenesis of neurodegenerative disorders (4). Different intracellular signaling pathways are activated in response to increases in IL-1β following brain damage, leading to more inflammatory damage in the brain tissue (5). Although there have been some contradicting results (6, 7), most studies showed a significant increase in serum levels of IL-1β in the IS patients (8-11). At the same time, the association of vitamin D (VitD) deficiency with the evolution and severity of IS has been vastly studied (12). It seems that inflammatory processes may be suppressed following an increase in VitD serum level (13). In the current study, IL-1β and VitD in acute-stroke patients were compared with the controls, and their possible correlations were investigated. We hypothesized that VitD deficiency might affect stroke evolution through inflammatory pathways. Our findings will help identify the benefit of treatment of acute stroke with VitD supplementation in countries with a high prevalence of VitD deficiency.

## MATERIALS AND METHODS

*Study Subjects*

This study was performed at Shiraz Namazi Hospital between August 2020 -2021. Our study included those over 18 years old who were 0-24 h from symptom onset (*n*=102) and 102 age-sex-matched controls. Brain non-contrast computed tomography (CT) or diffusion-weighted magnetic resonance imaging (MRI) confirmed IS. We excluded patients with severe inflammation, malignancy, transient ischemic attack, and immunosuppressive therapy from this study. Stroke severity was scored upon admission using the National Institutes of Health Stroke Scale (NIHSS), while the higher scores indicated greater severity (14, 15). Hypertension and diabetes were diagnosed based on defined criteria (16, 17).

The type of IS was determined based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification: cardioembolic (CE), large-artery atherosclerosis (LAA), small vessel diseases (SVD), and stroke of undetermined etiology (UD) (18). Our study was ethically approved by the local Ethics Committee of Shiraz University of Medical Sciences with grant number (IR.SUMS. REC.1399. 050, 19560). All patients or their proxy respondents assigned written informed consent. Taking blood samples from patients was performed within 24 h after IS.

*ELISA Method*

After the centrifugation of the coagulated blood at 3000g for 10 min, the serum was stored at −70°C. 25(OH) VitD is considered the primary circulating metabolite and the best indicator of VitD (19). The serum levels of 25-OH VitD were assessed in all the patients with an enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc.®, United States). Based on the manufacturer's instructions, serum levels of IL-1β were measured with specific ELISA kits (Kermania Pars Gen, Kerman, Iran).

*Statistical Analysis*

The chi-square test compared two categorized variables, and an independent two-sample t-test was used to compare the two numeric variables. The association between VitD and IL-1β levels with the risk of IS (between the case and the

controls) was assessed by logistic regression analyses. We used subgroup analysis and linear regression to identify the relationship between VitD and IL-1β levels with clinical parameters. The correlation between IL-1β and VitD with stroke severity was analyzed by the Spearman correlation. The IL-1β and VitD levels were shown as the mean±SE. The analyses were done using the SPSS software (version 19.0) and GraphPad Prism 5.0. The $p<0.05$ was considered a significant value.

## RESULTS

*Demographic and Clinical Characteristics of the Patients and the Controls*

This study included 102 patients and 102 age-sex-matched controls with demographic and clinical aspects, shown in Table 1. The prevalence of diabetes among the IS patients was significantly higher than in the controls (72.7% *vs.* 27.3%, $p=0.001$). We could also identify a significant difference in serum levels of IL-1β and VitD between the cases and the controls.

*Association of IL-1β and VitD with Risk of IS*

Once adjusted for significant covariates, the multiple logistic regression model showed a significant positive association between IL-1β level and evolution of IS (adjusted $OR_{IL1\beta}=1.006$, 95% CI=1.000-1.012, $p=0.041$), while showing the non-significant negative association between VitD and IS risk (adjusted $OR_{VitD}=0.98$, 95% CI=0.961-1.001, $p=0.06$, data not shown).

**Table 1. Demographic and clinical characteristics of the patients and controls.**

| Characteristics | IS patients (n=102) | Controls (n=102) | *p* |
|---|---|---|---|
| Male, n (%) | 42 (50%) | 42 (50%) | 1[a] |
| Female, n (%) | 60 (50%) | 60 (50%) | |
| Age, years | 67.6±1.33 | 67.5±1.3 | 0.9[b] |
| BMI (kg/m$^2$) | 25.15±0.34 | 24.61±0.20 | 0.2[b] |
| Hypertension, n (%) | 42 (53.8%) | 36 (46.2%) | 0.2[a] |
| Diabetes, n (%) | 32 (72.7%) | 12 (27.3%) | 0.001[a] |
| Hyperlipidemia n (%) | 32 (54.2%%) | 27 (45.8%%) | 0.2[a] |
| Smoking, n (%) | 17 (56.7%) | 13 (43.3%) | 0.2[a] |
| Drinking, n (%) | 7 (36.8%) | 12 (63.2%) | 0.1[a] |
| WBC | 7808.53±166 | 7353.92±173 | 0.06[b] |
| BUN | 15.9±.45 | 16.3±0.44 | 0.4[b] |
| Cr | 1.11±.02 | 0.93±0.08 | 0.06[b] |
| AST | 20.50±.61 | 20.61±0.45 | 0.8[b] |
| ALT | 19.03±.70 | 20.04±0.48 | 0.2[b] |
| IL1β, pg/ml | 80.13±6.81 | 60.31±4.12 | 0.014[b] |
| VitD, ng/ml | 24.30±1.42 | | |
| <20 | 47(45.6%) | 29.91±1.56 | 0.009[b] |
| ≥20 | 56(54.45%) | | |
| Types of stroke | | | |
| LAA | 39 (38.2%) | | |
| SVD | 33 (32.4%) | | |
| CE | 16(15.7%) | | |
| UD | 14(13.7%) | | |
| NIHSS at admission | | | |
| ≤6 | 37 (36.3%) | | |
| ≥7 | 65 (63.7%) | | |

Data were shown as mean±SEM or as n (%). [a] Chi-square Test; [b] Independent two-sample t-test. IS, ischemic stroke; BMI, body mass index; WBC, white blood cell; Cr, creatinine; BUN, blood urea nitrogen; AST, Aspartate aminotransferase; ALT, Alanine Aminotransferase; IL-1β, interleukin-1β; VitD, vitamin D; LAA, Large artery atherosclerosis; SVD, Small-vessel disease; CE, cardiac embolism; NIHSS, National Institutes of Health Stroke Scale.

*Serum levels of IL-1β and VitD in the patients and the controls*

A significant increase was identified in the serum level of IL-1β in the IS patients compared with the controls (IS=80.14±6.8 *vs.* CTRL=60.32±4.1 pg/ml, $p<0.05$, Fig. 1A). There was a significant difference in the VitD level between the two groups (IS=24.3±1.4 *vs.* CTRL=29.9±1.5 ng/ml, $p<0.01$). Our patients showed a lower level of VitD compared with the controls (Fig. 1B). We also found no difference in serum levels of IL-1β between the patients with different types of stroke (Fig. 1C).

*Subgroup Analysis for the Association between IL-1β and VitD levels with Clinical Parameters in the IS Patients*

As shown in Table 2, the subgroup analysis for 102 IS patients showed that the level of IL-1β significantly increased in the female gender compared with the male ones (96.6±12.4 *vs*. 68.5±7.3, $p=0.04$). IL-1β significantly increased in the patients with NIHSS ≥7 compared with those with NIHSS ≤6. Also, in patients with VitD levels lower than 20 ng/ml, the serum level of IL-1β significantly increased in those with high VitD (>20 ng/ml). However, no statistically significant differences were observed in the other subgroups, such as diabetes, hypertension, hyperlipidemia, drinking, and smoking, in the levels of IL-1β and VitD. We also found a significant decrease in the VitD level of the IS patients with NIHSS

**Table 2. Association of IL-1β and VitD with demographic and clinical parameters in IS patients by Subgroup analysis**

| Characteristics | N | Mean±SE IL-1β (pg/ml) | *p* | Mean±SE VitD (ng/ml) | *p* |
|---|---|---|---|---|---|
| Sex | | | | | |
| Male | 60 | 68.5±7.3 | 0.04 | 24.2±1.7 | 0.9 |
| Female | 42 | 96.6±12.4 | | 24.3±2.4 | |
| Age | | | | | |
| <70 | 56 | 70.4±7.3 | 0.1 | 23.3±1.8 | 0.4 |
| ≥70 | 46 | 91.9±12.1 | | 25.4±2.1 | |
| NIHSS | | | | | |
| ≤6 | 37 | 57.1±8.1 | 0.01 | 30.8±2.6 | 0.000 |
| ≥7 | 65 | 93.2±9.2 | | 20.5±1.4 | |
| Smoking | | | | | |
| Negative | 89 | 84.6±7.5 | 0.08 | 23.8±1.5 | 0.4 |
| Positive | 13 | 49.06±8.9 | | 27.07±4.2 | |
| Alcoholism | | | | | |
| Negative | 95 | 81.1±7.1 | 0.5 | 24.04±1.4 | 0.5 |
| Positive | 7 | 65.7±19.1 | | 27.8±3.7 | |
| Hypertension | | | | | |
| Negative | 60 | 83±9.7 | 0.4 | 23.7±2.1 | 0.6 |
| Positive | 42 | 74±6.9 | | 24.8±1.9 | |
| Hyperlipidemia | | | | | |
| Negative | 69 | 83.9±8.8 | 0.4 | 24.8±1.8 | 0.6 |
| Positive | 32 | 73.09±10.1 | | 23.5±2. 1 | |
| Diabetes | | | | | |
| Negative | 70 | 78.90±7.5 | 0.8 | 25.3±1.9 | 0.2 |
| Positive | 32 | 82.6±14.3 | | 22.09±1.5 | |
| VitD level | | | | | |
| ≤20 | 47 | 96.7±11.9 | 0.02 | | |
| >20 | 55 | 65.9±7 | | | |

Data were shown as mean±SEM, independent two-sample t-test. NIHSS, National Institutes of Health Stroke Scale; IL-1β, interleukin-1β; VitD, vitamin D.

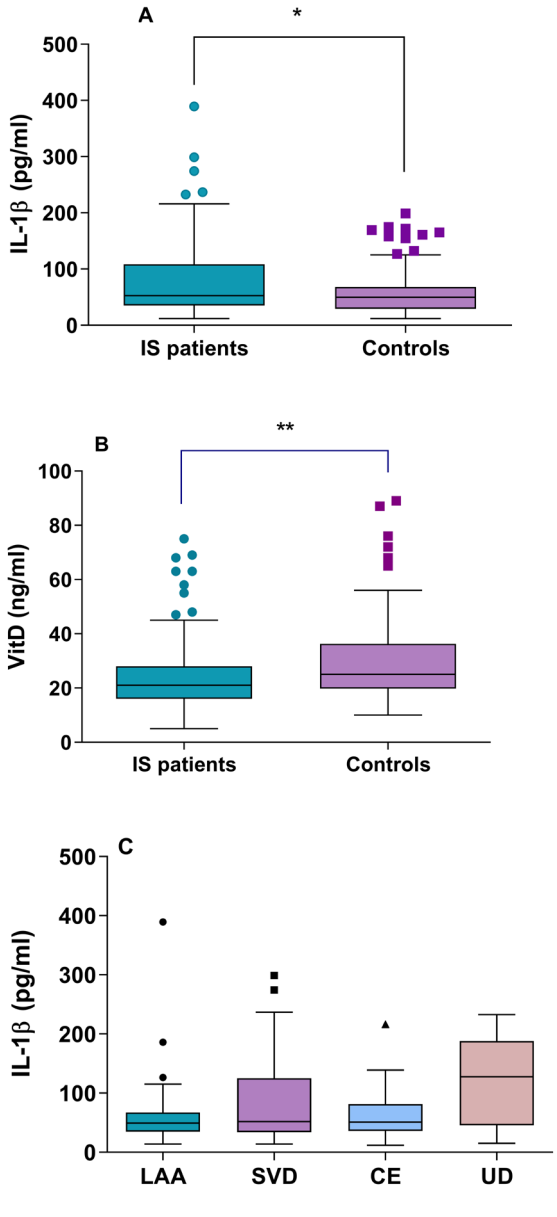


**Fig. 1.** The serum levels of IL-1β and VitD in patients and controls after stroke. Independent Student's t-test revealed that IL-1β was significantly higher in IS patients relative to controls, and patients had significantly lower serum levels of VitD relative to controls (A, B). The comparison of serum levels of IL-1β in different types of IS (C). Results were expressed as mean±SEM *$p$<0.05, **$p$<0.01, ***$p$<0.001. Abbreviations: IS, ischemic stroke; LAA, large-artery atherosclerosis; SVD, small-vessel disease; CE, cardiac embolism; UD, undetermined.

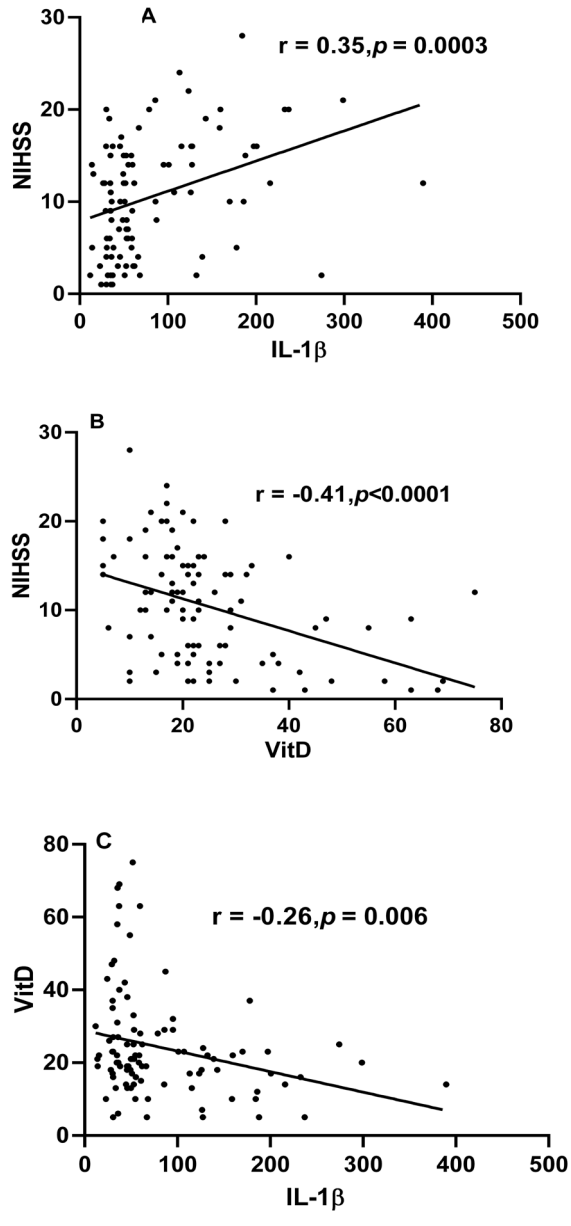


**Fig. 2.** The Spearman Correlation between serum levels of IL-1β and VitD with NIHSS in IS patients. The Spearman Correlation between IL-1β and NIHSS (A), between VitD and NIHSS (B), and between IL-1β and VitD (C) in IS patients after stroke. Abbreviations: NIHSS, National Institutes of Health Stroke Scale.

≥7 compared with those who had NIHSS≤6 (20.5±1.4 *vs.* 30.8±2.6 ng/ml, $p$=0.000).

*Linear Regression Analysis for the Association of IL-1β and VitD Levels with Clinical Parameters in IS Cases*

The linear regression showed that age, NIHSS, and type of stroke (LAA) were significantly associated with the level of IL-1β in IS patients. A significant positive association was observed between the age (beta=0.201, $p$=0.028, 95% CI=0.114-1.947) and stroke severity of patients (beta=0.255, $p$=0.014, 95% CI=0.565-4.998) with IL-1β

**Table 3. Linear regression analysis for the association between different parameters with IL-1β (A) and VitD (B) levels in ischemic stroke patients.**

| | Beta | *p* | 95.0%CI Lower Bound | Upper Bound |
|---|---|---|---|---|
| A | | | | |
| Age | 0.201 | 0.028 | 0.114 | 1.947 |
| Sex | 0.151 | 0.095 | -3.725 | 45.835 |
| NIHSS | 0.255 | 0.014 | 0.565 | 4.998 |
| VitD | -0.170 | 0.091 | -1.762 | 0.133 |
| LAA | -0.341 | 0.014 | -85.981 | -9.998 |
| SVD | -0.213 | 0.117 | -70.406 | 7.951 |
| CE | -0.146 | 0.239 | -73.582 | 18.571 |
| B | | | | |
| Age | 0.162 | 0.085 | -0.024 | 0.370 |
| Sex | 0.076 | 0.414 | -3.129 | 7.541 |
| NIHSS | -0.381 | 0.000 | -1.322 | -0.415 |
| IL1β | -0.177 | 0.091 | -0.080 | 0.006 |
| LAA | 0.026 | 0.855 | -7.579 | 9.124 |
| SVD | -.0117 | 0.401 | -11.995 | 4.841 |
| CE | -0.057 | 0.654 | -12.103 | 7.637 |

NIHSS, National Institutes of Health Stroke Scale; IL-1β, interleukin-1β; VitD, vitamin D; LAA, Large artery atherosclerosis; SVD, Small-vessel disease; CE, cardiac embolism

level and a negative association between LAA stroke (beta=-0.341, *p*=0.014, 95% CI=-85.981 to -9.998) and IL-1β level. However, we found a non-significant negative association between IL-1β and VitD level (*p*=0.09, Table 3).

In Table 3,VitD was considered a dependent factor, and the linear regression analysis showed a significant negative association between NIHSS with VitD level in the IS patients (beta=-0.381, *p*=0.000, 95% CI=-1.322 to -0.415).

*The Spearman Correlation between IL-1β and VitD with Stroke Severity*

We found a significant positive Spearman correlation between IL-1β and NIHSS score (*r*=0.35, 95% CI=0.1626-0.5166, *p*=0.0003, Fig. 2A). NIHSS showed a significantly negative Spearman correlation with VitD level (*r*=-0.41, 95% CI=-0.5826 to -0.2531, *p*<0.0001) (Fig. 2B). Our results also identified a significant negative correlation between IL-1β and VitD level (*r*=-0.26, 95% CI=-0.4449 to -0.07255, *p*=0.006, Fig. 2C).

## DISCUSSION

In this study, the serum IL-1β levels showed a significant increase in peripheral blood of the IS patients compared with the controls with a negative correlation with VitD level. Our patients also had a significantly lower level of VitD compared with the controls. We found a significant negative relationship between IL-1β and VitD levels. However, NIHSS showed a significant negative correlation with VitD and a positive correlation with IL-1β.

There is a controversy about the role of IL-1β in acute ischemic stroke. An increase in the level of IL-1β after IS was reported by some studies (8-11). In contrast, the others saw no increase in the IL-1β level (6, 7, 20, 21). The sample size and blood sampling time are the leading causes of controversies in IL-1β levels after IS. IL-1β plays essential roles in both acute and chronic inflammation, initiates the inflammatory responses when binding to the IL-1R1, activating the subcellular signaling pathways such as c-Jun N-terminal kinase (JNK), p38 MAP kinase, nuclear-factor kappa B (NF-κB), extracellular signal-regulated

kinases (ERKs), and mitogen-activated protein kinases (MAPKs) (22).

Several experimental and human studies reported that VitD deficiency was accompanied by a high plasma level of pro-inflammatory cytokines (23), and VitD supplementation could decrease the stroke severity (24).

At least two clinical trials have shown the beneficial effects of VitD in acute stroke. In a nonblinded randomized controlled trial, intramuscular injection of a single dose of vitamin D after stroke improved the three-month Scandinavian Stroke Scale (SSS) in the patients with vitamin D insufficiency/deficiency (25). However, they did not report any VitD intoxication. In another study, supplementation with oral vitamins A and D decreased IL-1β serum levels and improved the 3-month NIHSS in ischemic stroke patients (26).

It is suggested that the active form of VitD in the brain may be reduced after stroke by the upregulation of 24-hydroxylase (vitamin D inactivating enzyme) (24). Several mechanisms for the anti-inflammatory effect of vitamin D have been investigated, such as regulation of the immune system, (27) cytokine release (28), inhibiting NFκB activity (29), and up-regulating MKP5 (30). Vitamin D deficiency could increase the secretion and release of pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and IL-6 by stimulating the expression of NF-κB (31). In the stroke individuals, serum VitD levels are inversely associated with high-sensitivity C-reactive protein (hsCRP) and IL-6 (32). An animal study showed that VitD deficiency significantly reduced IL-2, IL-1α, IL-4, IL-1β, IL-10, and IFN-γ expression in the ischemic brain (33). Moreover, serum vitamin D has been shown to correlate with NIHSS and TNF-α in IS patients (34). Our study showed no significant difference between the patients with ischemic stroke subtypes in serum levels of IL-1β. Zeng et al. reported the association between CRP and LAA in the pathogenesis of stroke, while IL-1β could not show any association with stroke subtypes (35). However, high median plasma levels of IL-6, TNF-α, and IL-1β have been reported in the patients with the CE subtype. In contrast, the SVD subtype showed low median plasma levels of these inflammatory markers (36, 37).

## CONCLUSION

We found that ischemic stroke is correlated positively with IL-1β and negatively with VitD levels. Accordingly, the speculated role of VitD deficiency in the evolution and severity of stroke may be justified by its role in the modification of inflammation. Large clinical trials should examine the therapeutic effects of VitD in acute ischemic stroke as a cost-effective treatment.

## ACKNOWLEDGMENTS

This work was supported by Shiraz University of Medical Sciences (IR.SUMS.REC.1399.050) with grant agreement No. 19560.

## AUTHORS' CONTRIBUTION

All authors contributed to the study's conception and design. Material preparation, and data collection, were performed by Niloufar Razavi Mousavi, Najmeh Karimi, and Moosa Rahimi. The first draft of the manuscript was written by Mahnaz Bayat. All authors commented on previous versions of the manuscript and approved the final manuscript.

## CONFLICT OF INTEREST

None declared.

## REFERENCES

1. Tabrizi R, Lankarani KB, Kardeh B, Akbari

H, Azarpazhooh MR, Borhani-Haghighi A. A Comprehensive Systematic Review and Meta-analysis on the Risk Factors of Stroke in Iranian Population. Arch Iran Med. 2021;24(1):64-77.
2. safari A, safari, R., & Borhani-Haghighi, A. Immunology of stroke. Galen Medical Journal,. 2016;5( (51)):10-7.
3. Doll DN, Barr TL, Simpkins JW. Cytokines: their role in stroke and potential use as biomarkers and therapeutic targets. Aging Dis. 2014;5(5):294-306.
4. Nilupul Perera M, Ma HK, Arakawa S, Howells DW, Markus R, Rowe CC, et al. Inflammation following stroke. J Clin Neurosci. 2006;13(1):1-8.
5. Ahn KS, Aggarwal BB. Transcription factor NF-kappaB: a sensor for smoke and stress signals. Ann N Y Acad Sci. 2005;1056:218-33.
6. Ormstad H, Aass HC, Lund-Sorensen N, Amthor KF, Sandvik L. Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume. J Neurol. 2011;258(4):677-85.
7. Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, et al. Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. Stroke. 1995;26(8):1393-8.
8. Shademan B, Nourazarian A, Laghousi D, Karamad V, Nikanfar M. Exploring potential serum levels of Homocysteine, interleukin-1 beta, and apolipoprotein B 48 as new biomarkers for patients with ischemic stroke. J Clin Lab Anal. 2021:e23996.
9. Mazzotta G, Sarchielli P, Caso V, Paciaroni M, Floridi A, Floridi A, et al. Different cytokine levels in thrombolysis patients as predictors for clinical outcome. Eur J Neurol. 2004;11(6):377-81.
10. Sotgiu S, Zanda B, Marchetti B, Fois ML, Arru G, Pes GM, et al. Inflammatory biomarkers in blood of patients with acute brain ischemia. Eur J Neurol. 2006;13(5):505-13.
11. Wytrykowska A, Prosba-Mackiewicz M, Nyka WM. IL-1beta, TNF-alpha, and IL-6 levels in gingival fluid and serum of patients with ischemic stroke. J Oral Sci. 2016;58(4):509-13.
12. Siniscalchi A, Lochner P, Anticoli S, Chirchiglia D, De Sarro G, Gallelli L. What is the Current Role for Vitamin D and the Risk of Stroke? Current neurovascular research. 2019;16(2):178-83.
13. Safari A FN, Borhani-Haghighi A. The Association Between Inflammatory Biomarkers and Vitamin D Level With Evolution and Severity of Stroke. Basic and Clinical neuroscience.
14. Williams LS, Yilmaz EY, Lopez-Yunez AM. Retrospective assessment of initial stroke severity with the NIH Stroke Scale. Stroke. 2000;31(4):858-62.
15. Kwan J, Hand P. Early neurological deterioration in acute stroke: clinical characteristics and impact on outcome. QJM : monthly journal of the Association of Physicians. 2006;99(9):625-33.
16. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertension. 2020;75(6):1334-57.
17. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2004;27 Suppl 1:S5-S10.
18. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24(1):35-41.
19. Balvers MG, Brouwer-Brolsma EM, Endenburg S, de Groot LC, Kok FJ, Gunnewiek JK. Recommended intakes of vitamin D to optimise health, associated circulating 25-hydroxyvitamin D concentrations, and dosing regimens to treat deficiency: workshop report and overview of current literature. J Nutr Sci. 2015;4:e23.
20. Clausen BH, Wirenfeldt M, Hogedal SS, Frich LH, Nielsen HH, Schroder HD, et al. Characterization of the TNF and IL-1 systems in human brain and blood after ischemic stroke. Acta Neuropathol Commun. 2020;8(1):81.
21. Emsley HC, Smith CJ, Gavin CM, Georgiou RF, Vail A, Barberan EM, et al. Clinical outcome following acute ischaemic stroke relates to both activation and autoregulatory inhibition of cytokine production. BMC Neurol. 2007;7:5.
22. Kaneko N, Kurata M, Yamamoto T, Morikawa S, Masumoto J. The role of interleukin-1 in general pathology. Inflamm Regen. 2019;39:12.
23. Nowrouzi-Sohrabi P, Kalani M, Izadpanah P, Ahmadvand H, Fakhour M, Fadaei R, et al. Vitamin D status influences cytokine production and MALAT1 expression from the PBMCs of patients with coronary artery disease and healthy controls. Rev Assoc Med Bras (1992). 2020;66(12):1712-7.
24. Evans MA, Kim HA, Ling YH, Uong S, Vinh A, De Silva TM, et al. Vitamin D3 Supplementation Reduces Subsequent Brain Injury and Inflammation Associated with Ischemic Stroke. Neuromolecular medicine. 2018;20(1):147-59.
25. Narasimhan S, Balasubramanian P. Role of Vitamin D in the Outcome of Ischemic Stroke-A Randomized Controlled Trial. J Clin Diagn Res. 2017;11(2):CC06-CC10.
26. Kadri A, Sjahrir H, Juwita Sembiring R, Ichwan M. Combination of vitamin A and D supplementation for ischemic stroke: effects on

interleukin-1ss and clinical outcome. Med Glas (Zenica). 2020;17(2):425-32.
27. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. Immunology. 2011;134(2):123-39.
28. Roffe-Vazquez DN, Huerta-Delgado AS, Castillo EC, Villarreal-Calderon JR, Gonzalez-Gil AM, Enriquez C, et al. Correlation of Vitamin D with Inflammatory Cytokines, Atherosclerotic Parameters, and Lifestyle Factors in the Setting of Heart Failure: A 12-Month Follow-Up Study. Int J Mol Sci. 2019;20(22).
29. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor kappaB activation by interacting with IkappaB kinase beta protein. J Biol Chem. 2013;288(27):19450-8.
30. Nonn L, Peng L, Feldman D, Peehl DM. Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. Cancer Res. 2006;66(8):4516-24.
31. Rai V, Agrawal DK. Role of Vitamin D in Cardiovascular Diseases. Endocrinol Metab Clin North Am. 2017;46(4):1039-59.
32. Wang Q, Zhu Z, Liu Y, Tu X, He J. Relationship between serum vitamin D levels and inflammatory markers in acute stroke patients. Brain Behav. 2018;8(2):e00885.
33. Balden R, Selvamani A, Sohrabji F. Vitamin D deficiency exacerbates experimental stroke injury and dysregulates ischemia-induced inflammation in adult rats. Endocrinology. 2012;153(5):2420-35.
34. Omidvar Rezaei MR, Mehrdad Roozbeh,Bahareh Fazeli, Mohammadreza Hajiesmaeili, Hossein Pakdaman, and Leila Simani. Does vitamin D administration play a role in outcome of patients with acute ischemic stroke? A randomized controlled trial. current journal of neurology. 2021;20(1):8-14.
35. Zeng L, He X, Liu J, Wang L, Weng S, Wang Y, et al. Differences of circulating inflammatory markers between large- and small vessel disease in patients with acute ischemic stroke. Int J Med Sci. 2013;10(10):1399-405.
36. Tuttolomondo A, Di Raimondo D, Pecoraro R, Arnao V, Pinto A, Licata G. Inflammation in ischemic stroke subtypes. Curr Pharm Des. 2012;18(28):4289-310.
37. Licata G, Tuttolomondo A, Di Raimondo D, Corrao S, Di Sciacca R, Pinto A. Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. Thromb Haemost. 2009;101(5):929-37.