The Relationship between Vitamin D Serum Levels, Disease Severity, and Bleeding Volume in Patients with Intracerebral Hemorrhage

Irbab Hawari1 *, Muhammad Yunus Amran1, Muhammad Akbar1, Ashari Bahar1, Yudy Goysal1, Alfian Zainuddin2

1 Department of Neurology, Faculty of Medicine, Universitas Hasanuddin, South Sulawesi, Indonesia
2 Faculty of Medicine, Universitas Hasanuddin, South Sulawesi, Indonesia

ABSTRACT

Background: Stroke is the main cause of death and disability worldwide. Intracerebral hemorrhage (ICH) is a subtype that contributes to 10 to 20% of all stroke events. Meanwhile, several studies in Indonesia show that the rates of vitamin D deficiency and insufficiency are still high, at 60.6% and 33.3% respectively in young adults. Several studies have shown vitamin D deficiency during hospital admission is a potential biomarker for poor functional outcomes in patients with acute ischemic stroke or ICH. This research aimed to analyze the relationship between serum vitamin D levels, categorized as deficiency (<20 ng/mL), insufficiency (20-30 ng/mL), and normal (>30 ng/mL), with the severity of ICH as assessed by the NIHSS score and bleeding volume on head CT scans.

Methods: The study design was cross-sectional to assess the relationship between serum vitamin D levels, categorized as deficiency (<20 ng/mL), insufficiency (20-30 ng/mL), and normal (>30 ng/mL), with the severity of ICH as assessed by the NIHSS score and bleeding volume on head CT scans.

Results: From a total of 31 patients, the Spearman correlation test of the relationship between vitamin D levels and NIHSS scores obtained a weak correlation (r=0.127; p=0.494). The Kruskal Wallis test showed that there was no significant difference in the mean value of NIHSS scores in the vitamin D level categories (p=0.310). The Spearman correlation test of the relationship between vitamin D levels and bleeding volume obtained a weak correlation with an r=0.044; p=0.823. The Kruskal Wallis test showed there was no significant difference in the mean value of bleeding volume in the vitamin D level category (p=0.439).

Conclusion: There is no relationship between serum vitamin D levels, disease severity, and bleeding volume in ICH patients. Further research is needed to analyze the relationship between vitamin D serum levels and the severity of ICH with a retrospective cohort study design and discuss the effect of vitamin D on long-term functional outcomes.

Keywords: Vitamin D, 25(OH)D, NIHSS, Hematoma Volume, Intracerebral Hemorrhage, Stroke Functional Outcome, Stroke Prognosis

INTRODUCTION

Stroke is the leading cause of death and disability worldwide. Intracerebral hemorrhage (ICH) is one subtype that contributes to 10 to 20% of all stroke events. In Asian populations, the incidence of ICH is almost double that of other ethnicities (Ikram et al., 2012). Several studies have shown vitamin D deficiency during hospital admissions to be a potential biomarker of low functional outcomes in patients with acute ischemic stroke and ICH (An et al., 2017).

Several studies in Indonesia showed that although Indonesia is a tropical country with abundant sun exposure, most of the Indonesian population of adult and adolescent age has low vitamin D levels. Santoso et al. (2023), in their study of 33 people with an average age of 31.75 years, reported that 60.6% of the subjects were classified as
deficient in vitamin D levels and 33.3% were classified as insufficiency. Hence, the total number of subjects with low vitamin D levels reached 93.9%. Research conducted by Husna et al. (2021) in East Java, which assessed the relationship between sun exposure and vitamin D levels in elderly women with 40 subjects, reported that 70% of subjects had low vitamin D levels, with 30% classified as deficient and 40% classified as insufficiency.

Vitamin D refers to a group of fat-soluble vitamins derived from cholesterol. Vitamin D2 (ergocalciferol) and D3 (calcitriol) are known types that have biological effects on the human body. The skin synthesizes vitamin D when exposed to the sun. In fact, for most people, more than 90% of vitamin D3 comes from exposure to ultraviolet B rays in the sun. Vitamin D produced in the epidermis must be further metabolized in its active form. The first step, 25-hydroxylation, occurs in the liver. In any case, for the metabolite form of vitamin D to achieve maximum biological activity, it must be hydroxylated at position 1α by the enzyme CYP27B1; 1.25 (OH)2D is the most potent and known metabolite of vitamin D due to its various biological effects. Hydroxylation 1α occurs mostly in the kidneys (Ramasamy, 2020).

Various studies have shown a correlation between vitamin D levels as a risk factor for stroke, and the severity and outcome of stroke, especially in acute ischemic stroke. Research conducted by Liu et al. (2022) showed that vitamin D has a protective effect on ICH by accelerating macrophage differentiation and accelerating neurological recovery and hematoma clearance by increasing the total number of macrophages and erythrocytosis of red blood cells. Vitamin D also acts as an anti-inflammatory in stroke. Several studies reported that vitamin D deficiency increases levels of inflammatory mediators such as IL-1β, TNFα, and IL-6 which can increase the inflammatory process and damage to the blood-brain barrier after stroke (Ashouri et al., 2021). Results of a prospective study by Tony et al. (2017) in Egypt showed a significant relationship between serum vitamin D levels and the severity of ICH, where lower vitamin D levels indicated high severity. Meanwhile, studies on the relationship between serum vitamin D levels, and the severity of hemorrhagic stroke patients, especially intracerebral hemorrhage in Indonesia, are still very limited. The existence of a significant association with the study may help to reduce the severity of ICH in at-risk populations. Therefore, the researchers tried to conduct an analytical study of the relationship between vitamin D levels and disease severity measured by NIHSS score and bleeding volume using a CT scan of the head in patients with intracerebral hemorrhage by cross-sectional study design. The results of this study will add to the understanding of the role of vitamin D in the pathophysiology of ICH and may influence future clinical interventions.

**RESEARCH METHOD**

This study is a cross-sectional study conducted at Wahidin Sudirohusodo Hospital and other network hospitals in Makassar. The study population was all intracerebral hemorrhage patients. The subjects of the study were ICH patients who were undergoing hospitalization. This study has received a feasibility permit from the ethics commission with number 727/UN4.6.4.5.31/PP36/2023. Faculty of Medicine, Hasanuddin University. Data collection was carried out from September to December 2023 using the total sampling technique.

The inclusion criteria in the study were patients with intracerebral hemorrhage onset of 1-7 days, aged 18-80 years, and willing to participate in the study by signing informed consent. Meanwhile, the exclusion criteria are secondary ICH patients with other causes such as head injuries, vascular malformations, malignancy, and cerebral aneurysms. patients with infectious diseases, patients with subarachnoid hemorrhages, and patients with a history of blood clotting disorders.

The collection of subject demographic data was carried out using the subject biodata form. Assessment of the degree of disease is assessed using the Indonesian version of the NIHSS form. Blood for vitamin D level examination was taken in a vein in the subject's forearm and vitamin D levels were measured by the Enzyme-linked Immunosorbent Asays (ELISA) method. Subsequently, the vitamin D levels in the samples obtained were classified according to the guidelines the Endocrine Practice Guidelines and the Australian Working Group into the deficiency group (<20 ng/mL), insufficiency (20-30 ng/mL), and normal (>30 ng/mL) (Holick, 2009). Assessment of bleeding volume is measured through a CT scan of the head. The volume of bleeding in mL is determined by the formula ABC/2, where A is the largest bleeding diameter on the CT scan, B is the bleeding diameter with an angle of 90° to A, and C is the estimated number of pieces on the CT scan multiplied by the thickness of the cut.
The data obtained was then analyzed using the Statistical Package for Social Sciences software version 29. The results of the analysis are presented in the form of a table including percentages, mean values, medians, standard deviations, Pearson correlation if the data is normal or Spearman correlation if the data is not normal, and the results of the Kruskal Wallis differential test to determine the differences between groups of vitamin D categories.

**RESULTS**

31 subjects who met the inclusion criteria were obtained. The average age of the subjects was 53.55 years. Based on sex, there were more males than females (20 males vs 11 females or 64.5% vs 35.50%). Most of the subjects were in the age range of 46-55 years (10 subjects), and at least 1 subject was in the age range of 26-35 years.

The mean of NIHSS score was 10.58 (moderate). There were 25 subjects (80.60%) with a moderate degree and only 3 subjects (9.70%) with a mild and severe degree. The average vitamin D level of the study sample was 17.13 ng/mL, with most of them (24 samples or 77.40%) having vitamin D deficiency and only 3 subjects having insufficiency. The range of vitamin D levels was 0 ng/mL to 69.66 ng/mL (Table 1).

<table>
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<th>Characteristic</th>
<th>(n)</th>
<th>(%)</th>
<th>Mean</th>
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<td>64.50</td>
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<tr>
<td>Female</td>
<td>11</td>
<td>35.50</td>
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<tr>
<td><strong>Age</strong></td>
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<tr>
<td>36-45 years old</td>
<td>7</td>
<td>22.60</td>
<td>53.55</td>
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<tr>
<td>46-55 years old</td>
<td>10</td>
<td>32.30</td>
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<td>Moderate</td>
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<tr>
<td>Severe</td>
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<td>12.90</td>
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<tr>
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<td>Deficient</td>
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<td>77.40</td>
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<tr>
<td><strong>Bleeding volume (mL)</strong></td>
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The analysis using the Spearman correlation test of the relationship between vitamin D levels and NIHSS score obtained a value of r 0.127, indicating a weak correlation with a positive direction (Figure 1), with a p-value of 0.494 which showed that there was no correlation between vitamin D levels and NIHSS scores. The results of the Kruskal Wallis test to show the comparison of vitamin D levels with the NIHSS score obtained from the deficiency group had an average NIHSS score of 10.21, the insufficiency group of 13.00, and the normal of 11.00 with a p-value of 0.310 which showed that the difference between the three groups was not significant (Figure 2).
Figure 1. Spearman correlation test of the relationship between serum 25(OH)D levels and NIHSS score ($r = 0.127$, $p=0.494$)

Meanwhile, the analysis using the Spearman correlation test to determine the relationship between vitamin D levels and bleeding volume obtained a value of $r = 0.044$ which indicates a weak correlation (Figure 3), with a $p$-value of 0.823 which indicates that there was no correlation between vitamin D levels and bleeding volume. The results of the Kruskal Wallis test to show the comparison of vitamin D levels with bleeding volume obtained from the deficiency group had a bleeding volume of 17.70 mL, the insufficiency group of 32.76, and the normal of 12.91 with a $p$-value of 0.439 which showed that the average difference between the three groups was not significant (Figure 4).
DISCUSSION

This study was observational analytical research with a cross-sectional design conducted to determine the relationship between vitamin D levels and disease severity and bleeding volume in intracerebral hemorrhage patients.

In the characteristics of the research sample, the number of males (20 subjects) was more than females (11 subjects). These results are in line with various findings in epidemiological studies in general. Gokhale et al. (2015) in an analysis of 17 studies on sex differences in ICH, found that the overall incidence of ICH was higher in males than in females (RR=1.60, 95% CI=1.47-1.74).

The next characteristic is in the age group, where in this study, the age group of 46-55 years was obtained as many as 10 subjects (32.3%), followed by the age group of 56-65 years and the group of 36-45 years each as many as 7 subjects (22.6%). This result is different from the results obtained by Wang et al. (2022). The analysis of 52 studies showed that the highest number of subjects in the age group of 65-74 years was 1,159 people, followed by the age group of 75-84 years as many as 919 people.
Serum 25(OH)D levels are an option for detecting vitamin D levels in the human body because this form is the most circulating form of vitamin D in the body which has a half-life of about 2-3 weeks. The level of 25(OH)D in the body is a direct sum of vitamin D obtained from food intake and vitamin D obtained from sun exposure (Jukic et al., 2018).

From the results of statistical tests using the Spearman correlation test to assess the relationship between serum vitamin D levels and NIHSS scores, a correlation coefficient value of 0.127 with a p-value of 0.494 was obtained. This indicates a very weak and insignificant positive correlation. The results of the statistical test using the Kruskal Wallis differential test, obtained an average NIHSS score of in the vitamin D deficiency group 10.21, in the vitamin D insufficiency group 13.00, and in the normal group 11.00 with a p-value of 0.310. This indicates that there is no significant difference in the average vitamin D levels in the group. This result is different from the results of a study conducted by Tony et al. (2017), which was carried out in Egypt. This prospective study in 125 patients showed a correlation between serum vitamin D levels and the severity of intracerebral hemorrhage in the acute phase. Patients with ICH and low vitamin D levels had a heavier stroke presentation (16.74±8.08) than ICH patients with normal vitamin D levels (29.50 ± 12.41; p=0.020). ICH patients with low vitamin D levels also had worse functional outcomes (mRS 4.89±0.31 higher) compared to ICH patients with normal vitamin D levels (Tony, 2017).

Another factor that can affect the degree of disease in ICH is blood pressure. Wang et al. (2023), in a study of 1416 patients in various hospitals in Beijing, showed that blood pressure and high heart rate are associated with poor severity and prognosis in ICH patients. The higher the blood pressure and heart rate, the lower the GCS and the higher the NIHSS score during treatment. The incidence of death is also higher along with increasing blood pressure. High blood pressure increases are associated with autoregulatory disturbances and disturbances in the autonomic nervous system’s response. Increased blood pressure and heart rate are markers of increased sympathoadrenal tone and indicate the presence of severe neurological disorders. Early bleeding can compress areas of the brain that mediate autonomic responses and result in detrimental effects on autonomic dysfunction in ICH patients (Wang et al., 2023).

Saxena et al. (2016) In their study of 2653 patients involved as study subjects INTERACT2 (Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage Trial 2) shows that hyperglycemia and DM are also factors that can aggravate ICH severities. Patients with hyperglycemia and DM tend to have higher NIHSS scores and a worse prognosis than patients with normoglycemia status (Saxena et al., 2016).

Regarding functional outcomes, Zeng et al. (2021) examined the effect of vitamin D on functional outcomes after stroke over 5 years. In his study of 668 stroke patients, low vitamin D levels were associated with poor functional outcomes in stroke overall. However, a pool analysis of 75 patients with ICH, showed no significant association between vitamin D levels and functional outcomes of ICH patients over 5 years (Zeng et al., 2021).

The results of the Spearman correlation statistical test to assess the relationship between vitamin D levels and bleeding volume obtained correlation coefficient values of 0.044 and p=0.823 which showed a low positive correlation value and was not statistically significant. The results of the Kruskal Wallis differential test obtained a mean bleeding volume score of 17.70 in the vitamin D deficiency group, 32.76 in the insufficiency group, and 12.91 in the normal group with p=0.439. This indicates that there are no significant differences between the three groups. Until now, no research has been conducted on the relationship between vitamin D levels and the volume of lesions in humans.

Liu et al. (2022) showed a correlation between hematoma volume and vitamin D supplementation with experimental animals. Interventions with vitamin D administration were associated with a significant reduction in bleeding volume compared to controls at day 3 (9.8 versus 16.5 mm³, p <0.01) and day 5 (1.6 versus 4.1 mm³, p <0.001). It is associated with increased red blood cell clearance (erythrophagocytosis) mediated by monocyte-derived macrophages and the elimination of other toxic metabolic substances. At the time of ICH, RBCs undergo extravasation more than the phagocytes. Of all phagocyte cells, the observation results showed better phagocytosis ability compared to microglia. These results emphasize macrophages as a promising therapeutic target for the elimination of RBCs (Liu et al., 2022).

Another factor that affects the extent of the lesion is systolic blood pressure (SBP). SBP positively correlates with the volume of initial bleeding in patients and patients with SBP of more than 160 mmHg have a higher risk of hematoma expansion at the start of admission (Li et al., 2020). In another study by Rodríguez-Luna (2013), SBP 180-
load, SBP greater than 180 mmHg on most measurements within 24 hours, can predict hematoma expanders (OR 1.05, 95% CI 1.001-1.097, p 0.016). This is because, in the TDS range, there is a continuous rupture of the walls of small blood vessels. Mean arterial pressure High MAP is also positively correlated with hematoma expansion (Rodriguez-Luna et al., 2013).

Hyperglycemia after ICH is also associated with the expansion of the hematoma. In one study, Chu et al. (2022) show that the expansion of the hematoma increases with increasing hyperglycemia stress. SHR (stress hyperglycemia ratio) value in patients with ICH was higher in patients with hematoma expansion compared with SHR values in patients without hematoma expansion (OR 16,535, 95% CI 3,572-76,543). Hyperglycemia after ICH can induce an inflammatory reaction that exacerbates secondary brain injury through increased TNFα and High Mobility Group Box 1 Expression. Hyperglycemia also disrupts the integrity of the blood-brain barrier at the site of ICH initials and increases the expression of NFKB and MMP-9 (Chu et al., 2022).

The limitation of this study was that the measurement of the influence of other factors on the degree of severity and volume of bleeding, such as high blood pressure, DM, and dyslipidemia, was not conducted.

CONCLUSION

In this study, there was no relationship between vitamin D levels. the degree of disease, and the volume of bleeding in ICH patients. Further research is needed to analyze the relationship between vitamin D serum levels and the severity of ICH with a retrospective cohort study design and discuss the effect of vitamin D on long-term functional outcomes.

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CONFLICTS OF INTEREST STATEMENT

The author states that there is no potential conflict of interest about the authorship and publication of this article.

REFERENCES


