



# OPEN ACCESS

Key Words

CVA, vitamin B12, stroke, risk factors

### **Corresponding Author**

Dr. Monoj Sharma, Department of Biochemistry, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal 713104, India alamgir.cnmch10@gmail.com

#### Author Designation 1,2-

<sup>8</sup>Post Graduate Trainee
<sup>3</sup>Assistant Professor
<sup>4</sup>Demonstrator <sup>5</sup>Professor

Received: 6 September 2024 Accepted: 7 September 2024 Published: 11 September 2024

**Citation:** Dr. Md. Alamgir Perwana, Dr. Ramprasad Atta, Dr. Dinobandhu Naga, Dr. Srabani Ghosh, Dr. Goutam Chakraborti, Dr. Joyanti Mahata Sarkar, Dr. Shreya Halder and Dr. Monoj Sharma 2024. Assessment of Serum Vitamin B12 and Homocysteine Levels in Case of Cerebrovascular Accident. Res. J. Med. Sci., 18: 470-474, doi: 10.59218/makrjms.2024.3.18.470. 474

# Assessment of Serum Vitamin B12 and Homocysteine Levels in Case of Cerebrovascular Accident

<sup>1</sup> Dr. Md. Alamgir Perwana, <sup>2</sup> Dr. Ramprasad Atta, <sup>3</sup> Dr. Dinobandhu Naga, <sup>4</sup> Dr. Srabani Ghosh, <sup>5</sup> Dr. Goutam Chakraborti, <sup>6</sup> Dr. Joyanti Mahata Sarkar, <sup>7</sup> Dr. Shreya Halder and <sup>8</sup> Dr. Monoj Sharma

<sup>1,2,4-8</sup>Department of Biochemistry, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal 713104, India

<sup>3</sup>Department of General Medicine, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal 713104, India

## ABSTRACT

A stroke is characterized as "a clinical syndrome consisting of rapidly developing clinical signs of focal disturbance of cerebral function lasting more than 24 hrs or leading to death with no apparent cause other than a vascular origin" (American Heart Association, 1991). To ascertain the homocysteine and serum vitamin B12 status of individuals who have had cerebrovascular accidents (CVAs). The present study was a Hospital based observational analytical study. This Study was conducted from one and half year (December, 2022 to May, 2024) at the departments of Biochemistry and Medicine. Total 232 patients were included in this study. In the cases, 34 (29.3%) patients had Hemorrhagic and 82 (70.7%) patients had Ischemic CVA. In the cases, the mean Vitamin-B12 value (pg mLG<sup>1</sup>) (Mean±S.D.) of patients was 395.6466±137.7770. In the controls, the mean Vitamin-B12 value (pg mLG<sup>1</sup>) (Mean±S.D.) of subjects was 538.3621±111.8301. Level of mean Vitamin-B12 value in cases was lower with respect to controls and was statistically significant (p<0.0001). In the cases, the mean Homocysteine value (umole  $LG^1$ ) (Mean $\pm$ S.D.) of patients was 12.5884±9.2961. In Controls, the mean Homocysteine value (umole LG<sup>1</sup>) (Mean±S.D.) was 7.1704±0.8847. Level of mean Homocysteine value in cases was higher with respect to controls and was statistically significant (p<0.0001). In conclusion, measuring blood homocysteine and vitamin B12 levels is a critical step toward understanding potential causal factors of cerebrovascular accidents. The findings suggest a potential correlation between elevated homocysteine levels and low vitamin B12 levels and an increased risk of cerebrovascular accidents. This highlights how important it is to routinely check for these biomarkers in at-risk individuals, since doing so allows for early intervention and management strategies to lessen the possibility of such debilitating events.

Copy Right: MAK HILL Publications

### INTRODUCTION

The World Health Organization states that noncommunicable diseases such as heart disease, chronic obstructive pulmonary disease and cerebrovascular accidents are replacing infectious diseases as the leading causes of sickness worldwide. The most common preventable and treatable cause of death and disability worldwide today is Cerebrovascular Accident (CVA), which is primarily brought on by atherosclerosis in the arteries. CVA is characterized by syncopal attacks, limb weakness or paralysis, speech slurring, disturbed mental function, abnormal bowel and bladder function and other symptoms.

A stroke is characterized as "a clinical syndrome consisting of rapidly developing clinical signs of focal disturbance of cerebral function lasting more than 24 hrs or leading to death with no apparent cause other than a vascular origin" (or global in the event of a coma)<sup>[1]</sup>. The 85% of all strokes are caused by ischemic stroke, commonly referred to as cerebral infarction, which is one of the most prevalent cerebrovascular illnesses. Patients' lives and health are at risk because to its high incidence, morbidity and death. It is the second greatest cause of mortality worldwide and the main cause of disability. Ischemic stroke is classified into five categories: Large-Artery Atherosclerosis (LAA), Small-vessel Occlusion (SAO), Cardioembolism (CE), Stroke of Other Determined etiology (SOE) and Stroke of Uncertain Etiology (SUE) based on the criteria of the trial of org 10172 in acute stroke treatment (TOAST). Apart from the conventional risk factors including smoking, diabetes and hypertension, research has shown that homocysteine (Hcy) is a separate risk factor for disorders of the heart and brain. rial and veinous systems, primarily impacting the brain-spinal cord system.

Cerebrovascular accidents (CVAs) are associated with low serum vitamin B12 and high homocysteine levels. Vitamin B12 is a co-factor for two enzymes, namely methylmalonoyl-CO-A synthase and methionine synthase, which are essential for the biosynthesis of methionine and nucleotides. A deficiency in vitamin B12 causes a build-up of metabolic substrate, such as homocysteine, which alters immune homeostasis and contributes to atherosclerotic disorders, including ischemic strokes. It also supports cellular function and acts as a metabolic co-factor for gut microbes, which has an additional effect on local and peripheral immunity. Myelopathy (SACD), ataxia, peripheral neuropathy, optic neuropathy and dementia with psychosis are all treatable with vitamin B-12 supplementation in cases of severe vitamin B12 insufficiency.

#### MATERIALS AND METHODS

**Study type:** Hospital based observational analytical study.

Study design: case control study.

Study setting: Hospita1 based study.

**Place of study:** This study was conducted in the Depanment of Medicine and Department of Biochemistry of Burdwan Medical College and Hospital.

**Study population:** Patients admitted with CVA through emergency and OPD to Medicine ward (Male and Female). And control cases was taken from medicine OPD or emergency.

Sample size: 116 cases and 116 controls.

**Sample design:** Previous study showed that In Indian population prevalence of vitamin B 12 deficiency in all CVA patient is approximately 8.20/0.6 1 will take 95% precision in my study. So my level of error was 5%. Level of significance was considered at 95%. Considering all the facts sample size was 116. Similar number of controls added in the study.

**Study period:** One and half year. (December, 2022 to May, 2024).

#### Inclusion criteria:

- For cases
- Male or female aged 60 years and above
- Hypertension
- New Case of CVA
- For control group
- Male or female aged 60 years and above
- Hypertension

## Exclusion criteria:

- Male or Female aged below 60 years
- Previous history of stroke/TIA
- Previous Angina or Heart Failure or AMI
- Presence of Psychiatric disorder
- Diabetes mellitus
- Megaloblastic Anaemia
- Malignancy
- Chronic Alcoholism
- Malnutrition
- Any malabsorption syndrome
- Chronic diseases like TB, Liver diseases, CKD
- DRUGS like anticancer drugs, methotrexate

#### Parameters to be studied:

- Age, Height, Weight, BMI, Blood pressure
- Clinical presentation of patient
- Blood sample for investigation: serum Vit-B12, serum Homocysteine
- Radiological imaging (CT and or MRI)

## Study tools:

- Pre designed, pre tested schedule
- Weighing machine, Measuring tape for height
- Centrifuge machine REMI R-8C
- Routine laboratory glass wares
- Plain vials without any coagulant
- Distilled water, Normal saline (0.9%), acetic acid
- Rubber tourniquet, Disposable syringes cotton swab
- Sphygmomanometer, Stethoscope Nephrometer

**Study techniques:** Proper counselling and informed written consent was takenfrom each Cerebrovascular accident (CVA) patients/relative and control. Detailed history and clinical examination.

**Blood sample collection:** To estimates serum Vitamin B12 and Homocysteine, 5 mL of Venous-blood was drawn from each patient in a plain vial without any anti-coagulant through aseptic condition.

Procedure (parameters estimation):

- Estimation of Serum Vit-B12, Microbiological assay (CLIA)
- Estimation of Serum Homocysteine; Enzymeimmune assay (CLIA)

Plan of study: This Descriptive analytical study will includes strokes patients (CVA), age group about 60 years or above, both male and female with Hypertension without pre-existing strokes, myocardial infarction, Diabetes mellitus with or without history of smoking attending to the OPD Or Emergency for admission at Department of Medicine Burdwan medical college and Hospital 2022-24. The study was done after approval by the Ethics Committee of medical faculty, informed consent have to be taken from patients or patients party. The study group was consisted of 116 patients of CVA. Needed information was collected through careful history and complete physical examination, Blood pressure of all patients was checked and complete laboratory study including serum Vit-B12 and homocysteine. Radiological imaging was in consideration of this study. Details history was taken and existing documents was checked to excludes previous such morbidities. Blood pressure was measured through sphygmomanometer through proper way. The diagnosis of Hypertension was confirmed in hospital as Blood pressure above 140/90 mm of Hg. Details history of Hypertension and antihypertensive medication and duration was taken. History of other medication also be excluded-whether patients have been suffering from any other chronic illness will have to be reviewed. Patients of Diabeuxs mellitus and intake of medication on regular basis

or not was detailed at the lime of examination, laboratory finding including blood glucose level, urine examination to exclude Diabetes mellitus, History of smoking was detailed along with duration and frequency. GCS (Glasgow coma scale) of every patients was noted at the time of admission and subsequent as it will give a prognostic picture of each patients. Details history of psychiatric disorder and whether patients is on medication along with duration was taken Blood sample of each patient will collected for requisite blood parameter, checking along with blood Vitamin B12 and homocysteine level. The study group will consist of 116 cases of CVA Patients and 116 controls that was selected randomly from admission of OPD keeping all parameters of inclusion and exclusion criteria in mind. Needed information was collected through careful history and complete physical examination.

**Plan for analysis:** Data entry was done right after collection of relevant data (Oral questionnaires, blood samples) for a given patient is completed. Data analysis was done after completion of data collection from all patients. Data was analysed for any significant correlation between the parameters from the data collected on regular intervals. SPSS software was used for data analysis,

**Ethical considerations:** The study proposal along with other relevant documents would be submitted to the Institutional Review committee for review and approval. The study will commence after such approval is obtained in writing.

Statistical analysis: For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. A chi-squared test ( $\chi^2$  test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chisquared test' often is used as short for Pearson's chisquared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

Explicit expressions that can be used to carry out various t-tests are given below. In each case, the formula for a test statistic that either exactly follows or

closely approximates a t-distribution under the null hypothesis is given. Also, the appropriate degrees of freedom are given in each case. Each of these statistics can be used to carry out either a one-tailed test or a two-tailed test.

Once a t-value is determined, a p-value can be found using a table of values from student's tdistribution. If the calculated p-value is below the threshold chosen for statistical significance (usually the 0.10, the 0.05, or 0.01 level), then the null hypothesis is rejected in favour of the alternative hypothesis.

 $p \le 0.05$  was considered for statistically significant.

#### RESULTS

In Case, 84 (72.4%) patients were 61-70 years of age, 24 (20.7%) patients were 71-80 years of age, 8 (6.9%) patient were 81-90years of age (Table 1).

In Control, 82 (70.7%) patients were 61-70 years of age, 22 (19.0%) patients were 71-80years of age, 12 (10.3%) patient were 81-90 years of age.

Association of Age between case and control groups was not statistically significant (p = 0.6341).

In Case, 33 (28.4%) patients were female, 25 (21.6%) patients were male.

In Control, 83(71.6%) patients were female, 91 (78.4%) patients were male.

Association of Sex between cases and controls was not statistically significant (p = 0.2251).

In Case, the mean Age (Years) (Mean±S.D.) of patients was 68.2155±5.7903.

In Control, the mean Age (Years) (Mean±S.D.) of patients was 69.3707±5.8926.

Distribution of mean Age in between case and control Groups was not statistically significant (p = 0.1334).

In Case, the Vitamin B12 value (pg mLG<sup>1</sup>) (Mean±S.D.) of patients was 395.6466±137.7770.

In Control, the Vitamin B12 value (pg mL $G^1$ ) (Mean±S.D.) was 538.3621±111.8301.

Level of mean Vitamin B12 value in cases was lower with respect to controls and was statistically significant (p<0.0001) (Table 2).

In Case, the mean Homocysteine value (umole LG<sup>1</sup>) (Mean±S.D.) was 12.5884±9.2961.

In Control, the mean Homocysteine value (umole  $LG^1$ ) (Mean±S.D.) was 7.1704±0.8847.

Level of mean Homocysteine value in cases was higher with respect to controls and was statistically significant (p<0.0001).

#### DISCUSSION

The present study was a Hospital based observational analytical study. This Study was conducted from one and half year (December, 2022 to May, 2024) at department of Biochemistry and Medicine. Total 232 patients were included in this study.

- C Group-I-116 patients with case group
- C Group-II- 116 patients with control group

Ahmed *et al.*<sup>[2]</sup> examined that Age group and stroke subtype-specific metabolic B12 deficit (MetB12Def, serum B12 <258 pmol LG<sup>1</sup> and HHcy) between 2002 and 2017. Data for 4055 patients were available. Overall, 8.2% of patients had B12Def; this represents a decrease from 10.9% of patients referred before to 2009 to 5.4% of patients subsequently (p = 0.0001) also Prajapati and Ghosh<sup>[3]</sup> observed that the mean age in case group was 62.49 (SD: 12.45 years) and 56.62 (SD: 13.05 years) in control group with p = 0.001. Consequently, the sixth and seventh decade of life is when stroke prevalence is highest; however, in our study, of 232 patients, the majority were between the ages of 61 and 70 [166 (71.6%)] but this was not statistically significant (p = 0.6341).

Prajapati and Ghosh<sup>[3]</sup> observed that The purpose of this study was to assess the connection between acute cerebral stroke and vitamin B12. blood samples

Table 1: Association of age between demographic parameter with groups

	0.0495				
Age in group	Case	Control	Total	p-value	
61-70	84	82	166	0.6341	
Row (%)	50.6	49.4	100		
Col %	72.4	70.7	71.6		
71-80	24	22	46		
Row (%)	52.2	47.8	100		
Col (%)	20.7	19	19.8		
81-90	8	12	20		
Row (%)	40	60	100		
Col (%)	6.9	10.3	8.6		
TOTAL	116	116	232		
Row (%)	50	50	100		
Col (%)	100	100	100		
Sex	Case	Control	Total	0.2251	
Female	33	25	58		
Row (%)	56.9	43.1	100		
Col (%)	28.4	21.6	25		
Male	83	91	174		
Row (%)	47.7	52.3	100		
Col (%)	71.6	78.4	75		
Total	116	116	232		
Row (%)	50	50	100		
Col (%)	100	100	100		
		Mean	SD	0.1334	
Age (years)	Case	68.2155	5.7903		
	Control	69.3707	5.8926		

Table 2: Distribution of mean vitamin B12 value (pg mLG $^1$ ) and Homocysteine value (umole LG $^1$ ) in cases and controls										
	Number	Mean	SD	Minimum	Maximum	Median	p-value	T statistic		
Vitamin B12 value (pg mLG <sup>1</sup> )										
Case	116	395.6470	137.7770	150	700	397.50	< 0.0001	8.6621		
Control	116	538.3620	111.8300	321	784	515.50				
Homocysteine value (umole LG <sup>1</sup> )										
Case	116	12.5884	9.2961	5.42	50.56	9.25	< 0.0001	6.2489		
Control	116	7.1704	0.8847	5.38	9.25	7.13				

taken from hospitalized patients within 24 hrs of the stroke (n = 100) and from 100 control instances that were matched for age, sex and other changeable stroke risk variables, we discovered that the proportion of men was greater [174 (75.0%)] than the female population [58 (25.0%)]. Male: Female ratio was 3:1 but this was not statistically significant (p = 0.2251).

Yahn *et al.*<sup>[4]</sup> showed that When it comes to stroke, ischemic stroke is more common than hemorrhagic stroke and it affects older persons more frequently. Although diet is a modifiable risk factor for stroke, our research revealed that the majority of patients in the case group experienced ischemic stroke [82 (70.7%)] but this was statistically significant (p<0.0001).

Ahmed *et al.*<sup>[2]</sup> examined that age group and stroke subtype-specific metabolic B12 deficit (MetB12Def, serum B12 <258 pmol LG<sup>1</sup> and HHcy) between 2002 and 2017. Data for 4055 patients were available. Overall, 8.2% of patients had B12Def, this represents a decrease from 10.9% of patients referred before to 2009 to 5.4% of patients subsequently (p = 0.0001) but in our study, Age was higher in Control [69.3707±5.8926] compared to Case [68.2155±5.7903] but this was not statistically significant (p = 0.1334).

Prajapati and Ghosh<sup>[3]</sup> observed that the purpose of this study was to assess the connection between acute cerebral stroke and vitamin B12. blood samples taken from hospitalized patients within 24 hrs of the stroke (n = 100) and from 100 control cases matched for age, sex and other modifiable risk factors of stroke were analyzed also Yahn *et al.*<sup>[4]</sup> showed that Elderly people frequently have vitamin B12 deficiencies, which have been linked to ischemic stroke. The purpose of this review was to determine if vitamin B12 deficiency affects ischemic stroke risk and outcome. A vitamin B12 deficiency is a risk factor for ischemic stroke and a potential consequence, according to clinical evidence from their literature research. However, we discovered that the Vitamin-B12 value (pg mLG<sup>1</sup>) was higher in Control [538.3621±111.8301] compared to Case [395.6466±137.7770] and this was statistically significant (p<0.0001).

Al Baklawy *et al.*<sup>[5]</sup> found that serum. There have been reports of homocysteine increase in certain patients with cerebral vinous sinus thrombosis. Cerebrovascular illness and homocysteine levels have a complicated relationship and there is ongoing debate on the source and relevance of the homocysteine surge in cerebral vinous sinus thrombosis. Rabelo *et al.*<sup>[6]</sup> found that Increased risk of ischemic stroke has been linked to elevated homocysteine (Hct) levels. There is still some debate, though. To evaluate the levels of Hct between patients with ischemic stroke and controls, they conducted a systematic review and meta-analysis; nevertheless, in our investigation, the homocysteine value (umole  $LG^1$ ) was greater in Case [12.5884±9.2961] compared to Control [7.1704±0.8847] and this was statistically significant (p<0.0001).

# CONCLUSION

In conclusion, measuring blood homocysteine and vitamin B12 levels is a critical step toward understanding potential causal factors of cerebrovascular accidents. The findings suggest a potential correlation between elevated homocysteine levels and low vitamin B12 levels and an increased risk of cerebrovascular accidents. This highlights how important it is to routinely check for these biomarkers in at-risk individuals, since doing so allows for early intervention and management strategies to lessen the possibility of such debilitating events. In order to prevent and treat cerebrovascular accidents, more study is necessary to determine the specific processes behind this association as well as to investigate the possible therapeutic benefits of raising homocysteine levels and improving vitamin B12 levels. These initiatives have the potential to improve patient outcomes and lessen the toll that cerebrovascular illness has on patients and healthcare systems.

# REFERENCES

- 1. Hatano, S., 1976. Experience from a multicentre stroke register: A preliminary report. Bull. World Health Org., 54: 541-553.
- 2. Ahmed, S,. C. Bogiatzi, D.G. Hackam, A.C. Rutledge and L.A. Sposato *et al.*, 2019. Vitamin B 12 deficiency and hyperhomocysteinaemia in outpatients with stroke or transient ischaemic attack: A cohort study at an academic medical centre. BMJ Open., Vol. 9, No. 1.
- Prajapati, M. and L.M. Ghosh, 2020. Vitamin B 12: The hidden ingredient of stroke. Int. J. Res. Med. Sci., 8: 644-646.
- 4. Yahn, G.B., J.E. Abato and N.M. Jadavji, 2021. Role of vitamin B12 deficiency in ischemic stroke risk and outcome. Neural Regen. Res., Vol. 16, No. 3.
- Al Baklawy, M.A., R.M. Khodair, M.K. Faheem and I.A. Abd Elrassoul, 2020. Serum homocysteine level in patients with cerebral venous sinus thrombosis (CVST): Relation to initial thrombosis severity and outcome. Benha. J. Appl. Sci., 5: 121-127.
- Rabelo, N.N., J.P. Telles, L.Z. Pipek, R.F.V. Nascimento, R.C. Gusmão, M.J. Teixeira and E.G. Figueiredo, 2022. Homocysteine is associated with higher risks of ischemic stroke: A systematic review and meta-analysis. Plos One, Vol. 17, No. 10.