

# Prevalence of Serum Vitamin-B12 Level and its Association Among the Patients of Transient Ischemic Attack/Ischemic Stroke

ABDULLAH KHAN<sup>1</sup>, SHAHIDA HASAN MEMON<sup>2</sup>, RUKHSANA SABOOR<sup>3</sup>, ANEEB MENGAL<sup>4</sup>, NAILA MEMON<sup>5</sup>, MUHAMMAD ALI SOOMRO<sup>6</sup>, KIRAN AAMIR<sup>7</sup>, AAMIR RAMZAN<sup>8</sup>

<sup>1</sup>Demonstrator in biochemistry department jhalawan medical college khuzdar, Balochistan

<sup>2</sup>Professor, Head of biochemistry department Liaquat university of Medical and health sciences jamshoro Hyderabad

<sup>3</sup>Assistant professor, Pathology Department, Ghulam Muhammad mahar medical college Sukkur

<sup>4</sup>Demonstrator in physiology Department jhalawan medical college Khuzdar, Balochistan

<sup>5</sup>Senior WMO, consultant physician, Liaquat university hospital Hyderabad.

<sup>6</sup>Lecturer, Department of Pathology, Bilawal Medical College for Boys Liaquat university of Medical and health sciences jamshoro Hyderabad

<sup>7</sup>Associate professor, Pathology Department Liaquat university of Medical and health sciences jamshoro Hyderabad

<sup>8</sup>Lecturer in pathology Department Liaquat university of Medical and health sciences jamshoro Hyderabad

Corresponding author: Abdullah Khan, Email: [abdullahkhanrind@gmail.com](mailto:abdullahkhanrind@gmail.com), Cell: 03013696798

## ABSTRACT

**Objective:** To determine the hospital-based prevalence of Vitamin-B12 deficiency in ischemic stroke patients and to compare the association of VitB-12 among patients of Ischemic stroke and controls.

**Study Design:** Comparative study

**Place and Duration:** Neurology and Medicine wards at the Liaquat University Hospital Hyderabad/Jamshoro. From Dec 2021 to June 2022

**Methods:** It was a non-probability convenience sample that comprised 263 individuals, of whom 132 were cases and 131 were controls. Among the participants, there were no missing data. The completion of a standardized questionnaire allowed for the collection of information about socio-demographic factors. Serum levels of VitB-12 were measured for all of the individuals aged 18 to 60 years old and of both genders

**Results:** The gender distribution of cases and control groups, as well as the sociodemographic characteristics of the study population, have significant differences, in gender ( $p = 0.012$ ) and socioeconomic status ( $p = 0.016$ ). However, there was not a significant difference found in residence (0.82), nor age (0.46), in either of the groups. The average body mass index (BMI) was calculated to be  $24.1 \pm 6.1$ , while the average weight and height were determined to be  $62.8 \pm 12.3$  kg and  $158.62 \pm 5.21$ cm, respectively. The incidence of ischemic stroke was recorded in 84 patients, whereas transient ischemic attack was documented in 48 patients. The percentage of instances that were affected by risk factors such as smoking, using paan gutka, or having high blood pressure was much greater than the percentage of cases that were affected by obesity, diabetic patients, or vegetarians. In all, there were 132 people out of 263 who were checked for their blood VitB-12 levels who were determined to have suffered from a stroke or transient ischemic attack (TIA). The cases comprised of both ischemic stroke or TIA. In a comparison of cases (ischemic stroke/TIA) and controls, it was shown that the patients had significantly lower mean levels of blood VitB-12 than the controls did ( $132.7 \pm 2.41$  vs.  $272.1 \pm 5.17$  pg/mL respectively).

**Conclusion:** The patients reported to neurology ward of Liaquat University hospital Hyderabad and Jamshoro, we found an unacceptably high burden of stroke and transient ischemic attack. There is a significant association between VitB-12 and ischemic stroke as compared to healthy controls.

**Keywords:** Vitamin B-12, Ischemic Stroke, Risk Factors

## INTRODUCTION

Around the world, stroke is consistently ranked as one of the leading causes of death. 15 million individuals throughout the globe experience a stroke each year, and of those, 5 million people pass away and another 5 million become handicapped, placing a strain not just on their families but also on their communities. Ischemic stroke is a significant issue in the healthcare industry, and it is the leading cause of morbidity as opposed to fatality. (1) Ischemic stroke (IS) is mostly avoidable, and the risk factors for getting it are the same in industrialized nations as they are in less developed ones. Around ninety percent of all risk factors are conditions that can be controlled. These conditions include arterial hypertension, diabetes mellitus, cardiovascular diseases, smoking cigarettes, obesity, hyperlipidemia, physical inactivity, excessive alcohol use, an unhealthy diet, psychosocial stress, and depression. The blood VitB-12 level is a novel possibly treatable factor that contributes to the risk of stroke. Although this list is broad, it does not include every risk factor. (2) Strokes caused by ischemic conditions are quite frequent in Pakistan. Research that was conducted with the help of the local population found that the prevalence of stroke in the urban slums of Karachi is around 21.8%. It was determined that 105 patients, or 70%, had had an ischemic stroke, while 45 patients, or 30%, had suffered a hemorrhagic stroke. (3) VitB-12, or cobalamin, is a water-soluble vitamin that also goes by the name VitB-12. It has a role in the metabolism of each and every cell in the human body and is a cofactor in the process of synthesising DNA. (4) VitB-12 is an essential component of the one-carbon metabolism, which is a

major metabolic network that combines nutritional signalling with biosynthesis, redox homeostasis, and epigenetics. This metabolic network is responsible for the maintenance of epigenetic information. Meat, dairy products, eggs, and fish are all examples of meals that come from animal sources and provide humans with VitB-12. There are bacteria in the digestive tract, such as *Escherichia coli*, that are capable of producing VitB-12; however, the quantity produced is not adequate to meet the body's needs. (5) Methionine synthase is the enzyme that catalyses the metabolic (methylation) process that transforms homocysteine to methionine. This pathway needs folic acid (5-methyl tetrahydrofolate) and VitB-12 (cobalamin) as important cofactors in order to function properly. (6) In 2006, Spence reported that metabolic B12 deficiency was present in 10% of patients with stroke/transient ischemic attack (TIA) aged of homocysteine may lead to the formation of blood clots as well as damage to blood vessels. (8) It is possible that nutritional variables, notably a lack of VitB-12, contribute to elevated blood homocysteine levels in a population. This means that nutritional factors may potentially be a risk factor for intracranial sclerosis (IS). This could be significant as an acquired risk factor for stroke, which is also something that can be readily changed. (8) Patients who are old or vegetarian tend to have lower amounts of VitB-12 in their bodies, which may be an unrecognised factor in homocysteine levels and vascular disease. There are neurologic consequences associated with a lack of VitB12, such as neuropathy, dementia, and subacute combined degeneration of the spinal cord. This is in addition to the rise in total homocysteine that occurs as a result of the lack of VitB-12. It

is commonly observed that these risk factors are overlooked in older age patients. (9) A non-vegetarian diet, which may lead to a lack of VitB-12, is one of the less well-known risk factors that can add to the chance of having a stroke. (10) It is reported that patients of stroke have low blood levels of vitamin B-12. In addition, it has been shown that avoiding VitB-12 deficiency is an excellent method for lowering the chance of having a stroke. (11) The Heart Outcomes Prevention Evaluation 2 Trial was a large study that examined the stroke rate as a response to VitB-12 injection supplements. The findings of the study demonstrated that supplementation with VitB12 effectively reduced the risk of stroke among the participants of the study. (12)

## MATERIALS AND METHODS

This Study was conducted at department of Biochemistry with collaboration of Medicine and Neurology department at LUM&HS Jamshoro/Hyderabad and comprised of 260 patients.

Male or female 18-60 years of age, known case of ischemic stroke reporting to Medicine and Neurological departments at LUMHS Jamshoro/Hyderabad were included. Patients above 60 or below 18 years of age, pregnant females, patients taking drugs that might affect VitB-12 metabolism were not included. Patients were divided in two groups. Group (A) including cases of Ischemic stroke/TIA (132) and Group (B) including controls (131). The fully automated cobas-e 601 module uses proprietary electrochemiluminescence (ECL) technology for immunoassay analysis. It has all the equipment needed for quantitative and qualitative in vitro test determinations for a variety of applications. The Cobas e 601 analyser can measure blood and plasma VitB-12 levels.

All participants gave their consent. Detailed questionnaires were employed to collect demographic data on weight, height, BMI, nutrition, supplements, smoking history, and co-morbidities such diabetes and hypertension. Before signing up for this study, participants were given a volunteer form. Each person's height and weight were measured using an analogue floor scale with a height measuring scale. Before being translated to metres, respondents' heights were measured in centimetres. Test volunteers were instructed to sit up straight to take their blood pressure. Sphygmomanometer measurements were taken every five minutes for two readings. At regular intervals, glucometers measured participants' blood glucose levels. Self-reported strokes and TIAs were validated using medical records and a standard form filled out by the treating or research physician. This followed the protocol. We previously reported on this research's stroke incidence rate, which was comparable to that of Pakistan's relevant age group. TIAs were neurologic impairments that lasted less than 24 hours, while strokes were neurologic deficits that persisted longer and had a vascular aetiology. Two specialists at the research institution classified strokes as transient ischemic attack (TIA), ischemic stroke, intracerebral stroke, subarachnoid haemorrhage, and unexplained stroke.

SPSS 22 analysed the data. The mean  $\pm$  SEM represented quantitative data. Frequency and proportion represented qualitative data. Chi square was used to compare proportions between two qualitative criteria. An independent sample T-test compared case and control group mean values. The confidence interval was 95% and the margin of error was 5% for P-values less than 0.05.

## RESULTS

A total of 263 study participants; 132 cases of ischemic stroke/TIA and 131 control groups were included in this study. Table 1 shows the gender distribution of cases and control groups as well as the sociodemographic characteristics of the study population with significant differences, in Gender ( $p=0.012$ ) and socioeconomic status ( $p=0.016$ ) and there was no significant difference found in residence (0.82) and age (0.46) in both groups. Study results

showed that there was significance of male predominance and low socioeconomic status among the stroke/TIA cases groups.

Table 1: Sociodemographic Characteristics of Study Population

Variables	Cases (%)	Controls (%)	P value
Gender			
Male	94 (71%)	73 (56%)	
			0.0126
Female	38 (29%)	58 (44%)	
Age			
18-34	55 (42%)	52 (40%)	
			0.4610
35-60	77 (58%)	79 (60%)	
Residence			
Rural	51 (39%)	58 (44%)	
			0.8274
Urban	81 (61%)	73 (56%)	
Socio-economic status			
Low	82 (62%)	60 (46%)	
			0.0162
Moderate	50 (38%)	71 (54%)	

**Anthropometric Variables of Study Subjects:** There were 263 participants enrolled in the study who met the inclusion criteria. The mean body mass index (BMI) was  $24.1 \pm 6.1$  and mean weight and height were found to be  $62.8 \pm 12.3$  and  $158.62 \pm 5.21$  respectively (Table-2).

Table 2: Anthropometric measurements of study subjects

Variables	Mean $\pm$ SEM
Height (cm)	$158.62 \pm 5.21$
Weight (Kg)	$62.8 \pm 12.3$
BMI (Kg/m <sup>2</sup> )	$24.1 \pm 6.1$

**Vitals of Study Subjects:** Mean and SEM of vitals of patients were as follows; pulse  $98 \pm 14.2$  (50-130) beats per minute, systolic blood pressure (SBP)  $162 \pm 32.5$  (180-90) mmHg, diastolic blood pressure (DBP)  $82 \pm 25.0$  (40-110) mmHg, temperature  $99.5 \pm 3.4$  (97.4-101.5) °F and respiratory rate  $19 \pm 8.1$  (8-30) breaths per minute. (Table 3)

Table 3: Vitals of Study Subjects

Variables	Mean $\pm$ SEM
Pulse (beats per minute)	$98 \pm 14.2$
Blood Pressure (mmHg)	
Systolic Blood Pressure (mmHg)	$162 \pm 32.5$
Diastolic Blood Pressure (mmHg)	$82 \pm 25.0$
Temperature (°F)	$99.5 \pm 3.4$
Respiratory Rate (breaths per minute)	$19 \pm 8.1$

**Stroke/TIA Risk Factors of the Study Population:** Table 4 shows the risk factors for ischemic stroke and Tia. The proportion of Smoking, Paan gutka and hypertension was much higher among cases, whereas the proportions of Obesity, patients with diabetes, and vegetarians were comparable.

Table-4: Stroke Risk Factors of the Study Population

Risk factors	Cases (132)	Controls (131)	P-value
Hypertension (%)	89 (67)	55 (42)	0.023
Smoking (%)	105 (80)	82 (63)	0.156
Pan Gutka (%)	92 (70)	64 (49)	0.054
Obesity (%)	88 (67)	72 (55)	0.219
Diabetics (%)	25 (19)	12 (9)	0.193
Vegetarian (%)	39 (30)	32 (24)	0.165

**Prevalence Of Stroke and Transient Ischemic Attack (TIA) In Study Groups:** Ischemic Stroke was reported in 84 individuals while TIA was reported in 48 individuals. Overall, stroke and/or TIA were found in 132 individuals out of 263 individuals which were screened for serum VitB-12 levels. The prevalence of Ischemic Stroke and TIA across gender, age groups and ethnicities has been shown in table 5.

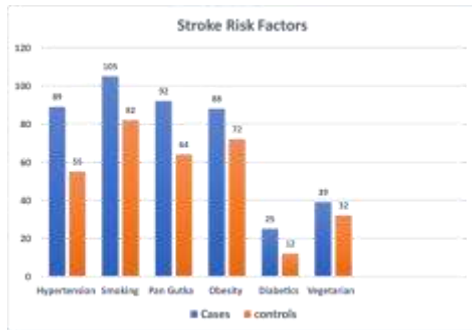


Fig.1: Stroke Risk Factors of the Study Population

Table-5: patient of stroke and transient ischemic attack (TIA) in study groups

Variables	TIA (48)	Ischemic stroke (84)
	N (%)	N (%)
Gender		
Male	25 (52%)	55 (66%)
Female	38 (48%)	29 (34%)
Age		
18-34	20 (42%)	32 (38%)
35-60	28 (58%)	52 (62%)
Ethnicity		
Sindhi	14 (39%)	24 (29%)
Urdu	17 (35%)	22 (26%)
Baloch	07 (15%)	17 (20%)
Pathan	10 (21%)	21 (25%)

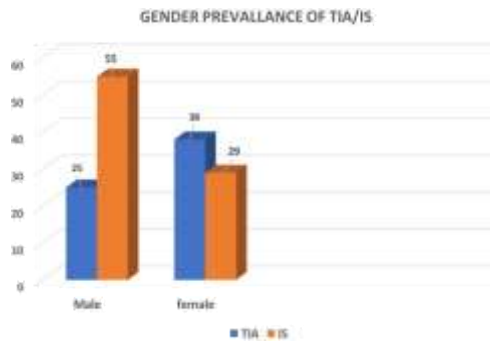


Fig.2: patient of stroke and transient ischemic attack (TIA) in study groups

**Serum VITB-12 levels:** On comparing cases (Ischemic Stroke/TIA) with controls, the mean levels of serum VitB-12 ( $132.7 \pm 2.41$  pg/mL vs.  $272.1 \pm 5.17$  pg/mL respectively) were significantly lower in the patients as compared to controls.

Table 6: Serum VitB-12 levels among cases and Controls

Variables	CASES (M $\pm$ SEM)	CONTROLS (M $\pm$ SEM)	P value
Serum B12 (pg./ml)	$132.7 \pm 2.41$	$272.1 \pm 5.17$	0.012

Values are expressed as Means  $\pm$  SEM. VitB-12 – range :234–894pg/mL

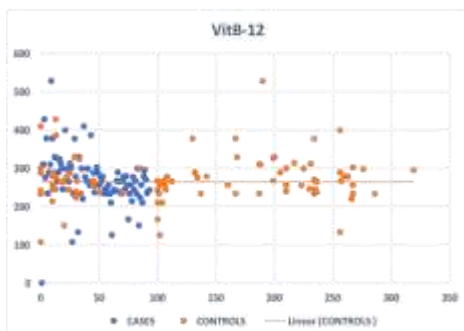


Fig.5: Serum VitB-12 levels among cases and Controls

## DISCUSSION

This Hospital-based, comparative research's primary conclusion was that low levels of VitB-12 were linked to an increased risk of cerebral ischemia. In the current investigation, a lack of VitB-12 was shown to be a substantial risk factor for the development of stroke. As was mentioned before, the aetiology of VitB-12 insufficiency may be complex and include several factors. Patients with renal failure, hypothyroidism, and other serious systemic disorders, as well as those using medicines known to induce B12 deficiency, were not included in the study from the beginning because we wanted to reduce the likelihood of the presence of confounding variables. A significantly higher proportion of B12 deficiency in our cases compared to controls suggests that B12 deficiency was one of the major contributors to IS/TIA in our patients and can therefore be regarded as a potential risk factor for IS. This finding was based on the fact that the proportion of cases with B12 deficiency was significantly higher than the proportion of controls with B12 deficiency. A previous study by Wadia et al. examining the association of B12 deficiency with Ischemic stroke/TIA had also suggested that nutritional B12 deficiency was the primary cause of HHC in their patients of IS, both arterial and venous. This conclusion was based on the findings of the study that examined the association of B12 deficiency with IS. (1)

After analysing the blood vitamin B levels of a number of individuals, researchers discovered a strong association between those levels and an elevated risk of cerebrovascular ischemia. According to one study, an increased risk was connected to having low levels of VitB-12 and folate. The researchers believe that the impact of these vitamins may be mediated in part by their influence on the metabolism of homocysteine. (2) The decrease in the mortality rate caused by strokes that occurred following widespread VitB-12 fortification of grain products in the United States and Canada provides evidence for the hypothesised influence that VitB-12 has on the development of cerebrovascular disorders. The levels of VitB-12 in the body may be a factor in determining homocysteine levels and the risk of cerebrovascular disease, as suggested in a study that was published not too long ago by QIN X et al. (3) Our findings lend credence to the idea that VitB-12 may play a preventative role in the onset of stroke. Even if VitB-12 is of special significance, the protective impact of B vitamins may still rely on adequate plasma levels of all three vitamins. This is the case even though VitB12 is the most essential of the B vitamins. The medical community in Pakistan has a general lack of awareness of the prevalence of VitB-12 insufficiency in the country's population as well as the myriad of consequences this condition may have. The incidence of B12 insufficiency has only been investigated by a few numbers of large-scale investigations in Pakistan up to this point. In their study, Refsum et al. discovered that almost half of their subjects had an insufficient level of B12, and they also discovered a significant inverse relationship between homocysteine and B12 ( $r = -0.59$ ). Despite the fact that 62% of the individuals were not vegetarians, the average level of B12 in their blood was 154 pg/ml. (5) Yajnik et al. observed a B12 deficit in 67% of their community-based participants and a substantial negative connection between B12 and homocysteine ( $r = 0.41$ ). This study also revealed a correlation between B12 and homocysteine. (6) Both of the authors did an in-depth analysis of the dietary habits of their participants and came to the conclusion that, despite the fact that B12 deficiency was more commonly associated with vegetarianism, the food habits could only partially explain the high prevalence of B12 deficiency. There is not much of a difference between the proportion of vegetarians in our cases and controls and the proportion of people who are B12 deficient in the two groups, which again suggests that there must be additional factors at play in the population group that our participants came from, such as impaired intestinal absorption of B12. Because of the high cost and limited availability of meat, nonvegetarians may also be at risk for B12 insufficiency for the same reasons as vegetarians.

In conclusion, the current research does demonstrate a connection between a B12 deficiency and I.S; but, just like any other observational study, it is unable to prove an epidemiological cause and effect relationship. Only if correcting the suspected risk factor, in this example a B12 deficiency, may result in a subsequent decrease of cerebro-vascular risk can it be shown that the condition was caused by the condition. The question of whether or not multivitamin supplementation has a role to play in decreasing cardiovascular risk has been investigated in a number of very large, international trials over the past decade. Some of these trials, such as HOPE-2, VISP, and VITATOPS, have attempted to answer this question. However, the results that were initially published were largely disappointing. However, there have been concerns voiced about the interpretation of their findings as well as the possible existence of variables that might cloud the outcomes of the investigation. Even though there was no decrease in the overall composite main outcome, the HOPE-2 study did show a substantial relative risk reduction in nonfatal strokes. This was one of the major outcomes being studied. (8) In a second effectiveness analysis of the VISP trial, after eliminating patients from either arm of the study who had received B12 injections, as well as patients who were suffering from renal failure, high-dose treatment with VitB12 was shown to be more effective than low-dose treatment.

It was shown that taking vitamins orally considerably reduced the chance of having a stroke or having a coronary event. (9) Additionally, in VITATOPS, there was a significant reduction in the primary outcome among patients with normal creatinine levels, but not among their counterparts. This raises the question of whether or not the final results have been confounded by the inclusion of patients with renal dysfunction. (10)

## CONCLUSIONS

Participants reporting to Neurology and Medicine department LUMHS Hospital Jamshoro/Hyderabad, we found a high burden of

stroke and transient ischemic attack. Both the clinical data and the findings of our study provide significant evidence to support the hypothesis that low levels of VitB-12 are a risk factor for ischemic stroke.

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