

**International Journal of Biomedical Research**

ISSN: 0976-9633 (Online); 2455-0566 (Print)

Journal DOI: <https://doi.org/10.7439/ijbr>

CODEN: IJBRFA

Original Research Article

**Evaluation of Serum Vitamin B12, Folic acid and Homocysteine levels in Stroke Patients**Jyothi D.N<sup>\*1</sup>, Anita R. Bijoor<sup>2</sup> and Gosala R K Sarma<sup>3</sup><sup>1,2</sup>Department of Biochemistry, St. John's Medical College and Hospital, Bengaluru 560034, Karnataka, India<sup>3</sup>Department of Neurology, St. John's Medical College and Hospital, Bengaluru 560034, Karnataka, India

QR Code

**\*Correspondence Info:**

Dr. Jyothi D.N

Department of Biochemistry

St John's Medical College and Hospital

Sarjapur Road, Bangalore-560034 Karnataka, India

**\*Article History:****Received:** 27/09/2017**Revised:** 07/10/2017**Accepted:** 07/10/2017**DOI:** <https://doi.org/10.7439/ijbr.v8i10.4419>**Abstract**

Homocysteine, vitamin B12 and folate have been associated with Cerebrovascular Accidents. A number of cross-sectional, case controlled cohort studies have demonstrated an association between high homocysteine level and increased risk of cardiovascular and cerebrovascular diseases, and there are equal numbers of studies that show no increase in risk, and there is still a debate as to the strength and validity of the association. This study was done with the objective to estimate the serum levels of vitamin B 12, folic acid and homocysteine in normal healthy controls and patients with stroke and to compare and correlate the values between these two groups. A total of 30 patients with acute ischemic stroke and 30 apparently healthy individuals were included in the study and their serum levels of vitamin B12, folic acid and homocysteine were estimated and statistically analyzed. On analysis; it was found that median serum level of vitamin B12 and folic acid in stroke patients was significantly lower than the controls ( $p < 0.001$ ) and median serum level of total homocysteine in stroke patients was significantly higher than the controls ( $p = 0.009$ ). It was also noted that there was a significant negative correlation between homocysteine and folic acid in controls while there was no significant correlation between homocysteine and vitamin B12 in both the groups. In conclusion, these results show that low levels of serum vitamin B12, folic acid and high levels of serum homocysteine are significantly associated with acute ischemic stroke.

**Keywords:** Ischemic Stroke, Homocysteine, B12 Vitamin, Folic acid.**1. Introduction**

Stroke is a global health problem. Worldwide, stroke is the third most common cause of death after coronary heart disease and all cancer deaths and it is the most important single cause of disability in the Western world [1]. Low and middle - income countries also account for 85.5% of total stroke deaths worldwide hence its prevention is a key strategy in reducing the rate of mortality and morbidity.

Several risk factors for stroke have been identified, which are the target of both primary and secondary preventive strategies. In 1969, McCully showed that elevated total Homocysteine (tHcy) levels were the cause of vascular diseases in children with inborn error of B12 vitamin metabolism. Since then, many studies have been

performed to understand the effect of hyperhomocysteinemia in cerebrovascular diseases. While some studies indicated that tHcy is an independent risk factor, others didn't confirm it [2].

There is growing evidence that high homocysteine levels contribute to the pathogenesis of ischemic stroke. Homocysteine is believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation and coagulation abnormalities. High homocysteine levels are associated with increased risk of cardiovascular and cerebrovascular disease, although there are studies that show no increase in risk, and there is still debate as to the strength and validity of the association [3].

Several factors can influence tHcy level, among which the most important are the concentrations vitamin B12 and folate. Decreased serum levels of these vitamins result in high plasma tHcy levels [4].

Homocysteine metabolism is dependent on folate and vitamin B12. Deficiencies of these vitamins would result in hyperhomocysteinemia. Several studies have demonstrated an association of deficiency of B12 and folate with raised plasma homocysteine concentration [5]. This may procure much importance in India where the cumulative consequence of vascular disease is augmented by the increased nutritional deficiencies. In such countries, it would, therefore, be relevant to introduce measures to reduce both risks of vascular disease as well as nutritional deficiencies.

In view of these findings a study was taken up to estimate the serum levels of vitamin B 12, folic acid and homocysteine in normal healthy controls and patients with stroke and to compare and correlate the values between these two groups.

## 2. Materials and Methods

In this descriptive study, 30 acute ischemic stroke cases diagnosed by CT/MRI findings in the age group 18-80 years, admitted as inpatients in St. John's Medical College and Hospital between April 2013 to July 2014, and 30 age and sex matched apparently healthy controls were measured. Written informed consent was obtained from the subjects, following which fasting blood sample was collected and analyzed for serum levels of vitamin B12, folic acid and homocysteine.

### 2.1 Inclusion criteria

A. For cases

- a. Male and female patients from 18 to 80 years of age were included.
- b. Only first ever ischemic stroke cases proven by history, clinical examination and confirmed by CT scan / MRI brain were included.

B. For controls

Age and sex matched apparently healthy individuals attending the hospital for health check-ups were included in the study.

### 2.2 Exclusion criteria

A. For cases

- a. Patients on vitamin B12, folic acid supplements within last 3 months
- b. Patients with stroke where CT/ MRI has not been.
- c. Patients with hemorrhagic stroke.
- d. Patients with venous stroke.
- e. Pregnant and lactating women
- f. Patients on certain drugs such as oral contraceptive pills, antiepileptics, nitrous oxide anesthesia and antagonists of vitamin B6.

g. Patients with liver and renal disease were excluded from the study.

B. For controls

- a. Subjects with history of previous cerebrovascular disease.
- b. Subjects on vitamin B12, folic acid supplements within last 3 months
- c. Subjects suffering from any disease at the time of sampling

The study protocol was approved by the Institutional Ethical Committee at the St John's Medical College and Hospital, Bangalore, India and informed consent was obtained from the subjects for the study.

### 2.3 Blood Sample Collection:

Informed consent was taken from patients and control subjects. Selected subject's fasting blood samples were collected with all aseptic precautions. 3 ml of blood was collected from the median cubital vein in the vacutainer. It was allowed to clot for 30 minutes and was centrifuged to separate the serum. The serum samples were stored at -20°C till they were analyzed.

### 2.4 Biochemical Analysis

Serum Vitamin B12 and Folic acid were estimated by chemiluminescent immunoassay. Serum Homocysteine was estimated by particle enhanced immunonephelometry. Vitamin B12 levels less than 148 pmol/L (200 pg/ml) was considered deficient [6]. A folate concentration less than 4ng/ml [7] was considered deficient. Hyperhomocysteinemia was defined as values >15µmol/l [8].

### 2.5 Statistical Analysis

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Median (25 percentile - 75 percentile) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Non-parametric Mann-Whitney test has been used to find the significance of study parameters on continuous scale between two groups. Effect size has been computed. Pearson correlation is used to find the correlation between Homocysteine and Vitamin B12, Folic acid levels.

## 3. Results

A total of 30 patients with acute ischemic stroke (16 male and 14 female) and 30 apparently healthy controls (16 male and 14 female) were evaluated in the study. Both the groups were well matched for age and gender. (Table 1 & 2).

**Table 1: Comparison of cases and controls according to broad age groups**

| Age Group        | Cases              |            | Controls           |            |
|------------------|--------------------|------------|--------------------|------------|
|                  | No                 | %          | No                 | %          |
| 21-35            | 2                  | 6.7        | 2                  | 6.7        |
| 36-50            | 4                  | 13.3       | 4                  | 13.3       |
| 51-65            | 16                 | 53.3       | 16                 | 53.3       |
| 66-80            | 8                  | 26.7       | 8                  | 26.7       |
| <b>TOTAL</b>     | <b>30</b>          | <b>100</b> | <b>30</b>          | <b>100</b> |
| <b>Mean ± SD</b> | <b>58.87±12.65</b> |            | <b>55.47±10.99</b> |            |

Sample are age matched with p=0.27

**Table 2: Gender distribution among cases and controls**

| Sex          | Cases     |            | Controls  |            |
|--------------|-----------|------------|-----------|------------|
|              | No        | %          | No        | %          |
| Male         | 16        | 53.3       | 16        | 53.3       |
| Female       | 14        | 46.7       | 14        | 46.7       |
| <b>Total</b> | <b>30</b> | <b>100</b> | <b>30</b> | <b>100</b> |

Sample are gender matched with p=1.00

Table 3 shows the median and 25<sup>th</sup> percentile - 75<sup>th</sup> percentile (P [25-75]) of Vitamin B12, Folic acid and Homocysteine of the Study groups.

As can be seen, significant increase (p=0.009) were observed in serum homocysteine whereas, vitamin B12 and folic acid levels showed significant decrease (< 0.001).

**Table 3: Comparison of serum vitamin B12, folic acid and homocysteine in cases and controls**

| Groups                 | Vitamin B12 |                 | Folic acid |               | Homocysteine |              |
|------------------------|-------------|-----------------|------------|---------------|--------------|--------------|
|                        | Median      | P [25-75]       | Median     | P [25-75]     | Median       | P [25-75]    |
| <b>Cases (N=30)</b>    | 196.00      | 161.75 - 381.00 | 5.54       | 2.32 – 11.08  | 15.10        | 7.99 – 22.95 |
| <b>Controls (N=30)</b> | 602.50      | 398.81 - 889.25 | 17.9       | 12.93 – 20.59 | 8.71         | 6.77 – 11.07 |
| <b>p value*</b>        | <0.001      |                 | <0.001     |               | 0.009        |              |

\* By using Mann Whitney U test

Table 4 shows the Pearson correlation of homocysteine versus vitamin B12 and homocysteine versus folic acid. There was no significant correlation observed between Homocysteine and Vitamin B12 in cases and

controls, however there was a significant negative correlation observed between Homocysteine and Folic acid in controls.

**Table 4: Pearson correlation of Homocysteine versus Folic acid and Vitamin B12 in cases and controls**

| Pair : Pearson correlation   | Cases   |         | Controls |         |
|------------------------------|---------|---------|----------|---------|
|                              | r value | p value | r value  | p value |
| Homocysteine vs. Vitamin B12 | -0.199  | 0.293   | -0.35    | 0.06    |
| Homocysteine vs. Folic acid  | -0.131  | 0.49    | -0.57    | 0.001   |

**Figure 1: The correlation between serum homocysteine and folic acid in Controls and Cases**

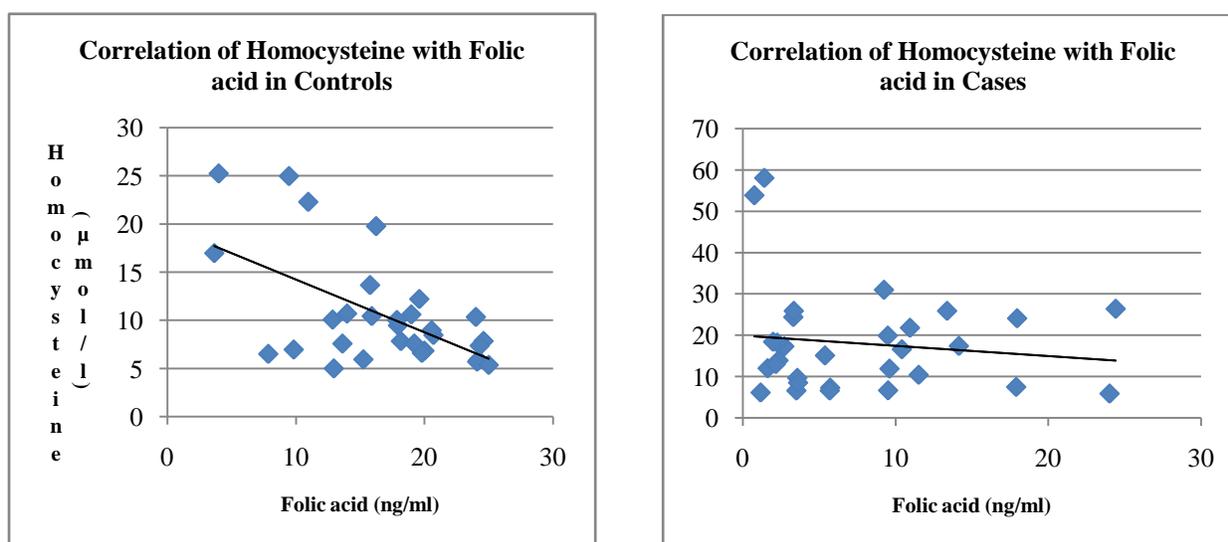
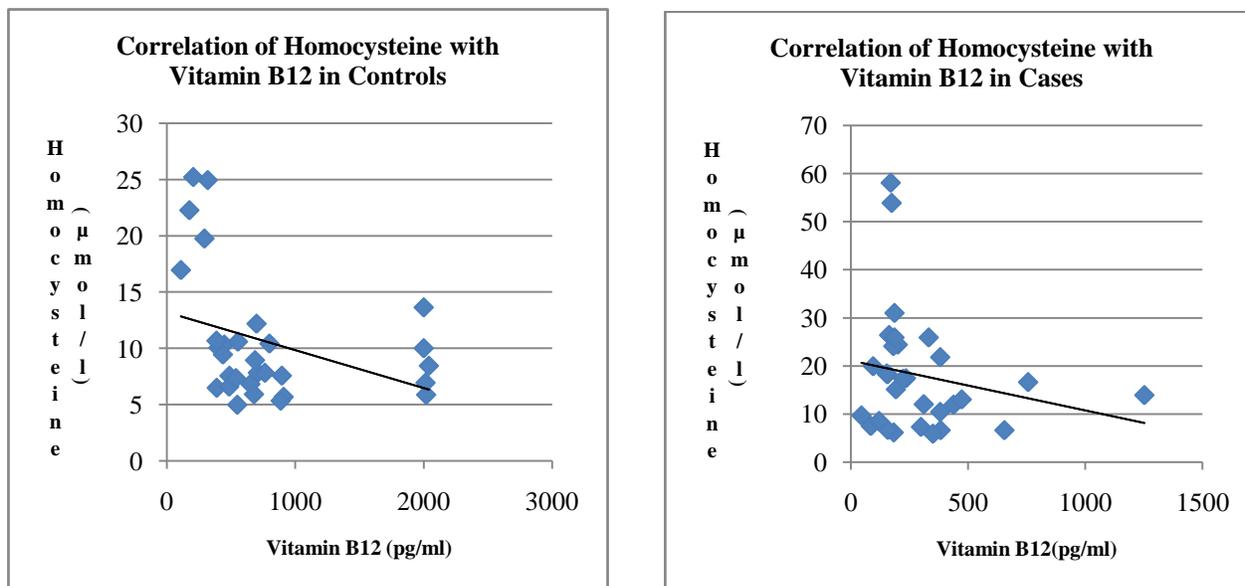


Figure 2: The correlation between serum homocysteine and Vitamin B12 in Controls and Cases



#### 4. Discussion

In the present study, Serum Vitamin B12, Folic Acid and Homocysteine levels in 30 Stroke patients were compared with 30 healthy age and sex matched controls. The study shows that there was significant hyperhomocysteinemia and deficiency of folic acid and vitamin B12 in patients with acute ischemic stroke when compared to controls.

The levels of vitamin B12 in cases (median-196pg/ml; 25<sup>th</sup>-75<sup>th</sup> percentiles [P25-P75], 161.75 – 381) were found to be lower when compared to the controls (median- 602.5pg/ml; 25<sup>th</sup>-75<sup>th</sup> percentiles [P25-P75], 398.75 – 889.25). This difference between the two groups was statistically significant, p<0.001. This is in good agreement with the study done by Kocer A *et al*[9]. Several studies have demonstrated the low levels of vitamin B12 in stroke patients [10,11]. Ischemia was because of increased homocysteine levels which damage the vascular endothelium and associated with cerebrovascular disease, coronary artery disease and periventricular white matter lucencies [WML] in patients with small vessel stroke.

The levels of folic acid in cases (median-5.54ng/ml; 25<sup>th</sup>-75<sup>th</sup> percentiles[P25-P75], 2.32ng/ml – 11.08ng/ml) were found to be lower when compared to the controls (median-17.9ng/ml; 25<sup>th</sup>-75<sup>th</sup> percentiles[P25-P75], 12.93 – 20.59) This difference between the two groups was statistically significant, p<0.001. This study goes in agreement with by Lu-chen Weng *et al*[12] which shows low folate intake was significantly and independently associated with ischemic stroke and a study by Wayne *et al*[13] where the patients with serum folate concentration <9.2 nmols/L were at increased risk for ischemic stroke.

Declining folate concentrations may result from inflammation and lymphocytic proliferation and could be responsible for the enhanced formation of total homocysteine.

Consequently the relatively strong association between homocysteine and neopterin concentrations and kynurenine/tryptophan implies that immune activation is related to the development of moderate hyperhomocysteinemia [14].

The levels of homocysteine in cases (median-15.10µmol/l; 25<sup>th</sup>-75<sup>th</sup> percentiles [P25-P75], 7.99µmol/l – 22.95µmol/l) were found to be higher when compared to the controls (median-8.71µmol/l; 25<sup>th</sup>-75<sup>th</sup> percentiles [P25-P75], 6.77µmol/l – 11.07µmol/l). This difference between the two groups was statistically significant. (p=0.009).

Several studies have shown the association of homocysteinemia in stroke patients [15-17]. Hyperhomocysteinemia in the stroke patients might be due to modulation in homocysteine metabolism [18-20]. Several factors may increase homocysteine levels in acute ischemic stroke patients. Elevated levels of Hcy can be primary - the commonest cause being due to cystathionine beta synthase deficiency. Also MTHFR gene polymorphism can increase the homocysteine level especially in the presence of low serum levels of folic acid. Secondary causes include low B12, B6 levels and many lifestyle factors such as smoking, coffee consumption; excessive alcohol intake, lack of exercise, obesity, stress and a host of systemic diseases are also associated with hyperhomocysteinemia (HHCy) [21].

There is evidence that hyperhomocysteinemia is both atherogenic and prothrombotic, operating through a

variety of potential mechanisms including direct endothelial injury, mitogenic effect on smooth muscle cells, impaired endogenous fibrinolysis, endothelial nitrous oxide response, and alteration in arachidonic acid metabolism [22, 23].

Elevated levels of the Hcy, called hyperhomocysteinemia (HHcy), are associated with higher risk of neurovascular diseases. They represent a risk factor for neurotoxicity and lead to brain damage in humans. Though mutations or polymorphisms in the key genes of Hcy metabolism pathway have been well elucidated in stroke, emerging evidences suggested that epigenetic mechanisms, such as DNA methylation, chromatin remodeling, RNA editing, noncoding RNAs (ncRNAs), and microRNAs (miRNAs) might equally play an important role in the stroke development. Genetic regulation of enzymes involved in the Hcy metabolism and levels of the vitamin cofactors (folate, B6 and B12) determine the level of Hcy. Clinical studies suggest that genetic variations of genes involved in these pathways, such as methyltetrahydrofolate reductase (MTHFR), cystathione-beta-synthase(CBS), DNA methyltransferase (DNMT) and nicotinamide N-methyl-transferase (NNMT) might increase the risk of stroke during hHcy. Nutritional supplements, e.g. folic acid (a cofactor in one-carbon metabolism), regulate epigenetic alterations and may play an important role in the maintenance of neuronal integrity [24].

Vitamin B12 and folate are the co-factors in homocysteine metabolism and have been documented to be strong correlates of plasma homocysteine in many studies and review articles [25,26].

The serum homocysteine was found to have negative and insignificant correlation with serum folic acid in control group but no significant correlation in patients. For cases the values were as follows:  $r = -0.131$  and  $p = 0.49$  and for controls,  $r = -0.57$  and  $p = 0.001$  (Figure 1). There was no significant correlation observed between Homocysteine and Vitamin B12 in cases: ( $r = -0.199$  and  $p = 0.293$ ) and controls ( $r = -0.35$  and  $p = 0.06$ ) (Figure 2)

Associations between homocysteine, low vitamin concentrations and ischemic stroke were seen. The rises as the levels of folate, vitamin B12 and vitamin B6 fall and high homocysteine concentrations are often seen with deficiency of these vitamins [27].

Experimental evidence indicates that the activities of remethylation and transsulfuration are coordinated.

According to Finkelstein and Martin [28], these two pathways can be considered to be competing for available homocysteine; reduction of activity in one pathway will lead to the more effective use of homocysteine by the second pathway.

Their studies reveal the capacity of the body to adapt to varying amounts of methionine in the diet and

suggest that homocysteine, which is derived solely from methionine, should not accumulate when only one of its metabolic pathways is impaired.

The fact that it does accumulate points to the existence of conditions whereby a defect in one pathway will lead to the impairment of the other. Therefore, the hypothesis that homocysteinemia is the result solely of inhibited synthesis of methionine methyl groups and/or cystathionine is not sufficient to explain all the observations that have been made.

Impairment of the remethylation pathway will thwart the induction of transsulfuration and prevent catabolism of excess homocysteine.

Similarly, an impairment of the transsulfuration pathway which requires B6 will prevent the induction of remethylation and inhibit disposal of homocysteine through its conversion to methionine.

In conclusion, it is observed that low levels of serum vitamin B12, folic acid and high levels of serum homocysteine are significantly associated with acute ischemic stroke. Effectiveness of supplementation with folate and B 12 in such patients needs further prospective studies with larger sample size. Further studies are required to assess if serum levels of vitamin B12, folic acid & homocysteine can be used as a predictor or risk marker for stroke.

**Conflict of Interest: Nil**

**Acknowledgment: Nil**

## References

- [1]. David A, Warrell DA, Cox TM, Firth JD, Edward J, Benz MD. Oxford Textbook of Medicine. 4<sup>th</sup> ed. Oxford: Oxford Press; 2003 Mar. p. 3239.
- [2]. Hippocrates. The Genuine Works of Hippocrates: Translated From the Greek With a Preliminary Discourse and Annotations by Francis Adams. Adams F, trans-ed. Baltimore, MD: Williams & Wilkins; 1939.
- [3]. Water soluble vitamins In: D M Vasudevan, Sreekumari S, Kannan Vaidyanathan. Textbook of Biochemistry for Medical Students. 6<sup>th</sup> ed. Kochi: Jaypee Brothers Medical Publishers (P) Ltd; 2011. p. 400-403.
- [4]. The Stroke/Brain Attack, reporters hand book. National Stroke Association, Englewood, CO, 1997.
- [5]. <http://inventors.about.com/library/inventors/inventors/bvitamins>, Accessed on 22.08.2014
- [6]. Carmel R. Biomarkers of cobalamin (vitamin B12) status in the epidemiological setting: a critical overview of context, applications and performance characteristics of cobalamin, MMA and holotranscobalamin II. *Am J Clin Nutr* 2011; 94:348S-58.

- [7]. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food and Nutrition Bulletin*, 2008; 29: S238–244.
- [8]. Refsum H, David Smith A, Per MU, Nexo E, Clarke R, McPartlin J, et al. Facts and Recommendations about Total Homocysteine Determinations: An Expert Opinion. *Clinical Chemistry* 2004; 50(1):3–32.
- [9]. Kocer A, Ince N, Canbulat CE, Sargin M. Serum vitamin B12 and folic acid levels in acute cerebral atherothrombotic infarction. *Tohoku J Exp Med* 2004 Oct; 204(2):155-61.
- [10]. Weikert C, Dierkes J, Hoffmann K, Berger K, Drogan D, Klipstein-Grobusch.K, Spranger J. B Vitamin Plasma Levels and the Risk of Ischemic Stroke and Transient Ischemic Attack in a German Cohort. *Stroke* 2007; 38(11):2912-8.
- [11]. Van Oijen MGH, Laheij RJF, Jansen JBMJ, Verheugt FWA. The Predictive Value of Vitamin B12 Concentrations and Hyperhomocysteinemia for Cardiovascular Disease. *Neth Heart Journal*. 2007; 15:291-4.
- [12]. Weng LC, Yeh WT, Bai CH, Chen HJ, Chuang SY, Chang HY, et al. Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake. *Stroke* 2008 Dec; 39(12):3152-8.
- [13]. Wayne HG, Steven JK, Robert FA, Janet BC, Michele LC. First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Stroke*.1995; 26:1166-70.
- [14]. Houcher B, Candito M, Gibelin P. Plasma Total Homocysteine, Folate and Vitamin B12 status and Hospitalization for Cardiovascular Disease. *International Society of Pteridinology*. 2007; 18(4):122-27.
- [15]. Omrani HQ, Shandiz EE, Qabai M, Chaman R, Fard HA, Qaffarpoor M. Hyperhomocysteinemia, folate and B12 vitamin in Iranian patients with acute ischemic stroke. *ARYA Atheroscler* 2011; 7:97–101.
- [16]. Fatima S, Memon SF., Ansari AK, Baloch AH. Hyperhomocysteinemia as a Risk Factor for Ischemic Stroke. *J Liaquat Uni Med Health Sci* 2012; 11(03):158.
- [17]. Moghaddasi M, Mamarabadi M, Mirzadeh S, Freydoonnejad AA, Razjouyan H et al. Homocysteine, vitamin B12 and folate levels in Iranian patients with ischemic stroke. *Neurol Res* 2010 Nov; 32(9):953-6.
- [18]. Giuseppe S, Gueli MC, Amelio MD, Saia V, Mangiapane P, Aridon P, et al. Blood levels of Homocysteine, Cysteine, Glutathione, Folic acid and Vitamin B12 in the Acute Phase of Atherothrombotic Stroke. *Neurological Sciences*.2007; 30:4:361-64.
- [19]. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994 Jul; 60(1):2-11.
- [20]. Nafija serdarevic, Lejla Begic, Adaleta Mulaomerovic-Softic. The concentration of homocysteine in patients after ischemic brain stroke and vascular dementia. *Journal of Health Sciences* 2011; 1(1):1-9.
- [21]. DeGraba TJ. The role of inflammation after acute stroke, utility of pursuing anti-adhesion molecule therapy. *Neurology*. 1998 ;( Suppl 3):S62-S68.
- [22]. Diaz-Arrastia R. Homocysteine and neurologic disease. *Arch Neurol* 2000; 57:1422-7.
- [23]. Evers S, Koch HG, Grottemeyer KH, Lange B, Deufel T, Ringelstein EB. Features, symptoms, and neurophysiological findings in stroke associated with hyperhomocysteinemia. *Arch Neurol* 1997; 54:1276-82.
- [24]. Petras, M, Tatarkova Z, Kovalska M, Mokra D, Dobrota D, Lehotsky J et al. Hyperhomocysteinemia as a risk factor for the neuronal system disorders. *J Physiol Pharmacol* 2014; 65:15-23.
- [25]. Spence JD. Nutrition and stroke prevention. *Stroke* 2006; 37: 2430-5.
- [26]. Thompson J. Vitamins and minerals 4: overview of folate and the B vitamins. *Community Pract* 2006; 79:197-8.
- [27]. Killian R, Kristopher A, Helga R, Lars Br, Godfried B, Per U, Paolo R, Roberto P. Low Circulating Folate and Vitamin B6 Concentrations. Risk Factors for Stroke, Peripheral Vascular Disease, and Coronary Artery Disease. *Circulation*.1998; 97:437-43.
- [28]. Finkelstein JD, Martin JJ. Methionine metabolism in mammals: distribution of homocysteine between competing pathways. *J Biol Chem* 1984; 259:9508-13.