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# EXPLORING SERUM HOMOCYSTEINE LEVELS IN NON-DIABETIC ISCHEMIC STROKE PATIENTS. A CASE-CONTROL STUDY.

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## Abstract.

### Aim:

The purpose of the present study was to evaluate the serum levels of homocysteine in individuals diagnosed with idiopathic ischemic stroke, and to establish a correlation between its clinical relevance and other risk factors associated with ischemic stroke,

### Methods:

The study utilized a case-control study design and was conducted at the Department of Medicine, DMCH, Lehariasarai. The study comprised a total of 21 individuals diagnosed with non-diabetic ischemic conditions, alongside 21 controls. Inclusion criteria included individuals of adult age, who exhibited no prior diagnosis of diabetes and presented with first-ever ischemic stroke. Confirmation of the ischemic stroke was confirmed through CT scanning of the cranial region. Exclusion criteria consisted of individuals diagnosed with diabetes mellitus, hemorrhagic stroke, individuals who encountered an embolic stroke, receiving vitamin supplementation, and phenytoin usage. The patient underwent regular examinations, including serum homocysteine level and fasting serum lipid profile assessment.

### Results:

The research showed a statistically relevant elevation in the mean serum homo-cysteine level among patients diagnosed with ischemic stroke, aged above 60 years, male gender, diagnosed with hypertension, and exhibiting a normal fasting lipid profile as compared to the controls. The statistical analysis was employed to determine the significance of the observed difference by utilization of the Chi-Square test, and Yate's correction.

### Conclusions:

A statistically relevant correlation was found among all the investigated factors for risk in non-diabetic individuals and the levels of serum homocysteine.

### Recommendation:

The assessment of serum homocysteine levels is strongly advised for the comprehensive evaluation of idiopathic ischemic strokes, to conduct a prognostic assessment.

*Keywords:* Homocysteine, Ischemic stroke, Nondiabetic, Risk factors, Submitted:2023-09- 26, Accepted: 2023-09-28

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## 1. INTRODUCTION.

Stroke is a significant contributor to both mortality and morbidity on a global scale. Stroke, also known as cerebrovascular accident, is characterized by a sudden appearance of a neurological deficit resulting from a localized vascular etiology [1]. Among all stroke cases, about 85 per- cent are classified as ischemic strokes, while the remaining 15 percent are categorized as hemorrhagic strokes [2]. Ischemic stroke syndromes encompass a diverse array of potential etiologies, exhibiting a distribution pattern commonly referred to as the rule of quarters. Specifically, around 25 percent of cases are attributed to cardioem- bolic origins, another 25 percent are associated with arteroembolic etiologies that involve large- artery disease, another 25 percent are classified as lacunar strokes resulting from small-vessel disease, and the remaining 25 percent are ascribed to various causes. It is interesting to note that the proportions of these stroke subtypes may ex- hibit regional disparities across different popula- tions [3].

Ischemic stroke is associated with atherosclerotic risk factors, which can be categorized into conventional and non-conventional factors. The conventional risk variables encompass hypertension, type 2 diabetes mellitus, smoking, and hypercholesterolemia [4]. Insulin resistance, fibrinolysisabnormalities, endothelial dysfunction, inflammation, hyperhomocysteinemia, microalbuminuria, vascular wall abnormalities, and postprandial glucose abnormalities, are among the non-conventional risk variables related to the development of atherosclerosis [5].

Homocysteine is an endogenous non-protein sulfhydryl amino acid that arises from the metabolic transformation of methionine. Approximately seventy percent of plasma homocysteine is found in a bound state with albumin, while thirty percent undergoes oxidation to form disulphides. Additionally, around one percent of homo-cysteine exists in a free form within the plasma [6].

The word "hyperhomocysteinemia" does not encompass a clinically significant pathological state. The term is employed for defining a biochemical aberration that may arise as a direct result of diverse pathological states. Each incremental rise of 2.5 $\mu$M in plasma homocysteine levels may be correlated with a corresponding elevation in the risk of stroke by around 20 percent [7]. Furthermore, it is noteworthy that elevated levels of plasma Total homo-cysteine exceeding 20 micromolar are strongly correlated with a significant 9-fold rise in the risk of myocardial infarction and stroke, in comparison to concentrations below 9 micromolar [8].

Hyperhomocysteinemia is a prevalent condition that serves as the primary prothrombotic factor linked to cerebrovascular accidents. Hyperhomocysteinemia is a medical ailment characterized by elevated levels of homocysteine in the blood. This condition has been associated with the development of cerebrovascular accidents, also known as strokes.

The mechanism by which hyperhomocysteinemia contributes to these strokes involves multiple factors. One of the key mechanisms is the effect of elevated homocysteine on blood pressure. Hyperhomocysteinemia has been shown to increase blood pressure, which in turn can lead to the occurrence of cerebrovascular accidents. Additionally, this condition can induce oxidative injury to the vascular endothelial cells. Specifically, it damages the production of nitric oxide (NO), which is an important vascular relaxing factor produced by the endothelial cells. The damage of endothelial NO production caused by hyperhomocysteinemia can further contribute to the development of cerebrovascular accidents. This is because NO plays a vital role in sustaining the usual function of blood vessels, including their ability to relax and dilate. When the production of NO is compromised, it disrupts the normal vascular tone and function, potentially leading to the occurrence of strokes [9].

The primary purpose was to explore the potential correlation between homocysteine levels and

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a range of conventional risk elements related to ischemic stroke. Additionally, the study aimed to investigate any potential associations between serum homocysteine levels and a range of risk factors, both modifiable and non-modifiable.

## 2. METHODS.

### *2.1. Study Design.*

The current investigation was carried out on non-diabetic individuals who experienced ischemic stroke and were admitted to the Department of Medicine at DMCH, Lehariasarai. The study was conducted for two years with a control group consisting of ordinary individuals of similar age and gender. The present investigation adopts a case-control study design.

### *2.2. Data Collection.*

The present work encompassed a cohort of 21 adult individuals, all of which were above the age of 18, and were diagnosed with ischemic stroke. A cohort of 21 individuals, who were determined to be within the normal range in terms of age and sex, were selected as control subjects for the study.

### *2.3. Exclusion criteria.*

The study excluded individuals with the following conditions: stroke, diabetic stroke, cerebrovascular accidents related to head trauma or brain tumors, individuals receiving vitamin supplementation, chronic use of anti-convulsant medication such as phenytoin, and patients with suspected cardiac emboli.

The data was meticulously gathered through a comprehensive assessment of the patient's medical history, thorough clinical examination, and detailed neurological evaluations. Additionally, a computed tomography (CT) image of the brain was done to definitively diagnose the presence of an ischemic stroke. A meticulously designed questionnaire was employed to collect comprehensive data about the familial background of diabetes mellitus, the occurrence of hypertension in the family, previous and current medical conditions, substance dependencies, and medication usage.

Blood samples are obtained to perform a complete blood count (CBC), measure fasting and postprandial blood glucose levels, assess HbA1c levels, evaluate urea in blood and serum creatinine levels, determine fasting lipid profile, analyze serum electrolyte levels, conduct an electrocardiogram (ECG), and obtain a computed tomography (CT) examination of the brain.

Serum homocysteine levels were assessed via the collection of approximately 2 ml of blood sample, subsequently drawn into a tube containing the anti-coagulant substance Ethylene Diaminetetraacetic acid (EDTA). The specimen was promptly placed in a cryogenic container and subsequently subjected to centrifugation for 30 minutes to mitigate the potential for inaccurate quantification of homocysteine concentration resulting from its liberation from erythrocytes. The plasma was subsequently refrigerated and maintained at a temperature of -80ºC until the completion of the analysis. The quantification of total plasma homocysteine was accomplished through the utilization of HPLC methodology.

### *2.4. Statistical Analysis.*

The statistical analysis was conducted to assess the significance of the differences observed in age, gender, and the prevalence of risk variables, including hypertension, and dyslipidemia. The Chi-Square test with Yate's correction was employed for this purpose.

## 3. RESULTS.

The current investigation encompassed a cohort of 21 individuals diagnosed with ischemic stroke, while an additional 21 patients were selected as a control group. The findings obtained from this study are presented in Table 1. The male patient population exhibited a higher prevalence (61.9%) in comparison to the female patient population (38.1%). The majority of patients (62.3%) fell in the age group of less than 60 years. The prevalence of male patients was higher across all age groups, except the age group spanning from 30 to 40 years.

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The study yielded a spectrum of results. The average serum homo-cysteine concentration in individuals with ischemic stroke was significantly elevated (12.61 $\mu$M/L) in comparison to the control group (4.89 $\mu$M /L) ($p<0.0001$). About age, the study revealed a higher concentration of 9 $\mu$M /L in individuals aged over 60 years, as compared to individuals aged under 60 years who exhib- ited a concentration of 6.4 $\mu$M /L. However, in the control group, the mean serum homo-cysteine levels exhibited a near-equivalence between the two age groups. The study observed a signifi- cant disparity in mean serum homocysteine levels between the cases and controls. Specifically, the cases exhibited higher levels of homocysteine (9 micro mol/L) compared to the controls (5.05 micro mol/L) ($p<0.0003$).

About gender, it was observed that male patients exhibited higher mean serum homocysteine levels (9.68 $\mu$M/L) compared to female patients (6.6 $\mu$M/L). Conversely, in the control group, male patients displayed higher mean serum homocysteine levels (4.55 $\mu$M /L) as compared to female patients (3.6 $\mu$M /L). Overall, the mean serum homocysteine levels were observed to be greater (8.14 micro mol/L) in the cases group compared to the control group (4.07 $\mu$M /L). The observed statistical disparity among the case group and the control group is highly significant ($p< 0.0001$).

Elevated serum homocysteine levels were observed in individuals presenting with a normal fasting lipid profile (9.3 $\mu$M /L), as compared to patients exhibiting fasting dyslipidemia (7.89 $\mu$M /L). In every phase of the study, it was observed that the average serum homocysteine levels were greater (8.59 $\mu$M /L) in the cases group as compared to the control group (6.05 $\mu$M /L). The observed statistical disparity between the case and control groups is deemed to be statistically significant, with a p-value of less than 0.0322.

The hypertensive patients exhibited elevated mean serum homocysteine levels (9.7 $\mu$M /L) as compared to the normotensive patients (9 $\mu$M /L). The study revealed the average serum homocysteine levels were significantly greater in the cases group (9.35 $\mu$M /L) in comparison to the control group (2.15 $\mu$M /L). The observed statistical disparity between the case group and the control group is highly significant, with a p-value less than 0.0001.

The study revealed that patients with ischemic stroke who also had hypertension exhibited the highest mean serum homocysteine level (19.4 $\mu$M /L). Additionally, male patients displayed elevated mean serum homocysteine levels (19.36 $\mu$M /L). Among the risk factors investigated, it was observed that individuals below the age of 60 years exhibited a mean serum homocysteine level of 12.8 $\mu$M /L, while female patients demonstrated a mean serum homocysteine level of 13.2 micromol/L. These findings suggest that younger age and female gender may be associated with comparatively lower levels of serum homocysteine.

## 4. DISCUSSION.

The current investigation comprised a cohort of 42 individuals who were admitted to the Department of Medicine, DMCH, Lehariasarai. Among these participants, 21 were diagnosed with non-diabetic ischemic stroke, while the remaining 21 individuals served as age and gender-matched non-diabetic controls. The age group of 51-60 years exhibited the highest prevalence of cases, while the age group of 30-40 years demonstrated the lowest frequency of cases.

The mean serum homo-cysteine level was found to be higher in individuals diagnosed with ischemic stroke when equated to the control group. The results of our investigation were in line with the research conducted by Datta *et al.* [10] and Boysen *et al.* [11], wherein they similarly observed a notable elevation in serum homocysteine levels among individuals with ischemic cerebrovascular accidents (CVA). The study conducted by Modi *et al.* [12] yielded the conclusion that hyperhomocysteinemia represents a significant risk factor for the occurrence of ischemic stroke. Boushley *et al.* [13] conducted a meta-analysis encompass- ing numerous observational studies investigating the correlation between total homo-cysteine conc. and atherosclerotic vascular disease. Among these

Table 1: **Analyzing Average Serum Homocysteine Levels Across Study Groups.**

| | **No. of patients** | | **Mean of serum homocysteine** | | **Total mean of serum homocysteine** | | **P-value** |
|---|---|---|---|---|---|---|---|
| | **Case** | **Control** | **Case** | **Control** | **Case** | **Control** | |
| Age less than 60 years | 14 | 14 | 6.4 | 5.1 | 9 | 5.05 | < 0.0003 |
| Age more than 60 years | 7 | 7 | 9 | 15 | | | |
| Male | 13 | 13 | 9.68 | 4.55 | 8.14 | 4.07 | <0.0001 |
| Female | 8 | 8 | 6.6 | 3.6 | | | |
| Dyslipidemia | 12 | 0 | 7.89 | 0 | 8.59 | 6.05 | <0.0322 |
| Normal | 9 | 21 | 9.3 | 6.05 | | | |
| Hypertensive | 5 | 0 | 9.7 | 0 | 9.35 | 2.15 | <0.0001 |
| Normotensive | 16 | 21 | 9 | 4.25 | | | |

studies, eleven specifically examined the relationship between homo-cysteine and risk variables, while 9 case-control works provided evidence supporting the hypothesis that homo-cysteine acts as an independent risk variable for ischemic stroke.

Among a cohort of 21 individuals diagnosed with ischemic stroke, it was observed that 14 patients were below the age of 60, while 7 patients were above the age of 60. Elevated serum homocysteine levels were observed in people aged 60 years and above (9 $\mu$M /L), in comparison to patients aged below 60 years (6.4 $\mu$M /L). The findings of our study were by the investigation conducted by Zongte *et al.* [14]. This study exhibited dissimilarities in comparison to the studies conducted by Narang *et al.* and Modi *et al.* [15,16].

The present study consisted of a cohort of 13 male participants and 8 female participants. The study findings revealed that male patients exhibited elevated mean serum homocysteine levels (9.68 $\mu$M /L) compared to their female counterparts (6.6 $\mu$M /L). The present study demonstrated concordance with the investigations conducted by Bogdan *et al*, Narang *et al*, and Modi *et al*, and [12, 15, 16]. In a study conducted by Kang *et al.* [17], it was observed that young women who are in good health exhibit lower levels of homocysteine compared to their male counterparts who

are also in good health. The observed difference tends to decrease as individuals progress in age. A sudden rise in serum homocysteine levels among women aged 50 and above indicates the potential disappearance of sex-based disparities in homocysteine concentrations as age progresses.

Dyslipidemia was detected in a cohort of 12 patients, while 9 patients exhibited a fasting lipid profile within normal limits. Elevated serum homocysteine levels were observed in individuals with a normal fasting lipid profile (9.3 $\mu$M /L) compared to patients with fasting dyslipidemia (7.89 $\mu$M /L). The results of our investigation were in alignment with the research conducted by Narang *et al.* and Modi *et al.* [15,16].

The blood pressure readings were within the normal range in 16 patients, while 5 patients exhibited hypertension. The hypertensive patients exhibited elevated mean serum homocysteine levels (9.7 $\mu$M /L) in contrast to the normotensive patients (9 $\mu$M /L). In the study led by Kit- tner *et al*, no conclusive evidence was discovered regarding the presence of elevated homocysteine levels in hypertensive individuals suffering from ischemic stroke [18]. The current study demonstrated concordance with the investigations conducted by Modi *et al.*, Narang *et al.*, and Graham *et al.* [16, 15, 19].

## 5. CONCLUSION.

A statistically relevant relationship was observed among all the investigated risk variables in individuals without diabetes and their corresponding serum homocysteine levels. Hyperhomocysteinemia is a condition characterized by elevated levels of homocysteine in the blood. It has been observed to potentially exert a more significant influence as a risk element in the growth of ischemic stroke in individuals without dia- betes. Timely identification of this condition, followed by appropriate pharmacological inter- ventions, can effectively mitigate the chances of stroke.

## 6. RECOMMENDATION.

The measurement of serum homocysteine is recommended in all instances of idiopathic ischemic strokes for prognostic evaluation. Additional investigation involving a larger cohort of patients and an expanded assessment of risk factors is needed to yield better findings

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