A CROSS SECTIONAL STUDY OF COMPARISON OF ELEVATED LEVELS OF SERUM HOMOCYSTEINE IN INTRACRANIAL AND EXTRACRANIAL ATHEROSCLEROTIC STENOSIS GROUPS

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ABSTRACT

Introduction: The primary goal of this study was to compare serum homocysteine levels in acute ischemic strokes caused by extracranial atherosclerotic stenosis (ECAS) and intracranial atherosclerotic stenosis (ICAS) groups in order to see if there was a link between elevated serum homocysteine levels and ICAS. Methodology: The present study was a cross-sectional study done by the department of neurology at a tertiary level hospital in Mumbai. Clinical, radiological & laboratory parameters, including serum Homocysteine, were compared between patients with ECAS and ICAS stroke. Results: The study enrolled 150 patients, 110 in the ECAS group and 40 in the ICAS group. We observed that mean serum Homocysteine levels were 2.42 µg/ml in the ECAS group and 4.88 µg/ml in the ICAS group (p=0.01). High serum Homocysteine levels were more common in the ICAS group than in the ECAS group (55 percent versus 33.6 percent, p = 0.01). The lipid profile was found to be similar in the two study groups, except for low HDL, which was observed in 70 (64%) of the ECAS patients as compared to 17 (43%) of the ICAS (p-value < 0.05). There was no statistical difference in demographic and other risk factor profile caparison between ECAS and ICAS patients. Conclusions: High serum Homocysteine levels were found to be more common in the ICAS group than ECAS group.

KEYWORDS Serum Homocysteine, Risk factors, Intracranial Atherosclerosis, Stroke

Introduction

Intracranial atherosclerotic disease, ICAD, is atherosclerosis of the large arteries at the base of the brain. It preferentially affects Asians, Hispanics, Far East Asians, and Blacks.1 Intracranial atherosclerotic stenosis of the major arteries (intracranial internal carotid artery, middle cerebral artery, vertebral artery, and basilar artery) is the most common proximate mechanism of ischemic stroke worldwide. It causes 30% to 50% of strokes in Asians and 8% to 10% in North American Caucasians.2 The accumulation of lipids and inflammatory cells along the inner walls of arteries is the hallmark of atherosclerosis. Atherosclerotic plaques are mostly seen around arterial bifurcations, branch points, and vessel curvatures. In contrast, straight non-branching arterial segments are usually spared.3

The predisposition toward more intracranial occlusive lesions in the Asian population remains unclear. However, various past two decades have shown that coronary heart disease, stroke, hypertension, and diabetes mellitus are associated with more extensive cerebral atherosclerosis. In addition, risk factors associated with intracranial atherosclerosis are certain races (Hispanic Americans, blacks, and Asians), female sex, young age, hypertension, smoking, diabetes mellitus and lipid disorders.4 The exact mechanisms why intracranial atherosclerotic stenosis is more common in some ethnic groups are still unclear.

Homocysteine is a sulphur-containing amino acid formed...
during the metabolism of methionine, an essential amino acid derived from dietary protein. Elevated serum homocysteine is an independent risk factor for atherothrombotic disease and stroke.\(^3\) To our knowledge, no Indian study has looked into the link between serum homocysteine levels and intracranial atherosclerosis. This led us to conduct the current research, hoping to establish a link that will allow us to form new treatment and preventive strategies for such patients.

**Methodology**

This study was done at PD Hinduja National Hospital & Medical Research Centre, Mumbai, from January 2017 to December 2018 after obtaining approval from the Institutional Ethics Committee. It was a cross-sectional observational study with a sample size of 150 patients divided into the ECAS and ICAS groups, with 110 and 40 patients, respectively. Before study enrollment, informed consent was obtained from the patients or their legal representatives.

**Inclusion criteria**

ICAS was defined as significant stenosis in the intracranial arteries, including anterior cerebral artery, middle cerebral artery, posterior cerebral artery, basilar artery and internal intracranial carotid, vertebral artery, without significant stenosis of the extracranial carotid and vertebral arteries. ECAS was defined as significant stenosis in the common carotid, extracranial internal carotid and vertebral arteries without significant stenosis of the intracranial arteries. Significant stenosis was defined as more than 50% stenosis of the arterial lumen assessed by 1.5 Tesla MR Angiography.

**Exclusion criteria**

Patients who had a stroke caused by mechanisms other than atherosclerosis of the large arteries were excluded. These patients had a lacunar ischemic stroke, a cardio-embolic stroke, or clinical or radiological evidence of cancer. Patients with clinical or serological evidence of CNS vasculitis and those with clinical or biochemical signs of hepatic, renal, or thyroid disease. Patients with significant (>50%) atherosclerotic stenosis of both extracranial and intracranial arteries on CT or MRI were also excluded.

The patients went through a recording of detailed history and physical and neurological examination. Their detailed clinical and laboratory data were recorded. MRI brain (1.5 Tesla) with angiography was done in all patients. CT angiography of head and neck vessels was done in selected patients to confirm the presence of atherosclerotic arterial stenosis, excluding non-atherosclerotic diseases of vessels like vasculitis.

Our laboratory did a serum homocysteine level through Chemiluminescent Microparticle Immunoassay (CMLA). The normal values of the level of serum Homocysteine as per our biochemical laboratory were 0.79 to 2.03 µg/ml for males and 0.60 to 1.80 µg/ml for female patients. The levels of serum Homocysteine greater than 2.03 µg/ml are considered as high as per our laboratory; this level is equivalent to 15 µmol/L per international standards.\(^6\) The data were compiled using a Microsoft Excel sheet and analysed using SPSS 23 software. Descriptive analysis for numerical data consisted of mean + SD (if normally distributed) / median & range (if not normally distributed), and categorical data included frequencies and percentages for various parameters. Normality of data was checked using Kolmogorov Smirnov test. A p-value of less than 0.05 was considered statistically significant.

**Results**

150 patients were included in this study; 110 were assigned to the ECAS group and 40 to the ICAS group. Table 1 compares demographic and risks factor profiles in the above groups. In the ECAS group, the median age of patients was 62 years with a range of 31-89 years; the ICAS group was 67 years with a range of 34-87 years. No statistical difference was found in comparing age in the study groups (p value= 0.59). 76 males and 34 females were in the ECAS group, compared to 34 and 6 in the ICAS group. The comparison of gender distribution was equal in both groups (p=0.051). Hypertension was detected in 72 (65%) of ECAS patients and 30 (75%) of ICAS patients. In comparison, diabetes mellitus was found in 52 (47%) ECAS patients and 22 (55%) ICAS patients. These observations were statistically not significant, with p values of 0.26 and 0.41 respectively. Abdominal obesity was identified in 45 (41%) of ECAS patients and 19 (48%) of ICAS patients, and the difference was statistically insignificant, with a p-value of 0.47. The ECAS group had 15 (14%) smokers as compared to 9 (23%) in the ICAS group (p-value = 0.19). A history of alcohol abuse was present in 13 (12%) and 9 (23%) patients of ECAS and ICAS groups, respectively. These observations were statistically insignificant, with p values of 0.19 and 0.11, respectively.

Table 2 compares Lipid profiles in the ECAS and ICAS groups. It was found to be similar in the two study groups, except for low HDL, which was observed in 70 (64%) of the ECAS patients as compared to 17 (43%) of the ICAS patients with a statistically p value < 0.05.

Table 3 compares serum homocysteine levels in ECAS and ICAS groups. The mean levels of serum Homocysteine were 4.88 µg/ml (range:0.7 to 54.1 µg/ml) in the ECAS group and 2.42 µg/ml (range:0.40-25.6 µg/ml) in the ICAS group (p value=0.01). Serum homocysteine levels > 2.03 µg/ml (equivalent to > 15 µmol/L) were considered as high levels. High serum Homocysteine levels were more common in the ICAS group than in the ECAS group [22 patients (55%) versus 18 patients (33.6%)]. It was more common in the ICAS group than in the ECAS group. This observation was statistically significant with a p-value of 0.01.

**Discussion**

Intracranial atherosclerosis, the leading cause of stroke in Asian and Indian populations, is still unclear. The primary aim of our study was to find out the link between elevated serum homocysteine levels and Intracranial atherosclerotic stenosis. Hence, we compared the number of patients with elevated levels of serum Homocysteine in ECAS and ICAS groups. We also compared these groups’ risk factors, radiological profiles and other biochemical parameters.

In our study, 27% of the patients had ICAS. In previous research in Asian populations, intracranial atherosclerosis was found to be the primary cause of ischemic strokes in roughly 33%-50% of patients. ICAS was also described as the most prevalent mechanism of stroke by Kaul et al. in the Hyderabad Stroke Registry.\(^8\) The lower number of patients with ICAS in our study could be due to the exclusion of patients with simultaneous significant (>50%) atherosclerotic stenosis of both extracranial and intracranial arteries.
### Table 1 Comparison of demographic and risk factor profile between ECAS & ICAS groups

| Clinical variables | ECAS (n=110) | ICAS (n=40) | p value |
|--------------------|--------------|-------------|---------|
|                    | N | % | N | % |   |
| 1) Gender          |   |   |   |   |   |
| Females            | 34 | 31% | 6 | 15% | 0.051 |
| Males              | 76 | 69% | 34 | 85% |   |
| 2) Past medical history |   |   |   |   |   |
| Hypertension       | 72 | 65% | 30 | 75% | 0.26 |
| Diabetes mellitus  | 52 | 47% | 22 | 55% | 0.41 |
| Smoking            | 15 | 14% | 9  | 23% | 0.19 |
| Alcohol            | 13 | 12% | 9  | 23% | 0.11 |
| Abdominal obesity  | 45 | 41% | 19 | 48% | 0.47 |
| 3) Age             |   |   |   |   |   |
| Median value       | 62 years | 67 years |   |   | 0.593 |
| Range              | 31-89 | 34-87 |   |   |   |

### Table 2 Comparison of Abnormal Lipid profile between ECAS and ICAS groups

| Laboratory variables          | ECAS (n=110) | ICAS (n=40) | p value |
|-------------------------------|--------------|-------------|---------|
|                               | N | % | N | % |   |
| High Cholesterol              | 30 | 27% | 8 | 20% | 0.36 |
| High low-density lipoprotein  | 35 | 32% | 8 | 20% | 0.15 |
| Low high-density lipoprotein  | 70 | 64% | 17 | 43% | <0.05 |
| High very low-density lipoprotein | 26 | 24% | 5 | 13% | 0.26 |
| High triglycerides            | 25 | 23% | 5 | 13% | 0.31 |

### Table 3 Comparison of serum Homocysteine levels between ECAS and ICAS groups

| Serum Homocysteine levels     | ECAS (n=110) | ICAS (n=40) | p value |
|-------------------------------|--------------|-------------|---------|
|                               | N | % | N | % |   |
| High                          | 37 | 33.6 | 22 | 55% | 0.01 |
| Normal                        | 73 | 66.4 | 18 | 45% |   |
| Mean value                    | 2.42 µg/ml | 4.88 µg/ml |   |   | 0.01 |
| Range                         | 0.40 – 25.60 µg/ml | 0.70 – 54.10 µg/ml |   |   |   |
Our study found that older age (> 60 years) is a risk factor for both intra and extracranial stenosis. The median age of patients was 67 and 62 years in ICAS and ECAS groups, respectively. As per Srivastava et al., intracranial stenosis was more commonly associated with younger age (age < 60 years) than extracranial stenosis with older age group (age > 60 years).\(^9\) However, Uehara et al., in another study, discovered that older age is a risk factor for both extracranial and intracranial stenosis.\(^10\)

Kim JS et al. investigated risk variables between patients with ICAS and those with ECAS; they discovered that patients with ICAS were more likely to be young and female than those in the ECAS group.\(^11\) We found more males than females in both ECAS and ICAS groups. However, this observation was statistically insignificant. This could be related to the fact that in most of India, men are the breadwinners and are more likely to seek medical attention, implying cultural prejudice.

It should be noted that we could not find a significant association between any comorbid conditions like hypertension, diabetes mellitus, abdominal obesity, smoking and drinking habits with both ECAS and ICAS. Li et al. found that hypertension was more frequently associated with ECAS than ICAS (67.5% vs 53.8%, \(p < 0.001\)). In contrast, Jin et al. reported that hypertension was a significant risk factor for ECAS than ICAS (56.9% vs 28.6%, \(p < 0.001\)).\(^12,13\) Keheya et al. found no statistically significant link between diabetes mellitus and the ICAS or ECAS groups in their study (53.9% Vs 55.2%).\(^14\) Rincon F and colleagues have reported DM as more prevalent and important for prognosis in ICAS.\(^15\) DM is an important component of metabolic syndrome and a well-known risk factor for ECAS.\(^16\) However, its involvement in ICAS has yet to be determined, and past research has shown mixed results.

Abdominal obesity was observed to affect both ECAS and ICAS groups equally in our study. In a similar study, Jin et al. discovered no significant differences in BMI or waistline between ICAS and non-ICAS groups.\(^13\) Although obesity is a known risk factor for stroke, no other studies have looked into its relationship with ICAS or ECAS. Future research should look into this link, as the South-East Asian population has a higher risk of abdominal obesity and metabolic syndrome.

The lipid profile was similar in the two study groups, except for low HDL, which was observed more commonly in ECAS than in the ICAS group. High levels of LDL-C (low-density lipoprotein cholesterol) have also been associated with ECAS as reported by Ritz K et al.\(^17\) High-density lipoprotein-cholesterol (HDL-C), non-high-density lipoprotein- cholesterol (non-HDL-C), and total cholesterol (TC) levels have all been linked to an elevated risk of ICAS in Chinese populations.\(^18\) The exact role of lipid profile parameters in ICAS remains controversial.

In our study, we observed that higher serum Homocysteine levels were more common in ICAS patients than in ECAS patients. This was similar to a study done by Kim JM and colleagues, who found that elevated serum homocysteine levels were independently associated with intracranial arterial calcification and atherosclerosis burden.\(^19\) Elevated serum homocysteine leading to endothelial dysfunction is well known. WKC Lai and MY Kan have postulated the three mechanisms explaining hyperhomocysteinemia-related endothelial dysfunction and atherosclerosis: (1) oxidative stress, which includes the disruption of NO synthase function, (2) endoplasmic reticulum stress, which leads to endothelial cell apoptosis; and (3) chronic inflammation and prothrombotic conditions.\(^20\)

There were a few limitations of the study. This was a hospital-based study. This might introduce some sampling bias. We excluded patients with the simultaneous presence of significant (>50%) extracranial and intracranial stenosis. Our sample did not include asymptomatic patients with cerebral artery stenosis or patients with transient ischemic events.

**Conclusion**

High serum Homocysteine levels were observed to be more common in the ICAS group than ECAS group.

**Study Funding**

None.

**Conflict of interest**

None.

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