ORIGINAL ARTICLES

Human carcinoembyronic antigen is a useful biomarker for diagnosis of acute ischemic stroke – A pilot study

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Abstract

Background: Human carcinoembryonic antigen (CEA) is widely used as tumor marker. Striking similarities have been reported between process of tumor genesis and atherosclerotic diseases. CEA has been reported as a useful biomarker for diagnosis of acute coronary syndrome whose main pathogenesis mechanism is rupture of atherosclerotic plaque. Although same pathogenic mechanism leads to ischemic stroke, the role of CEA in stroke has not been studied. This study was aimed at evaluating role of CEA as diagnostic marker for acute atherosclerotic ischemic stroke. *Methods:* Sixty male subjects between 40 to 60 years of age were divided into 2 groups with 30 subjects each: Group A (ischemic stroke) and Group B (age and sex matched healthy controls). Exclusion criteria for Group A included diabetes mellitus, heavy smoking, recent cerebrovascular or myocardial events. The blood sample was taken not later than 24 hours of onset of stroke in Group A. In Group B, the blood samples were drawn at the time of enrollment in the study. CEA levels were estimated using double sandwich ELISA method. *Results:* The mean (SE) serum CEA levels in healthy controls and ischemic stroke patients were 0.95(0.11) and 5.57 (0.20) ng/mL respectively (p<0.001). After adjusting for confounders such as total leucocytes count, waist hip ratio, hypertension and smoking mean (SE) were 1.38 (1.23) and 5.26 (0.85) ng/ml respectively (p<0.001).
Conclusions: Patients with ischemic stroke have raised level of CEA. CEA is a promising novel biomarker for diagnosis of acute ischemic stroke.

INTRODUCTION

Although, CT scan and MRI have taken a big step forward towards the diagnosis of acute ischemic stroke, early diagnosis is still a major challenge. Any cost effective bedside biomarker which can diagnose ischemic stroke earlier than imaging methods should be welcoming. Like acute coronary syndrome (ACS), rupture of atherosclerotic plaque in the arteries supplying brain is a major pathogenic mechanism for the development of acute ischemic stroke and constitutes 32% of all strokes.[1]

Carcinoembryonic antigen (CEA) is a tumor marker associated with various malignancies such as carcinoma colon, lung and breast cancer. Some non neoplastic conditions such as obesity, smoking, ACS, inflammatory bowel disease, chronic liver and kidney diseases are also associated with higher CEA level.[2] This molecule has also been reported as a useful biomarker for

early diagnosis of ACS, as its level rise with rupture of atherosclerotic plaque in coronary arteries.[3] As most of ischemic strokes also develop due to rupture of atherosclerotic plaques in extra or intra cranial arteries, this molecule may also prove to be a useful marker for early diagnosis of acute ischemic stroke.

Imaging modalities like CT and/ or MRI are time consuming. It is also not always easily available or sensitive. A bedside biomarker such as CEA may thus be of value in early diagnosis. This study was aimed at evaluating role of CEA as diagnostic marker for atherosclerotic ischemic stroke.

METHODS

This analytical cross sectional study was conducted at a tertiary care hospital in North India from year 2012 to 2014. It comprised of two groups with 30 males between 40 to 60 years of age in each

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group. Each patient was age matched with a healthy control (±2 years). Group A comprised of patients with acute atherosclerotic ischemic stroke (presenting within 24 hours of onset of symptoms) based on TOAST criteria.[4] Group B were healthy controls. Informed consent was taken from each individual before enrollment in the study. Subjects in Group A were recruited as early as possible after the onset of symptoms of stroke but not later than 24 hours. Group B included age and sex matched healthy attendants of patients and our hospital staff members not belonging to our own department. The exclusion criteria were: Acute ischemic or hemorrhagic stroke in the last 3 months, chronic inflammatory diseases, past or present malignancy, liver disease, kidney disease or inflammatory bowel disease, cardiac source of thromboembolism such as atrial fibrillation, myocardial infarction and dilated cardiomyopathy valvular lesions, ACS, acute pancreatitis, hypothyroidism, diabetes mellitus or impaired fasting or post prandial glucose levels (fasting blood sugar >100mg/dL and postprandial>140mg/dL), chronic obstructive airway disease, those who were positive for stool for occult blood, history of radiotherapy or chemotherapy, smokers with history of smoking for more than 50 pack years and obesity (BMI>25kg/m²).

Acute ischemic stroke was diagnosed based on CT scan and/or MRI brain.[4] Echocardiography was done wherever required based on clinical evaluation to exclude cardiac thromboembolism. Two ml venous blood sampleswere withdrawn as early as possible but not later than 24 hours of onset of symptoms in patients group for estimation of CEA levels. In controls group the blood samples were drawn at the time of enrollment in study. Serum samples were stored in deep freezer at -80°C for further processing. Commercially available kits (Weldon Biotech.) using double sandwich ELISA for quantitative assessment of CEA levels was used for estimation of its concentrations. Software SPSS 20.0 was used to analyse the data. Paired Student's T- Test was applied to compare age and sex matched groups. To adjust plausible confounders such as total leucocytes count (TLC), waist hip ratio (WHR), smoking and hypertension (SBP ≥140 and/or DBP≥90 mm of Hg) we applied generalized estimation equation with Robust estimation. P value< 0.05 was considered statistically significant and < 0.001 as highly significant.

RESULTS

Pair age matching was done. Mean age was 51.0±4.8 and 51.7±4.8 years respectively .Range of age was 42 to 59 years in both group. There was no statistically significant difference of body mass index (BMI) between the two groups. The mean WHR was significantly higher in patients with ischemic stroke as compared to controls (p<0.001). Also 23(76.6%) patients with acute ischemic stroke were hypertensive, while none of the controls was hypertensive (p < 0.001). Twenty two out of 30 (73.3%) patients were smokers while none of the controls was smoker (p<0.001) (Table 1).

Mean total leucocyte count (TLC) was found to significantly higher in patients group as compared to controls (p<<0.001). Other hematological parameters including hemoglobin, blood urea,

Table 1: Demographic data of the study groups

Parameters	Group A Mean±SD (range) (n=30)	Group B Mean±SD (range) (n=30)	p-value*
Body mass index (kg/m²)	22.37±1.76 (18.9-24.9)	22.36±1.75 (18.9-24.9)	0.326
Waist Hip Ratio	0.90±0.03 (0.84-0.98)	0.87±0.02 (0.78-0.90)	**<0.001**
Hypertensive	23 (76.6%)	0 (0%)	**<0.001**
Smokers	22 (73.3%)	0 (%)	**<0.001**

*p- value significant at < 0.05 and highly significant at < 0.001.

serum creatinine, liver function testsand lipids parameters were comparable in both groups (Table 2).

Serum CEA levels were found to be significantly higher in patients with acute ischemic stroke as compared to controls (p<0.001). Even after adjusting for confounding variables like TLC, WHR, smoking and hypertension the difference was statistically highly significant (p<0.001) (Table 3 and fig.1).

Discussion

Our case-control study shows that CEA is significantly elevated among male patients with atherosclerotic stroke after adjustment for confounders such as hypertension, smoking, WHR and total leucocytes count. Prevalence of hypertension, high WHR and smoking was significantly higher in our patients with acute atherosclerotic ischemic stroke. This observation is in concordance with previous major prospective epidemiological studies like Framingham heart study which suggest that central obesity and hypertension are risk factors for atherosclerosis. Smoking is a well known major risk factor for atherosclerosis.

CEA is a glycoprotein involved in cell adhesion. It is normally produced during fetal development, but the production of CEA stops before birth. CEA is a glycosyl phosphatidyl inositol (GPI)-cell surface anchored glycoprotein whose specialized sialofucosylated glycoforms serve as functional colon carcinoma L-selectin and E-selectin ligands, which may be critical to the metastatic dissemination of colon carcinoma cells.[5-7] CEA has a molecular weight of 180 to 200kDa. It is over expressed in adenocarcinoma in the colon and other organs including pancreas, lung, prostate, urinary bladder, ovary, and breast; therefore it is widely used as a serological marker of malignant tumors. On the other hand, serum CEA levels may increase under some nonmalignant conditions such as, chronic renal failure, hypothyroidism, cigarette smoking[2], and some chronic inflammatory diseases. However, the extent of CEA elevation in such nonmalignant conditions, when present, is usually only modest. CEA is one of the most widely used tumor marker. An increasing amount of scientific and clinical evidence has established striking parallels in the etiology, induction and pathogenesis of cancer and atherosclerosis.[8-9] The biological properties and function of CEA , which facilitate the diffusion and metastasis of neoplasia and adhesion and induction of proliferation, chemotaxis and angiogenesis, also allows one to speculate about its putative involvement in atherosclerotic processes.[10-11]

Role of CEA has been evaluated in pathogenesis of atherosclerosis and ACS. Ishizaka et al. have reported a significant association between CEA concentration and carotid atherosclerosis independent of other risk factors in Japanese males[12], suggesting its role in pathogenesis of atherosclerosis. Additionally CEA may affect other different mechanisms which drive atherosclerotic plague evolution towards acute complications, these include promotion of hypercoagulable state and deregulation of matrix metalloproteinase as observed in cancer. Vassalle et al. established role of CEA in causation of plaque disruption/rupture which might precipitate ACS.[3]

Leucocytosis has been reported in patients with acute events related to atherosclerotic plaque. It is well accepted that atherosclerosis is a chronic inflammatory state and various inflammatory markers such as hs-CRP and TLC have been reported to be raised in patients with ACS.[13] Inflammatory markers such as higher TLC and ESR have been correlated with poor outcome in patients with ischemic stroke.[14] So the presence of inflammatory state akin to ACS in our patients with stroke might have resulted in higher TLC observed in our study.

Striking parallels can be drawn in pathogenesis of coronary artery disease and atherothrombotic stroke. Firstly, both are atherosclerotic diseases, hence, risk factors and process of atherosclerotic plaque development is similar in both. Secondly, as described below the process of development of stable atherosclerotic plaque into vulnerable plaque, and its rupture is also similar in both. However, in CAD the plaque is in coronaries and in atherothrombotic stroke it is in extra/ intracranial arteries. Once the plaque ruptures in any of these arteries territories, the process of thrombosis, its propagation, and embolization is similar in both. Thirdly, the treatment modalities including use of antiplatelet drugs, thrombolytic and preventive strategies are also similar. The role of CEA in plaque disruption/rupture has been established in pathogenesis of ACS. However, no study has been done to study correlation of CEA with cerebrovascular diseases which have the same mechanism of acute event that is plaque instability, rupture, thrombosis and embolization.

Various mechanisms have been proposed regarding role of CEA in atherosclerosis and acute event like ACS. Stimulation of monocytes and

Table 2: Routine parameters of the study groups

Parameters	Group A Mean±SD (Range) (n = 30)	Group B Mean±SD (Range) (n = 30)	p- value
Hemoglobin (g/dl)	13.5±1.4 (11-16)	13.6±1.4 (11-16)	0.161
Total leucocyte Count (cellsg/mm^3)	9400.0±2456.8 (4700-14000)	7373.3±1802.6 (4000-9900)	<0.001
Blood urea (mg%)	27.8±8.1 (10-39)	27.9±8.2 (10-39)	0.662
Serum creatinine (mg%)	0.79±0.21 (0.4-1.2)	0.80±0.17 (0.5-1.1)	0.552
Serum sodium (meq/L)	139.7±5.3 (131-150)	138.8±4.7 (131-149)	0.285
Serum potassium (meq/L)	4.05±0.50 (3.1 − 5.0)	4.02±0.50 (3.1 − 5.0)	0.199
Total bilirubin (mg%)	1.03±0.29 (0.4-1.6)	1.01±0.27 (0.4-1.5)	0.312
Direct bilirubin (mg%)	0.53±0.16 (0.3-0.9)	0.53±0.14 (0.3-0.9)	0.677
Alkaline phosphatase (U/L)	103.4±15.4 (86-134)	101.2±15.2 (86-134)	0.145
Alanine aminotransferase (ALT) (U/L)	20.3±4.2 (14-29)	21.6±5.2 (10-26)	0.792
Aspartate aminotranferase (AST) (U/L)	21.5±5.9 (10-30)	21.6±5.2 (10-26)	0.851
Serum albumin (mg/dl)	3.92±0.40 (3.1-4.6)	3.94±0.38 (3.1-4.6)	0.556
Serum globulin (mg/dl)	3.3±0.3 (3-4)	3.2±0.2 (3-4)	0.124
Total cholesterol (mg/dl)	153.2±20.0 (100-190)	(106-190) 151.2±21.2	0.497
Triglycerides (mg/dl)	111.0±22.1 (71-154)	108.8±24.3 (70-154)	0.059
High density lipoprotein-cholestrol (mg/dl)	30.8±5.9 (20-45)	30.6±6.2 (20-45)	0.553
Low density lipoprotein- cholesterol (mg/dl)	101.6±6.8 (82-125)	100.9±7.9 (82-125)	0.184

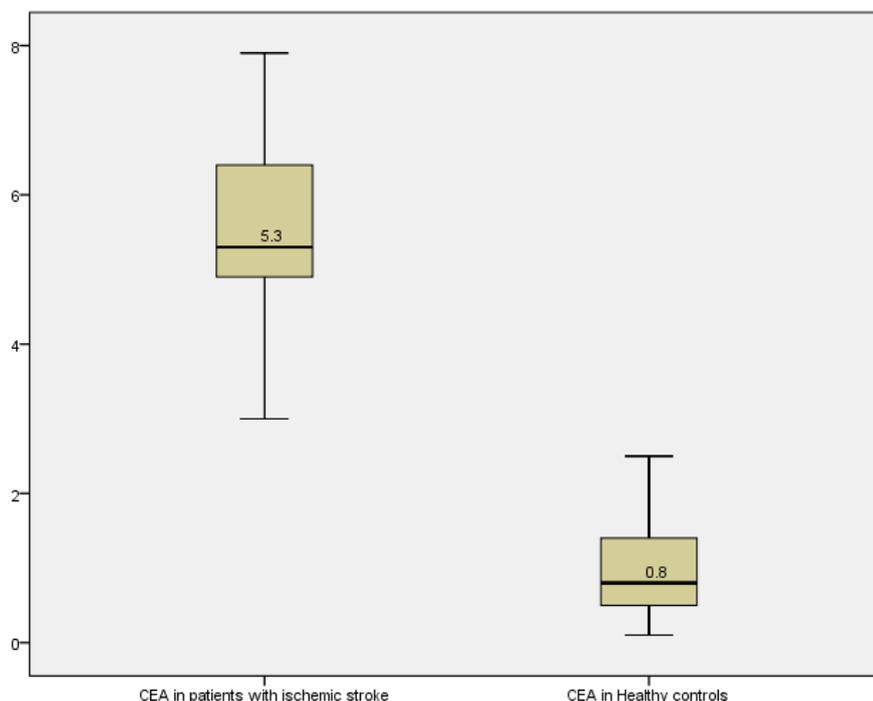*p- value significant at < 0.05 and highly significant at < 0.001*

Fig.1: Distribution of CEA in patients with ischemic stroke and healthy controls

macrophages with CEA results in an increase in the production of proinflammatory cytokines[12], which subsequently upregulate adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, on the surface of vascular endothelial cells.[15] These processes are thought to play a role in facilitating the metastasis of cancer cells. Early stages of atherosclerosis also involve recruitment of inflammatory cells and their transendothelial migration, which is mediated by such cellular adhesion molecules on the surface of vascular endothelial cells.[5] The somatic mutation theory theorized a common pathogenic pathway for atherogenesis and carcinogenesis, the atherosclerotic plaque being considered pathogenically comparable to a benign cancer form involving smooth muscle cells.[15] Role of CEA is also implicated in plaque rupture, thrombosis and occlusion of blood vessels including promotion of a hypercoagulative state as well as the dysregulation of matrix metalloproteinases and tissue inhibitors of metalloproteinases, as observed in cancer.[16-18] CEA also acts as an inducer of

Table 3: Mean serum CEA levels in study groups

Parameter Mean (SE) (Range)	Group A (n = 30)	Group B (n = 30)	p- value*
Serum CEA (ng/ml) unadjusted mean (SE)	5.57 (0.20) (3.0-7.9)	0.95 (0.11) (0.1-2.5)	**< 0.001**
Serum CEA (ng/ml) Adjusted mean (SE) After adjusting for TLC, WHR, hypertension and smoking	5.26 (0.85)	1.38 (1.23)	**< 0.001**

p- value significant at < 0.05 and highly significant at < 0.001.

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CEA-related cell adhesion molecule 1-mediated apoptosis.[19] Apoptosis is recognized as a key event in atherosclerotic plaque instability and rupture.[19] The above mentioned mechanism of plaque rupture may be responsible for ischemic stroke in our subjects explaining the elevated serum CEA levels in them.

Even after adjusting for confounding variables, our patients with ischemic stroke had significant higher mean concentration of CEA ($p < 0.001$). Our study is the first showing higher concentration of this biomarker in patients with acute ischemic stroke. Imaging modalities like CT scan may not normal if done within 6 hours of stroke. Moreover, CT scan and MRI may not be available. In these situations CEA, which can be estimated at bedside, may prove to be useful biomarker for early diagnosis of ischemic stroke.

Our study has many limitations. Firstly, we had a very small sample size. Secondly, we analysed only men in our study and thirdly, because of cross-sectional nature of our study we cannot determine whether there is a causal relationship between rise in CEA and acute ischemic stroke. Moreover, the study findings can not be extrapolated to those who were excluded, i.e., females, diabetics, heavy smokers, recent cerebrovascular or cardiovascular events. However, the reliability of our results is reinforced by strict criteria used to select our patients and strengthened by relationship between clinical phenotypes and correlating CEA concentration.

In conclusion, our study indicates that CEA may prove to be a promising biomarker for early diagnosis of acute ischemic stroke. It may have very useful diagnostic value in situations where imaging gives ambiguous results. However, further studies in larger cohorts are required to establish such a relationship.

DISCLOSURE

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