Original article:

Carotid artery bifurcation and incidence of carotid plaque formation: Histological analysis based on cadavers: an eastern Indian study

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Abstract

Introduction: This study is based on observing the position of bifurcation of CCA, with respect to anteriorly placed structures of neck, in cadavers as well as measurement of c-IMT at carotid bifurcation histologically.

Materials & Methods: 60 freshly embalmed cadavers (38 male and 22 female) were used for the present study.

Results: High bifurcation level of CCA was observed in 31% cadavers without much gender variation ((Males showing 33% and Females 27%). The c-IMT mean was more in females (0.71 ± 0.03 mm) than males (0.65 ± 0.07mm) bilaterally. The c-IMT increased with higher levels of bifurcation on the left side, the mean measurements being 0.63 ± 0.14 mm & 0.69 ± 0.01mm in male and female cadavers respectively. The maximum c-IMT was observed between 40-60 years in both male and female cadavers bilaterally.

Discussion: Our study has depicted the cIMT to be more in females and increasing with the height of carotid artery bifurcation in left side of both genders. Since the maximum cIMT was recorded in age group of 40-60 years, it can be concluded that the maximum risk for atherosclerotic complications lies with this age group.

Key words: Common Carotid Artery (CCA), Carotid artery - Intima media thickness (c-IMT)

INTRODUCTION

The incidence rate of Stroke is 119-145/100,000 based on the recent population based studies and case fatality rates are being the highest 42% in Kolkata1. Based on CT scan, ratio of infarction: haemorrhage was 1.86 in a study by Das SK et al2. Large vessel intracranial atherosclerosis is the commonest cause of ischemic stroke in India3. The extracranial carotid disease is the etiological factor in 25-26% and intracranial carotid disease in 30% of ischemic stroke cases4. It is, therefore, very much necessary to interrupt the pathological basis of stroke by implementing primordial or at most, primary levels of prevention, so that we can reduce the burden of this disease amongst high risk groups. The CCA intima-media thickness (IMT) measurement is a surrogate marker and rather an indicator of atherosclerosis in our body and is well correlated to incidence of cardiac thromboembolisms5. Duplex-B mode scan is the benchmark investigating procedure for measuring the IMT, as mentioned above6. Previous studies have
convincingly proved the role of this c-IMT (carotid intima-media thickness) in early prevention of ischaemic stroke among western population. The CCA bifurcates at upper border of thyroid cartilage, most commonly. However, considerable variations are possible in its mode and sites of bifurcation. Furthermore, the length of common carotid artery and position of its terminal bifurcation have a negative correlation to extent of atherosclerotic involvement of Internal Carotid artery (ICA), as noted by Beiles et al.

Management of stroke has also been challenging to clinicians as well as surgeons. Recombinant-tissue plasminogen activator (r-tpa) is being randomly used for thrombolysis in acute stroke patients. Surgeons should consider CEA in patients who have made a good recovery from their stroke after thrombolysis and who have a 50-99% internal carotid artery stenosis. Carotid End-Arterectomy (CEA) for severe carotid stenosis is a safe procedure with good protection from ischemic events. Detailed preoperative cardiac evaluation and appropriate patient selection is essential. The procedure of CEA can pose difficulty on basis of site and geometry of bifurcation of the common carotid artery (CCA). Higher bifurcation of CCA presents a tough challenge for the surgeons to operate on the subject and complications are consequently more.

Our study focuses on observing the position of bifurcation of CCA, with respect to anteriorly placed structures of neck, in cadavers as well as measurement of c-IMT at carotid bifurcation histologically, which is the most common site for plaque formation. Eventually, the study also aims at hypothesising, whether site of bifurcation of CCA can affect the propensity to develop atherosclerosis, in an individual. Further, the study can substantiate previous findings of Duplex-B Scan of c-IMT and this is of its first kind among Eastern Indian population.

AIMS & OBJECTIVES

This is an observational cross sectional study which focuses on the following:

1. Determination of height or level of bifurcation of common carotid arteries in human cadavers.
2. Histological carotid artery intima-media thickness estimation around the point of bifurcation.
3. Analysis of any gender or age variation as well as correlation between the first two parameters.

MATERIALS AND METHODS

60 freshly embalmed cadavers (38 male and 22 female) were used for the present study. Each cadaver was placed in supine position with neck extended at 30° with the horizontal plane. A vertical incision was given, along the midline, extending from symphysis menti up to the suprasternal notch. Another horizontal incision was given from the symphysis menti, along the lower border of body of the mandible, up to angle of mandible and then extended up to the tip of the mastoid process. By means of subsequent soft tissue dissection the carotid triangle was exposed and the common carotid artery (CCA) was identified. The upper extent of CCA i.e point of its bifurcation into Internal Carotid Artery (ICA) and External Carotid Artery (ECA), was exposed and noted, with respect to surrounding soft tissue landmarks (Thyroid Cartilage = TC, Hyoid Bone = HB and Angle of Mandible = AM), as shown in Figures 1 & 2. This process was done on both sides of each cadaver, since the CCA is a bilateral structure. Thus 120 specimen were obtained and analysed for the present study.
After exposing and identifying the point of bifurcation, 1 centimeter segment of the CCA, proximal to this point, was obtained for histological study. The segments of CCA were collected in 10% formalin solution. They were washed in water. Graded concentrations of alcohol for dehydration were used. Alcohol was removed using Xylol. Subsequently, paraffin blocks were prepared and 5 micron thick sections were made with a microtome. Finally staining was done with Hematoxylin & Eosin as per standard staining protocol. Paraffin was removed using xylol, followed by hydration using reducing concentrations of alcohol viz absolute, 90%, 70%, 50% alcohols. Final change was made in tap water followed by haematoxylin. Blueing was performed subsequently using light microscopy (to visualise optimum staining of basophilic parts) and acid alcohol (for optimum decolourisation, if necessary). Finally counter-staining was done using Eosin and finally, dehydration was performed using increasing concentrations of alcohol. Each slide was viewed under compound light binocular microscope and the intima-media of each arterial slide was identified with 10X objective. Then, pictures of the part of arterial wall, which showed maximum intima-medial thickness, were obtained with digital camera. The images were transferred to a personal computer (PC) and the images were analysed using IMAGE-J software, as shown in Figure 3. The software was calibrated using Neubauer’s Chamber (Haemocytometer) and pixels were converted to micro-meters, while recording the c-IMT.
RESULT ANALYSIS

In this study, high bifurcation level (defined as located above the upper border of thyroid cartilage) was observed in 31% cadavers without much gender variation ((Males showing 33% and Females 27%), as in Figure 4.

The high bifurcation levels were mostly located in between HB and TC (23% in females and 26% in males). An interesting observation in this study was that, high bifurcation was seen mostly in age group of 40-60 years on right side compared to younger population (less than 40 years) on left side. The c-IMT\text{mean} was more in females (0.71 ± 0.03 mm) than males (0.65 ± 0.07mm) bilaterally. In both genders, the c-IMT increased with higher levels of bifurcation on the left side, the mean measurements being 0.63 ± 0.14 mm & 0.69 ± 0.01mm in male and female cadavers respectively. However, on the right side, the c-IMT was maximum for bifurcation at level of thyroid cartilage in males (0.69 ± 0.06 mm) as opposed by females, which revealed maximum c-IMT for higher bifurcations (0.74 ± 0.03 mm). When age-wise variation of c-IMT was considered, it was noted that c-IMT was increasing with age, the maximum being between 40-60 years in both male and female cadavers bilaterally, as shown in Tables 1 & 2.
Table 1: Age-wise c-IMT variations in Male cadavers (all measurements are in millimetres)

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<th>Less than 40 years</th>
<th>40-60 years</th>
<th>More than 60 years</th>
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<td>Right</td>
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<td>Right</td>
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<tr>
<td>0.65 ± 0.09</td>
<td>0.57 ± 0.11</td>
<td>0.67 ± 0.08</td>
<td>0.63 ± 0.08</td>
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Table 2: Age-wise c-IMT variations in Female cadavers (all measurements are in millimetres)

<table>
<thead>
<tr>
<th></th>
<th>Less than 40 years</th>
<th>40-60 years</th>
<th>More than 60 years</th>
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<td></td>
<td>Right</td>
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<tr>
<td>0.73 ± 0.02</td>
<td>0.68</td>
<td>0.73 ± 0.06</td>
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DISCUSSION

The increasing burden of carotid artery stenosis has posed a serious threat to our society and their management are even more crucial and challenging to clinicians as well as surgeons. Per-operative difficulties are main drawbacks of higher levels of bifurcation of the CCA. That is why, the dilemma between medical or surgical options for severe CCA stenosis becomes more annoying at times. Most of the previous studies have described the levels of bifurcation of CCA on basis of posteriorly placed landmarks i.e. the cervical vertebrae and their surrounding structures11-15. Lu’cev et al16, Lo et al17 and Al-Rafiah et al18 have tried to define the positions of bifurcation of CCA with respect to anterior soft tissue landmarks on cadavers while McNamara et al19 have demonstrated the same with help of Angiography on living subjects. Irrespective of the methods of defining high bifurcation levels of CCA, the incidence on the same ranged from 22.5% - 60% and it corroborates well with our study, as we have seen 31% cadavers showed higher levels of bifurcation than normal position. Adamantios Michalinos et al20 mentioned in their review article that the bifurcation level of CCA is not affected by age or gender. However, there is notable asymmetry between sides ranging between 50 and 75% across various studies13,14,16,21. Our study results are consistent with these findings as the bifurcation positions were unaffected by age or gender, but the left side (53.9%) showed CCAs with higher bifurcations significantly more as compared to right side (46.3%). This finding is consistent with the work of S. K. Klosek and T. Rungruang14. The higher levels of bifurcation can be explained embryologically, as the internal & external carotids have different origins. Moreover, the knowledge of high bifurcation is necessary to decide the procedure of management of carotid artery stenosis. As endarterectomy of high bifurcating carotid arteries can lead to disastrous complications including hypoglossal or marginal mandibular nerve injuries22,23 or osteomyelitis (as it requires mandibulotomy25 or styloidotomy24), the procedure of choice has become carotid artery stenting26.

Carotid artery intima-media thickness (cIMT) has been a well known indirect marker for cardiovascular disease progression5 and the mean cIMT for south Kannada population is 0.59 mm5. However, for Rajasthani population27 the value is somewhat higher (1.05 – 1.07mm). In Hungarian population28 the normal cIMT is around 0.97 mm. Among eastern Indian population there has been no consensus regarding the normal range of cIMT. Our study has depicted the cIMT to be more in females (0.69 mm) and increasing with the height of carotid artery bifurcation in left side of both
genders. Since the maximum cIMT was recorded in age group of 40-60 years, it can be concluded that the maximum risk for atherosclerotic complications lies with this age group. The positive correlation between cIMT & bifurcation height also establishes a positive correlation between incidence of carotid artery atherosclerosis & its bifurcation level.

**CONCLUSION**

The pertinent results of this study clearly indicate that cIMT increases with age and more so on left side, concluding the predilection for developing atherosclerosis and its complications. Overall, females are more prone to atherosclerotic disease as per this study and this contradicts the previous studies which have shown male predilection for the same. However, further studies (both in vivo & in vitro) should be invited to substantiate these findings.

**REFERENCES**


