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Morphometric measurements of extracranial and intracranial atherosclerotic disease: A population-based autopsy study

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Background and aims: Intracranial (IAD) and extracranial atherosclerotic diseases (EAD) have been mostly investigated using imaging methods. Autopsy studies allow for a direct and complete evaluation of the atherosclerotic disease. We aimed to investigate the frequency of IAD and EAD, their association, and related risk profiles in a large cross-sectional autopsy study.
Methods: We measured the intima-media thickness and stenosis of the common (CCA) and internal carotid arteries (ICA), using morphometric measurements. The main outcome was stenosis (≥50%) in the artery with the largest obstruction among the 12 cerebral arteries. We used multivariable logistic regression models to investigate the association between EAD and IAD.
Results: In 661 participants (mean age = 71.3 ± 11.7 y, 51% male), stenosis was more common in IAD than in EAD (59% *vs.* 51%). EAD was associated with Caucasian race, hypertension, and smoking, while IAD was associated with older age, less years of education, hypertension, diabetes, and a previous history of stroke. Stenosis in CCA and ICA was associated with more than two times the odds of having stenosis in the intracranial arteries (CCA: OR = 2.32, 95% CI = 1.64; 3.28; ICA: OR = 2.51, 95% CI = 1.76; 3.57).
Conclusions: In this population-based autopsy study, IAD was common, even more common than EAD, but correlated with EAD.

1. Introduction

Stroke is the second leading cause of mortality and the 7th leading cause of disability-adjusted life years (DALY) lost worldwide [1,2]. Nearly 90% of strokes are ischemic, and large artery atherosclerosis is one of the main causes of ischemic stroke [3]. Extracranial atherosclerotic disease (EAD) has been extensively associated with a higher risk of ischemic stroke in longitudinal studies, and it is more frequent in Caucasians [4,5]. On the other hand, less is known about intracranial atherosclerotic disease (IAD), probably because more accurate IAD detection was only recently possible with high-resolution magnetic resonance imaging and intravascular ultrasound [6]. IAD has also been associated with a higher risk of stroke recurrence [7], and it is more common among individuals from Asian, African, and Hispanic ancestries [8]. Moreover, IAD and EAD have been associated with different risk factors; age, race, education, metabolic syndrome, and stroke have been associated with IAD, while race, male sex, hypertension, heart disease, smoking, and alcohol use are associated with EAD [9].

The association between IAD and EAD has been evaluated in white and Asian patients with previous cardiac diseases or stroke [10–12] using imaging methods, which only allow for indirect measurements of atherosclerosis. Autopsy studies allow for the

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direct visualization of atherosclerotic plaques, and they could contribute to understand the true prevalence of IAD and determine the correlation with EAD and associated risk factors. This information may help target individuals at risk for ischemic stroke, which is important to develop new diagnostic techniques and/or new treatment paradigms. However, few autopsy studies have been conducted [13–15]. Mazighi et al. found an association between proximal EAD and intracranial internal carotid artery stenosis in subjects with fatal stroke; however, its association with atherosclerosis in other intracranial arteries was not investigated [13]. Therefore, we investigated the prevalence of IAD and EAD, their association, and their related risk factors using direct morphometric measurements of atherosclerotic disease in a large population-based autopsy study including diverse races.

2. Materials and methods

2.1. Participants

In the city of Sao Paulo (Brazil), subjects dying from natural death of an unknown cause undergo an autopsy exam to elucidate the cause of death. Autopsies are performed at the Sao Paulo Autopsy Service (SPAS) at the University of Sao Paulo within a short post mortem interval (mean of 10 h). Consistently, the main cause of death is cardiovascular related (60% of cases), with stroke being responsible for 2% of deaths [16]. Details about our study design can be found elsewhere [17]. While the next of kin (NOK) waited for the autopsy, our team explained the study aims and asked for donations of the cerebral and carotid arteries. The NOK signed informed consent, allowing material collection and data provision. This study was approved by the local ethics committee. We collected samples from 2005 to 2008. We included subjects who were aged 50 years or older at the time of death. We excluded cases with post mortem interval >24 h (n = 120), and cases where NOK who did not see the deceased at least weekly or could not provide reliable clinical information (n = 88). We also excluded cases in which we could not collect the internal carotid artery due to anatomical difficulties in dissecting this artery (n = 247).

2.2. Atherosclerosis evaluation

Cerebral arteries were dissected from the brain, washed in water to remove blood clots, and stored in 70% alcohol for 24 h. We then injected gelatin inside the vessel lumen to prevent artery collapse and fixed it in 10% formalin. After fixation, we cut the artery into 3-mm thick cross-sections to evaluate the presence of atheromatous plaques in each of the 12 arteries from the Circle of Willis (CW) [right and left anterior cerebral arteries, anterior communicating artery, right and left middle cerebral arteries, right and left internal carotid arteries (close to the CW), right and left posterior communicating arteries, right and left posterior cerebral arteries, and the basilar artery]. In each artery, we selected the cross-section with the largest lumen obstruction and photographed it using a stereomicroscope (Nikon SMZ 1000; Nikon Inst., New York, USA). Using an image processor (ImageJ®), we measured the area delineated by the outer arterial wall and the lumen area (Fig. 1A). For each of the 12 cross-sections, we calculated a CW stenosis index by subtracting the lumen area from the outer area, dividing this difference by the outer area and multiplying it by 100.

Carotid arteries were dissected from the aortic arch and processed similarly as the CW arteries. After fixation in 10% formalin, carotid arteries were cut into 5-mm thick cross-sections. We selected the section of the common (CCA) and internal carotid arteries (ICA) with the largest atheroma plaques. Common carotid artery intima-media thickness (C-IMT) was measured 1 cm below the artery bifurcation, and internal carotid artery intima-media thickness (I-IMT) was measured 1 cm above the bifurcation (Fig. 1B a) following standard procedures. We prepared histological slides of the three selected sections with hematoxylin-eosin and Verhoeff's staining and photographed them with the stereomicroscope. Using ImageJ®, we measured the area delineated by the internal elastic lamina and the lumen area (Fig. 1B b). The carotid stenosis index was calculated similarly to the CW stenosis index. C-IMT and I-IMT were calculated by dividing the intima-media area (i.e. the area delineated by the external elastic lamina and the lumen) by the media perimeter (delineated by the external elastic lamina) (Fig. 1B c). We described additional details about the morphometric measurement of atherosclerosis elsewhere [18,19]. Atherosclerosis evaluation was performed by one rater (CKS) independently from clinical and statistical analysis.

2.3. Sociodemographic and clinical variables

Sociodemographic variables included age at death, sex, race (white, black, and Asian), and education. Clinical variables included a previous history of diabetes, hypertension, dyslipidemia, stroke, heart disease, smoking (never, current, or past smokers), alcohol use (never, current, or past use), physical activity, and body mass index (BMI). Age information was retrieved from government-issued documents. Race and other clinical variables were reported by the NOK. The deceased was weighed in the supine position without clothes using an electronic scale, and height was measured using a stadiometer. BMI was calculated by dividing the weight in kg by the square of the height in meters.

2.4. Statistical analysis

We used the mean and standard deviation to present descriptive statistics of quantitative variables and relative frequencies for categorical variables. We compared the included and excluded subjects from this study using unpaired *t*-test for continuous variables, and Chi square test or Fisher's exact test for categorical variables. The association of stenosis (obstructions ≥50%) in EAD and in IAD with sociodemographic and clinical variables was investigated using multivariable logistic regression models including all variables. We used Venn diagrams to describe the relative frequency of stenosis in the EAD and IAD in our sample.

We used logistic regression models to examine the association between stenosis in the cerebral and carotid arteries. The dependent variable was the presence of stenosis (≥50%) in the cerebral artery with the largest obstruction among the 12 CW arteries [20]. The independent variables were C-IMT and I-IMT (in mm) and the presence of stenosis (obstruction≥50%) in the CCA and in the ICA. The multivariable logistic regression models were adjusted for age at death, sex, race (white *versus* non-white), and education. We used Stata 12.0 (StataCorp LP, College Station, Texas, USA) for statistE analyses.

3. Results

Included participants (n = 661) were older, and had less diabetes and stroke than excluded subjects (n = 455) (Table 1). Among the included participants, the mean age was 71.3 ± 11.7 years, 51% were male, and 68% were white. Regarding cardiovascular risk factors, 65% had hypertension, 28% had diabetes, and 19% had heart disease. We evaluated 12 CW arteries from each of the 661 participants (total of 7932 arteries), and we selected the artery with the largest obstruction. Among the CW arteries with the largest obstruction, 387 (59%) had intracranial stenosis (obstruction ≥50%). We also selected the segment with the largest obstruction