

Evaluation of Some Risk Factors for Atherosclerosis in the Circle of Willis Observed at Autopsy in University College Hospital, Ibadan, Nigeria

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Abstract: There has been a steady change in lifestyle of the Nigerians causing the increase in vulnerability of cerebrovascular disease like atherosclerosis. This study was aimed at evaluating some risk factors for atherosclerosis in the circle of Willis autopsy. The clinical record of patients were retrieved from their case files in order to obtain information on the sex, age, clinical diagnosis, past medical history and presence or absence of cardiovascular risk factors such as cigarette smoking, alcohol intake, diabetes, and hypertension. Samples were obtained from 44 consecutive brains in autopsies conducted on subjects aged 15 years and above at the University College Hospital, Ibadan. With ethical clearance, the subject's case files were retrieved for some data. Twenty-six (59.1%) of the subjects had no identifiable risk factors for atherosclerosis in their medical history. Eighteen (40.9%) of the subjects had at least one risk factor of atherosclerosis in their history. The risk factors identified were hypertension in 10 (55.6%) cases, hypertension combined with previous alcohol ingestion in 3 (16.7%) cases, hypertension combined with diabetes mellitus in 3 (16.7%) cases, and alcohol ingestion in 2 (11.1%) cases (Thus, a total of 16 (61.5%) patients had hypertension, 6 (33.3%) patients had a history of alcohol ingestion.

Key words: Atherosclerosis, autopsy, cerebrovascular, circle of willis, diabetes mellitus, hypertension

INTRODUCTION

It has been documented that the larger arteries are more likely to be atherosclerotic. (Pasterkamp and Falk, 2000). Studies on a large number of sections of cerebral arteries and quantitative assessment of degree of atherosclerosis showed that there was an almost linear relationship between the degree of atherosclerosis and the radius of the artery. (Pasterkamp and Falk, 2000). Further studies showed that the incidence of plaque formation was higher in arteries of larger lumen than in those with smaller lumen. (Toschi *et al.*, 1997).

The bifurcations of arteries have also been described as common sites of atherosclerosis due to a local increase in the vessel radius existing at the bifurcation (Banning, 2001). However, results of some studies conducted have shown that the Internal Carotid Arteries (ICAs), Basilar Artery (BA) and Middle Cerebral Artery (MCA) are most involved and that the smaller caliber vessels such as the Vertebral Arteries (VAs), Posterior cerebral arteries(PCAs), Anterior cerebral arteries(ACAs), and the communicating arteries, are less severely involved (Resch

et al., 1970). This would be in agreement with Laplace's law.

Studies examining the severity and extent of atherosclerosis have shown that atherosclerosis is due to a variety of factors which are mutually interrelated. Among these are the effect of hormones, nutritional state, sex, age and race (Resch *et al.*,1970). Results of such studies show that a single lesion is an evidence of the site of origin of atherosclerosis in a given case especially if the criterion is a macroscopic lesion. It has been documented that the site of predilection are the upper and lower BA, the ICA, the MCA, the PCA, the ACA and the communicating arteries (Resch *et al.*,1970). Also, the severity of atherosclerosis was found to be greatest in the vessels with largest radius (Resch *et al.*, 1970).

Cerebral atherosclerosis in different races: It has been reported that cerebrovascular disease is a main cause of death in Japan. (Pasterkamp and Falk, 2000). The death rate from cerebrovascular disease was 171.6 per 100,000 population in Japan as compared to 106.7 in the U.S.A, while death rate due to arteriosclerotic heart disease was

51.1 and 319.7, respectively in 1964 (Pasterkamp and Falk, 2000). From the statistics above, it is seen that the technologically advanced country like the U.S.A have a lower cerebrovascular disease rate but a higher rate of arteriosclerotic heart disease as compared to the Japan. A survey of the incidence of cerebrovascular disease in Japan revealed that the incidence of cerebral thrombosis is much higher than that of cerebral hemorrhage. (Pasterkamp and Falk, 2000). Similar studies on autopsied cases from Hiroshima and Nagasaki demonstrated that the presence of occlusive vascular disease as the cause of stroke in Japan does not differ appreciably from that in the United States (Pasterkamp and Falk, 2000). The comparative clinical study on cerebrovascular disease between Japanese and Americans demonstrated that the lesions of the smaller cerebral arteries in the Japanese tended to be much more severe than those in the Americans (Pasterkamp and Falk, 2000). Intracranial arteries investigated in each instance where the arteries forming the circle of Willis, BA, VA and cerebellar arteries.

It was reported that the sites of bifurcation of the larger vessels were the most frequent sites of early mild cerebral atherosclerosis in the Japanese (Pasterkamp and Falk, 2000). The most common sites for severe atherosclerosis were in the order of: the proximal portion of the PCA, the peripheral portion of the MCAs, VAs at their fusion site with the inferior part of the BA, and the anterior and proximal portion of the MCAs. It was noted that the prevalent sites of advanced severe cerebral atherosclerotic changes differed from the common sites for early mild cerebral atherosclerosis. This finding was contrary to the findings obtained on Americans and Norwegians, in which the ICA to the trifurcation of the MCA was also one of the most common sites for severe lesions (Adami, 1909).

Cerebral atherosclerosis appeared as early as the second decade and increased in severity with age, particularly in the fifth decade in males and during the sixth decade in females. (Pasterkamp and Falk, 2000). Severe stenosis of the ICA, and the proximal portion of the MCA, appeared to be less common in Japanese than in Americans (Pasterkamp and Falk, 2000).

In females, the intensity and frequency of atherosclerotic lesions in scores (Baker's method) (Adami, 1909) was slow up to the fifth decade compared to males, but was very steep after the sixth decade, becoming equal to those of males. Severities of cerebral atherosclerosis in both sexes were compared within each age group. Although the numbers of cases in each age group were too small to be significant, the preponderance of lesions in males began in the third decade and extends to the fifth (Pasterkamp and Falk, 2000).

Some morphological studies of cerebral arteries in different population groups in Africa, carried out to evaluate the extent and severity of atherosclerosis and

certain diseases associated with atherosclerosis showed that cerebral atherosclerosis is less in the different African population groups living in Nigeria, Uganda or Senegal when compared with American Caucasians and Blacks (Resch *et al.*, 1970).

Association of waist circumference with low density lipoprotein (LDL-C): Oxidation of LDL-C is a hallmark of atherosclerosis development. (Boucek, 1965) Studies that used circulating oxidative LDL-C (ox-LDL-C) to investigate oxidative stress induced by obesity have shown decreased circulating ox-LDL-C concentrations in morbidly obese patients after weight loss following surgery. (Duguid, 1926) Concentrations of circulating ox-LDL-C and ox-LDL-C antibodies did not vary significantly between overweight and normal-weight subjects in a Japanese population, however, significantly higher serum ox-LDL-C were observed in both obese females and in obese subjects with hypertension (Duff, 1951).

Higher serum concentrations of C-reactive proteins (CRP) have been found in obese patients Body mass index (BMI >30) (Stephenson *et al.*, 1962) and have been shown to be directly related with malondialdehyde (MAD)-modified LDL-C concentrations (Texon, 1957) Adipose tissue is a highly metabolic active organ. Hence, it might be reasonable to assume that abdominal fat is closely involved in the production of oxidative stress (Forbus, 1930). In a study, high plasma concentrations of circulating ox-LDL-C and CRP were directly associated with waist circumference (Forbus, 1930). Increase amount of abdominal fat was associated with biomarkers of oxidative stress and inflammation, which are known risk factors of atherosclerosis development (Forbus, 1930).

Association of body height with cardiovascular risk factors: The importance of development in utero and in infancy is indicated by associations of both low birth weight and weight at one year with a high risk of Coronary Heart Disease (CHD). The role of exposures in the first two decades of life in CHD development is supported by post-mortem studies that reveal that around 50% of young men have some evidence of atherosclerosis (fatty streaks or plaques) in their blood vessels (Gubner and Ungerleide, 1949). Blood pressure, blood lipids, body mass index, and smoking in childhood predict the extent of such post-mortem atherosclerotic changes. (Schwartz and Mitchell, 1962; Wesolowski *et al.*, 1965)

Experiments suggested that height and Forced Expiratory Volume (FEV) may be used as biomarkers for a range of exposures influencing post-natal growth such as diet, exposure to infection, and stress. (Schwartz and Mitchell, 1962; Wesolowski *et al.*, 1965) These factors in turn may have long term influences on the risk of developing atherosclerosis as indicated by height-CHD associations (Baker *et al.*, 1969).

Association of cerebral atherosclerosis and some cardiovascular risk factors: Diabetes has been shown to be a predisposing factor for stroke and there is evidence that a high blood glucose level confers a poor prognosis on those who suffer a stroke. (Burton, 1951). In the Framingham study conducted in 1981, it was shown that diabetes doubled the risk of atherothrombotic brain infarcts. Some investigators have shown that increased transvascular lipoprotein transport in diabetes may be further accelerated if hypertension or albuminuria is present, possibly explaining increased intimal lipoprotein accumulation and thus atherosclerosis (Lansing *et al.*, 1950).

Published findings on the relationship between alcohol consumption and atherosclerosis are conflicting (World Health Organization, 1958). The doubt and discrepancies are to a considerable extent the result of methodological difficulties such as variation in the definition of alcoholism, lack of reliable quantitative information on alcohol consumption, inadequate control groups, confusion between the effect of alcohol consumption itself and the influence of disorders (emaciation, obesity, avitaminoses) associated with alcoholism (World Health Organization, 1958). The results of a study carried out on people taking alcohol on 16 or more days per month showed extensive atherosclerosis in the aorta as compared to people drinking not more than 3 days per month. This difference was most significant in the case of calcified lesions. Fatty streak in the aorta was more extensive in the medium group (drinking 4-15 days per month) than in either of the extreme groups. The few differences concerning myocardial and cerebral infarction had a negative association with alcohol consumption (World Health Organization, 1958).

Rationale for study: Many studies have been done on atherosclerosis of the cerebral vasculature. Some investigators studied the grading of atherosclerotic lesions in different races and in Africans before the introduction of the AHA classification (Stary *et al.*, 1995). Other studies have proposed correlation between variants of the circle of Willis and some cerebrovascular diseases (Uston, 2005). Differences in the incidence of these diseases in different populations have also been documented (Pasterkamp and Falk, 2000). Few studies have been done to address the various artery segments of the circle of Willis and their vulnerability to specific atherosclerotic lesion types. No known study has addressed some of the risk factors for atherosclerosis in the circle of willis in Nigerians.

The present study describes some of the risk factors for atherosclerotic lesions in the circle of Willis used seen

at autopsy in the University College Hospital (UCH), Ibadan.

MATERIALS AND METHODS

Study design: This is a descriptive, cross-sectional study carried out on autopsy subjects at the University College Hospital Ibadan in July, 2009. We studied 44 consecutive patients (aged ≥ 15 years) that underwent post-mortem examination at the University College Hospital, Ibadan. Ethical approval was obtained from the joint University of Ibadan/University College Hospital Ethical Review Committee. Informed consent was also obtained from the next of kin along with the consent for autopsy.

Data collection procedure: The clinical record of patients was retrieved from their case files in order to obtain information on the sex, age, clinical diagnosis, past medical history and presence or absence of cardiovascular risk factors such as cigarette smoking, alcohol consumption, diabetes, and hypertension. The post-mortem pathological findings including height (cm), waist circumference (cm), maximum anterior abdominal wall fat thickness (cm) and pathological diagnosis were also noted.

Sample collection procedure: Brain collection followed the conventional autopsy procedure. Fresh brains with no macroscopically evident pathological findings were dissected out and fixed in 10% formalin. For the brains with pathological findings especially at the base, the circles of Willis were dissected out after the brains have been fixed and reviewed by a pathologist (usually 3-5 days after the autopsy).

After fixation in 10% formalin for at least 24 h, the arteries were sectioned and macroscopically examined for the presence or absence of fatty streaks, fatty plaques, fibrous plaques, complicated plaques and thrombi. Thereafter, tissue processing was done at the histochemistry laboratory of the Pathology department of the UCH, Ibadan.

Cross sections of about 4 mm thickness from each artery type were placed in disposable plastic tissue cassettes, (model: S-LT EC6) which were numbered based on the artery type and post-mortem numbers. For each of the 44 subjects, there were 14 cassettes containing the sectioned artery segments. In total there were 616 tissue cassettes for processing. These cassettes were put in stainless steel baskets and inserted into the bath of an automated Leica tissue processor (model no. EM TP4C). After sectioning, the sections were stained using Haematoxylin and Eosin (H&E).

Table 1: Comparison of means of anthropometric variables of subjects with no risk factor and of subjects with at least one risk atherosclerosis

	No risk factor (n = 26) Mean±SD	At least one risk factor (n = 18) Mean±SD	p-value
Age (years)	36.5±11.9	53.6±12.9	<0.0001*
Height (cm)	164.0±9.51	63.7±7.3	0.9
Waist circumference (cm)	42.1±6.	156.6±23.7	<0.005*
Abdominal wall fat thickness	2.3±1.2	4.0±2.0	<0.001*(cm)

*:Statistically significant p value

Table 2: Comparison of means of anthropometric variables in subjects with hypertension, alcohol use, diabetes with subjects with no risk factors

	Age MEAN±SD	Height MEAN±SD	Waist Circumference MEAN±SD	Abdominal wall fat thickness MEAN±SD
No risk factor (n = 28)	36.5±11.9	164.0±9.5	42.1±6.1	2.3±1.2
Hypertensive (n = 16)	54.2±13.6	162.1±6.1	59.1±23.9	4.3 ± 1.9
p-value	0.002	0.5	0.001	0.0001
No risk factor (n = 39)	36.5±11.9	164.0±9.5	42.1±6.1	2.3±1.2
Alcohol use (n = 5)	52.6±6.3	167.8±8.8	42.8±12.8	3.0±1.5
p-value	0.007	0.4	0.9	0.2
No risk factor (n = 41)	36.5±11.9	164.0±9.5	42.1±6.1	2.3±1.2
Diabetes (n = 3)	57.3±16.1	159.7±4.7	58.7±8.1	4.7±5.8
p-value	0.01	0.4	0.0001	0.003

Slides of the different arteries from the circle of Willis were examined under a light microscope (model no. Olympus CHT Binocular) and a histological grading based on the AHA method of classification (appendix IV) was done with the assistance of a pathologist. Normal intimal thickening was graded Type O. Intima with a single layer of isolated foam cells was graded Type I. Intima with multiple foam cell layers usually greater than two layers was graded Type II. Intima with a pool of extracellular lipid was graded Type III. The presence of Type III lesions with cholesterol crystals was graded Type IV. Presence of fibrous cap over extracellular lipid in the intima was graded Type V. Intima with surface defect and (or) thrombosis was graded Type VI. Intima with plaque area calcified (with or without lipid core) was graded Type VII. Intima with hyalinised fibrous plaque with no lipid core was graded Type VIII. A completely occluded vessel lumen was graded IX. For the purpose of description and comparisons with studies conducted in other races, the Gore method was also employed in scoring atherosclerotic vessels, that is: AHA lesion I-III were described as mild; AHA lesion IV-V were described as moderate; and AHA lesion VI-IX were described as severe.

Statistical analysis: The data obtained was entered into a database and analysed using the SPSS 16 statistical software programme. Pearson test was used to determine correlation between variables. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Twenty-six (59.1%) of the subjects had no identifiable risk factors for atherosclerosis in their medical history. Eighteen (40.9%) of the subjects had at least one

risk factor of atherosclerosis in their history. The risk factors identified were hypertension in 10(55.6%) cases, hypertension combined with previous alcohol ingestion in 3(16.7%) cases, hypertension combined with diabetes mellitus in 3(16.7%) cases, and alcohol ingestion in 2(11.1%) cases. Thus, a total of 16(61.5%) patients had hypertension, 6 (33.3%) patients had a history of alcohol ingestion.

(Eleven (61.1%) of the 18 subjects with at least one risk factor were males and seven (39.9%) were females. This difference was not statistically significant ($p = 0.5$).

The mean age of subjects with at least one risk factor (53.6±12.9 years) was significantly greater than the mean age of subjects with no identifiable risk factor 36.5±11.9 years), ($p < 0.0001$) (Table 1).

The mean height of subjects with at least a risk factor of atherosclerosis (163.7±12.9 cm) was not significant when compared with subjects with no risk factor (164.0±9.5 cm), ($p = 0.9$) (Table 1).

The mean waist circumference in subjects with at least a risk factor (56.6±23.7 cm) was significantly greater than the mean waist circumference of subjects with no risk factor (42.1±6.1 cm), ($p < 0.005$) (Table 1).

The mean abdominal wall fat thickness in risk factor subjects (4.0±2.0 cm) was significantly greater than subjects without risk factors (2.3±1.2 cm), ($p < 0.001$) (Table 1). Nine (56.3%) of the hypertensive subjects were males, and seven (43.8%) were females. This gender distribution was not statistically significant ($p = 0.9$). In the hypertensive subjects, the mean age (54.2±13.6 years) was significantly greater than those without hypertension (36.5±11.8 years), ($p < 0.0001$) (Table 2). The mean height of hypertensive subjects (162.1±6.1 cm) was not significant when compared to the mean of those without hypertension (164.0±9.5 cm), ($p = 0.5$) (Table 2). Mean waist circumference in the hypertensive subjects

(59.1±23.9 cm) was significantly greater than the mean of the subjects without hypertension (42.1±6.1 cm), ($p < 0.001$) (Table 2). The mean abdominal wall fat thickness in the hypertensive subjects (4.3±1.9 cm) was significantly greater than the mean of the non-hypertensive subjects (2.83±1.2 cm), ($p < 0.001$) (Table 2).

Four (80%) of the 5 subjects with a history of alcohol ingestion were males and one (20%) was a female. This gender difference was not statistically significant, ($p = 0.2$). The mean age of the subjects who used alcohol (52.6±6.3 years) was significantly greater than the mean age of those who did not use alcohol (36.5±11.9 years), ($p < 0.007$) (Table 2). The mean height of the subjects who used alcohol (167.8±8.8 cm) was not significantly greater than the mean height of subjects who did not use alcohol (164.0±9.5 cm), ($p = 0.4$) (Table 2). The mean waist circumference in subjects who used alcohol (42.8±12.8 cm) was not significantly greater than the mean of the subjects who did not use alcohol (42.1±6.1 cm), ($p = 0.2$) (Table 2). The mean abdominal wall fat thickness in the subjects who used alcohol (3.0±1.5 cm) was also not significantly greater than the mean abdominal wall fat thickness in the subjects who did not use alcohol (2.3±1.2 cm), ($p = 0.2$) (Table 2).

All of the three subjects with diabetes mellitus in the present study were females. This gender distribution was statistically significant, ($p < 0.05$). In the diabetes subjects, the mean age (57.3±16.1 years) was significantly greater than the mean age of the subjects without diabetes (36.5±11.9 years), ($p < 0.01$) (Table 2). For the mean height in the subjects with and without diabetes, there was no significant difference in the mean height of the diabetes subjects (159.7±4.7 cm) when compared to the mean of the non diabetes subjects (164.0±9.5 cm), ($p = 0.4$) (Table 2). The mean waist circumference in the diabetes subjects (58.7±8.1 cm) was significantly greater than the mean of the non diabetes subjects (42.1±6.1 cm), ($p < 0.0001$) (Table 2). The mean abdominal wall fat thickness in the diabetes subjects (4.7±5.8 cm) was also significantly greater than the mean of the non diabetes subjects (2.3±1.2 cm), ($p < 0.003$) (Table 2).

DISCUSSION

Subjects having risk factors like hypertension, diabetes, alcohol use were noted in this study. From results of this study, there was a consistently higher frequency of cerebral atherosclerosis in subjects with hypertension. The severity of cerebral atherosclerosis using vessel grading was also greater in hypertensive subjects when compared to normotensives. This is in keeping with Laplace's law which clearly implies that an increase in radius, or in pressure, or in both would increase the tension which produces the injury to the vessel wall (Resch *et al.*, 1970), though this was not

statistically significant implying that there are other underlying risk factors of cerebral atherosclerosis that were not considered in this study but were common in the entire population. These could include the dietary habits and lifestyle of this selected population. Hypertension is known to increase the severity of atherosclerosis throughout the decades (Resch *et al.*, 1970). The combination of hypertension and diabetes mellitus increases the progression of atherosclerosis in the cerebral vessels. Experiments have shown that transvascular LDL transport is increased in patients with diabetes mellitus, especially if hypertension is present. Therefore, lipoprotein flux into the arterial wall could be increased in these patients, possibly explaining accelerated development of atherosclerosis. (Jensen *et al.*, 2005) Subjects with a history of alcohol usage had fewer cerebral lesions; this was also seen in subjects with hypertension and alcohol usage. Even though we could not quantify the extent of alcohol usage by subjects in this study, studies have shown that moderate usage of alcohol was protective of coronary and cerebral atherosclerosis progression, (Lifsic, 1976) we could say that alcohol somewhat reduces the extent and severity of cerebral atherosclerosis.

CONCLUSION

Studies of atherosclerosis in the circle of Willis of 44 consecutive autopsy subjects in the UCH, Ibadan revealed that higher frequency of cerebral atherosclerosis in subjects with hypertension. Atherosclerosis of the circle of Willis is more common in older subjects and frequently severe in those aged 55-64 years and males are at a greater risk of developing cerebral atherosclerosis than females.

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