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Serum Lipid Profiles and Homocysteine Levels in Adults with Stroke or Myocardial Infarction in the Town of Gombe in Northern Nigeria

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ABSTRACT

While the incidence of infectious diseases has been on the decline in developing countries, the toll of cardiovascular diseases, including stroke and myocardial infarction, has been increasing. The impression of physicians in certain regions of the western Sahel, including the state of Gombe in northeastern Nigeria, is that macrovascular disease in the indigenous population is on the rise. This study was, therefore, undertaken to compare well-established risk factors for cardiovascular disease in a group of 53 men (n=34) and women (n=19) in the town of Gombe who had suffered a stroke or heart attack with the corresponding parameters in 48 age- and gender-matched healthy adults living in the same environment. The parameters of cardiovascular diseases considered were: overweight and obesity, blood pressure, lipid profiles, and homocysteine. While the male and female patients who had suffered stroke (n=48) or heart attack (n=5) were borderline hypertensive, their mean body mass index values were not different from the corresponding values of their control groups. Although the serum homocysteine levels of the patients and controls were not significantly different, 85% of the stroke patients had serum homocysteine levels greater than 10 μM. These high homocysteine levels could not be accounted for by sub-optimal folate or vitamin B12 status. The serum levels of HDL-cholesterol and triglyceride were not significantly different between the male and female patients and their respective controls. However, the males, but not the females, with macrovascular disease had significantly higher levels of total cholesterol (161 vs 137 mg/dL, p=0.04) and LDL-cholesterol (91 vs 70 mg/dL, p=0.02). In addition, both female and male stroke/myocardial infarction patients exhibited an elevated LDL-cholesterol/HDL-cholesterol ratio. These results indicate that blood pressure and the LDL-cholesterol/HDL-cholesterol ratio are associated with stroke and myocardial infarction in adults in northern Nigeria, thereby creating potential opportunities for possible public-health interventions.

***Key words*:** Cardiovascular diseases; Cardiovascular accident; Myocardial infarction; Cholesterol; Lipoproteins, LDL; Homocysteine; Folate; Vitamin B12; Nigeria

INTRODUCTION

The incidence of stroke and heart attack in the developing regions of the world has been increasing steadily over the past several decades (1-3). This increasing trend has been attributed, in part at least, to migration of populations from rural areas to urban centres where the lifestyle is often more sedentary and where stress is greater (4,5). The perception of physicians in northern Nigeria, where we recently conducted several studies on risk factors of cardiovascular disease, among men and women (6,7), is that stroke and heart attack in the local population are on the increase.

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Elevated serum triglycerides, total cholesterol, and LDL-cholesterol are well-established risk factors of cardiovascular disease (8-12). HDL-cholesterol, in contrast, is widely regarded as protective. A low serum HDL-cholesterol level is, therefore, thought to be an independent cardiovascular risk factor that leads to the development of atherosclerosis and related cardiovascular events (13). Moreover, numerous clinical trials have shown that cholesterol-lowering statins reduce the relative risk of major cardiovascular events, including stroke and myocardial infarction, by 25-40% (13). In a study conducted in Australia, Simons and colleagues found that total cholesterol above a threshold level of 7.06 mmol/L (274 mg/dL) in men and above 7.80 mmol/L (300 mg/dL) in women predicted coronary heart disease, but with stroke the prediction was incremental (14). They found that only HDL-cholesterol and hypertension predicted ischemic stroke. These authors argue that their findings provide a basis for recommending that cholesterol testing and treatment that is designed to lower LDL-cholesterol in individuals at risk of coronary heart disease or stroke. In their review of several HDL-cholesterol and infarction prevention trials, Rizos and Mikhailidis concluded that lowered HDL-cholesterol and/or raised total cholesterol, LDL-cholesterol and triglyceride levels are associated with an increased risk of a cerebrovascular event (15). However, the relationship of serum lipids to ischemic stroke has yet to be universally accepted. For example, in their study of stroke patients in Scotland, Misirli and co-workers failed to find statistically significant differences in the total cholesterol, HDL-cholesterol, LDL-cholesterol or triglyceride levels between patients and control subjects (16). The only independent risk factors they found for ischemic stroke were hypertension and diabetes mellitus. Numerous published reports have established that moderate hyperhomocysteinaemia is an important risk factor of cardiovascular disease (17-21). Homocysteine is toxic to the vascular endothelium and can promote the peroxidation of LDL (22).

The literature is lacking in reports relating serum lipid profiles or homocysteine levels and coronary heart disease and stroke in indigenous sub-Saharan populations. We were, therefore, interested in inquiring as to which of the following well-established risk factors for cardiovascular disease might be associated with cardiovascular disease in Gombe, Nigeria, a town located in the northeastern part of the country: serum lipids (including total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), homocysteine, overweight and obesity, and blood pressure. To this end, we studied 53 consecutive men and women who were admitted in January-April 2002 to a tertiary care centre in Gombe, namely the Federal Medical Centre-Gombe (FMC-G), due to stroke or heart attack. This report contains the results of our comparison of the lipid profiles and homocysteine levels in the 53 stroke or heart attack patients and 48 healthy control men and women in Gombe.

MATERIALS AND METHODS

Subjects

During January-April 2002, 53 consecutive patients who were admitted to the Federal Medical Centre, Gombe, due to stroke (n=48) or myocardial infarction (n=5) were enrolled in the study: 34 patients were male and 19 were female. Six patients died: 2 women from stroke, 3 men from stroke, and 1 man from myocardial infarction. The 48 healthy controls—21 women and 27 men—were recruited from the town of Gombe for the study. The incentive for individuals to serve as controls in the study was the promise that each would be informed of the overall results of the study and any information that was pertinent to them.

Ten mL of venous blood was obtained within two hours of admission and allowed to clot at room temperature for 45 minutes. The clotted specimens were centrifuged at 5,000xg in a clinical centrifuge, and the serum fraction was aliquoted into cryovials and stored at -43 ^{o}C until which time they were transported in the frozen state to New Mexico where various biochemical analyses were performed in September 2002. The study was approved by the Human Research Review Committee of the University of New Mexico and the Human Ethics Committee of the Federal Medical Centre-Gombe.

Biochemical analyses

Total cholesterol was determined by the end-point colorimetry method of Allain *et al*. (23) using a Vitros 950 analyzer. HDL-cholesterol was determined using Kodak Vitros Cholesterol slides and a Vitros 250 analyzer (24). Triglycerides were determined by the method of Spayd *et al*. (25) using a Vitros Analyzer Clinical Chemistry Slide (TRIG) and a Vitros 950 analyzer. LDL-cholesterol was calculated using the

following equation: LDL-cholesterol=total cholesterol-(HDL-cholesterol+triglycerides/5). Serum folate and vitamin B12 were determined immunologically by competitive magnetic separation assays using kits purchased from Technicon (Tarrytown, NY, USA). Homocysteine was determined using the Imx Homocysteine assay kit (Abbott Diagnostics Division, Abbott Laboratories, Columbus, OH, USA).

RESULTS

Comments on the study population

The average age of the 34 male patients was 53.5 years (Table 1), which was not significantly different from that of the male controls (51.1 years). In addition to age, the male patients and the controls were closely matched for height, weight, and body mass index (BMI). In general, neither the male patients nor their controls were overweight (as defined by a BMI greater than 25 kg/m^2) (26). Although the male patients in the stroke/heart attack group were not severely hypertensive, they did qualify as having borderline hypertension on the grounds that their mean systolic pressure was 133 mmHg and their mean diastolic pressure was above 90 mmHg. (Hypertension is defined as blood pressure >140/90 mmHg.) The mean systolic and diastolic pressures of the control males were in the normotensive range (122/78 mmHg). Both systolic and diastolic pressures of the male patients and male controls were statistically different, the former having the higher values.

As was the case for the males in this study, the average ages of the female patients (57.2 years, Table 2) and female controls (52.7 years) were not significantly different. Similarly, the mean heights, weights, and BMI values were not significantly different between the women who had experienced stroke or heart attack and those in the healthy control group. However, like the male patients, the female patients had borderline hypertension (131/88 mmHg) at the time the study was conducted. The difference between the diastolic pressure, but not the systolic pressure, of the female patients and controls was statistically significant (p=0.001).

Biochemical analyses

Since there were no statistically significant differences in any of the biochemical variables between the patients with stroke versus myocardial infarction, data for the two patient groups were pooled. As shown in Table 1, while the mean serum homocysteine concentration of men who had suffered a stroke or heart attack (19.6 μM) was not statistically different from that of the control group (17.9 μM), it was, nevertheless, considerably greater than the upper limit of the reference range for healthy adults (12.4 μM) (27). The status of the male patients and male controls with respect to vitamin B12 and folate, two vitamins required for homocysteine assimilation, was adequate as evidenced by the fact that the mean concentrations of these vitamins were well within the range of values seen in healthy adults in the USA (28).

Table 1. Characteristics of male stroke and myocardial infarction patients and healthy controls in Gombe, Nigeria

Characteristics	Controls (n=27) (mean±SD)	Patients (n=34) (mean±SD)	p value
Age (years)	51.1±9.8	53.5±15.1	NS
BP systolic (mmHg)	122±12	133±15	0.004
BP diastolic (mmHg)	78±8	91±14	<0.001
Height (m)	1.68±0.08	1.67±0.06	NS
Weight (kg)	61.6±10.6	65.7±12.1	NS
BMI (kg/m^2)	21.7±3.6	23.6±3.8	NS
Triglycerides (mg/dL)	134±73	166±104	NS
Cholesterol (mg/dL)	137±39	161±49	0.04
HDL (mg/dL)	40±14	36±11	NS
LDL, calculated (mg/dL)	70±30	91±36	0.02
LDL/HDL ratio	1.8±0.5	2.5±0.7	<0.001
Homocysteine (μM)	17.9±10.1	19.6±10.3	NS
Vitamin B12 (pg/mL)	545±283	433±218	NS
Folate (ng/mL)	4.5±2.2	4.4±2.9	NS

BMI=Body mass index
BP=Blood pressure
NS=Not significant

The triglyceride and HDL-cholesterol levels of the stroke/heart attack group of males were not significantly different from the corresponding control values. However, the two lipid parameters that did distinguish the male patients from the healthy controls were the total cholesterol and LDL-cholesterol concentrations. The total cholesterol concentration and the LDL-cholesterol concentration were both 15-20% higher in the patients compared to the controls, and the differences in the mean values were statistically significant ($p<0.05$).

suffered a major macrovascular accident, such as stroke or myocardial infarction, from their healthy counterparts. We found three particular parameters that accomplished this goal for men: namely blood pressure, serum total cholesterol concentration, and LDL-cholesterol concentration. In contrast, for the female stroke/myocardial infarction group, none of the four serum lipids nor homocysteine distinguished them from the female control subjects. However, for both the gender groups that had experienced stroke or heart attack, the LDL/cholesterol

Table 2. Characteristics of female stroke and myocardial infarction patients and healthy controls in Gombe, Nigeria

Characteristics	Controls (n=21) (mean±SD)	Patients (n=19) (mean±SD)	p value
Age (years)	52.1±9.6	57.2±16.0	NS
BP systolic (mmHg)	122±9	131±22	NS
BP diastolic (mmHg)	78±7	88±14	0.001
Height (m)	1.6±0.05	1.59±0.05	NS
Weight (kg)	64.5±17.7	62.6±8.1	NS
BMI (kg/m^2)	25.8±6.4	24.9±2.9	NS
Triglycerides (mg/dL)	135±49	178±118	NS
Cholesterol (mg/dL)	169±36	191±62	NS
HDL (mg/dL)	43±10	42±12	NS
LDL, calculated (mg/dL)	99±29	114±44	NS
LDL/HDL ratio	2.3±0.6	2.7±0.7	0.02
Homocysteine (μM)	13.7±6.3	17.4±9.7	NS
Vitamin B12 (pg/mL)	547±249	497±344	NS
Folate (ng/mL)	4.5±1.8	4.8±3.2	NS

BMI=Body mass index
BP=Blood pressure
NS=Not significant

In addition, the LDL-cholesterol/ HDL-cholesterol ratio was elevated to a significant extent in the male patients relative to their control group (2.5 vs 1.8, $p<0.001$).

Different results were obtained for the female subjects (Table 2): relative to healthy women, the serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels of women who had experienced a stroke or heart attack were not significantly different from the corresponding values of the female controls. However, the LDL-cholesterol/ HDL-cholesterol ratio in the female patients was significantly increased relative to the same ratio for the female controls (2.7 vs 2.3, $p=0.02$). In general, the serum homocysteine concentrations of the women in our study were generally lower than they were in the males; however, the mean homocysteine values of the female patients and the female controls were not significantly different from each other.

DISCUSSION

We set out to identify risk factors of cardiovascular disease that might distinguish adults in Gombe who had

ratio was significantly increased relative to their respective control groups. This ratio is widely regarded as an index of risk for cardiovascular disease (29).

Forty-four of the 53 (85%) patients had serum homocysteine concentrations greater than 10 μM. Our finding that the mean homocysteine levels of all the four study groups in the present study, while being in excess of the upper limit of the reference range for healthy adults in the USA (27), were not statistically different from one another indicates that homocysteine is probably less important than the LDL-cholesterol/ HDL-cholesterol ratio when it comes to ranking cardiovascular disease or stroke risk factors for the population of Gombe.

The relatively high homocysteine concentrations we observed in many of the 101 total subjects who were involved in this study and which confirm our previous findings in northern Nigeria (6,7) were probably not the result of inadequate folate or vitamin B12 nutrition, since the serum levels of these two vitamins are well within the reference range for healthy adults in the USA (28). One

hypothesis we are planning to test to explain the unusually high incidence of elevated serum homocysteine in the Gombe region of northern Nigeria is that there is a high prevalence among the population of a mutation in the gene encoding 5,10-methylene tetrahydrofolate reductase (MTHFR). Mutations affecting the activity or expression of MTHFR have been shown to be associated with hyperhomocysteinaemia (30).

Since the male and female controls in the present study were of approximately the same age as the patients who had experienced a stroke or heart attack, we did not have to be concerned that our conclusions regarding lipid profiles or homocysteine levels in the patient and control groups may have been confounded by age considerations. Furthermore, the possible confounding variable of age appears not to have been a factor in our study because when we tested for possible correlations between LDL-cholesterol, total cholesterol, HDL-cholesterol or triglycerides versus age in both female and male pairs, none was found (data not shown).

Despite the relatively low number of patients investigated in the present study, it is nevertheless noteworthy that the distribution of cardiovascular events between stroke (n=48) and myocardial infarction appears to be very different from what would be expected in a more developed western country. This distinction raises the possibility that the underlying aetiology of these cardiovascular diseases in northern Nigeria might not be the result of typical atherosclerotic disease. Future extensions of our study should take into consideration additional risk factors of cardiovascular disease, including exercise level, diet, and chronic inflammation. With regard to diet, we recently documented the intake of relatively large amounts of cholesterol and saturated fats by the predominant ethnic group in the Gombe region, namely the Fulani (7). The economy and culture of the Fulani are centred on cattle and, as such, dairy products contribute significantly to their diet. Dairy products, such as milk, butter, and cheese, are excellent sources of trans fatty acids that decrease HDL-cholesterol, raise LDL-cholesterol, and increase the risk of cardiovascular disease (31-34). We plan to measure the levels of trans fatty acids in blood of the adult population of Gombe and the surrounding towns and villages. As for the role inflammation might play in explaining the unusual distribution we observed between stroke and myocardial infarction in the patients in the present study, in future research of this kind, we plan to measure the levels of C-reactive protein which is a marker of inflammation (35).

Having identified an association between serum total cholesterol, particularly LDL-cholesterol, and macrovascular disease in men in northern Nigeria and having found the LDL-cholesterol/HDL-cholesterol ratio to be significantly increased in both men and women who experienced myocardial infarction or stroke in Gombe, one may raise the obvious question of what might be done to reduce the risk of cardiovascular disease in this population. Our findings have two practical implications. The first pertains to education. It is reasonable to propose that public-health officials in the region inform adults of the value of daily exercise, since moderate exercise for 30 minutes per day has been shown to significantly reduce the risk of heart attack and stroke (29). A second action that should be entertained is that of screening hypertensive adults for their lipid profiles, since the results of the present study revealed that the LDL-cholesterol/HDL-cholesterol ratio distinguished the stroke and heart attack groups from the healthy controls. The deep and pervasive poverty of Gombe and the surrounding states renders it unrealistic to propose that the costly cholesterol drugs, such as statins that are widely used for reducing the risk of people living in the economically-prosperous parts of the world, such as Europe and North America, will be available any time soon to many people in northern Nigeria. Nevertheless, the relatively inexpensive drugs that have been used for lowering cholesterol levels for decades (e.g. gemfibrozil, fenofibrate) may be within economic reach of individuals at an increased risk of cardiovascular disease in Nigeria. Gemfibrizol, for example, was shown to increase HDL by 6% and decrease triglycerides by 31% (15), while bezafibrate increases HDL-cholesterol by 18% and decreases triglyceride levels by 21%.

The results of the present study should stimulate a discussion of possible educational and economically feasible pharmacological interventions that might be realistically considered by government officials and non-profit organizations interested in improving the health and longevity of the people of northern Nigeria and perhaps other regions of sub-Saharan Africa as well.

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