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Original Article

Chlamydia pneumoniae as an Independent Risk Factor for Acute Myocardial Infarction among Central Africans

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ABSTRACT

Background: Cardiovascular diseases are rising in low-income countries. We aimed to determine the association between high titers to *C. pneumoniae* IgG and acute myocardial infarction (MI). **Methods:** This nested case-control study was conducted from December 2007 to December 2009. Patients diagnosed with acute MI were matched to controls by their age, sex, area of residence, and also their socio-economic status. **Results:** A total of 72 patients with acute MI were matched to 32 controls. After adjusting for confounding factors, only left ventricular hypertrophy (RR = 30.5; 95% CI *P* = 0.003), previous exposure to *C. pneumoniae* (RR = 43.5; 95% CI *P* = 0.004), and blood concentrations of Gamma-glutamyltransferase ≥ 30 UI /L (RR = 124.1; 95%CI *P* < 0.0001) were significantly and independently associated with acute MI. **Conclusion:** Findings confirmed the potential aetiologic relationship between *C. pneumoniae* and acute MI among Central Africans.

Keywords: *Chlamydia pneumoniae* IgG; left ventricular hypertrophy; GGT ≥ 30 IU/L; acute myocardial infarction.

RÉSUMÉ

Contexte : Les maladies cardiovasculaires sont en hausse dans les pays à faible revenu. Cette étude vise à déterminer l'association entre un taux élevé d'IgG *Chlamydia pneumoniae* et l'infarctus aigu du myocarde (IAM). **Méthodologie :** Cette étude cas témoin imbriquée dans une cohorte a été menée de Décembre 2007 à Décembre 2009. Les patients ayant reçu un diagnostic d'IAM ont été jumelés aux cas témoins selon l'âge, le sexe, la zone de résidence et le statut socioéconomique. **Résultats :** Un total de 72 patients diagnostiqué d'IAM ont été jumelés à 32 cas témoins. Après rajustement des facteurs confusionnels, seul l'hypertrophie ventriculaire gauche (RR = 30.5; 95% CI *P* = 0.003), une exposition antérieure à *C. pneumoniae* (RR = 43.5; 95% CI *P* = 0.004) et une concentration sanguine de Gamma-glutamyltransferase ≥ 30 UI /L (RR = 124.1 ; 95%CI *P* < 0.0001) étaient associés significativement et indépendamment à un IAM. **Conclusion :** Les résultats confirment la relation étiologique potentielle entre *C. pneumoniae* et l'IAM parmi les Centrafricains.

Mots clés : *Chlamydia pneumoniae* IgG; hypertrophie ventriculaire gauche; GGT ≥ 30 IU/L, infarctus aigu du myocarde.

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INTRODUCTION

Cardiovascular diseases (CVD), although previously considered rare in the African continent, are currently among the leading causes of deaths (1). Progressive urbanization and the westernization of lifestyle, leading to an epidemiological transition of developing countries can be mentioned as possible reasons (1, 2).

Recent epidemiological studies have shown the significant associations between different infections, including *C. pneumoniae* and atherosclerotic diseases (stroke, peripheral arterial diseases, and coronary heart diseases) (3, 4). Infections caused by cytomegalovirus, herpes simplex virus, *Helicobacter pylori* and *Chlamydia pneumoniae* are among the most commonly incriminated (5). However, some controversies have failed to establish a significant relationship of infectious agents such as *Helicobacter pylori* and cytomegalovirus with atherosclerotic diseases (6). *C. pneumoniae* is an emerging cause of community-acquired pneumonia, pharyngitis, bronchitis and sinusitis, and can account for approximately 5-10% of all these cases (5, 7). Seroprevalence studies have shown that this infection is more prevalent in men than women, and this prevalence increases with age (8). There is also evidence to suggest that the *C. pneumoniae* could play a role in all stages of atherosclerosis, from the initial inflammatory lesion to plaque rupture (9, 10).

A previous study on acute MI in the Democratic Republic of Congo (DRC), a country located at the heart of Central Africa, showed that acute MI was more observed among young adults with a history of tobacco smoking. Among other identified risk factors for acute MI in DRC is hyperuricemia and infection due to *H. pylori* were also mentioned (11). Although still rare in the DRC, the emergence and rapid growth of CVD accounts for the strong increase in coronary-related morbidity and mortality predicted cases and further underlines the need for an epidemiological control plan aimed at preventing CVD in DRC in particular and developing countries in general.

Hence, the aim of this study was to determine the association between high titers to *C. pneumoniae* IgG and acute MI among patients' attendees in Kinshasa, DRC.

MATERIALS AND METHODS

Study Design and Sampling Strategy:

The study was nested within a prospective cohort of 205 patients whose characteristics were published elsewhere (4). From 2007 to 2009, patients were assessed on a monthly basis for the outcome of myocardial infarction and blood samples were drawn and stored. Medically documented patients with first-time admission for acute MI were individually matched with study population-based controls selected from the same cohort. Seventy-two cases of acute MI and 32 controls (ratio of 2 cases: 1 control) were matched by age, sex, area of residence, socio-economic status, and time of biological sample collection.

LOMO clinic, a specialized clinic for the management of CVD in Kinshasa (DRC), was the study setting. A clinical examination was performed by the attending physician and patients were included if they had given informed consent, had no history of trauma, and if no other non-cardiac cause of chest pain had been diagnosed. In addition, patients' chest pain should have begun within the previous 24 hours, had been of greater than 10 minutes' duration, and accompanied by an abnormal resting 18-lead ECG (ST elevation and no ST elevation). Patients were finally retained as study participants only after confirmation of a diagnosis of acute MI. Controls were individuals who never been diagnosed as having stable or unstable angina pectoris, acute MI or any other cardiovascular disorder. Even if their ECG was normal on admission, biochemical tests were conducted to exclude the diagnosis of acute MI.

The attending physician also recorded information on subjects' demographics and clinical characteristics. Risk factors commonly associated with CVD were also identified. The characteristics of interest included sociobiographical factors (age, gender, area of residence, socio-economic status, history of hypertension, type 2 diabetes mellitus, tobacco use, alcohol intake, and physical inactivity), body mass index (BMI = weight in Kg / height in m squared), waist circumference, hip circumference, systolic blood pressure, diastolic blood pressure, left ventricular hypertrophy (Sokolow-Lyon Index ≥ 35 mm using ECG), blood total cholesterol, high density lipoprotein-cholesterol (LDL-C), creatinine, urea, total creatinine phosphokinase (CPK), the cardiac fraction of CPK (CK-CPK), Troponin I, triglycerides, glucose, and gamma-glutamyltransferase (GGT).

Definitions used for physical inactivity, tobacco use, alcohol consumption, area of residence, and socio-economic status were from the 2003 World Health Organization (WHO) stepwise approach to surveillance of non-communicable diseases (STEPS) – a framework for surveillance. The study protocol was reviewed and approved by the relevant Ethics Committee in DRC.

Ascertainment of Cases of Acute Myocardial Infarction:

The criteria established by the 2007 Joint Commission of the European Society of Cardiology, the American College of Cardiology Foundation, the American Heart Association, and the World Heart Federation was used to ascertain the diagnosis of acute MI (12). These criteria combined the presence of ischaemic acute chest pain with changes in the ECG and the rise and/or fall of biochemical markers of myocardial necrosis (preferably troponin or MB fraction of creatine kinase) with at least one value above the 99th percentile of the upper reference limit.

Changes in the ECG included either (i) new ST segment elevation at the J-point in two contiguous leads with the cut-off points ≥0.2 mV in men or ≥0.15 mV in women in leads V2-V3 and/or ≥0.1 mV in other leads; or (ii) new

horizontal or down-sloping ST segment depression ≥0.05 mV in two contiguous leads; and/or T wave inversion ≥0.1 mV in two contiguous leads with prominent R-wave or R/S ratio >1; (iii) any Q-wave in leads V2-V3 ≥0.02 s or QS complex in leads V2 and V3.

Whilst the first 2 types of ECG changes only reflected myocardial ischemia and were not sufficient by themselves to define acute MI, the final diagnosis of myocardial necrosis was dependent on the detection of elevated levels of cardiac biomarkers in the blood. In summary, blood samples were collected on admission and again at 12 to 24 h if the earlier samples were negative and the clinical index of suspicion of acute MI was high. However, in most situations, elevated values for biomarkers were recorded from two successive blood samples in order to diagnose acute MI. Levels of the myocardial fraction of creatine kinase (CK-MB) and troponin I were measured in blood samples of all patients to support the diagnosis of acute MI. Plasma CK-MB was measured using the quantitative immunofluorescence assay (Dade-Behring, Marburg, Germany) with the analytic sensitivity of the assay being 0.6 ng/mL and the upper normal limit considered to be 5.0 ng/mL. Other laboratory tests were included in the diagnostic protocol to assess risk factors commonly associated with CVD. Those tests involved the measurement of total cholesterol, high-density and low-density lipoproteins, total creatine phosphokinase, triglyceride, gamma-glutamyl transferase (GGT), and fasting glucose levels in the blood samples obtained from all study participants.

Serological Diagnosis of Previous Infection due to Chlamydia pneumoniae:

Serum samples were obtained from blood collected by venepuncture from all study participants (cases and controls) on admission. The samples were kept at 2-8°C if the assay was performed within 5 days after sample collection; otherwise samples had been aliquoted and frozen at -70°C until use.

To detect IgG to *C. pneumoniae*, Novagnost ™ (Germany) ELISA system was used. Briefly, 100µL of controls (positive, negative and cutoff) and diluted samples were dispensed into a 96 microwell plate coated with *C. peumoniae* antigens following manufacturer's instructions. After incubation (1 hour ± 5 min at 37±1°C), wells' contents were aspirated followed by washing steps, and the addition of 100µl *C. peumoniae* anti-IgG conjugate into indicated wells. After 30 min incubation at room temperature, 100µl TMB substrate solution was added into all wells, followed by 15 min incubation at room temperature in the dark and subsequent addition of 100µl of stop solution, and measurement of the absorbance (30 min later) of the specimen at 450/620 nm using ELISA microwell plate reader.

In order for an assay to be considered valid, the following criteria were met: (i) negative control in A1: absorbance value < 0.200 and < cut-off; (ii) cut-off control in B1 and C1: absorbance value 0.250 –0.900; positive control in D1: absorbance value > cut-off. Samples were considered positive if the absorbance value was higher than 15% over the cut-off. A sample with an absorbance value of less than 15% above or below the cut-off was not considered as clearly positive or negative, and was said to be in the "grey zone". The tests were repeated after 2-4 weeks for samples that were in the "grey zone". If the results of the second test were again in the grey zone the sample was considered to be negative. Samples were also considered negative if the absorbance value was lower than 15% below the cut-off.

Statistical Analysis:

Descriptive measures such as mean, median, variance, range and standard deviations were used to summarize the study results. The Student's T test, Mann-Whitney U test and the Pearson's chi-square test (with application of exact Fischer's test) were used to compare cases of acute MI and control subjects; and logistic regression analysis was performed to study the relationship between case-control status, previous *C. pneumoniae* infection status, and demographic and cardiovascular risk factor covariates. The Receiver Operating Curves (ROC) were used to discriminate cases of acute MI and controls by the levels of continuous variables not yet described by the diagnostic performance (Area under the Curve with its 95% CI, Standard Error, Sensitivity, Specificity, Optimal Cut-off points, and P-values). A p value of < 0.05 was considered as statistically significant. All analyses were performed with the statistical package IBM, SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Data were obtained from 72 cases of acute MI and 32 matched controls. Table 1 depicts the mean levels of selected characteristics of patients with acute MI as compared with those from controls. Except for age, hip circumference, waist circumference, body mass index, total cholesterol, HDL-C, LDL-C, and fasting glucose, the mean values of systolic BP, diastolic BP, CPK, CK-MB, Troponin I, triglycerides, and GGT were significantly (P < 0.05) higher in cases of acute MI than controls. The rest of the study characteristics were similar between both study groups (results not shown).

Left ventricular hypertrophy, positive IgG anti- *C. pneumoniae*, hypertension, and type 2 diabetes mellitus were significantly (P < 0.05) associated with acute MI, whereas age, gender, physical inactivity, tobacco use, alcohol intake did not impact on the onset of acute MI (Table 2)

Table 1: Selected characteristics of patients with acute myocardial infarction as compared to the controls

variables	acute MI (n= 72) (mean ± SD)	control (n= 32) (mean ± SD)	ρ value
Age (years old)	59±13	59.8±10	0.694
Hip circumference (cm)	98.8±11.8	99.4±13.9	0.83
Waist circumference (cm)	97.8±10.6	98.8±11.8	0.658
Body mass index (Kg/m2)	29.1 ± 5.5	27.4 ± 4.8	0.144
Systolic BP (mmHg)	152.8±28.5	170.9±33.5	0.005
Diastolic BP (mmHg)	91.1±16.2	98.2±16.6	0.041
Total cholesterol levels (mg/dl)	225.7±60.4	236.5±67	0.419
HDL - C (mg/dl)	61.7±25.3	66.1±33.2	0.459
LDL - C (mg/dl)	141.3±49.8	145±52	0.725
Total creatine phosphokinase (CPK: IU/L)	342.6±121	136.7±136.3	<0.0001
Cardiac fraction of CPK (CK-MB: ng/ml)	114.9±31.7	58.3±52.4	<0.0001
Log Troponin I (µg/ml)	2.31±0.35	0.015±0.002	<0.0001
Triglyceride (TG: mg/dl)	127.8±69.3	103.2±49.2	0.044
Gamma-glutamyltransferase (GGT: IU/L)	65.8±31.6	31.4±32.5	<0.0001
Fasting glucose levels in the blood (mg/dl)	109.8±57.7	112±52.2	0.848

In multivariate analysis (logistic regression model), after adjusting for confounding factors (triglycerides, hypertension, and type 2 diabetes mellitus), presence of left ventricular hypertrophy, positive IgG anti- *C. pneumoniae*, and GGT ≥ 30 UI /L were identified as the most important ($p < 0.05$) and independent risk factors (predictors) of acute MI (**Table 3**).

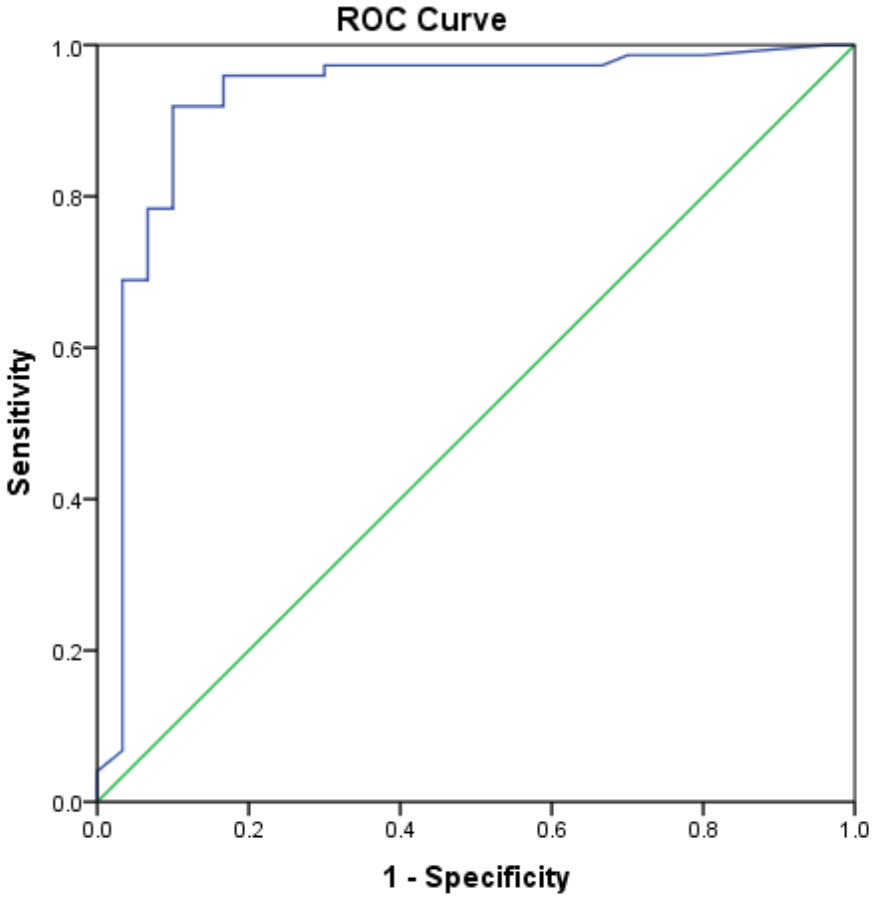
Gamma-glutamyltransferase ≥ 30 UI /L was the optimal cut-off point to discriminate between the presence and absence of acute MI by the following features of the diagnostic performance using ROC: AUC = 0.927 (95% CI 0.858 – 0.996), Standard Error = 0.035; $P < 0.0001$ (**Figure 1**).

Table 2: Results of univariate analysis of general characteristics of the study population

variables	acute MI n (%)	controls n (%)	ρ value
Gender			0.973
male	47 (65.4)	21 (65.6)	
female	25 (34.7)	11 (34.4)	
Physical inactivity	70 (97)	30 (94)	0.956
Tobacco use	16 (22.2)	9 (28.1)	0.516
Alcohol consumption	44 (61.1)	20 (62.5)	0.893
Age ≥ 40 years old	67 (93.1)	31 (96.9)	0.441
Left ventricular hypertrophy	57 (79.2)	7 (21.9)	<0.0001
Positive IgG anti-*C.pneumoniae*	33 (45.8)	3 (9.4)	<0.001
Arterial hypertension	53 (71.6)	13 (43.3)	0.007
Type 2 diabetes mellitus	33 (44.6)	3 (10)	< 0.001

Table 3: Independent predictors (risk factors) of acute myocardial infarction

	B	SE	Wald	RR (95% CI)	P-value
Independent predictors					
Left ventricular hypertrophy					
Yes vs. No	3.419	1.133	9.108	30.5 (3.3 - 281.2)	0.003
Positive IgG anti- *C.pneumoniae*					
Yes vs. No	3.774	1.308	8.329	43.5 (3.4 - 564.8)	0.004
Gamma-glutamyltransferase ≥ 30 UI / L					
Yes vs. No	4.821	1.135	18.053	124.1 (13.4 - 1147.7)	< 0.0001
Constant	-4.003	1.147	12.182		< 0.0001



Diagonal segments are produced by ties.

DISCUSSION

The study showed a significant and independent association between left ventricular hypertrophy, high titers to *C. pneumoniae* IgG, gamma-glutamyltransferase ≥ 30 UI /L and acute MI. This association was maintained even after adjustment for cardiovascular risk factors, suggesting that chronic infections could be associated with an increased risk for acute MI.

C. pneumoniae Infection:

Numerous reports have shown some evidence to suggest that *C. pneumoniae* could play a role in all stages of atherosclerosis, from the initial inflammatory lesion to plaque rupture (13, 14). It has been hypothesized that *C. pneumoniae* gains their entry into the vessel wall by extravasation of infected blood monocytes followed by an inflammatory response with production of cytokines and leukocyte adhesion molecules, facilitating the formation of an atherosclerotic plaque (13, 14) . The infection by *C. pneumoniae* leads to the development of specific antibodies that can be detected to indicate an exposure to this pathogen (3, 15).

Although acute MI remains a problem of individuals aged > 40 years, the condition was diagnosed among 6% of our study population aged <40 years (data not shown) supporting previously published reports about acute MI rates ranging from 4% to 10% in young adults aged <40 years (16-18). There are many published studies from the literature that have explored the relationship between *C. pneumoniae* IgG and acute MI, and the results remain controversial (5, 14-16, 19, 20) . Differences in used study designs, lack of standardization in the adjustment of cardiovascular risk factors and other confounding factors, methods used to measure Chlamydia infection and particularly lack of general consensus about the definition of seropositivity might account for these differences.

Left Ventricular Hypertrophy:

This study confirmed a published report from the literature about the significant association between left ventricular hypertrophy and acute MI among African-

American patients (21). There was another report on the univariate significant association between hypertension, type 2 diabetes mellitus, and acute MI among black Bantu Africans (22). Hypertension, often severe and uncontrolled and associated with type 2 diabetes mellitus, can lead to left ventricular hypertrophy (23).

Gamma-glutamyltransferase:

Amongst the independent risk factors of acute MI in this study, the increase in the concentrations of serum GGT was the most important predictor. This finding is consistent with publications among Europeans with and without diabetes mellitus (24, 25). Whilst risks for developing acute MI were increased by 30-fold and 43-fold respectively for, left ventricular hypertrophy and past exposure to *C. pneumoniae*, the risk was 124-fold higher when serum levels of GGT were ≥ 30 IU/L. Different reasons may explain such higher risk for acute MI. Gamma-glutamyltransferase is known as a potential marker of left ventricular function (25) so that both left ventricular hypertrophy and GGT ≥ 30 IU/L may interact in the development of acute MI. The stronger power of the revealed cut-off optimal point of GGT in this study was similar to the median GGT ≥ 32 IU/L which was higher in patients with acute coronary syndrome as compared to their control group median GGT < 16 IU/L among patients from Turkey (24). Biological plausibility reported that serum GGT, an enzyme mostly derived from the liver, impacts on the extracellular catabolism of glutathione (an antioxidant marker), and is associated with metabolic syndrome and type 2 diabetes mellitus (26).

Clinical Implications:

Although tobacco use, alcohol intake, lipid-lipoprotein profile, fasting glucose, physical inactivity, body mass index, hip circumference and waist circumference were not significantly associated with acute MI, the present findings will have significance in terms of the pathophysiology, the diagnosis, and the prevention of atherosclerotic diseases including myocardial infarction across different ethnic groups in general and in black Bantu Africans in particular. Nature and nurture will be studied among Africans who experience health transitions (aging, increase in non-communicable diseases associated with physical inactivity, urbanization, migration, smoking, salt, fats, obesity, and excessive alcohol intake). Sanitation and antibiotics will control *C. pneumoniae* infection. The signalling events stimulated by GGT catalysis of glutathions might become a therapeutic target as animal studies reported on a redox-metabolic-electrical remodelling in the diseased left ventricle and focus on the role of GGT in post myocardial infarction rat hearts (27).

We need clarity on different stages of infection (acute, chronic, latent or recurrent infections) which may initiate atherosclerotic plaque instability towards the rupture.

Limitations and Strengths of the Study:

Limitations to this study involve the fact that ELISA test was used without being associated with a gold standard test (micro-immunofluorescence test) that has a higher specificity. However, despite the small size of this study, the use of randomization and a prospective cohort allowed the selection of closely matched control population-based participants (nested case-control design) on the basis of time of variables collection in order to establish the temporality of exposures and acute MI (outcome).

CONCLUSION

In conclusion, this is the first study to our knowledge in the Democratic Republic of the Congo supporting the association between left ventricular hypertrophy, high titers to *C. pneumoniae* IgG, serum GGT levels ≥ 30 IU/L, and acute MI. However, traditional risk factors of acute MI in this study were not shown to be associated with acute MI either in univariate or multivariate analysis.

Competing Interest:

Authors declare that they have no competing interest.

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