ORIGINAL ARTICLE

Prevalence of *Helicobacter pylori*, cytomegalovirus, and *Chlamydia pneumoniae* immunoglobulin seropositivity in coronary artery disease patients and normal individuals in North Indian population

Lt Col Rohit Tewari*, Col VS Nijhawan†, Col MN Mishra#, Lt Col Puja Dudeja**, Brig TK Salopal‡

ABSTRACT

BACKGROUND

In present day atherosclerosis is perceived as a chronic inflammatory vascular condition and infectious diseases are believed to contribute to its pathophysiology. In this context, the microorganisms which are believed to play a role in the pathophysiology include *Chlamydia pneumoniae,* cytomegalovirus (CMV), and *Helicobacter pylori*.

METHOD

A case control study (retrospective) was conducted over a two-year period. The study population was divided into two groups with 200 individuals in each group. The first group comprised cases of coronary artery disease (CAD) and the second comprised healthy controls selected from the general population after matching for age and sex. Enzyme-linked immunosorbent assay (ELISA) was done for immunoglobulin (IgG) antibodies to *H. pylori, C. pneumonia*, and CMV. They were also evaluated for conventional risk factors including hypertension, diabetes, obesity, and dyslipidaemia. Epi Info™ version 6 six software was used for analysis of data. Odds ratio, χ^2 for trend and multiple logistic regression analysis were used to find out statistically significant results.

RESULTS

Seropositivity for *H. pylori* was present in 119 patients of CAD (59.5%) but it was present in only 76 controls (38%) ($P=0.001$). There was a statistically significant association between seropositivity for *H. pylori* and CAD. There was no statistically significant association between *C. pneumoniae* and CMV seropositivity with CAD. Multiple logistic regression analysis was done with CAD as the outcome (dependent variable). The predictor covariates (independent) variables were seropositivity to *H. pylori, C. pneumoniae,* and CMV, hypertension, obesity, diabetes, and dyslipidaemia. It was found that seropositivity to *H. pylori,* hypertension, obesity, and dyslipidaemia were significant risk factors for CAD.

CONCLUSION

Our study shows an association between IgG antibody response to *H. pylori* and CAD. Multiple logistic regression analysis showed that this association was retained even on comparison with the other risk factors.

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Key Words: *Chlamydia pneumoniae*; coronary artery disease; cytomegalovirus; *Helicobacter pylori*

INTRODUCTION

Atherosclerosis is the main underlying cause of cardiovascular diseases. Framingham's heart study and other studies have shown main risk factors of coronary artery disease (CAD) including hypertension, hypercholesterolaemia, diabetes, smoking, environmental factors (such as unsuitable diets, less physical activity), and hereditary factors.[1,2] One of the first published texts related to the atherogenic role of virus, namely, Marek's disease herpervirus, appeared in 1978.[3] At present, several infectious agents resulting in chronic infections have been specified as probable contributors in generation of atherosclerosis.[4] Microorganisms believed to play a role include *Chlamydia pneumoniae,* cytomegalovirus (CMV), and *Helicobacter pylori.*

The conventional atherosclerosis risk factors, including hypertension, diabetes mellitus (DM), smoking, and cardiac diseases, do not fully account for the risk of atherosclerosis, and cases, especially young subjects, often do not have any of these factors.[5] Atherosclerosis is today perceived as a chronic inflammatory vascular condition.[6]

MATERIALS AND METHOD

A case control study (retrospective) was conducted. The study population was divided into two groups. The first group comprised the cases of CAD selected on a combined account of

*Classified Specialist (Pathology), Level 3 Hospital (MONUSCO), Congo, †Director MS (PS), O/o DGMS, L Block, MOD, New Delhi, #Classified Specialist (Pathology), Command Hospital (SC), Pune – 40, **DADH, HQ Bengal Area, Kolkata, ‡Brig Med HQ UB Area.

Correspondence: Lt Col Rohit Tewari, Classified Specialist (Pathology), Level 3 Hospital, MONUSCO, Congo.
E-mail: rohittewariaa@rediffmail.com

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clinical history, electrocardiographic (ECG) changes, and treatment records. The second group comprised healthy controls selected from the general population after matching for age and sex. Both groups together comprised 200 individuals. The study was conducted over a period of two years at a zonal hospital in the Northern sector.

The subjects were interviewed in the outpatient department (OPD) of the hospital. Before the interview the subjects were informed about the scope and nature of the study and were fully assured strict confidentiality. Factual data was recorded by the investigator. For physical examination and blood sample collection for biochemical and serological testing, subjects were called to the hospital laboratory and at that time the data was cross checked and corroborated with the available records with the patient.

For purpose of data collection, age was defined in years. It was ascertained by dependent card/service identity card. History of hypertension was asked for and the patient's notebook containing treatment details was seen for confirmation of diagnosis. Three successive readings were taken at 5 minute intervals and the average was recorded as the blood pressure. The scale used for detection was according to the Seventh Report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure.[7]

A cut-off fasting plasma glucose level of ≥126 mg% was taken as diabetic. The subject was also considered diabetic if he/she was already a known diabetic under treatment.

World Health Organization (WHO) classification was used to define obesity. Height of the subject measured in nearest cm and weight in Kg was recorded. Body mass index (BMI) was ascertained and values above 25 were considered as obese.[8]

The subjects were stratified based on lipid values as per the adult treatment panel (ATP) III classification according to Third Report of the National Cholesterol Education Programme (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults.[9]

Fasting blood samples were collected for testing after consent. Kits used for biochemical testing (plasma glucose and serum lipid profile) were manufactured by Transasia Biomedicals Ltd in technical collaboration with Erba Diagnostics, Germany. For enzyme-linked immunosorbent assay (ELISA), serum from blood samples of control and study groups were stored at –20°C until analysis. All serum samples were evaluated for presence of IgG antibodies to *H. pylori* by ELISA using kits manufactured by Biotecx Laboratories, Houston, Texas. The manufacturers claimed a sensitivity and specificity of 100%. Immunoglobulin G antibodies to CMV was tested by ELISA (kits manufactured by Biotecx Laboratories, Houston, Texas) and the manufacturers claimed a sensitivity and specificity of 100%. Immunoglobulin G antibodies to *C. pneumoniae* were detected by ELISA using kits manufactured by Novatech Immunodiagnostica, Germany.

Data was compiled on an excel sheet. Epi Info™ version 6 software was used for analysis of data. Odds ratio, χ^2 for trend, and multiple logistic regression analysis were used for statistical analysis.

RESULTS

The median age for cases and controls was 51 was and 54 years, respectively. Around 72% of the cases and 75% of the controls were in the age group of 46–85 years (Table 1); 53% of cases and 46% of controls were males.

Immunoglobulin G (IgG) seropositivity for *H. pylori* was present in 119 patients (59.5%) but it was present in only 76 controls (38%) ($P=0.001$; Table 2). There was a statistically significant association between seropositivity for *H. pylori* and CAD. Odds that an individual with CAD had *H. pylori* seropositivity were 2.40 times greater than for an individual without CAD. The prevalence of *H. pylor*i seropositivity was seen to increase with increasing age of the subjects with CAD and this relation was statistically significant. The prevalence of *H. pylori* seropositivity in controls decreased initially from the age group up 30–45 (33.33%) to 46–60 (22.20%), however it increased in the group of 61–87 years of age (64.51%) and no linear trend was seen.

Immunoglobulin G seropositivity for *C. pneumoniae* was present in 130 patients (65%) and in 128 controls (64%). There was no statistically significant association between the presence of Chlamydia infection and CAD. The prevalence *C. pneumoniae* seropositivity was seen to increase with increasing age of the subjects from age group of 30–45 years (64.58%) to 46–60 years (73.33%); however, it declined in the age group of 61–85 years (50%). There was no linear trend observed between increasing age and chlamydia seropositivity.

Table 1 Distribution of cases and controls with respect to age.

Age group (yr)	Cases (% of total number) *n*=200	Controls (% of total number) *n*=200
30–45	52 (26)	48 (24)
46–60	86 (43)	90 (45)
61–85	58 (29)	60 (30)
>85	4 (2)	2 (1)

Table 2 Distribution of cases and controls with respect to *Helicobacter pylori* infection.

	Seropositive for *H. pylori*	Seronegative for *H. pylori*
Cases of CAD (*n*=200)	119	81
Controls (*n*=200)	76	124
Proportion of cases exposed: 0.595		
Proportion of controls exposed: 0.38		

CAD: coronary artery disease, *H. pylori*: *Helicobacter pylori*.
χ^2 value 18.5
P value (0.000); <0.05
Odds ratio 2.40 (95% CI: 1.57–3.66)
Seropositivity for *H. pylori* was present in 119 patients (59%) but it was present in only 76 controls (38%) ($P=0.001$).

Immunoglobulin G seropositivity for CMV was present in 122 patients (61%) while it was present in 130 controls (65%). There was no statistically significant association between the presence of CMV infection and CAD. The prevalence of CMV seropositivity in controls was static, about 75%, in the age groups 30–45 and 46–60 years. Seropositivity to CMV infection declined in controls in the age group 60–87 years (40.6%).

Multiple logistic regression analysis was done with CAD as the outcome (dependent variable; Table 3). The predictor covariates (independent) variables were IgG seropositivity to *H. pylori, C. pneumoniae*, and CMV, hypertension, obesity, diabetes, and dyslipidaemia. It was found that IgG seropositivity to *H. pylori*, hypertension, obesity, and dyslipidaemia were significant risk factors for CAD.

DISCUSSION

Infection with *H. pylori* is common in the Indian subcontinent. Exposure occurs in childhood and approximately 80% of adults have been infected at some time. Prasad et al had demonstrated a seroprevalence of 80% in asymptomatic healthy individuals in the community.[10]

Seropositivity for IgG antibodies to *H. pylori* was seen in 59.5% of the cases as against 38% of the controls. A significant association was present between presence of *H. pylori* infection and CAD. This is similar to the findings of Mendall et al[11] and Patel et al.[12] This suggests that *H. pylori* may be involved in the pathogenesis of atherosclerosis. A study has confirmed identification of *H. pylori* DNA in atherosclerotic plaques of patients with severe CAD. This supports the hypothesis that *H. pylori* may be involved in the pathogenesis of atherosclerosis.[13] Another study suggested that in younger individuals in Japan, *H. pylori* infection is significantly associated with acute myocardial infarction (AMI) independent of the classic coronary risk factors.[14] This was also seen in our study where *H. pylori* seropositivity proved to be an independent risk factor for CAD on multiple logistic regression analysis independent of other conventional risk factors. Rajasekhar et al had shown higher levels of lipids, lipoproteins, C-reactive protein and higher percentage of coronary risk factors in patients seropositive to *H. pylori* in unstable angina suggesting that *H. pylori* may influence coronary risk factors and may play a role in pathogenesis of atherosclerosis.[15] Infection with *H. pylori* may influence atherogenesis through low grade, persistent inflammatory stimulation.[16] Other mechanisms for the development of atherosclerosis in relation to *H. pylori* infection have also been studied. Tamura et al from Japan suggested that *H. pylori*-induced chronic atrophic gastritis decreases plasma vitamin B_{12} and folic acid levels, thereby increasing homocysteine levels, which is a known risk factor for atherosclerosis.[17]

Investigators looked at serological markers of infection with *H. pylori* in a group of patients with unstable angina. However, no relationship was found between unstable angina and *H. pylori* infection, though relationship with *C. pneumoniae* and CMV was demonstrable.[18] Eradication of *H. pylori* infection does not appear to influence any changes of coronary risk factors, as reported by one study that compared sugar, lipid, and fibrinolytic profiles before and after *H. pylori* eradication.[19] It has been shown that no benefit of treatment with antibiotics was observed in high-risk patients as reported by trials examining whether treatment of infection can prevent the complications of CAD.[20]

Sero-surveys indicate a seroprevalence of 22–57% in children under the age of five, increasing to 80–90% by the age of 20, and remaining constant thereafter.[21–23] The prevalence in our study was lower than that demonstrated in these studies. This may be due to the fact that the prevalence of *H. pylori* varies with geographic area, ethnicity, race, age, and socioeconomic status.[21,22]

Immunoglobulin G antibody seropositivity for *C. pneumoniae* was present in 130 patients (65%) and in 128 controls (64%). There was no significant association between presence of Chlamydia infection as evidenced by presence of seropositivity to IgG antibodies and CAD. This finding is similar to the finding of Sotiropoulos et al.[24] In several epidemiological and experimental studies, *C. pneumoniae*, among other infectious agents, has been associated with the risk of ischaemic heart disease.[25,26] Serological association was also shown by

Table 3 Results of multiple logistic regression analysis of various risk factors for association with coronary artery disease.

Term	Odds ratio	95% CI		Coefficient	SE	Z statistic	*P* value
C. pneumoniae	1.9241	0.9032	4.0990	0.6545	0.3859	1.6961	0.0899
CMV	1.7332	0.7756	3.8732	0.5500	0.4103	1.3405	0.1801
H. pylori	2.1978	1.0758	4.4900	0.7874	0.3645	2.1603	0.0307
DM	0.6799	0.3325	1.3903	–0.3858	0.3650	–1.0572	0.2904
Hypertension	2.9977	1.4496	6.1992	1.0979	0.3707	2.9616	0.0031
Lipid profile	8.2611	3.8953	17.5201	2.1116	0.3836	5.5049	0.0000
Obesity	3.2546	1.5560	6.8076	1.1801	0.3765	3.1342	0.0017

CMV: cytomegalovirus, *C. pneumoniae*: *Chlamydophila pneumoniae,* DM: diabetes mellitus, *H. pylori*: *Helicobacter pylori*, SE: standard error.
Multiple logistic regression analysis was done with coronary artery disease (CAD) as the outcome (dependent variable). The predictor covariates (independent variables) were *C. pneumoniae*, CMV, *H. pylori,* DM, hypertension, dyslipidaemia, and obesity. It was found that *H. pylori*, hypertension, obesity, and dyslipidaemia were significant risk factors for CAD.

Agarwal et al.[27] They showed a seroprevalence of 64% in the cases (similar to ours) but a lower seroprevalence of 38% in the control group. The prevalence of *C. pneumoniae* seropositivity was seen to increase with increasing age of the subjects from age group of 30–45 years (64.58%) to 46–60 years (73.33%); however, it declined in the age group of 61–85 years (50%). There was no linear trend observed between increasing age and seropositivity for IgG antibodies to *C. pneumoniae*. A study by Koh et al demonstrated *C. pneumoniae* seropositivity of 74.6% in males and 59.4% in females.[28] They also demonstrated rising seropositivity with increasing age from childhood to adulthood, with subsequently stable levels. This pattern of rising and levelling off of seropositivity with age suggests that *C. pneumoniae* infection occurs early in life, and in older ages the high level of seropositivity is probably maintained by re-infections or chronic infections.

Seropositivity for CMV was present in 122 patients (61%) and it was present in 130 controls (65%). There was no significant association between presence of CMV infection and CAD.

Our study demonstrated a prevalence of CMV infection as evidenced by elevated IgG antibodies of 65% in the control group. The prevalence of CMV seropositivity in controls was static, about 75%, in the age groups 30–45 and 46–60 years. Seropositivity to CMV infection declined in controls in the age group 60–87 years.

The prevalence of IgG antibodies to CMV in Indian population has earlier been shown to be around 95%.[29] These studies did not demonstrate any significant difference of seropositivity to CMV based on age. This differs from Western studies which show a significantly increased seropositivity with age.[30] This may be due to the early acquisition of the infection in India in childhood compared to Western populations leading to a higher prevalence in younger adults.

We were able to demonstrate association of CAD with dyslipidaemia, hypertension, and obesity. However, we were not able to demonstrate any association with DM. The independent risk on logistic regression analysis was found to be highest for dyslipidaemia.

CONCLUSION

Our study showed a statistically significant association between seropositivity for IgG antibodies to *H. pylori* and CAD. Associations of CAD with dyslipidaemia, hypertension, and obesity were also demonstrable.

On the basis of current knowledge, chronic infections like *H. pylori* appear to be risk factors that may act in association with conventional risk factors and genetic predispositions and are not sufficient for disease development. In such a concept, Koch's postulates for causality, which require a specific link between the microbial agent and the disease, among others, cannot be satisfied. However, more general criteria for causality are still insufficiently fulfilled, and the role of chronic infection and inflammation in atherosclerosis pathogenesis is still incompletely defined. Future studies need to address whether inhibition of inflammation or longstanding antimicrobial therapies will reduce the risk of atherosclerosis and CAD and thus offer effective adjuncts to treatment already in clinical use.

Intellectual Contributions of Authors

Study concept: Lt Col Rohit Tewari
Drafting and manuscript revision: Lt Col Rohit Tewari, Col MN Mishra, Lt Col Puja Dudeja
Statistical analysis: Lt Col Puja Dudeja
Study supervision: Brig TK Salopal, Col VS Nijhawan

CONFLICTS OF INTEREST

None identified.

REFERENCES

1. Ridker PM, Genest G, Libby P. Risk factors for atherosclerotic disease. In: *Textbook of Heart Disease* 6th ed. Braunwald E, Zipes DP, Libby P, Bonow R, eds. Philadelphia: WB Saunders 2001:1028–1031.
2. Wayne AR, Pratt CM, Ryan TG, Roberts R. Diagnosis and management of patients with acute myocardial infarction. *Hurts the Hearts* 10th ed. McGraw-Hill 2001:1277–1278.
3. Fabricant CG, Fabricant J, Litrenta MM, Minick CR. Virus induced atherosclerosis. *J Exp Med* 1978;148:335–340.
4. Kuvin JT, Kimmelstiel CD. Infectious causes of atherosclerosis. *Am Heart J* 1999;137:216–226.
5. Goyal P, Kalek SC, Chaudhry R, Chauhan S, Shah N. Association of common chronic infections with coronary artery disease in patients without any conventional risk factors. *Indian J Med Res* 2007;125: 129–136.
6. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 1999;340:115–126.
7. Chobanian AV, Bakris GL, Black HR, et al. Seventh Report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* 2003;42:1206–1210.
8. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. Geneva: World Health Organization 2000;894:i–xii, 1–253.
9. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–3421.
10. Prasad S, Mathan M, Chandy G, et al. Prevalence of *Helicobacter pylori* in southern Indian controls and patients with gastroduodenal disease. *J Gastroenterol Hepatol* 1994;9:501–506.
11. Mendall MA, Goggin PM, Molineaux N, et al. Relation of Helicobacter pylori infection to coronary artery disease. *Br Heart J* 1994;71: 437–439.
12. Patel P, Mendall MA, Carrington D, et al. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. *BMJ* 1995;311:711–714.
13. Kowalski M, Rees W, Konturek PC, et al. Detection of Helicobacter pylori specific DNA in human atheromatous coronary arteries and

its association to prior myocardial infarction and unstable angina. *Dig Liver Dis* 2002;34:398–402.
14. Kinjo K, Kurtobi T, Sasaki T, Kohama A. Prevalence of Helicobacter pylori infection and its link to coronary risk factors in Japanese patients with acute myocardial infarction. *Circ J* 2002;66:805–810.
15. Rajasekhar D, Subramanyam G, Latheef A, et al. Infectious etiology in acute coronary syndromes. *Indian J Med Microbiol* 2002;20:83–87.
16. Tamer GS, Tengiz I, Ercan E, Duman C, Alioglu E, Turk UO. Helicobacter pylori seropositivity in patients with acute coronary syndromes. *Dig Dis Sci* 2009;54:1253–1256.
17. Tamura A, Fujioka T, Nasu M. Relation of *Helicobacter pylori* infection to plasma vitamin B12, folic acid, and homocysteine levels in patienrets who underwent diagnostic coronary arteriography. *Am J Gastroenterol* 2002;97:861–866.
18. Altannavch TS, Roubalova K, Broz J, Hruba D, Andel M. Serological markers of *Chlamydia pneumoniae, Cytomegalovirus* and *Helicobacter pylori* infection in diabetic and nondiabetic patients with unstable angina pectoris. *Cent Eur J Public Health* 2003;11:102–106.
19. Lu YH, Yen HW, Lin TH, et al. Changes of coronary risk factors after eradication of *Helicobacter pylori* infection. *Kaohsiung J Med Sci* 2002; 18:266–272.
20. Frishman WH, Ismail AA. Role of infection in atherosclerosis and coronary artery disease: a new therapeutic target? *Cardiol Rev* 2002;10: 199–210.
21. Graham DY, Adam E, Reddy GT, et al. Seroepidemiology of *Helicobacter pylori* infection in India. Comparison of developing and developed countries. *Dig Dis Sci* 1991;36:1084–1088.
22. Gill HH, Majumdar P, Shankaran K, Desai HG. Age-related prevalence of *H. pylori* antibodies in Indian subjects. *Indian J Gastroenterol* 1994;13:92–94.
23. Jais M, Barua S. Seroprevalence of anti *Helicobacter pylori* IgG/IgA in asymptomatic population from Delhi. *J Commun Dis* 2004;36: 132–135.
24. Sotiropoulos A, Gikas A, Skourtis S, et al. Seropositivity to Chlamydia pneumonia or Helicobacter pylori and coronary artery disease. *Int J Cardiol* 2006;109:420–421.
25. Maia IL, Nicolau JC, Machado MN. Prevalence of *C. pneumoniae* and *M. pneumoniae* in different forms of coronary disease. *Arq Bras Cardiol* 2009;92:405–411.
26. Kalayoglu MV, Libby P, Byrne GI. Chlamydia pneumoniae as an emerging risk factor in cardiovascular disease. *JAMA* 2002;288: 2724–2731.
27. Agarwal A, Chander Y, Nagendra A. Serological evidence of chronic *Chlamydia pneumoniae* infection in coronary artery disease. *MJAFI* 2007;63:229–232.
28. Koh WP, Taylor MB. Seroprevalence of IgG antibodies against *Chlamydia pneumoniae* in Chinese, Malays and Asian Indians in Singapore. *Int J Epidemiol* 2002;31:1001–1007.
29. Kothari A, Ramachandran VG, Gupta P, Singh B. Seroprevalence of *Cytomegalovirus* among voluntary blood donors in Delhi, India. *J Health Popul Nutr* 2002;20:348–351.
30. Galea, Urbaniak SJ. *Cytomegalovirus* studies on blood donors in North-East Scotland and a review of UK data. *Vox Sang* 1993;64: 24–30.

Book review

Mastering rhinoplasty, second edition. Rollin K Daniel. Publisher: Springer-Verlag Berlin and Heidelberg GmbH & Co. KG, Berlin, Denmark. Publication: 2010. Price: €249 (with DVD, Hardcover). Pages: 460. Illustrations: 1408 colour illustrations, 8 colour tables. ISBN: 9783642014017.

This book guides the reader through a standard rhinoplasty operation, advanced techniques, and management of difficult cases. The book describes procedures developed exclusively by the author. The DVDs contains video clips that show the author performing the techniques in question. Every surgeon who carries out rhinoplasty procedures will learn a great deal from this book. The beginner is guided through the performance of a standard rhinoplasty operation that can be expanded to incorporate the described advanced techniques as experience is gained. Here, the emphasis is on the routine case that is too frequently overlooked in favour of the esoteric. For proficient surgeons the latest breakthroughs in the management of difficult cases, such as saddle nose, skin sleeve problems, and dorsal grafting, are clearly depicted. The text is complemented by a wealth of colour figures as well as by DVDs containing integrated video clips that transport the reader into the operating room with the author while he performs live surgery and demonstrates the technique in question. This book is useful for Otorhinolaryngologists, Maxillofacial Surgeons, and Plastic Surgeons.

Contributed by
Maj Rohit Sharma*, Maj DC Chaudhary†
*Graded Specialist (Oral & Maxillofacial Surgery),
HQ IMTRAT, C/o 99 APO, †Graded Specialist (Orthodontics),
11 CDU, C/o 56 APO.

