Original article

Role of CMV & Interferon-γ in Myocardial infarction, Angina and Hypertension

Haider M[1], Rizvi M[2], Malik A[3], Azam M[4], Rabbani MU[5]

Abstract:

Aims: There is limited epidemiologic evidence relating CMV specifically to primary coronary atherosclerosis. Its association with atherosclerosis and restenosis appears to have merit and needs to be studied further. Cardiovascular disease being an inflammatory process leads to detectable rise in inflammatory markers like Interferon-γ. The aim of this study was to evaluate the role of CMV and Interferon-γ in cardiovascular disease. ***Methods***: Study was conducted on 63 randomly selected cardiovascular disease patients and 29 healthy controls. ELISA for detection of IgG antibodies against CMV were detected (Calbiotech Diagnostics, USA). Interferon-γ levels were determined by ELISA (Diaclone, USA). Relevant investigations, clinical history & examination were recorded. ***Results***: Of 63 cases 41 (65.08%) were positive for IgG antibodies against CMV and 8 (27.58%) of 29 controls were IgG positive ($p<0.001$). Among CMV seropositives 19 (46.34%) were MI patients, 13 (31.70%) were hypertensives and 9 (21.95%) were angina patients. The mean value of Interferon-γ for cases was 32.13pg/ml, the mean of controls was 11.32pg/ml ($p<0.0001$). Among CMV IgG seropositives the mean value of Interferon-γ in hypertensives was 12.76pg/ml, in angina patients was 32.48pg/ml and in MI patients was 67.10pg/ml. ***Conclusion***: In our study CMV seropositivity was significantly associated with CVD cases. CMV seropositivity increased with severity of disease. Mean value of Interferon-γ was higher among cases reflecting role of inflammatory aetiology in CVD. Mean value of Interferon-γ increased with severity of disease infection clearly indicating the role of inflammatory markers along with CMV in cardiovascular disease.

Keywords: cardiovascular disease; Cytomegalovirus; Interferon- γ; ELISA

Bangladesh Journal of Medical Science Vol. 14 No. 04 October'15. Page: 339-345
DOI: http://dx.doi.org/10.3329/bjms.v14i4.25764

Introduction

The known risk factors of Cardiovascular Disease (CVD) are not sufficient to explain all the epidemiological variables and fluctuations of the disease. These observations fuel renewed interest in a link between CVD and infectious agents.

Infectious etiology with regard to CVD has been in the limelight for more than 20 years [1]. The hypothesis of infectious aetiology of atherosclerosis is one of the most interesting areas of vascular research, it states that more than one infectious agent could play a role in atherogenesis and atherothrombosis. This entire process may involve pro-inflammatory mechanisms like interleukins and cytokines.

Infectious agents are considered as significant factors in the pathogenesis of atherosclerosis because atherogenic processes resemble chronic inflammation [2] – a process that may be promoted by microorganisms like cytomegalovirus (CMV) [3-5]. CMV is a relatively newer pathogen with regard to atherosclerosis. This association was proposed in the 1980's. The hypothesis of infectious aetiology is very attractive because it would provide us with a powerful platform for prevention and treatment of a disease which represents an escalating public health problem worldwide and especially in India. Even after extensive research the role of infectious agents in CVD still remains an enigma.

1. Mehvash Haider
2. Meher Rizvi
3. Abida Malik
4. Md Azam
5. M.U. Rabbani

Department of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh

Corresponds to: Mehvash Haider, Mailing address: Room 316, Dept of Microbiology, GB Pant Hospital, Delhi, 110001, INDIA, E-mail address: mehvashaider@gmail.com

Cytomegalovirus is a member of Betaherpesviridae in the subfamily of Herpesviridae. Most people are infected with CMV at some point in life although the age of infection varies worldwide. As with other Herpes viruses CMV establishes a latent infection in the host and may reactivate later during a period of immunosuppression. CMV commonly causes intrauterine infection and prenatal damage to foetus, leading to congenital abnormality. In adults and older children it may cause a syndrome resembling EBV mononucleosis. In adults it is usually asymptomatic. Almost two decades ago several investigators suggested a role for herpes viruses in CVD. The Atherosclerosis Risk in Communities Study in 2000 correlated pre-existing high CMV titres and traditional risk factors with carotid atherosclerosis, incident MI or CHD death [6]. Seroepidemiologic, histopathologic, in vitro and animal studies have investigated possible links between human atherosclerosis and human herpes viruses, primarily CMV [7].

Studies have linked Cytomegalovirus to three arterial diseases: Primary atherosclerosis, Post angioplasty restenosis and post transplantation atherosclerosis. An association has been observed between the presence of CMV and restenosis following coronary angioplasty. [8]There is limited epidemiologic evidence relating CMV specifically to primary coronary atherosclerosis. Its association with atherosclerosis and restenosis appears to have merit and needs to be studied further. Cardiovascular disease being an inflammatory process leads to detectable rise in inflammatory markers e.g. Interferon-γ. Over the last decade substantial advances in basic and experimental science have established the role of inflammation in CVD [9], from initiation to progression. Thus inflammation may be considered as a surrogate marker of increased risk of adverse cardiovascular events. The development of interest in inflammatory markers is also due to the fact that reduction in levels of systemic inflammation by medical intervention could lead to significant reduction in adverse cardiovascular events.

The aim of this study was to study the association of Cytomegalovirus with Cardiovascular Disease & Evaluation of inflammatory markers as potential predictors of risk for cardiovascular events.

MATERIAL AND METHODS

***STUDY GROUP*:**

The study was conducted overa period of 11 months. Consecutive cases fitting the selection criteria were includedin the study.

The study group consisted of a cohort of 63 Cardiovascular Disease patients. Cardiovascular Disease includes a constellation of diseases, namely Coronary Artery Disease, Hypertension, Transient Ischemic attack, Stroke, Congenital Heart Disease & Peripheral Vascular Disease.

We studied patients with Primary hypertension and Acute Coronary Syndrome. A detailed clinical history was recorded. A written and informed consent was obtained from all subjects.

Acute Coronary Syndrome is an umbrella term used to cover any group of clinical symptoms compatible with acute myocardial ischemia. Patients with ACS include those whose clinical presentations cover the following range of diagnoses: unstable angina, non–ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI) [10].

Primary Hypertension:

Primary Hypertension was defined as Blood pressure >140/90mm Hg on 2 or more occasions with at least a gap of 8 hours between the 2 occasions.

Angina:

Angina is a subset of Coronary Artery Disease which was defined as progressive reduction in blood flow to myocardium due to build-up of atheromatous plaques. It manifests as stable angina, unstable angina and myocardial infarction.

Unstable angina was defined as one of the following in presence of chest pain

- Chest pain occuring at rest and lasts > 10 minutes
- Chest pain of severe and new onset (within prior 4-6 wks)
- Chest pain occuring with a crescendo pattern (more severe and more frequent)

Stable angina was defined as chest pain occurring on physical activity, begins slowly and gets worse and is relieved on medication or rest.

Myocardial Infarction:

Myocardial infarction was defined as chest pain lasting more than 30 minutes with ST segment elevation/ depression, evolving Q waves, symmetric inversion of T waves & elevation of cardiac markers.

Exclusion Criteria:

The following exclusion criterion was followed:

1. Patients of secondary hypertension (renal causes, endocrine causes, drug induced)
2. Patients with other concomitant infectious disease.
3. Patients with any other septic foci.
4. Patient with *history of antibiotic intake in the last 1 month.*

CONTROL GROUP*:*

Control group consisted of 29 age, sex and other variable matched healthy volunteers. A detailed

clinical history & physical examination of all cases was recorded.

***ETHICAL CLEARANCE*:**

Ethical clearance was obtained from Institutional Ethical Committee Jawaharlal Nehru Medical College, AMU, Aligarh.

***STATISTICAL ANALYSIS*:**

Each parameter as it was measured was compared between cases and controls to see if their mean level was similar. For this a two group Students's t test or Wilcoxon Ranksum test was applied as appropriate.

***SAMPLE COLLECTION AND TRANSPORTATION*:**

5 ml blood was obtained by venepuncture taking all aseptic precautions. Serum was separated from sample by centrifugation and stored at-20ºC.

Enzyme immunoassay was performed for detection of IgG antibodies against CMV (Calbiotech Diagnostics, USA).

Levels of Interferon-γ were estimated by Enzyme immunoassay (Diaclone, USA) following manufacturer's instructions. Standard curve was prepared against which the sample readings were plotted.

Results

3.1 *Demography of the study & control group*:

Of the 63 cardiovascular disease cases, majority of patients 45 (71.43%) were in the age group 41-60 years with maximum clustering 22 (34.92%) in the age group 56-60 years followed by 12 (19.05%) in the age group 46-50 years. The mean age distribution was 52.7±8.9 years in cases and 54.01±8.1 years in controls. In the cases 38 (60.31%) were male and 25 (39.68%) were female. In the control group 18 (62.5%) were male and 11 (37.5%) were female. Out of 63 cases, highest number of patients were of myocardial infarction 28 (44.4%) followed closely by primary hypertension 21 (33.33%) and 14 (22.2%) angina patients.

3.2 *Role of conventional risk factor in CVD*:

On analysing conventional risk factors in the study group a majority, 35 (55.55%) had past history of a cardiac episode, 20 (31.75%) were obese (BMI>30), 19 (30.15%) were smokers, 18 (28.57%) had deranged lipid profile, 12 (19.05%) patients had family history of cardiac problem and only 5 (7.81%) were diabetics. None of the traditional risk factors were significantly associated with cardiovascular disease. Table 1 shows distribution of risk factors in relation to spectrum of CVD.

3.3 *CMV in study group*:

Out of 63 cases 41 (65.08%) were positive for IgG antibodies against CMV and 8 (27.58%) of 29 controls were IgG positive($p<0.001$). Of 41 CMV IgG seropositive, 26 (63.41%) were males and 15 (36.58%) were females. Majority 9 (21.95%) cases were in the age group 46-50 years with scattering of cases 23 (56.09%) in the age group 46-60 years as shown in Table 2. Unexpectedly CVD manifested in a relatively younger age group with the youngest patient being 35 years old. There were 3 & 5 patients in the 36-40 and 41-45 yr age group.

The relatively young population of 30-50 years (43.9%) was associated with CMV followed by 51-55 years and 56-60 years. This shift in age could be attributed to a change in lifestyle compounded by CMV reactivation. Detection of CMV was significantly more ($p<0.001$) with CVD. Sensitivity of this test was 65.08%, specificity was72.41%, positive predictive value 83.67%, negative predictive value 48.84% and Odds ratio was 4.89. On evaluating the role of conventional risk factors in CMV infected individuals 21 (51.2%) had past history of cardiac event, 14 (34.1%) were smokers, 11 (26.8%) were obese, 10 (24.4%) had deranged lipid profile, 7 (17.1%) had family history and only 5(12.2%) had diabetes as shown in Table 3. The conventional risk factors with or without CMV infection were not strongly associated with CVD. After adjusting for traditional risk factors using multiple logistic regression CMV independently emerged as a single significant risk factor in causation of CVD

Cases in each study group were also analysed with respect clinical details such as haemogram, neutrophilia, ESR, renal function test, liver function test, ECG, cardiac enzymes Echo, ultrasound and angiography was performed. However no clear association was observed except for the presence of ECG findings and cardiac enzymes in patients of angina and MI respectively. These clinical variables along with the traditional risk factors were also compared among the CMV seropositive and seronegative populations and it was observed that there was no significant difference in the prevalence of traditional risk factors in either group. On analysing the role of CMV seropositivity in the study groups majority 19 (46.34%) were MI patients, 13 (31.70%) were hypertensives and 9 (21.95%) were angina patients.

Value of Interferon-γ ranged from 5.46-17.05 pg/ml among controls and 8.09-638.84 pg/ml among cases. The mean value of Interferon-γ for cases was much higher at 32.13 than the mean of controls which 11.32pg/ml ($p=0.026$). Among the cases there were two groups; 41 (65.07%) were CMV seropositive and 22 (34.92%) were CMV seronegative. Distribution

of CMV seropositives and seronegatives according to study group is given in Table 4.

On comparing the value of Interferon-γ in the study groups between CMV seropositives and seronegatives the following results were obtained. Among hypertensives CMV seropositives had marginally higher value at 12.76 pg/ml whereas seronegatives had 10.06pg/ml.Among angina patients CMV seropositives had 32.48 pg/ml IFN- γ whereas seronegatives had 9.73 pg/ml IFN- γ. Finally among MI patients CMV seropositives had a much higher level of IFN- γ at 67.10 pg/ml whereas seronegatives

Table1. Distribution of risk factors in the study groups

Study group	Hypertension	Angina	MI
Smoking(n=19)	3(15.79%)	6(31.57%)	10(52.63%)
Diabetes(n=5)	4(80%)	1(20%)	-
Obesity(n=20)	11(55%)	1(5%)	8(40%)
Family history(n=12)	4(19.05%)	1(7.14%)	7(25%)
Past history(n=35)	11(52.38%)	6(42.85%)	18(64.29%)
Dyslipidemia(n=18)	6(33.33%)	4(22.22%)	8(44.44%)

Table 2. Cytomegalovirus IgG Seropositivity according to age

AGE (in years)	Cytomegalovirus IgG Seropositivity	
	CASES n=63	CONTROLS n=29
30-35	1(2.43%)	-
36-40	3(7.31%)	-
41-45	5(12.19%)	1(12.5%)
46-50	9(21.95%)	3(37.5%)
51-55	7(17.07%)	1(12.5%)
56-60	7(17.07%)	2(25%)
61-65	5(12.19%)	-
66-70	4(9.76%)	1(12.5%)
TOTAL	**41(65.08%)**	**8(27.58%)**

Table 3. Distribution of Risk factors in seropositive and seronegatives population.

Risk Factors	CMV IgG		
	sero+ n=41	sero- n=22	
Smoking	14 (34.1%)	5 (22.7%)	
Diabetes	5 (12.2%)	0	
Obesity	11 (26.8%)	9 (40.9%)	
Past History of Cardiac Event	21 (51.2%)	14 (63.6%)	
Family History of Cardiac event	7 (17.1%)	5 (22.7%)	
LIPID PROFILE	10 (24.4%)	8 (36.4%)	

Table 4. Number of CMV seropositive & seronegatives patients in each study group whose IFN- γ was estimated

Study group	CMV seropositive (N=41)	CMV seronegatives (N=22)
Hypertension	12 (29.26%)	6 (27.27%)
Angina	9 (21.90%)	7 (31.81%)
MI	20(48.78%)	9 (40.90%)

Table 5. Comparision of value of Interferon-γ in the study groups between CMV seropositives ans seronegatives.

Value of Interferon - γ in the study group (pg/ml)	CMV seropositive	CMV seronegatives	P value
Hypertension	12.76	10.06	0.5
Angina	32.48	9.73	0.023
MI	67.10	11.81	0.047

had 11.81pg/ml (Table 5).

Discussion & conclusion

In today's world where most deaths are attributed to non–communicable chronic diseases, more than half of these are as a result of cardiovascular disease (CVD). Deaths from CVD are often premature and millions of non-fatal events result in disability. The known risk factors of cardiovascular disease are not sufficient to explain the current epidemiology of the disease. These facts renew interest in a hypothesis linking CVD and infectious agents. The increasing association of CVD with the younger age group in India needs to be explored. It could be related to the drastic change in lifestyle of the younger generation with the incumbent ever escalating stress. The conventional risk factors need a relatively long lag phase before symptoms of CVD manifest & thus the usual age of developing CVD is usually after the fifth decade. We explored whether there were other factors riding in tandem with the lifestyle changes which may lead to shifting of the age group of developing CVD to a younger population.

Out of 63 cases majority of cases 18 (28.57%) were in the age group 30-50 years followed by 14 (22.22%) in the age group 51-60 years. In Western countries where CVD is considered a disease of the aged, 23 per cent of CVD deaths occur near the age of 70; this compares with 52 per cent of CVD deaths occurring among people under 70 years of age in India [11,12]. As a result, the Indian subcontinent suffers from a tremendous loss of productive working years due to CVD deaths: an estimated 9.2 million productive years of life were lost in India in 2000, with an expected increase to 17.9 million years in 2030 (almost ten times the projected loss of productive life in the United States) [13].

Strangely the traditional risk factors were not significant contributors to CVD in the present study while CMV emerged as a significant risk factor for CVD. This startling observation strengthens the role of CMV alone and more so when it activates proinflammatory cytokines like Interferon- γ in pathogenesis of cardiovascular disease over and above the traditional risk factors.

MANOVA test was used to study CMV seropositivity, Interferon-gamma and traditional risk factors as independent variables and CVD outcome as dependent variable. CMV seropositivity and Interferon-gamma In our study CMV was not only significantly associated with CVD cases ($p<0.001$) but CMV seropositivity increased with severity of disease (from hypertension to MI) underlining its role in progression of the disease. The results of a study [14] demonstrated a similar prevalence of CMV seropositivity in patients with coronary artery disease and in an age and sex matched control group.

Earlier studies have mostly reported association of CMV infection with coronary atherosclerosis in transplanted hearts, following coronary angioplasty, or in carotid arteries [17,18]. In a previous investigation from the Framingham Heart Study, no association between clinically apparent herpes virus infection and coronary heart disease was documented [19]. Recent prospective studies have also failed to demonstrate an association of CMV infection with risk of cardiovascular disease [20-23]. Our study contrasts with these observations from population-based studies, and it indicates a strong association between CMV infection and risk of cardiovascular disease. This could be due to cryptic factors in the Indian population possibly genetic or racial or even related to increasingly stressful lifestyles.

In another study among control patients matched to surgically treated cases of atherosclerosis, high CMV antibody titres were more common in those who subsequently developed coronary events than in the remainder(45% v 26%), but this comparison was not adjusted for potential confounding variables[24].

Detection of CMV IgG in hypertensives in our study had a good Sn of 61.90%, a high Specificity of 72.41%, PPV 61.90%, NPV 72.41% and OR of 4.27. In Angina patients Sensitivity was 64.68%, Sp was 72.41%, PPV 52.94%, NPV 80.77%, OR was 4.73, whereas in MI patients Sensitivity was 67.86%, Sp was 72.41%, PPV 70% and OR was 5.54. The OR is extremely high in all the three subgroups,

highest being in MI and lowest in hypertension. The increasing sensitivity of CMV with worsening clinical spectrum points to it's role in initiation of the disease (presence of CMV in hypertensives) and in progression of the disease as is witnessed by incremental increase in sensitivity in angina patients followed by MI patients. Such a comparision between these study groups has not been made before. This comparision shows better sensitivity and Odds ratio for MI patients.

Of 41 CMV seropositive cases traditional risk factors did not play a significant role: 13 (31.70%) were smokers, 11 (30.64%) had past history, 8 (19.51%) each were obese and had deranged lipid profile, 6 (14.63%) had family history and 4(9.76%) had diabetes. None of the traditional risk factors came out significantly with CMV seropositivity or CMV seronegativity in cardiovascular disease patients. This implies there are as yet other unexplored causes leading to CVD.

Mean value of Interferon-γ was higher among cases as compared to controls, reflecting the role of inflammatory aetiology in CVD[14,15]. Significantly large number of cases with elevated levels of Interferon- γ were CMV seropositive (65.07%). Also on comparing the number of patients in each study group, we observed that more of the seropositive patients developed myocardial infarction (48.78% were seropositive and 40.90% were seronegative). An interesting trend of increasing value of Interferon gamma with the severity and chronicity of cardiovascular disease is clearly visible in our study group both in seropositives as well as seronegatives. Patients of Myocardial infarction had the highest absolute value of Interferon- γ. This may suggest an increasing role of inflammation and inflammatory markers not only in the initiation but also in the progression of cardiovascular diseases. On comparing the absolute value of Interferon- γ in the individual study groups CMV seropositives had higher values than seronegatives. This observation again strengthens the hypothesis of infection and inflammation having a combined role in cardiovascular disease.The highest mean Interferon-γ levels were seen in MI patients with CMV infection clearly indicating an active role of CMV along with inflammatory markers in the pathogenesis of cardiovascular disease.

CMV infection itself has atherogenic potential [17] and being intracellular pathogen also had the ability to induce release of Interferon- γ [25] CMV being a chronic infection fluctutating between the poles of periods of latency and reactivation may lead to release of proinflammatory cytokines including IFN-γ which may accelerate atherogenesis ultimately causing plaque rupture.

In conclusion the inflammatory response to CMV should be studied in detail. It can be hypothesized that the adverse clinical outcome is not solely due to infection per se but also due to the inflammatory response to infection.

CMV, an omnipresent virus which infects practically every individual in childhood may be getting reactivated in a subset of individuals who are under stress due to modern work culture and stressful lifestyles. Herpes simplex 1 viruses are known to get reactivated under stress, CMV may also be getting reactivated under similar conditions. CMV on reactivation may be associated with hyperplasia and atherogenesis of the coronary arteries, the concomitant raised Interferon-γ leading to rupture of the plaque leading to MI.

In our study angina was associated with raised CMV titres and moderately elevated levels of Interferon-γ while MI was associated with raised CMV titres and highly elevated titres of Interferon-γ while least Interferon-γ titres were observed in hypertension which explains the preeminent role played by Interferon-γ in concert with CMV in the progression of cardiovascular disease. However this is just a baseline study and larger prospective studies need to be undertaken before any concrete conclusions can be made.

References:

1. Ngeh J. Chlamydia Pneumoniae in Elderly Patients with Stroke Study (CPEPS): A case-control study on the seroprevalence of Chlamydia pneumoniae in patients aged over 65 years admitted with acute stroke or transient ischaemic attack. MSc Dissertation, University of Keele, UK, 2000.
2. Ross R. Atherosclerosis- an inflammatory disease. *N Engl J Med* 1999;**340**:115-26. http://dx.doi.org/10.1056/NEJM199901143400207
3. Gayelos CA, Quinn TC. The role of Chlamydia pneumoniae in cardiovascular disease. *Adv Intern Med* 2000;**45**:139-73.
4. Muhlestein JB. Chronic infection and coronary artery disease. *Med Clin. North Am* 2000;**84**:123-48. http://dx.doi.org/10.1016/S0025-7125(05)70210-8
5. Kol A, Libby P. Molecular mediators of arterial inflammation: A role for microbial products? *Am Heart J* 1999;**138**:450-52. http://dx.doi.org/10.1016/S0002-8703(99)70273-5
6. Sorlie PD, Nieto FJ et al. A prospective study of Cytomegalovirus , herpes simplex virus type 1, and coronary heart disease. *Arch Intern Med* 2000;**160**:2027-32 http://dx.doi.org/10.1001/archinte.160.13.2027
7. Mattila KJ, Valtonen VV, Nieminen MS, Asikainen S. Role of Infection as a risk factor for atherosclerosis, myocardial infarction and stroke. *Clin Infect Dis* 1998;**26**: 719-34. http://dx.doi.org/10.1086/514570
8. Ebstein SE, Zhou YF, Zhu JH. Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation* 1999;**100**: 20-8. http://dx.doi.org/10.1161/01.CIR.100.4.e20
9. Libby P, Ridker PM et al. Inflammation and Atherosclerosis. *Circulation* 2002;**105**:1135-43. http://dx.doi.org/10.1161/hc0902.104353
10. Kristian A. Thygesen, and Joseph S. Alpert. The Definitions of Acute Coronary Syndrome, Myocardial Infarction, and Unstable Angina. *Current Cardiology Reports* 2001;**3**:268–272
11. Gupta R. Burden of coronary heart disease in India. *Indian Heart J* 2005;**57**: 632-8.
12. Ghaffar A, Reddy KS, Singhi M. Burden of noncommunicable diseases in South Asia. *BMJ* 2004;**328** : 807-10 http://dx.doi.org/10.1136/bmj.328.7443.807
13. Leeder SR, Raymond SU, Greenberg H, Lui H, Esson K. A race against time: the challenge of cardiovascular disease in developing economies. The Center for Global Health and Economic Development. New York: Columbia University; 2004
14. Grayston JT, Golubjatnikov R, Hagiwara T et al. Serologic tests for Chlamydia pneumoniae. *Pediatr Infect Dis J* 1 993;**12**:790- 1 .
15. Schroecksnadel K, Frick B, Winkler C, Fuchs D. Crucial role of interferon-gamma and stimulated macrophages in cardiovascular disease. *Curr Vasc Pharmacol* 2006 Jul;**4**(3):205-13. http://dx.doi.org/10.2174/157016106777698379
16. Vaddi K, F.A. Nicolini, P. Mehta, J.L. Mehta, Increased Secretion of Tumor Necrosis Factor-a and Interferon-y by Mononuclear Leukocytes in Patients With Ischemic Heart Disease Relevance in Superoxide Anion Generation. *Circulation* 1994;**90**:694-699)
17. Danesh J, Collins R, Peto R. Chronic Infection and coronary heart disease: is there a link ? *Lancet* 1997;**350**: 430-6. http://dx.doi.org/10.1016/S0140-6736(97)03079-1
18. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation* 1997;**96**:4095–103. http://dx.doi.org/10.1161/01.CIR.96.11.4095
19. Havlik RJ, Blackwelder WC, Kaslow R, Castelli W. Unlikely association between clinically apparent herpesvirus infection and coronary incidence at older ages. The Framingham Heart Study. *Arteriosclerosis* 1989;**9**:877–80. http://dx.doi.org/10.1161/01.ATV.9.6.877
20. Danesh J, Wong Y, Ward M, Muir J. Chronic infection with Helicobacter pylori, Chlamydia pneumoniae, or cytomegalovirus: population-based study of coronary heart disease. *Heart* 1999;**81**:245–7. http://dx.doi.org/10.1136/hrt.81.3.245
21. Ossewaarde JM, Feskens EJ, De Vries A, et al. Chlamydia pneumoniae is a risk factor for coronary heart disease in symptom-free elderly men,but Helicobacter pylori and cytomegalovirus are not. *Epidemiol Infect* 1998;**120**:93–9. http://dx.doi.org/10.1017/S0950268897008303
22. Strachan DP, Carrington D, Mendall MA, Ballam L, Morris J, Butland BK, et al. Relation of Chlamydia pneumoniae serology to mortality and incidence of ischaemic heart disease over 13 years in the Caerphilly prospective heart disease study. *BMJ* 1999;**318**:103540.
23. Ridker PM. Inflammation, infection and cardiovascular risk: How good is the clinical evidence? *Circulation* 1998;**97**: 1671-4. http://dx.doi.org/10.1161/01.CIR.97.17.1671
24. HemChandra Jha, Pragya Srivastava,Rakesh Sarkar,Jagdish Prasad, and Aruna singh Mittal. Association of Plasma Circulatory Markers, Chlamydia pneumoniae, and high Sensitive C-Reactive Protein in Coronary Artery Disease Patients of India. 2009.Hindawi Publishing Corporation Mediators of Inflammation Volume 9.
25. Hausen A, Fuchs D, Reibnegger G et al. Neopterin in clinical use. *Pteridines* 1989;**1**: 3-10. http://dx.doi.org/10.1515/pteridines.1989.1.1.3