Genetic Instabilities in H. pylori infected Cardiovascular Diseases

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Abstract - The association between Helicobacter pylori infection of the stomach and ischemic heart disease has been documented by many studies. The present study is focused to evaluate the role of H. pylori infection and genetic instability of the host genome and development of cardiovascular disease (CVD). Four groups each with 50 subjects were included and consists of H. pylori infected patients with CVD, H. pylori infected patients without CVD and also CVD patients without H. pylori infection. The results were compared with 50 normal, healthy age and sex matched controls. Total and LDL cholesterol values, blood sugar, Aspartate aminotransferase, and high sensitive CRP were statistically higher in H. pylori subjects than the controls. The mean break per cell value was found to have significant correlation with the above parameters and was higher in H. pylori infected patients with CVD. The lipid profiles of H. pylori patients with and without CVD were statically different from that of the controls. The study suggests that impaired DNA repair also is a hallmark of H. pylori infection in-vivo. Hence it was concluded that H. pylori contribute to the pathogenesis and progression of CVD and also the DNA repair efficiency plays a predictive role in the H. pylori seropositive subjects. The treatment for H. pylori should be initiated in all patients in order to prevent the progression to CVD.

Index Terms— Cardiovascular diseases, C-reactive Protein, DNA repair, Helicobacter pylori, Inflammation, Lipid profile

1 INTRODUCTION

Helicobacter pylori is one of various spiral, gram negative and microaerophilic bacteria which produces colonies in human and primates stomach and results in infectious and inflammatory diseases. The association between Helicobacter pylori infection of the stomach and ischemic heart disease has been documented by many studies [1, 2]. However, the position remains uncertain, an assessment supported by a recent review [3]. Since the risk of acquiring H. pylori infection in childhood increases with socioeconomic deprivation and overcrowding, the association may be indirect, H. pylori infection and ischemic heart disease are both related to social class. Some studies have shown little or no excess risk [4, 5] but others reported a four-fold to five-fold increased risk without adjustment for measures of social class and other risk factors for ischemic heart diseases [6, 7] or a two-fold to three-fold increased risk after such adjustment [7].

A high percentage of patients have none of traditional risk factors such as hypertension, smoking, obesity, hypercholesterolemia or genetic predisposition [8]. Accordingly, medical researchers are beginning to find other risk factors for coronary atherosclerosis. Clinical and experimental studies indicate that inflammatory conditions have a role in atherosclerosis [9]. Many epidemiological investigations have shown a significant relationship between coronary ischemia and various infectious agents such as bacterial and viral agents [10].

The role of inflammation in the pathogenesis and progression of coronary artery disease (CAD) has been increasingly discussed, but still remains unclear. Inflammatory changes in the vessel wall play an important role in the pathogenesis of atherosclerosis. Systemic inflammatory reaction can be detected by showing increased plasma levels of different proinflammatory cytokines and acute-phase proteins. Infectious agents have been linked to coronary heart disease on epidemiological and pathogenetic grounds. The prevalent condition and the exact mechanism of initiation of atherosclerotic vascular disease remain unclear.

However, many similarities exist between the processes of inflammation and atherogenesis. The evidence is growing for the role of an active inflammation in the atherosclerosis in the coronary circulation and elsewhere. In particular, monocytes and macrophages have long been recognized as components of atheromatous plaques. Elevated levels of the acute phase proteins, fibrinogen and C-reactive protein (CRP) and pro-inflammatory cytokines are known to be associated with an increased risk of cardiovascular events [11]. The possibility that an undetected chronic infection may be behind these changes in inflammatory markers is an attractive hypothesis. This has led to the spotlight falling on microorganisms, which is known to be commonly detectable in asymptomatic individuals. The direct proof of bacteria in arteries of affected organs seems to be more convincing.

Helicobacter eradication in patients [12] suggests

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that impaired DNA repair also is a hallmark of H. pylori infection in vivo. H. pylori directly damages DNA and triggers a DNA-damage response (DDR) in infected cells. Double stranded breaks (DSBs) accumulate in various cell lines and in primary gastric epithelial cells upon infection with H. pylori in a time and dose dependent manner. The fragmentation of host nuclear DNA requires direct contact of live bacteria with their host cells, is independent of the H. pylori virulence determinants vacuolating cytotoxin A (VacA) and the Cag PAI, and does not require ROS (Reactive oxygen species)-mediated DNA damage. Efficient repair of H. pylori-induced DSBs is apparent upon antibiotic killing of the bacteria, but prolonged infections lead to residual unrepaired breaks and negatively affect cell viability [13]. In conclusion, H. pylori have the unique ability to induce host cellular DNA damage directly, thus providing a mechanistic explanation for the development of coronary artery disease.

However, the predictive role of H. pylori infection in CVD is still a matter of debate. It has been found that patients with low serum triglycerides and H. pylori seropositivity might generate CVD both in general and particularly in men [14]. A causal association between H. pylori infection and CVD would be of major health importance, because the infection can be evaluated and eradicated [15] which showed prevention of cardiometabolic risk [16]. Therefore, it becomes essential to evaluate potential cross-sectional and prospective associations between H. pylori seropositivity and CVD risk factors.

Several epidemiological and clinical reports have suggested that seropositivity for H. pylori infection may be a risk factor for DNA repair mechanism. However, there has been no prospective study of this association involving H. pylori infection in DNA damage and CVD. Hence the present study is undertaken to evaluate CVD risk factors and their DNA repair efficiency in H. pylori infected subjects by investigating the physiological, biochemical and cytogenetic alterations.

MATERIALS AND METHODS

The study was carried out in 200 subjects who were divided into four groups namely those with or without H. pylori infection and also with or without CVD (50 each). Those without H. pylori infection and cardiovascular disease formed the fourth group as controls. All these subjects were recruited from General Hospital, Trivandrum with informed consent. Demographic information, medical history, including doctor-diagnosed cardiovascular disease was collected. The samples were analyzed for the presence of H. pylori IgM, IgG, IgA antibodies by ELISA. Blood sugar, lipid profile, hsCRP and ASO were measured by enzymatic method using Siemens Dade Dimension automatic analyzer. The mean break per cell value of all subjects was analyzed by bleomycin induced chromosome sensitivity analysis. The results were statistically analyzed using SPSS 9 version

For mutagen sensitivity analysis, set up the lymphocyte cultures using RPMI 1640 as the medium supplemented with 15% foetal bovine serum, 10µg/ml phytohaemagglutinin. 0.03 units/ml of bleomycin treatment was given 6 hrs before harvesting to induce chromosome breakage. At the end of 70^{th} hour, the culture was treated by colchicine (0.04µg/ml) to arrest the cell division at metaphase. Then the culture was incubated for 72 hours at 37^0C. For mutagen sensitivity, the slides were stained with Giemsa and look for chromosomal lesions such as breaks, gaps, accentric fragments, ring chromosome etc, were also scored. The frequency of chromatid breaks were considered as a measure of an individual's DNA repair capacity. For chromosome sensitivity analysis the mean number of break per cell (b/c) was calculated. The frequencies of breaks were expressed as b/c for comparison. Any individual expression <0.8 was considered hyposensitive, between 0.8 and 1.0 as sensitive and those >1.0 was considered hypersensitive. A minimum of 100 metaphases per culture was scored and data were analyzed.

RESULTS

The present study was undertaken to evaluate the role of H. pylori infection, if any, and genetic instability of the host genome and development of cardiovascular disease (CVD). An attempt was also made to correlate these findings with various immunological, biochemical and genetic characteristics of CVD in H. pylori infected subjects. The role of environmental factors and lifestyle associated risk factors in predisposing H. pylori positive and negative patients to CVD was also correlated. 200 subjects were included in the present study. They were divided into 4 groups.

Group 1A: H. pylori Positive with CVD

Group 1B: H. pylori Positive without CVD

Group 2A: H. pylori Negative with CVD

Group 2B: H. pylori Negative without CVD (Normal Controls)

Anthropometric, socioeconomic and physiological data of all subjects were collected. Blood samples were collected and analyzed for the presence of H. pylori IgM, IgG, IgA antibodies as well as blood sugar, lipid profile, hsCRP and ASO were also estimated. The mean break per cell value of all subjects was analyzed.

The distribution of the subjects according to age is given in Table 1. The age of the subjects ranged from 20 to 50 years with a mean age of 43.41 and the age of the control subjects ranged from 18 to 50 years with a mean age of 38.7. Among the subjects 62.5% (N= 125) comes under the age group of 41-50 yrs and 9.5% (N=19) were in the age group of 20-30. The remaining 28% (N=56) comes under the age group of 31-40 yrs. The distribution of subjects according to sex was also studied. In Group 1A there were 48% (N=24) males and 52% (N=26) females whereas in Group 1B there were 44% (N=22) males and 56% (N=28) females. In Group 2A and Group 2B the number of males were 60% (N=30) and females were 40% (N=20). The groups were compared according to sex.

H. pylori IgG, IgA and IgM antibodies were analysed using ELISA techniques. It was observed that 55 were positive to igG antibodies and 60 were positive to IgA antibodies. IgG and/or IgA antibodies were detected in 100 subjects. None of the subjects were positive to IgM antibodies.

The Serum lipid profile were determined in all the subjects by estimating the levels of triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol in the serum. The lipid profile value of H. pylori positive subjects were compared with that of H. pylori negative subjects. The mean values and the comparative evaluation are given in table 2. There was no significant difference in triglyceride values between H. pylori positive and negative subjects and significant differences was observed ($p < 0.001$) in total and LDL cholesterol values between these groups. The difference in the HDL cholesterol values are moderately significant ($p<0.05$).

The mean serum lipid profile value of all the 4 groups of subjects and the comparative evaluation of this value are given in table 2. The Post Hoc test- Multiple Comparisons is given. In the case of lipid profile, significant alteration was observed only in total cholesterol and LDL cholesterol. Triglyceride and HDL cholesterol did not show any alterations. The Post Hoc test revealed that the difference in total cholesterol was significant ($p < 0.001$) on comparison between all groups except between Group 1A and Group 1B. In the case of LDL cholesterol the difference were significant ($p < 0.001$) except between Group 2A and Group 2B. In the case of HDL cholesterol significant difference was observed only between Group 1A and Group 2A ($p < 0.001$).

The comparison of Mean value of random blood sugar, AST, hsCRP and break/cell value are given in table 3. All the values show significant alteration and comparison between groups. Post hoc test reveals that blood sugar values showed significant difference on comparison between any group except between Group 1A and Group 1B. Similarly AST also showed significant difference on comparison between any groups except Group 1A and Group 1B and Group 2A and Group 2B. High sensitive CRP showed significant difference only on comparison between group 1A and Group 1B. The difference in break/cell value also showed same pattern that of AST i.e. significant difference is shown on comparison between any groups except Group1A and group 1B and Group 2A and Group 2B.

The Total cholesterol and LDL cholesterol were found to have significant correlation with the mean break/cell value of the subjects ($p< 0.001$), but triglyceride had no significant correlation while HDL cholesterol was moderately correlating with mean break/cell value.

DISCUSSION

Saad et al [17] found that H. pylori seropositive subjects have higher serum triglyceride, total cholesterol and LDL-C concentrations when compared to healthy subjects. Earlier studies suggested that there is a significant association between H. pylori infection and cardiovascular diseases, this association due to the effect of the infection on lipid metabolism [18]. In the present study, the total cholesterol and LDL cholesterol were found to have significant correlation with the mean break/cell value of the subjects ($p< 0.001$), but triglyceride had no significant correlation.

Yudkin et al [19] showed that there is a significant decrease in HDL-C level in patients with gastritis and cardiovascular diseases. Furthermore, in Laurila study on patients with H. pylori a significant decrease in HDL-C level was reported. Studies of Scragg et al [20] and Kowalski [21], on the relationship between H. pylori and cardiovascular diseases showed that LDL-C concentration is increased by H. pylori infection. The present study observed only a moderate correlation between HDL and mean b/c value.

CRP is an easily measurable substance in blood. Increased levels of this protein indicate acute inflammation. Many studies have shown that high levels of CRP also indicate an increased risk of suffering from a heart attack or stroke. Ishida et al [22] demonstrated that H. pylori infection

may slightly elevate serum CRP, thereby may increase systemic disease risk. Another study reported that increasing age, smoking, symptoms of chronic bronchitis, H. pylori and Chlamydia pneumonia infections, and BMI were all associated with raised concentrations of CRP [23]. Wellen et al [24] reported that HP infection stimulates inflammatory responses leading to insulin resistance and persistent hyperglycemia by producing proinflammatory cytokines such as C-reactive protein and interleukin-6. The present study observed a statistically significant difference in high sensitive CRP among H. pylori subjects with and without CVD. It was reported that H. pylori induces a suboptimal activation of neutrophils that could potentially suppress the mounted innate immune response and help to establish a chronic inflammatory environment in the periphery, necessary for CAD development [25]. Mendall et al [23] demonstrated a strong association between H. pylori infection, increasing age and socio-economic deprivation. In the present study, AST, hsCRP as well as RBS showed significant correlation with mean break/cell value.

Elevated fibrinogen levels were independently associated with H. pylori infection compared with those free from infection [26]. By contrast, some found no association [27] between H. pylori and fibrinogen. Most of the previous studies measured only H. pylori IgG antibodies [28] while some measured both IgG and IgA antibodies to detect H. pylori infection [29]. Rupprecht et al [30], found no relationship between H. pylori IgG seropositivity and risk of fatal cardiovascular events, IgA seropositivity was significantly associated with fatal cardiovascular events, with a hazard ratio of 2.5. The present study measured IgG, IgA and IgM antibodies for the detection of H. pylori infection.

The present study found a positive correlation between DNA repair and degree of cardiovascular disease, meaning that DNA repair becomes defective with increased intensity of H. pylori infection. Hence the study can be concluded based on the significant association found between H. pylori infection and serum hsCRP levels, fibrinogen, blood sugar, lipid profiles supporting that H. pylori infection may increase the risk of cardiovascular disease. The predictive role of DNA repair proficiency is quite debatable.

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Table 1: Distribution of subjects according to age

Age in yrs	Category								Total	
	Group 1 A		Group 1 B		Group 2 A		Group 2 B			
	N	%	N	%	N	%	N	%	N	%
20-30	4	8.0	3	6.0	5	10.0	7	14.0	19	9.5
31-40	13	26.0	14	28.0	15	30.0	14	28.0	56	28
41-50	33	66.0	33	66.0	30	60.0	29	58.0	125	62.5
Total	50	100	50	100	50	100	50	100	200	100

Table no: 2 Distribution of lipid profile in H. pylori infected subjects

Lipids mg/dL	*H. pylori*				t	p
	Positive (N=100)		Negative (N=100)			
	Mean	SD	Mean	SD		
Triglyceride	128.6	63.5	116.6	39.4	1.599	0.112
Total C	236.1	55	167.3	32.3	10.791	<0.001
HDL- C	55.5	13.8	51.6	13.7	1.998	<0.05
LDL- C	152.7	47.4	87	18	12.938	<0.001

Table 3: Comparison of Mean Random blood Sugar (RBS), AST, hsCRP and Mean b/c Value in study and control subjects

Test		N	Mean	SD	Minimum	Maximum	F	p
RBS (mg/dL)	Group 1 A	50	127.24	48.17	60	268	7.853	<0.001
	Group 1 B	50	134.14	70.91	68	497		
	Group 2 A	50	101.18	13.7	67	128		
	Group 2 B	50	100.72	13.22	67	120		
	Total	200	115.82	46.12	60	497		
AST (IU/L)	Group 1 A	50	46.9	19.917	21	106	18.977	<0.001
	Group 1 B	50	45.24	22.915	19	134		
	Group 2 A	50	31.24	8.348	14	59		
	Group 2 B	50	26.02	11.388	11	48		
	Total	200	37.35	18.875	11	134		
hsCRP (mg/dL)	Group 1 A	50	4.2	4.8	1.01	26.21	28.217	<0.001
	Group 1 B	50	0.57	0.25	0.1	1.36		
	Group 2 A	50	0.91	0.4	0.09	2.04		
	Group 2 B	50	0.33	0.19	0.02	0.62		

	Total	200	1.5	2.87	0.02	26.21		
Mean b/c value	Group 1 A	50	0.815	0.06	0.691	0.927	49.384	<0.001
	Group 1 B	50	0.78	0.056	0.665	0.912		
	Group 2 A	50	0.71	0.045	0.602	0.804		
	Group 2 B	50	0.705	0.066	0.464	0.802		
	Total	200	0.75	0.075	0.464	0.927		