BIOCHEMICAL AND IMMUNOLOGICAL STUDIES IN CARDIOVASCULAR DISEASE PATIENTS WITH HELICOBACTER PYLORI INFECTION

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Abstract

This study was conducted to evaluate the biochemical and the immunological changes in Cardio Vascular Disease (CVD) patients with H. Pylori infection. Fifty H. pylori patients with CVD and fifty H.pylori patients without CVD were included in the study. For comparison of the result fifty normal, healthy age and sex matched controls were also included. The blood pressure, BMI and waist circumference of H. Pylori patients were higher than that of the controls. There was no statistically difference between the test subjects. The renal function of the test and control subjects were with in the normal limit whereas Aspartate amino transferase, blood sugar and high sensitive CRP were statistically higher in H.pylori subjects than the controls. The increase in the above parameters was higher in patients with CVD. The lipid profiles of H.pylori patients with and without CVD were statically different from that of the controls. It was concluded that H.pylori contribute to the pathogenesis and progression of CVD and hence the treatment for H.pylori should be initiated in all patients who are positive to H.pylori so that the progression to CVD can be prevented.

Abbreviations used:

H. Pylori – Helicobacter pylori; CVD- Cardiovascular disease; AST- Aspartate amino transferase, hsCRP- High sensitive C- reactive protein. HDL-C- High Density Lipoprotein Cholesterol; LDL-C- Low density lipoprotein cholesterol.

Introduction

The principal cause of death in the world is cardiovascular diseases (CVD) the majority of which are coronary heart disease or cerebrovascular disease with a pathogenic mechanism of atherothrombosis [1]. In recent years, a theory has been proposed as the inductor mechanism for atherothrombosis which states that inflammatory and immunological processes triggered by viral and/or bacterial infections are the underlying cause of the atherosclerotic process. Atherosclerosis is a very complex disease entity. The lesions of atherosclerosis take different forms, depending upon their anatomic site (coronary artery disease, cerebral arteries, and lower extremity arteries); the age, genetic and physiological status of the affected individual and presumably upon the risk factors to which each individual is exposed. It remains the major cause of premature death in Europe, even though CVD mortality has fallen considerably over recent decades in many European countries. It is estimated that 80% of all CVD mortality now occurs in developing countries [2].

The prevalent condition and the exact mechanism of initiation of atherosclerotic vascular disease remain unclear. Nevertheless, many similarities exist between the processes of inflammation and atherogenesis, and the evidence is growing for the role of an active inflammatory process in the pathogenesis of 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 [3]. The possibility

that an undetected chronic infection may be behind these changes in inflammatory markers is an attractive hypothesis, and has led to the spotlight falling on microorganisms, which is known to be commonly detectable in asymptomatic individuals. Despite of declining mortality from cardiovascular disease, these disorders still remain the leading cause of death in developed as well as developing countries. Understanding the pathophysiology of atherosclerosis is useful in treating its consequences [4,5].

Seroepidemiologic studies have demonstrated that atherosclerosis is associated with several infectious pathogens, including cytomegalovirus [6], H. pylori [7], and C. pneumonia [8] and both H. pylori infection and CVD are associated with the socioeconomic status of patients, and both increase with age [9]. Finding a causal association between the two diseases would be very important in that H.pylori can be screened for and it is amenable to treatment. The role of inflammation mechanisms in the pathogenesis and progression of CVD has been increasingly discussed, but still remains unclear. Epidemiological studies have suggested an association between atherosclerosis and chronic Helicobacter pylori (H. pylori) infection. The association of H. pylori to atherosclerosis, particularly to CVD, is based on serological findings [10].

Heterogeneous inflammatory infiltrates of T-lymphocytes and activated macrophages which could possibly be attracted by infectious agents are commonly encountered within atherosclerotic lesions and intimal fibrocellular proliferations. These histopathologic findings suggest the role of infectious agents and inflammatory processes in the pathogenesis of atherosclerosis [11]. The gram-negative bacillus Helicobacter pylori, which colonize the gastrointestinal tract of more than 50% of the adult population,

have been associated with chronic gastritis, peptic ulcer, and gastric cancer. Recently, several studies have described the association of H. pylori with extragastric diseases [12]. The putative role of H. pylori in cardiovascular system diseases has garnered much attention. In the carotid arteries, the serologic evidence of an H. pylori–atherosclerosis association has preceded the confirmation of H. pylori DNA in the plaques themselves [13,14,15].

Several epidemiological and clinical reports have suggested that seropositivity for Helicobacter pylori may be a risk factor for cardiovascular disease. However, there has been no prospective study of this association involving H.pylori infection and CVD. Hence the present study is undertaken to investigate a possible link between bacterial infection and development of cardiovascular disease and correlate with various physical, biochemical and immunological characteristics of CVD in H. pylori infected subjects by evaluating the various immunological abnormalities associated with H. pylori infection and CVD.

Materials and Methods

The study was carried out in 100 subjects with H.pylori infection who were divided into two groups namely those with or without CVD (50 each). All these subjects were recruited from General Hospital, Trivandrum with informed consent. Demographic information, medical history, including doctor-diagnosed coronary heart disease was collected. Fifty healthy age and sex matched controls from among the siblings or from the staff formed the control group. The height and weight of each patient were measured and

the body mass index (BMI) was calculated as kg/m^2. The blood samples were collected from all the subjects after 12 hours fasting. The blood glucose level, total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) and so on were measured by enzymatic method using Siemens Dade Dimension automatic analyzer. High sensitive C-reactive protein (hsCRP) was measured by Turbidimetric Immunoassay. H pylori antibody status was also tested by ELISA. The results were statistically analyzed using SPSS 9 version.

Results

The demographic data and blood pressure of all the subjects are given in table 1. The age of the H.pylori positive subjects with CVD ranged from 29 to 50 with a mean age of 44.2±6.2. The age of the H.pylori positive subjects without CVD ranged from 21 to 50 with a mean age of 44.6±5.0. The age of the control subjects ranged from 21 to 50 with a mean age of 41.2±8.2. The body mass index and the waist circumference of the subjects were recorded in Table 1. A statistically significant increase in BMI and waist circumference was observed among subjects with H pylori infection than the control subjects. Statistically significant difference in both systolic and diastolic blood pressure was observed in the test subjects compared to their normal counter parts between the study subjects and the control subjects. H.pylori infected subjects with or without CVD showed a mean Systolic pressure of 140.2±18.9 and 137.3±18.4 respectively than the control subjects (126.0±5.7) (p=<0.001). Similarly, H.pylori infected subjects with or without CVD showed a mean diastolic pressure of 77.8±11.9 and 75.4±15.4 respectively than the control subjects (68.0±8.8) (p=<0.001).

Biochemical analysis, reveled a statistically elevated blood sugar, AST, and hs CRP in the test subjects compared to the normal controls. The elevation being more in the test subjects with CVD rather than in the test subjects without CVD. The renal function assed by blood urea, serum creatinine and uric acid showed no difference between the test and control subjects. The elevation in AST, a cardiac marker, shows the involvement of the heart in H.pylori infection. (Table 2).

Dyslipedimia was observed among H.pylori infected subjects with or with CVD infection. Lipid profile estimation showed a statistically significant difference between the study subjects with H.pylori infection and the control subjects (Table 3). H.pylori infected subjects with or without CVD showed an elevated level of total cholesterol, LDL cholesterol and Triglyceride. The elevation in these parameters was slightly higher in the test subject with CVD than without CVD. H.pylori infected subjects with CVD showed a significant decrease in HDL cholesterol than that of the controls but the decrease in HDL cholesterol in H.pylori patient without CVD was lower but the difference was not significant.

Significantly elevated IgM antibody index was observed among H.pylori infected subjects with or without CVD and showed a mean value of 0.36 and 0.26 respectively ($p=0.027$). Similarly IgG antibody index and IgA antibody index were also increased among H.pylori infected subjects with or without CVD than the control subjects. Regarding the level of hsCRP, a statistically significant increase was observed among H.pylori infected subjects with CVD (4.02) and H.pylori infected subjects without CVD

showed a mean value of 0.83. The control subjects showed a mean hsCRP level of 0.38 (p=<0.001) (Table 4).

Table 1.

Comparison of the demographics data and Blood pressure of the test subjects with that of the controls.

		N	Mean	Std. Deviation	F	P
AGE (yrs)	H Pylori positive with CAD	50	44.2	6.2	3.898	.022
	H Pylori positive without CAD	50	44.6	5.0		
	Control	50	41.2	8.2		
	Total	150	43.3	6.7		
BMI (Kg/m^2)	H Pylori positive with CAD	50	25.4	4.4	12.107	.000
	H Pylori positive without CAD	50	24.3	3.2		
	Control	50	22.2	1.6		
	Total	150	24.0	3.5		
WAIST CIRCUMFERENCE (cms)	H Pylori positive with CAD	50	90.9	13.9	19.705	.000
	H Pylori positive without CAD	50	91.5	10.2		
	Control	50	79.6	6.7		
	Total	150	87.4	11.9		
Systolic blood pressure (mm/Hg)	H Pylori positive with CAD	50	140.2	18.9	11.688	.000
	H Pylori positive without CAD	50	137.3	18.4		
	Control	50	126.0	5.7		
	Total	150	134.5	16.6		
Diastolic blood pressure (mm/Hg)	H Pylori positive with CAD	50	77.8	11.9	8.668	.000
	H Pylori positive without CAD	50	75.4	15.4		
	Control	50	68.0	8.8		
	Total	150	73.7	12.9		

Table 2.

Comparison of the biochemical changes of the test subject with that of the controls.

		N	Mean	Std. Deviation	F	P
UREA (mg/dL)	H Pylori positive with CAD	50	24.4	8.3	.346	.708
	H Pylori positive without CAD	50	23.3	6.7		
	Control	50	23.4	6.1		
	Total	150	23.7	7.1		
CREATININE (mg/dL)	H Pylori positive with CAD	50	1.14	.25	.914	.403
	H Pylori positive without CAD	50	1.12	.27		
	Control	50	1.07	.32		
	Total	150	1.11	.28		
URIC ACID (mg/dL)	H Pylori positive with CAD	50	3.85	1.20	2.822	.063
	H Pylori positive without CAD	50	4.22	.97		
	Control	50	3.66	1.43		
	Total	150	3.91	1.23		
AST (IU/L)	H Pylori positive with CAD	50	46.9	19.9	11.19	.000
	H Pylori positive without CAD	50	45.2	22.9		
	Control	50	31.2	8.3		
	Total	150	41.1	19.4		
BLOOD SUGAR (mg/dL)	H Pylori positive with CAD	50	127.2	48.2	7.34	.001
	H Pylori positive without CAD	50	116.3	31.5		
	Control	50	101.2	13.7		
	Total	150	114.9	35.6		
hs CRP (mg/dL)	H Pylori positive with CAD	50	4.02	4.92	23.356	.000
	H Pylori positive without CAD	50	.83	1.04		
	Control	50	.38	.25		
	Total	150	1.74	3.31		

Table 3.

Comparison of the Lipid profile of the test subject with that of the controls.

		N	Mean	Std. Deviation	F	P
CHOLESTEROL (mg/dL)	H Pylori positive with CAD	50	236.8	52.9	42.653	.000
	H Pylori positive without CAD	50	235.4	57.5		
	Control	50	160.2	25.5		
	Total	150	210.8	59.3		
TRIGLYCERIDE (mg/dL)	H Pylori positive with CAD	50	130.0	61.6	3.037	.051
	H Pylori positive without CAD	50	127.1	66.0		
	Control	50	105.2	30.3		
	Total	150	120.8	55.7		
HDL-C (mg/dL)	H Pylori positive with CAD	50	48.5	14.4	4.064	.019
	H Pylori positive without CAD	50	54.3	13.8		
	Control	50	56.7	13.8		
	Total	150	53.2	15.0		
LDL-C (mg/dL)	H Pylori positive with CAD	50	152.5	44.9	76.573	.000
	H Pylori positive without CAD	50	157.3	32.7		
	Control	50	84.0	14.5		
	Total	150	131.3	47.0		

Table 4.

Comparison of the immunology of subjects

		N	Mean	Std. Deviation	F	p
IgM Antibodi index	H Pylori positive with CAD	50	.36	.23	5.056	.027
	H Pylori positive without CAD	50	.26	.24		
	Control	50	0.25	.		
	Total	100	.31	.24		
IgG anti body index	H Pylori positive with CAD	50	1.24	.80	5.327	.023
	H Pylori positive without CAD	50	.90	.69		
	Control	0	.	.		
	Total	100	1.07	.76		
IgA Antibody index	H Pylori positive with CAD	50	1.12	.45	.016	.900
	H Pylori positive without CAD	50	1.10	.75		
	Control	0	.	.		
	Total	100	1.11	.62		

Discussion

Infection by *H. pylori* induces an elevation of cholesterol and triglyceride levels with a decrease in HDL cholesterol [16] contributing to the development of dyslipidemia, a known cardiovascular risk factor. In the present study also observed a statistically significant increase in Total cholesterol, triglyceride and LDL cholesterol levels in H.pylori infected subjects. Moreover the level of HDL cholesterol was significantly reduced among subjects with H.pylori infected CVD subjects.

An earlier study conducted by us reveled a very high prevalence of IgA and IgG anti bodies against H.pylori in more than 40% of population in a coastal village [17]. There is growing evidence that inflammation plays an important role in atherosclerosis and

that some markers of inflammation are associated with a greater risk of coronary cardiopathy or a worse prognosis [18]. Birnie et al [19] detected an elevated hs-CRP which was found to be associated with a worse prognosis in patients with unstable angina or recent myocardial infarction. The present study also observed a statistically significant increased level of hsCRP among H.pylori infected subjects with CVD than the control subjects. Pellicano et al. [20] reported significantly higher prevalence of Hp infection in patients with CVD than in controls (77% vs 59%). Danesh et al [21] found, moderate association between hs-CRP and CVD.

The incidence of chronic diseases, particularly diabetes mellitus (DM) and coronary heart disease (CHD) are significantly higher among Asian Indians, living in the Indian subcontinent or abroad [22]. Xavier et al [23] reported that by the year 2020 India will bear 60% burden of the world's incident of CVD. The effects of a change in life-style due to migration, particularly from rural to urban India, have been predicted to be an etiological factor in CVD [24] . In addition, genetic predisposition, accentuated by change of life style, has also been hypothesized as a major risk factor for CVD in Asian-Indians [25]. However, accumulating evidence has also implicated inflammation in the pathogenesis of atherosclerosis [26]. Infection induced inflammation has recently been implicated strongly in atherogenesis [27]. One such infection is caused by Helicobacter pylori (H. pylori), which is contracted by more than 85% of the populations in the Indian subcontinent during their childhood [28].

H. pylori infection is already known as a causative factor for gastritis, gastroduodenal ulcerations, gastric adenocarcinoma, or mucosaassociated lymphoid tissue

lymphoma [29]. Rahman et al., [5] showed platelet activation/ aggregation measured by the blood level of thromboxane (TXB), which serves as an index of inflammation in CHD [30], was significantly higher in H. pylori infected CHD patients with diabetes mellitus compared to similar patients without diabetes. This observation raises the possibility that inflammation due to H. pylori infection could also be involved in the pathogenesis of Type 2 diabetes (T2DM), which is also highly prevalent in Asian Indians [22]. In the present study, a statistically increased blood sugar level was observed among subjects with H pylori infection than the control subjects.

H. pylori, give its widespread distribution in the world population and the high incidence of gastro duodenal disease and one of the most important microorganisms associated with illness that were previously considered to have a non-infectious etiology. Infection by *H. pylori* induces an elevation of cholesterol and triglyceride levels with a decrease in HDL cholesterol contributing to the development of dyslipidemia, a known cardiovascular risk factor, which is in agreement with the present finding. With respect to the association of this bacterium with coronary cardiopathy, the existing scientific evidence suggests that infection by *H. pylori* contributes to the genesis, progression, and severity of cardiovascular disease, although it is unlikely that it triggers cardiovascular disease on its own. Ultimately, it is the balance between the factors that favour cardiovascular disease and the host´s protective factors.

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