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***Helicobacter pylori* Infection and Inflammation: Implication for the Pathophysiology of Diabetes and Coronary Heart Disease in Asian Indians**

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Abstract

Asian Indians living in the Indian subcontinent or abroad experience high rate of coronary heart disease (CHD) and type 2 diabetes mellitus (T2DM). Asian Indians are also known to suffer from various infections, particularly during their childhood. One such chronic infection is with *Helicobacter pylori* (*H. pylori*). Since *H. pylori* with its specific virulence factor cytotoxin-associated gene A (*cagA*) has been suggested to be associated with CHD, a role of this *H. pylori* infection was investigated in the pathogenesis of CHD in Asian Indians living in Bangladesh. *H. pylori* (*CagA*) infected subjects with CHD (HP+ve cases, n=21), and without CHD (HP+ve controls, n=20), and non-infected without CHD (HP-ve normal controls, n=21) were included in this study. Thromboxane (TXB), an index of platelet activation, was found to be significantly higher in the HP+ve cases (p=0.05), but not in the HP+ve controls (p=0.88) when compared with HP-ve controls. Analyses of lipid profiles revealed that while triglycerides, total cholesterol and LDL did not show any significant changes, HDL was significantly lower in both the HP+ve cases (p=0.0003) and controls (p=0.005). The mean fasting glucose level in the HP+ve cases was markedly increased (p>0.0001), while it was intermediate in the HP+ve controls, and lowest in the HP-ve controls. HOMA-IR values, a measure of insulin resistance, did not reflect any substantial differences between the HP+ve and HP-ve controls, but they were highly significantly different between the HP+ve cases and HP-ve controls. HOMA-B, indicating insulin secretory dysfunction (ISD), was significantly higher in both the HP+ve groups when compared with the normal controls. The data indicate that *H. pylori* infection is associated with impaired insulin secretion, and that a component of insulin resistance that occurs independent of *H. pylori* can then lead to a worsening of glucose tolerance and the development of CHD. This is the first demonstration to our knowledge that *H. pylori* (*CagA*) infection is associated with insulin secretory dysfunction in human subjects. Since many Asian Indians contract various other chronic and acute infections, it is important to investigate the role of *H. pylori* and other infectious agents in the pathogenesis of T2DM and CHD.

Keywords

H. pylori; Inflammation; Heart Disease; Insulin Dysfunction; Asian Indians

INTRODUCTION

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 (Nath et al. 1998; McKeigue et al. 1991). Despite the fact that many Asian-Indians are vegetarians; the CHD incidence rate is very high in India itself (Raman 1993). In fact, Xavier et al. (2008) recently reported that by the year 2010 India will bear 60% burden of the world's incident of CHD. 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 CHD (Janus et al. 1993). In addition, genetic predisposition, accentuated by change of life style, has also been hypothesized as a major risk factor for CHD in Asian-Indians (Dhawan et al. 1994). However, accumulating evidence has also implicated inflammation in the pathogenesis of atherosclerosis (Radar 2000). Thus a role for the well-known vicious cycle of "Infections and Malnutrition", still unabated in the Indian subcontinent, can not be ruled out, since infection induced inflammation has recently been implicated strongly in atherogenesis (Libby et al. 1997). 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 (Sarker et al. 1997). This bacterium is harbored in the pyloric region (antrum) of the human stomach and persists lifelong, thus creating a state of chronic inflammation, albeit a low-grade one (Graham 1989). It is a gram-negative bacterium which is capable of invoking systemic host inflammatory responses including elevations in tumor necrosis factor-alpha (TNF- α), interleukin-1b, C-reactive protein (CRP), lipid-peroxides, hyperhomocysteinemia (HHcy) and intercellular- and vascular-cell adhesion molecules (ICAM-1 and VCAM-1, respectively) (Mc Gowan et al. 1996; Khaled and Mahalanabis 2000; Huhtinen et al. 1998; Khaled and Cornwell 2004; Siddiqui et al. 2009). Recent evidence also suggests that infection with *H. pylori*, particularly those strains containing the specific virulence factor cytotoxin-associated gene A (cagA), may be associated with human coronary heart disease (CHD) (Pasceri et al. 1998). *H. pylori* infection is already known as a causal factor for gastritis, gastroduodenal ulcerations, gastric adenocarcinoma, or mucosa-associated lymphoid tissue lymphoma (Graham 1989). Now an extra-gastric involvement of this bacterium appears to be an emerging area of further investigations.

The above mentioned considerations prompted us to investigate whether inflammation as induced by this chronic *H. pylori* infection has any role in the pathogenesis of CHD in Asian Indians living in Bangladesh. We observed (Rahman et al. 2007) that platelet activation/aggregation measured by the blood level of thromboxane (TXB), which serves as an index of inflammation in CHD (Antman et al. 2005), 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 (Nath et al. 1998). Further investigations were, therefore, undertaken to examine if *H. pylori* infection with its virulent strain, cytotoxic associated gene A (CagA), could be implicated in insulin resistance and/or insulin secretion along with other traits that comprise the Metabolic Syndromes, including obesity assessed as body mass index (BMI) and dyslipidemia reflected by the lipid profiles. Results of our investigations are presented here to show for the first time, to our knowledge, a statistically significant association between *H. pylori* (CagA) and dysfunctional insulin secretion.

MATERIALS and METHODS

This study was conducted at the National Institute of Cardiovascular Diseases (NICVD) with the approval from the Ethical Review Committee of the University of Dhaka. *H. pylori* (CagA) positive subjects who underwent coronary angiography for the first time and found to have coronary artery narrowing of more than 50% were considered as HP+ve cases (n=21), while subjects without CHD but with DM were grouped in HP+ve (n=20) and subjects without HP, DM and CHD were grouped in HP-ve (n=21), normal controls. Since there were about 7 CHD patients without this infection, they were not included in this study. The IgG antibodies to *H. pylori* were measured by an enzyme linked immunosorbent assay (ELISA) method and CagA was assessed by the Western blot analysis. Blood levels of fasting glucose, triglycerides, total cholesterol, high and low density lipoproteins (HDL and LDL) were measured by the enzymatic photometric method. Thromboxane B (TXB) was estimated using enzyme immunoassay kit.

Insulin resistance was determined by the Homeostasis Model Assessment (HOMA-IR) according to the formula: $\text{HOMA-IR} = [\text{fasting glucose (millimoles per liter)} \times \text{fasting insulin (milliunits per liter)}] / 22.5$. HOMA-CIGMA software (Levy et al. 1998) was used in these calculations. Insulin secretion was calculated as the HOMA-B (beta) cell index according to the equation: $\text{HOMA-B} = [\text{fasting insulin (milliunits per liter)} \times 20] / [\text{fasting glucose (millimoles per liter)} - 3.5]$. These models have already been validated against clamp measurements (Matthews et al. 1985).

Data were analyzed using SAS (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance was used to determine differences and pair-wise least square means were compared post hoc to determine if there were significant differences between the three groups. The p values less than 0.05 were considered statistically significant.

RESULTS

Demographic characteristics, metabolic features including lipid profiles, and thromboxane B levels are delineated in Table 1 for the HP+ve cases, HP+ve control, and HP-ve control subgroups. In this table, higher HOMA-B (beta) values are indicative of worsening insulin secretion dysfunction (ISD), and higher HOMA-IR values reflect more severe insulin resistance, as designated by earlier studies (Matthews et al. 1985). All three groups were well matched for age and BMI (p = NS). In order to investigate the effects of *H. pylori*-induced inflammation, HP+ve cases and HP+ve controls were compared with HP-ve controls, i.e. not infected with *H. pylori* and without CHD. Statistically significant differences were observed among the subgroups involving HOMA- B, HOMA-IR, fasting glucose, HDL and TXB.

The state of inflammation, as measured by the level of TXB, was not significantly different between the HP+ve and HP-ve controls (p=0.88); however, TXB level in HP+ve cases exceeded that in the HP+ve controls (p = 0.04) and HP-ve controls (p = 0.05) as depicted in Figure 1. This suggests that high TXB and concomitant platelet activation may be a property of CHD independent of *H. pylori* infection. Although triglycerides, total cholesterol, and LDL values were statistically similar among subgroups (p = NS; Table 1), HDL values were, however, significantly increased in HP-ve controls compared with either HP+ve cases (p=0.0003) or HP+ve controls (p=0.005), shown in Figure 2, suggesting that *H. pylori* infection is associated with reduced HDL whether or not infected patients have CHD. Fasting glucose values are represented by bar plots in Figure 3 which demonstrates statistically significant differences among the subgroups, with lowest glucose concentrations in HP-ve controls, intermediate values in HP+ve controls, and highest values in HP+ve

cases ($p = 0.04$ for all comparisons). Importantly, however, the mechanisms responsible for variable glucose tolerance did appear to differ among the subgroups. As shown in Figure 4A, HOMA-B was elevated in HP+ve controls compared with HP-ve controls ($p = 0.02$), while the difference between HP+ve controls and HP+ve cases did not achieve statistical significance ($p = 0.08$), indicating that *H. pylori* infection per se was associated with impaired insulin secretion. In contrast, in Figure 4B, HOMA-IR was similar in HP-ve controls and HP+ve controls ($p = \text{NS}$), but was significantly increased in HP+ve cases compared with either control group, indicating that patients with CHD were more insulin resistant independent of whether or not *H. pylori* infection was evident. This greater degree of insulin resistance could then explain why fasting glucose was higher in the HP+ve cases compared with the HP+ve controls (see Figure 3). Thus, the presence of *H. pylori* (CagA) led to impaired insulin secretion and moderate fasting hyperglycemia; however, those patients developing CHD were more insulin resistant and this seemed to combine with *H. pylori*-associated insulin secretory dysfunction to worsen the degree of fasting hyperglycemia.

DISCUSSIONS

Recently, Antman et al. (2005) clearly demonstrated that the pathogenesis of CHD involved interactions between inflammation and arachidonic acid metabolism, resulting in platelet activation. Thromboxane, measured as TXB, is considered as an index of inflammation-related platelet activation, particularly as relates to CHD risk. This seems to be well supported by our observations, as depicted in Figure 1, showing a significantly higher level of TXB in the HP+ve cases with CHD, but not with HP+ve and HP-ve controls. Since the TXB values for the HP+ve and HP-ve groups do not differ significantly ($p=0.88$), it appears that *H. pylori* infection may not have any influence on the prostaglandin biosynthesis from arachidonic acid metabolisms. On the other hand, *H. pylori* has been found by us and others to invoke cytokine proliferations, similar to any other infections, as shown by the *denovo* generation of increased levels of TNF- α and CRP (Mendall et al. 1994; Khaled and Mahalanabis 2000; Siddiqui et al. 2009).

Analyses of lipid profiles revealed that the levels of total cholesterol, triglyceride and LDL do not significantly differ between all of the three groups. HDL, on the other hand, was significantly decreased in both the HP+ve controls ($p=0.005$) and the HP+ve cases ($p=0.0003$) compared to the HP-ve normal controls. Since the reduced HDL levels did not differ in comparing HP+ve controls and cases, these findings are in agreement with an earlier report by Niemela et al. 1996), suggesting that *H. pylori* (CagA) per se may be responsible in modifying lipid milieu in the host, thus making them more susceptible to CHD.

It is clearly evident in Figure 3 that *H. pylori* (CagA) positive subjects with CHD (cases) have significantly higher fasting glucose than both the HP+ve and HP-ve controls. HP+ve controls also show significantly higher fasting glucose than the HP-ve controls. Fasting glucose values of 12.10 ± 1.09 mmol in the HP+ve cases and 8.22 ± 0.62 mmol in the HP+ve controls (Table 1) are indicative of the existence of diabetes in these two groups, while the mean value of 5.95 ± 0.38 mmol in HP-ve normal controls lies within the range defined by impaired fasting glucose. Corresponding to the subgroup with the highest fasting blood glucose levels, HOMA-IR values indicate that insulin resistance (IR) is significantly more severe in the HP+ve cases than both the control groups. It is, however, interesting to note that HOMA-IR values in the control subgroups without CHD, namely the HP+ve control group with more mild diabetes and the HP-ve control group with impaired fasting glucose, do not differ significantly. On the other hand, the HOMA-B (beta) index, reflecting insulin secretion dysfunction (ISD), showed progressive deterioration as a function of worsening

glucose tolerance among all three subgroups. Specifically, HOMA-B values were increased in both HP+ve groups compared with HP-ve normal controls, and this demonstrates, for the first time to our knowledge, an important potential sequella of *H. pylori* (CagA) infection, namely insulin secretion dysfunction (ISD) in human subjects. The data are consistent with the formulation that chronic *H. pylori* (CagA) infection is associated with impaired insulin secretion and mild diabetes, and that the presence of severe insulin resistance, occurring independently of *H. pylori* status, then leads to more severe degrees of diabetes and the development of CHD.

The relationship between inflammation and insulin resistance (IR) in the pathogenesis of type 2 diabetes, which most Asian Indians suffer from, is well known (Fernandez-Real JM and Ricart W. 1999). Although TXB, an index of platelet activation, appears to be a marker of inflammation in CHD, as explained by Antman et. al., it does not, however, indicate any link with the *H. pylori* infection in this study. *H. pylori* (CagA), on the other hand, is known to invoke cytokine production, particularly TNF- α and CRP (Mendall et al. 1997; Khaled and Mahalanabis 2000; Siddiqui et al. 2009). These proinflammatory cytokines have been suggested to be strongly associated with HOMA-IR (Pickup et al. 2000). Although these cytokines were not evaluated in this study, the *H. pylori* (CagA) infected patients with CHD were observed to have more markedly elevated fasting glucose and levels of insulin resistance. Thus, our data do not exclude a role for *H. pylori* infection in human insulin resistance,. Alternatively, the more severe insulin resistance in the HP+ve cases with CHD could have combined with *H. pylori*-associated impairments in insulin secretion to produce more severe state of glucose intolerance.

Indeed, a novel observation in the current study is the association between *H. pylori* infection and impaired insulin secretion. Multiple studies have previously documented a positive connection between diabetes mellitus and pancreatitis, and *H. pylori* infection has more recently been shown to participate in pancreatitis (Hart et al. 2008). Furthermore, it has been reported that insulin producing pancreatic beta-cells are especially susceptible to damage by inflammation and oxidative stress (Fosslien 2001). Since *H. pylori* (CagA) infection is capable of inducing inflammation, a deficit in insulin secretion as found in this study is, therefore, quite conceivable. It has also been reported recently that another chronic infection, namely with hepatitis C virus (HCV), is associated with elevations in HOMA-B, i.e. insulin secretory dysfunction (ISD), in humans (Lecube et al. 2006). Both HCV and *H. pylori* (CagA) infections are rampant in the Indian subcontinent (Nath et al. 1998). It is important, therefore, to conduct prospective studies to ascertain a link between these chronic infections with the Metabolic Syndrome trait complex, including visceral obesity, diabetes mellitus, and the pathogenesis of CHD. Based on these data, it is also conceivable that the prevention and/or treatment of *H. pylori* infection could prevent future diabetes and CHD.

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Figure 1.
Bar graph of the levels of Thromboxane (TXB) in HP-ve controls, HP+ve controls and HP +ve with CHD cases.

Figure 2.
Bar graph of the levels of HDL in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

Figure 3.
Bar graph of the levels of fasting glucose in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

Figure 4.

Bar graph of the levels of a) HOMA-B (ISD) and b) HOMA-IR in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

Table 1

Descriptive Statistics of the three groups

Demographics	HP-ve Controls	HP+ve Controls	HP+ve Cases
Age (yrs)	48.8 ± 2.2	43.7 ± 2.0	49.4 ± 2.1
Sex (% M)	100	75	95.2
Metabolic Features			
BMI (kg/m ²)	24.9 ± 0.9	25.3 ± 0.7	25.3 ± 0.7
HOMA-B (ISD)	79.9 ± 6.3	109.7 ± 9.1	132.7 ± 10.6
HOMA-IR	7.7 ± 0.4	7.5 ± 0.7	11.0 ± 0.6
Fasting Glucose (mmol)	5.95 ± 0.38	8.22 ± 0.62	12.10 ± 1.09
Triglycerides (mg/dl)	202.8 ± 22.2	244.6 ± 24.5	234.1 ± 28.8
Total Cholesterol (mg/dl)	191.6 ± 10.0	201.8 ± 8.8	215.2 ± 9.8
HDL (mg/dl)	41.8 ± 2.6	33.1 ± 1.9	30.7 ± 1.5
LDL (mg/dl)	123.7 ± 9.1	112.9 ± 8.0	132.8 ± 9.5
Inflammatory Marker			
Thromboxane B (TXB) (pg/ml)	493.8 ± 48.3	479.9 ± 75.2	670.9 ± 64.1