

***Helicobacter pylori* Infection and Inflammation: Implications for Pathophysiology of Diabetes Mellitus and Coronary Heart Disease**

Afzalur Rahman¹, Mark B. Cope², Shafique A. Sarker³, W. Timothy Garvey^{2,4},
Habib S. Chaudhury¹ and Mohammad A. Khaled²

¹National Institute of Cardiovascular Diseases (NICVD), ²Department of Nutrition Sciences,
University of Alabama at Birmingham (UAB), ³International Center for Diarrheal Disease
Research, Bangladesh (ICDDRDB), Dhaka, Bangladesh,

⁴The Birmingham Veterans Affairs Medical Center, Birmingham, AL 35233, USA Alabama, USA

KEYWORDS *Helicobacter pylori*. Inflammation. Heart Disease. Insulin Secretory Dysfunction. Asian Indians

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.

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*, 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

Corresponding Author:

Dr. M. A. Khaled

Department of Nutrition Sciences, University of Alabama
at Birmingham, Birmingham, AL 35294, USA

Fax: (205) 934-7049

Email: khaledm@uab.edu

region (antrum) of the human stomach and persists life-long, 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- α (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 Syndrome, 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)

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
<i>Metabolic Features</i>			
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	***ΔΔΔ11.0±0.6
Fasting Glucose (mmol)	5.95±0.38	*8.22±0.62	***ΔΔΔ12.10±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
<i>Inflammatory Marker</i>			
Thromboxane B (TXB) (pg/ml)	493.8±48.3	479.9±75.2	Δ*670.9±64.1

*P<0.05, **P<0.01 and ***P<0.001 vs HP-ve Controls; ΔP<0.05, ΔΔΔP<0.001 HP+ve Controls

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<0.05 for all

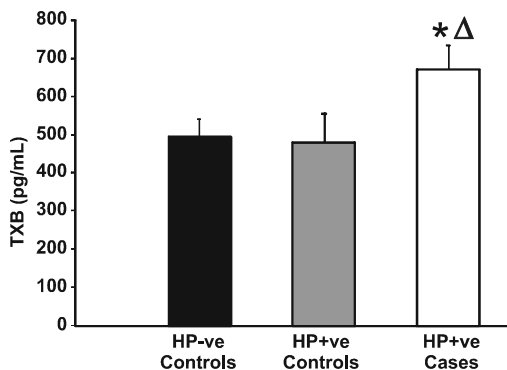


Fig. 1. Bar graph of the levels of Thromboxane (TXB) in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

*P<0.05 against HP-ve Controls
ΔP<0.05 against HP+ve Controls

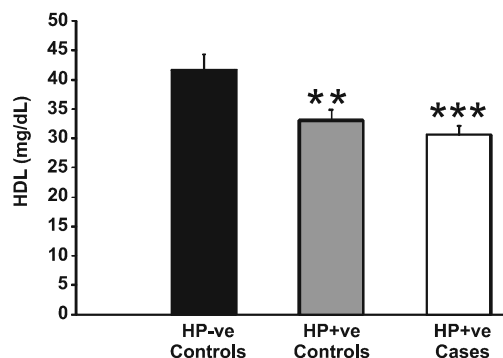


Fig. 2. Bar graph of the levels of HDL in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

P<0.01 and *P<0.001 against HP-ve Controls

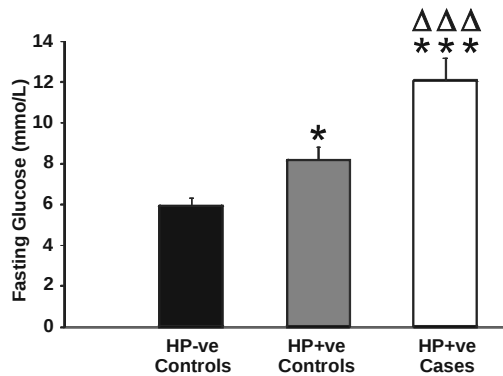


Fig. 3. Bar graph of the levels of fasting glucose in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

* $P < 0.05$ and *** $P < 0.001$ against HP-ve Controls;
 $\Delta\Delta\Delta P < 0.001$ against HP+ve Controls

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.

DISCUSSION

Recently, Antman et al. (2005) clearly demonstrated that the pathogenesis of CHD involved interactions between inflammation and arachidonic acid metabolism, resulting in platelet

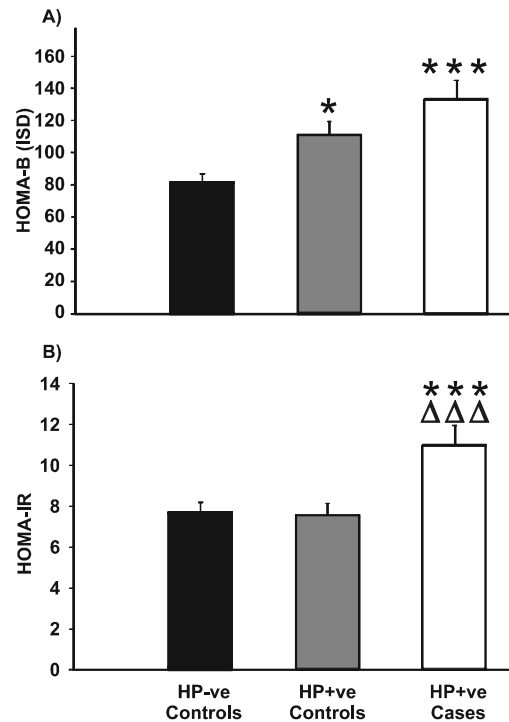


Fig. 4. Bar graph of the levels of a) HOMA-B (ISD) and b) HOMA-IR in HP-ve controls, HP+ve controls and HP+ve with CHD cases.

* $P < 0.05$ and *** $P < 0.001$ against HP-ve Controls;
 $\Delta\Delta\Delta P < 0.001$ against HP+ve Controls

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 *de novo* 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/L in the HP+ve cases and 8.22 ± 0.62 mmol/L 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/L 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 and Ricart 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.

ACKNOWLEDGEMENTS

We are grateful for all the facilities and support

provided by the NICVD for this study and to Dr. Jonathan Levy, Diabetes Research Laboratory, Oxford University, Oxford, UK, for supplying the HOMA-CIGMA Calculator Program v2.00. We acknowledge additional research support from the American Heart Association (AHA), National Institutes of Health (DK-38764), a merit review grant from the Department of Veterans Affairs, and core laboratory support from the UAB Diabetes Research and Training Center (P60 DK079626).

REFERENCES

- Antman EM, DeMets D, Loscalzo J 2005. Cyclooxygenase inhibition and cardiovascular risk. *Circulation*, 112: 759-770.
- Dhawan J, Bray CL, Warburton R, Ghambhir DS, Morris J 1994. Insulin resistance, high prevalence of diabetes, and cardiovascular risk in immigrant Asians. Genetic or environmental effect? *Br Heart J*, 72: 413-421.
- Fernandez-Real JM, Ricart W 1999. Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. *Diabetologica*, 42: 1367-1374.
- Fosslien E 2001. Mitochondrial medicine-molecular pathology of defective oxidative phosphorylation. *Ann Clin Lab Sci*, 31: 25-67.
- Graham DY 1989. Campylobacter pylori and peptic ulcer disease. *Gastroenterology*, 96: 615-625.
- Hart AR, Kennedy H, Harvey I 2008. Pancreatic cancer: a review of the evidence on causation. *Clin Gastroenterol Hepatol*, 6: 275-282.
- Huhtinen HT, Grönroos JM, Haapamäki MM, Nevalainen TJ 1999. Phospholipase A2 in gastric juice of Helicobacter pylori-positive and negative individuals. *Clin Chem Lab Med*, 37: 61-64.
- Janus ED, Postiglione A, Singh RB, Lewis B 1996. The modernization of Asia. Implications for coronary heart disease. *Circulation*, 94: 2671-2673.
- Khaled MA, Mahalanabis D 2000. Higher Serum Tumor Necrosis Factor in H. Pylori. Infected Humans with Higher Body Mass Index. *The FASEB J*, 14: A248.
- Khaled MA, Cornwell PC 2004. Hyperhomocysteinemia due to Helicobacter pylori? *Atherosclerosis*, 172: 199-200.
- Levy JC, Matthews DR, Hermans MP 1998. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*, 21: 2191-2192.
- Lecube A, Genesca J, Hernandez C, Simo R 2006. Proinflammatory Cytokines, Insulin Resistance, and Insulin Secretion in Chronic Hepatitis C patients. *Diabetes Care*, 29: 1096-1101.
- Libby P, Egan D, Skarlatos S 1997. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation*, 96: 4095-4103.
- Matthews DR, Hosker JP, Rudenki AS, Naylor BA, Treacher DF, Turner RC 1985. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28: 412-419.
- Mc Gowan CC, Cover TL, Blaser MJ 1996. Helicobacter pylori and gastric acid: biological and therapeutic implications. *Gastroenterology*, 110: 926-38.
- Mc Keigue PM, Shah B, Marmot MG 1991. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet*, 337: 382-386.
- Mendall MA, Patel P, Asante M, Ballam L, Morris J, Strachan DP, Camm AJ 1997. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart*, 78: 273-277.
- Nath I, Reddy KS, Dinshaw KA, Bhisey AN, Krishnaswami K et al. 1998. India. *Lancet*, 351: 1265-1275.
- Niemelä S, Karttunen T, Korhonen T, Läärä E, Karttunen R et al. 1996. Could Helicobacter pylori infection increase the risk of CHD by modifying serum lipid concentrations? *Heart*, 75: 573-575.
- Pasceri V, Cammarota G, Patti G, Cuoco L, Gasbarrini A et al. 1998. Association of Virulent Helicobacter pylori Strains with Ischemic Heart Disease. *Circulation*, 97: 1675-1679.
- Pickup JC, Chusney GD, Thomas SM, Burt D 2000. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci*, 67: 291-300.
- Rader DJ 2000. Inflammatory markers of coronary risk. *N Eng J Med*, 343: 1179-1182.
- Rahman MA, Chaudhury HS, Sarker SA, Khaled MA, Biswas KB et al. 2007. Helicobacter pylori Infection and Insulin Secretory Defect Are Associated with Angiographically Defined Coronary Artery Disease. *Amer J Cardiol*, 99: 83F.
- Raman KV 1993. Prevalence of coronary heart disease in the rural population of Thiruvananthapuram district, Kerala, India. *Int J Cardiol*, 39: 59-70.
- Sarker SA, Mahalanabis D, Hildebrand P, Rahaman MM, Bardhan PK et al. 1997. Helicobacter pylori: prevalence, transmission and serum pepsinogen II concentrations in children of a poor community in Bangladesh. *Clin Infect Dis*, 25: 990-995.
- Siddiqui NR, Garvey WT, Khaled MA 2009. Helicobacter pylori induces higher C-Reactive Protein in Obese African Americans. *Artery Res*, 3: 39-42.
- Xavier D, Pais P, Devereaux PJ, Xie C, Prabhakaran D et al. 2008. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. *Lancet*, 371: 1435-42.