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Role of microalbuminuria and hs-CRP in Helicobacter pylori infected type 2 diabetes mellitus in Eastern India

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ABSTRACT

Background: *Helicobacter pylori (HP) infections are one of the few commonest worldwide which affects approximately 50% of the overall population. It is reported that high serum levels of C-reactive protein (CRP) is a novel cardiovascular risk factor that impairs endothelial function. H. Pylori infection in gastric mucosa may cause systemic inflammatory reaction. This study aimed to examine the association between the infection and serum high sensitivity C-reactive protein (hsCRP). Thus our study attempts to find the levels of microalbuminuria between H. pylori infected patients and non-infected patients in diabetes mellitus and establish its association with blood glucose levels.*

Materials and Methods: *The case control study consists of 98 diabetic patients where 52 were clinically suspected case of H. Pylori infection and 46 without H. Pylori infection in medicine department. Serum hs CRP levels were determined with a high-sensitivity nephelometric method. Urinary albumin excretion was measured by nephelometric test for the measurement of microalbumin in urine. Three endoscopic biopsy samples were obtained from the antral mucosa of each patient. The first two biopsy samples were placed into 10% formol solution and dyed by Giemsa and hematoxylin-eosin while the third sample was given for rapid urease test and investigated for H. pylori.*

Results: *The presence of H. pylori infection was detected in 52 of 98 diabetic patients (53.06%). The ESR and hs-CRP levels were elevated in H. Pylori infected diabetes cases as compared to non-infected diabetics*

which were found statistically significant. The Microalbumin levels were higher in diabetic patients infected by H. pylori compared to non-infected patients (185.3 ± 90.5 vs 128.1 ± 42.6; p <0.001). However, a high positive correlation was observed between ESR with hs-CRP level (r = 0.825; P = <0.001) among H. Pylori infected diabetes subjects while no correlation was seen in non-infected diabetics (r = 0.279; P = 0.098).
Conclusions: *We have also observed an increase in hs-CRP and ESR levels in H.pylori infected diabetics which are also in confirmation with other fewer studies. Moreover, an association is observed between ESR and Hs-CRP in H. Pylori infected diabetics which suggest a plausible role in the pathogenesis and could be used as screening markers of the disease. hsCRP was higher among the infected individuals. The summary odd ratio indicated that H. pylori infection could influence the serum hsCRP level.*
Keywords: *Helicobacter pylori, Type 2 diabetes mellitus, Microalbuminuria, hs-CRP, ESR.*

INTRODUCTION

Helicobacter pylori (HP) infections are one of the few commonest worldwide which affects approximately 50% of the overall population, and are more prone in developing countries [1]. H. pylori is a gram-negative, spiral-shaped pathogenic bacterium that exclusively colonizes the gastric epithelium causing chronic gastritis, peptic ulcer disease, and/or gastric malignancy and is mainly acquired in childhood by the fecal-oral, oral-oral or gastro-oral route [1-3]. The infection provoke an acute polymorphonuclear infiltration in the gastric mucosa, which is gradually substituted by an immunologically-mediated, chronic, predominantly mononuclear cellular infiltration and characterized by the local production and systemic diffusion of pro-inflammatory cytokines affecting the remote tissues and organic systems [4-6]. On the other hand, Type 2 diabetes mellitus (T2DM) is an emerging pandemic group of metabolic syndrome characterised by hyperglycemia, responsible for an estimated 3.8 million adult deaths worldwide [7]. The pathogenesis of T2DM is multifarious, with risk factors associated with lifestyle (e.g., diet, obesity, physical activity), genetic background, and socioeconomic factors [8,9]. The defects in insulin secretion and/or insulin action by the pancreas results in the accumulation of sugar in the bloodstream [10]. Recent evidence implicates the pathological involvement of inflammation in T2DM, which is an important process induced by H. pylori infection[11]. However, the findings of few studies are inconsistent but the presence of H. pylori is found to be higher in diabetic patients compared to nondiabetic patients [12–14]. H. pylori colonizes in the gastric antrum in all diabetic patients with impaired metabolic control [12–15].

The existence of H. pylori and DM is one of the main causes which play a vital role in the development of gastrointestinal diseases [16]. The worsening of glycemic and metabolic control increases the prevalence of H. pylori infections and complaints of dyspepsia [12, 16]. Many studies have shown the association between dyslipidemia and H. pylori that H. Pylori eradication improves dyslipidemia and insulin resistance and lowers inflammation [17]. Moreover, it is shown that there is a significant relationship between microvascular complications of diabetes (nephropathy, neuropathy, and retinopathy) and H. Pylori but the results are incosistent [17,18,19].

Microalbuminuria may be considered as a marker of diabetic mellitus related complications where the appearance of albumin in urine is thought to be the consequence of generalized endothelial damage along the vascular area including the glomerulus [20]. Few of the infectious diseases caused by Hepatitis B and C virus, chlamydia, Epstein Barr virus, cytomegalovirus, Helicobacter pylori (H. pylori), may be the etiological factors related with this vascular endothelial damage and consequently developing atherosclerosis [21-24]. Many studies have attempted to explore various markers of H. Pylori infection in diabetes mellitus but it is still unclear. Thus our study attempts to find the levels of microalbuminuria between H.pylori infected patients and non-infected patients in diabetes mellitus and establish its association with blood glucose levels.

METHODS

The case control study consists of 98 diabetic patients where 52 were clinically suspected case of H. Pylori infection and 46 without H. Pylori infection in medicine department in ICARE Institute of Medical Sciences and Research, Haldia. Routine biochemical parameters and endoscopy were done for both the subjects. The exclusion criteria of the subjects were those previously diagnosed to have H. pylori infection or those who had undergone or were currently undergoing H. pylori eradication, those receiving anti-ulcer treatment in the last three months and still receiving proton-pump inhibitors (PPI) or H2 receptor blockers, diabetic patients with poor glucose regulation diagnosed previously and detected in laboratory parameters as having nephropathy, retinopathy, neuropathy, or ischemic cardiovascular disease, vascular or inflammatory disease, rheumatoid or immunological diseases, neurological diseases. Informed consent was taken by patients in both the groups. The study was approved by the Institution Ethics committee.

About 4 ml of venous blood was taken by arm venous puncture in sterile vials. 2 ml of blood was collected without anticoagulant and serum was separated by centrifugation at 3500 rpm for 15 - 20 mins and was used for measurement of hs CRP and routine biochemical parameters. Serum hs CRP levels was determined with a high-sensitivity nephelometric method. Urinary albumin excretion was measured by nephelometric test for the measurement of microalbumin in urine. Three endoscopic biopsy samples were obtained from the antral mucosa of each patient. The first two biopsy samples were placed into 10% formol solution and dyed by Giemsa and hematoxylin-eosin while the third sample was given for rapid urease test and investigated for H. pylori. The histological helicobacter pylori positive patients were confirmed by curved organisms seen under hematoxylin and eosin stained sections while a pink color change upto 24 h after the addition of gastric juice was urease positive. Culture was done on Skirrow's medium and the isolates were identified by standard methods.

The rest of the blood sample was collected in sterile tube containing potassium-EDTA anticoagulant for measurement of ESR by Wintergreen method.

Statistical analysis of different biochemical parameters was performed by Students't-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed t--tests and for nonparametric Mann-Whitney U "t" test. To find out the correlation between two variables, Pearson's product moment correlation coefficient was used. A value of $P < 0.05$ was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA).

RESULTS

The demographic and biochemical profile of the T2DM infected or noninfected with H. pylori is presented in Table 1. There was no significant difference in age, sex distribution or BMI in either of the two groups between H. Pylori infected and noninfected diabetes mellitus patients (Table 1). The presence of H. pylori infection was detected in 52 of 98 diabetic patients (53.06%). The ESR and hs-CRP levels were elevated in H. Pylori infected diabetes cases as compared to non-infected diabetics which were found statistically significant (Table 1). The Microalbumin levels were higher in diabetic patients infected by H. pylori compared to non-infected patients (185.3 ± 90.5 vs 128.1 ± 42.6; p <0.001) [Figure 1]. However, a high positive correlation was observed between ESR with hs-CRP level ($r = 0.825$; $P = <0.001$) [Figure 2] among H. Pylori infected diabetes subjects while no correlation was seen in non-infected diabetics ($r = 0.279$; $P = 0.098$) [Figure 3].

Table 1: Demographic and biochemical profile of the subjects

	T2DM with H. pylori (n= 52)	T2DM without H. Pylori (n= 46)
Age(in years)	54.68 ± 5.9	55.09 ± 6.3
Sex (M/F)	30/22	26/20
BMI (kg/m^2)	26.72 ± 2.34	25.96 ± 1.42
FBG (mg/dl)	129.23 ± 18.52	118.7 ± 27.17
HbA1c	6.52 ± 0.48	5.96 ± 0.84
Serum total CHL (mg/dl)	188.3 ± 23.44	194.9 ± 42.3
Serum HDL (mg/dl)	39.98 ± 6.12	40.22 ± 4.66
Serum TG (mg/dl)	192.7 ± 64.34	182.8 ± 59.2
ESR (mm/h)	138.4 ± 37.2	82.4 ± 23.1*
Hs-CRP (mg/L)	4.68 ± 1.82	3.42 ± 0.54*

[FBG, fasting blood glucose; CHL, cholesterol; TG, triacylglyceride; HDL, high density lipoprotein cholesterol; ESR, Erythrocyte sedimentation rate; hs-CRP, high sensitivity- C Reactive Protein. Age, BMI, and serum levels of biochemical parameters were expressed as the means ± SD. Statistically significant, * p < 0.001 vs Control.]

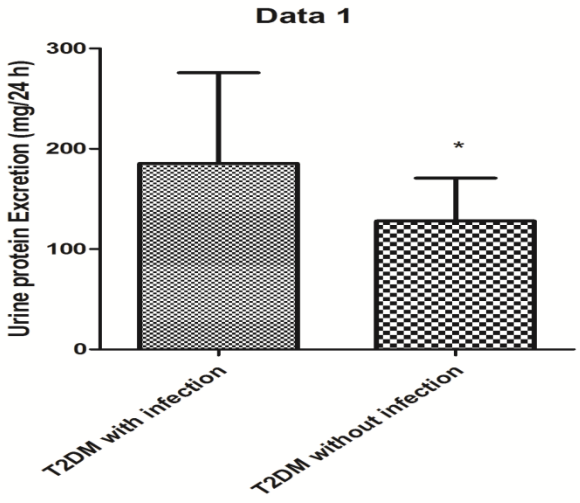

Figure 1: The Microalbumin levels in diabetic patients infected by H. pylori compared to non-infected patients.

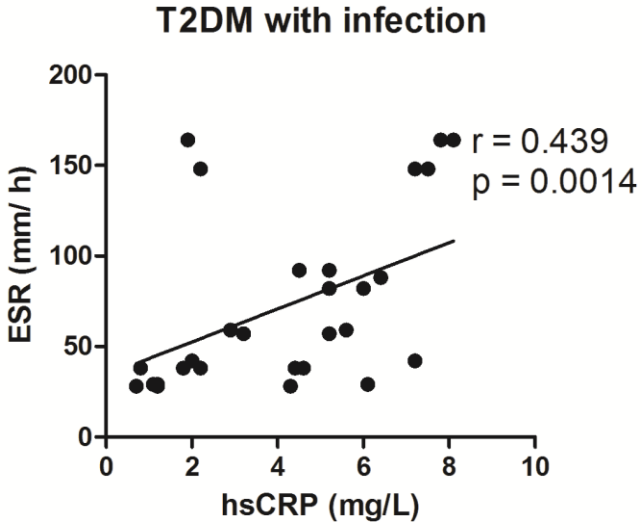

Figure 2: High positive correlation between ESR with hs-CRP level among H. Pylori infected diabetes subjects

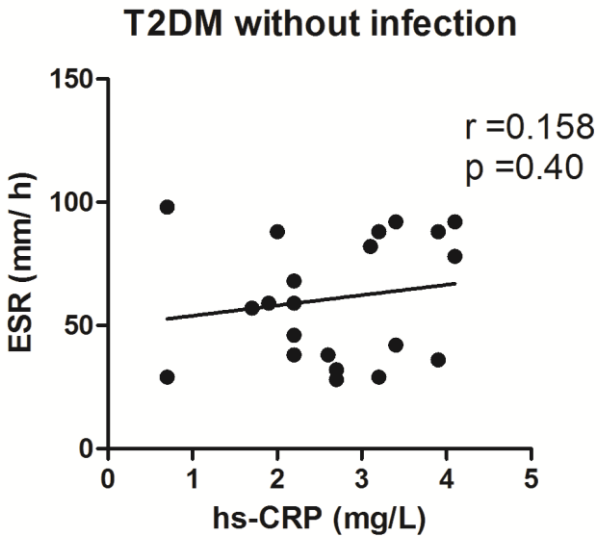

Figure 3: No correlation between ESR with hs-CRP level was seen in non-infected diabetics (r = 0.279; P = 0.098).

DISCUSSION

DM patients are generally prone to many chronic infections. The prevalence of H. Pylori in DM patients is very much contradictory as such in our study, H. pylori positivity was detected in 53.06% of the T2DM patients [25]. Fewer studies also observed that the prevalence of H. Pylori infection was significantly higher in the DM group compared to the control group while some studies showed no significant difference in the DM group and the control group with regard to H. pylori infections [26-28]. However, there is no substantial evidence indicating that H. pylori plays a role in diabetes, there are several lines of facts to associate increased susceptibility to infection in

diabetic patients. In T2DM there is a loss of cellular and humoral immunity which may boost patients' sensitivity to H. pylori infection [29]. Moreover, there is fall of gastrointestinal motility and acid secretion which leads to pathogen colonization and infection rate in the gut [35]. Additionally, altered metabolism of glucose may generate chemical changes in the gastric mucosa leading to H. pylori colonization [30]. Achlorhydria and reduced acid secretion are considered to be a negative factor for H. pylori infections. Moreover, hyperglycemia facilitates secondary H. pylori colonization to antibiotics taken and increases the prevalence of infection in T2DM [31]. Our study showed significant higher microalbuminuria in H. Pylori infected diabetics compared to nondiabetics which is also similar with fewer studies. Malb has been found to be associated with endothelial low-grade inflammation and vascular dysfunction [32, 33]. It has also been observed that persistent systemic inflammatory response related with H. pylori increases the vascular injury in diabetics and predisposes them to pulmonary, cardiovascular and cerebral diseases[34]. The infection contributes to strong inflammatory response, atherogenesis and plaque instability which might be due to pro-inflammatory factors that are produced at excessive amounts and cross-reaction between the released mediators and host antigens causes gastric injury and extra-digestive manifestations [34,35].

We have also observed an increase in hs-CRP and ESR levels in H.pylori infected diabetics which are also in confirmation with other fewer studies. Moreover, an association is observed between ESR and Hs-CRP in H. Pylori infected diabetics which suggest a plausible role in the pathogenesis and could be used as screening markers of the disease. There were few limitations such as small sample size and other associated factors with diabetes which might have interfered with the study. Despite these limitations, there shows an increase in microalbuminuria and inflammation markers and their association which play a vital role in the pathogenesis of the disease. A larger cross-sectional study needs to be done to conclude the fact.

Conflict of Interest: None Declared

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