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Prevalence of *H. pylori* Infection in Type 2 Diabetes mellitus patients in Rural Rajasthan – A Case Control Study

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Abstract
In patients with diabetes mellitus (DM), chronic infections are frequent and severe due to the impairment of their immune status. However, data on the prevalence of Helicobacter pylori (H. pylori) infection in DM type II (DMT2) are scanty and contradictory. Aim of this study was to evaluate the prevalence of H. pylori infection in DMT2 patients in Northern region of India (Rajasthan state, more specifically in and around the rural area of Pilani) and also to find if there exists any significant correlation between H. pylori infection and DMT2. The study was carried out in rural Rajasthan. A case control study of 72 patients (33 diabetics and 39 non-diabetic subjects) was designed for the period of six month. The study design was based on pre-decided inclusion and exclusion criteria. Blood samples were collected from controls and DMT2 patients after obtaining informed consent. Anti - H. pylori - IgG Microplate ELISA test was performed for presence or absence of H. pylori. Differences between DMT2 patients and controls were evaluated based on statistical analysis. Data was analyzed by using Chi-square test at 5 % level of significance. The difference of H. pylori prevalence between diabetics (88%) and control (67%) was significant ($p<0.05$) showing strong correlation between the association of H. pylori and DMT2.
Key Words: Diabetes Type II, H. pylori, Ulcer, Case Control Study

Introduction:
Acid peptic disorders are common in patients with or without diabetes. *Helicobacter pylori* (*H. pylori*) is responsible for most of the duodenal ulcers and many gastric ulcers in normal population. *H. pylori* negatively affects the protective mucous coating of the stomach and duodenum and allow gastric acid to get through to the sensitive lining beneath. Both, acid and the bacteria irritate the gastric lining and cause a sore, or ulcer. The organism is also able to survive in stomach acid and stomach pH, as it itself secretes enzymes that neutralize the acid. This mechanism allows *H. pylori* to make its way to the protective mucous lining.

H. pylori is a gram-negative, spiral, flagellate bacillus and is the single most common cause of peptic ulcer.[1] The high prevalence of this organism worldwide presents clinicians and other

health care workers a formidable challenge with regard to its control in a large population. *H. pylori* is S-shaped, curved rod (0.5-0.9 µm wide by 2-4 µm long) when observed in vivo. Because of it spiral shape, bacterium burrows through the delicate stomach lining and sustain its life instomach. These bacterial cells are actively motile in nature. The bacterium is microaerophilic, growing best at atmosphere of 5% oxygen, with 5-10% CO_2 on blood-containing media in vitro. The genomic DNA of bacterium is a single circular molecule with a mean size of 1.71 Mb (ranging from 1.40 to 1.73 Mb), and with a base composition in the range 35-37 mol % of G+C. DNA-DNA hybridizations show a high level (>65%) of sequence homology between strains, despite extensive sequence variation and gene rearrangements.[2]

Literature on prevalence of *H. pylori* infection shows that, about 30% of population in the United States is carrying the organism, which is higher as compared to most of the developing countries. Human beings are the major reservoir of *H. pylori*, but the exact source and route of infection is still unknown and further, it has been shown that water is not a vehicle of infection. [3-4]

Diabetes mellitus and *H. pylori*:

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from either absolute or a relative deficiency of insulin and/or action. The chronic hyperglycemia in DM patients is associated with long-term damage, dysfunction, and failure of various organs viz. eyes, kidneys, nerves, heart and blood vessels. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany the chronic hyperglycemia.

Two major forms of diabetes mellitus are Type 1, previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes (DMT1) and Type 2, previously called non-insulin dependent diabetes mellitus (NIDDM) or maturity-onset diabetes (DMT2). Many reports on *H. pylori* have indicated that the seroprevalence of *H. pylori* is high in DM patients.[5-6] An endoscopic

study for association of *H. pylori* and DM patients revealed the point prevalence (active disease: infection ratio) of active peptic ulceration of 4.7% and, a lifetime prevalence (lifetime disease: infection ratio) of 13% in DM patients.[7] Another study on association of H.Pylori and DM patients reported the point prevalence as 6.6% and lifetime prevalence of 13.2% in South India.[8]

Earlier published reports suggest a significant relationship between *H. pylori* and DMT2, indicating that the prevalence of the infection in the diabetic population is significantly higher.[9-11] Ricci et al.[12] have reported that upper gastrointestinal symptoms (abdominal pain or discomfort, bloating, early satiety, nausea and vomiting) are common in individuals with diabetes and more prevalent than in controls. These symptoms are very similar to those of a *H. pylori* infection. Studies have also indicated the possible role of *H. pylori* in causation or escalation of various diabetic complications like coronary artery disease and diabetic nephropathy in DMT2 patients.[13] Some studies have demonstrated a relation between *H. pylori* and autonomic neuropathy in DMT2.[14] A recent study demonstrates that *H. pylori* masks differences in homocysteine plasma levels between controls and DMT2 patients, which appears to be one of the factors responsible for the increased risk of vascular damage.[15]

Data concerning the prevalence of *H. pylori* in diabetic patients are scanty and controversial. Many studies have reported coherence of *H. pylori* with the DMT2. But there are other studies which proved poor correlation between the *H. pylori* and DMT2. Zelenkova J et al in a study of 195 diabetes patients (DMT1 and DMT2 combined) has found lower seroprevalence of *H. pylori* in diabetic patients of type I and II in comparison with the healthy population.[16] Xia et al showed that *H. pylori* infection is not associated with diabetes mellitus, not with upper gastrointestinal symptoms in DM patients.[17] Anastasios R et al studied *H. pylori* infection in DM patients for its prevalence using endoscopic findings. In their cross-sectional study of 172 dyspeptic patients (67 diabetics and 105 nondiabetic subjects) there was lack of difference between DM and non-diabetic patients in regard to the prevalence of both *H. pylori* infection and

H. pylori-related gastroduodenal disorders.[18] Jones et al reported similar results it has been showed that *H. pylori* infection is not associated with delayed gastric emptying or upper gastrointestinal symptoms in diabetes.[19]

Thus, aim of this study was to evaluate the prevalence of *H. pylori* infection in DMT2 patients in rural Rajasthan and also to find if there exists any significant correlation between *H. pylori* infection and DMT2.

Materials and Methods:

The study was planned for the period of 6 months. Inclusion and exclusion criteria were designed according to the need of the study. Validated questionnaire was given to all the participants so as to evaluate the prevalence and the intensity of gastrointestinal symptoms (epigastric pain, halitosis, bloating, postprandial fullness, nausea and vomiting). Information was also collected regarding any co-existing disease and details of the current therapy for any other element. Participants those complied with the requirement of the questionnaire were included in the study.

Age and sex matched controls were selected from same geographical location so as to reduce the bias of the study. Care was taken to include patients and controls with similar food habits and life style. The sample size was determined based on the total number of patients visiting the selected locations of the study and the newly diagnosed cases of *H. pylori* during the course of patient screening for the study. In the present study, total of 33 DMT2 patients (21 male and 12 female) and 39 of controls (22 male and 17 female) were enrolled from Pilani and other villages in Jhunjhunu district (Rajasthan). Written informed consent was obtained from each participant before inclusion in the study.

Details were explained to each patient, and the detailed history of the subject was collected using data sheet. The local language (Hindi) was also used while obtaining the information. Two milliliters of blood was drawn by vein puncture, from 33 diabetics and 39 healthy controls. After allowing the blood to clot in glass test tubes, the samples were centrifuged; the serum (volume ranging from 0.4 ml to 1.0 ml) was collected in micro-centrifuge tubes, labeled and stored at -20°C until assayed.

DM was diagnosed according to the American Diabetes Association (ADA) revised criteria and Blood sugar was estimated by enzyme method. Third generation immunienzymometric assay employing Streptavidin-Biotin-based system was used to perform ELISA for diagnosis of *H. pylori*. The commercially available Anti-*H. pylori*-IgG Microplate ELISA kit (Monobind Inc., USA) was used in this study. Non-isotopic immunometric assays are preferred to traditional isotopic ones in recent times. Streptavidin-biotin based advanced immunoenzymometric assays (IEMA) capitalizes on the extremely high binding constant (105 mol/L) of avidin-biotin complex to devise immunoassays that are much more sensitive than simple antibody systems.

The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate through the interaction of streptavidin coated on the well and exogenous added biotinylated *H. pylori* antigen. Upon mixing biotinylated antigen and a serum containing the antibody, reaction between the antigen and the antibody results to form an immune-complex. The interaction is illustrated by the following equation:

h-Ab(x-*H. pylori*) + BtnAg(*H. pylori*) ↔ h-Ab(x-*H. pylori*) - BtnAg(*H. pylori*)

h-Ab(x-*H. pylori*) = human auto-antibody (variable quantity)

BtnAg(*H. pylori*) = biotinylated antigen (constant quantity)

h-Ab(x-*H. pylori*) - BtnAg(*H. pylori*) = immune complex (variable quantity)

Simultaneously, the complex was deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. After an incubation period of 1 hour, the well was washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species specific antibody (anti-IgG) was then added to the mircowells. This conjugate binds to the immune complex that had formed earlier. The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation was separated from un-reacted materials by a wash step. The enzyme activity in this fraction was directly

proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown sample can be ascertained. Five calibrators of known concentrations were used (0, 10, 25, 50, 100 U/ml); 72 samples were loaded on the microtitre wells. The instructions and incubation times were strictly followed. ELISA Reader (Dr. Reddy's Laboratories, Hyderabad, India) was used to measure the absorbance of all the samples and calibrators. The standard curve was plotted and the concentrations of unknown samples were found from the equation. Samples whose concentration was more than 20 U/ml were taken to be positive for *H. pylori*.

Table 1: Demographics of the diabetic patients and controls

	DM	Control	P
Age(years)	46±8.2	49±9.3	NS
Sex			
Male	21	22	NS
Female	12	17	NS
Diabetes duration	7.5±4	-	

DM, diabetes mellitus type2; NS, not significant

Selection of population

Patients who had upper GI endoscopic confirmation of peptic ulcers, long term treatment of non-steroidal anti-inflammatory drugs, history of long term use of chemotherapy for GI diseases, GI malignancies, GI bleeding disorders, unstable cardiac or pulmonary disease, gastro-oesophageal reflux disease or those with past history of *H. pylori* infection or on drug regimen for *H. pylori* infection were excluded from the study. Persons with use of antibiotics or antisecretory therapy, congestive heart failure renal failure were not included. Height and weight were measured using

standard methods and BMI was calculated (data not shown).

The study was approved by the local ethics committee of BITS, Pilani

Results

The results of the analysis were as follows:

Table 2: Summary of ELISA results performed for identification of *H. pylori*

	Diabetic population(n=33)	**Control population(n=39)**	**Total population(n=72)**
***H. pylori* positive**	29(88%)	26(67%)	55
H.pylori negative	4(12%)	13(33%)	17

Table 3: Gender-wise statistics of total population

Category	**No. of males(n=43)**		**No. of Females(n=43)**		**Total**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Controls	14	8	12	5	26	13
Diabetes	19	2	10	2	29	4

Table 4: Analysis of the DMT2 population

Characteristic	***H. pylori* positive**	***H. pylori* negative**
Number	**29(88%)**	4(12%)
Sex(Male: Female)	19:10	2:2

Statistical Analysis:

Comparisons between data of categories were performed by using Chi-square test and alternative

hypothesis that "there is a significant difference between the prevalence of *H. pylori* infection among diabetics and controls" against the null hypothesis that "there is no such difference" was tested.

Among 33 diabetics, 4 were negative for *H. pylori* infection. The calculated value of χ^2 was found to be 4.459 and at degree of freedom=1. The calculated value of p was found to be less than theoretical value ($p < 0.05$). This statistically shows high prevalence of *H. pylori* among the screened DMT2 patients.

Results and Discussion:

H. pylori is one of the most common chronic infections worldwide in DM patients.[20] It also is the main etiologic agent of chronic gastritis and peptic ulcer, and is also related to gastric cancer.[21] Life style factors such as obesity, physical inactivity and a genetic predisposition are important risk factors for the development of DMT2. In addition, chronic infections are well known as determinants for the manifestation of DMT2.

Several studies have investigated the prevalence of H Pylori in diabetic patients and a possible role of the infection in their metabolic control with discordant results.[22-24] Some studies did not find a higher prevalence of *H. pylori* in diabetic patients and did not support any correlation between metabolic control and infection, while others have demonstrated a higher seroprevalence of the infection in diabetic participants and a reduced glycemic control in infected DM patients when compared with uninfected diabetic patients. Similar results were obtained by Berner Et al.[25]. Sargyn Et al. demonstrated that the eradication rate was 50% in diabetic group versus 85% in non diabetic group.[26] A statistically significant correlation was found between *H. pylori* infection and presence of neuropathy by Demir Et al.[27]

Moreover, a link between *H. pylori*, insulin and fasting serum glucose levels has recently been demonstrated.[28] These results could be explained by considering the evidence that some strains of *H. pylori* are considered more virulent; in particular, Cag-A-positive strains. They are presumed to have a higher pathogenic effect on gastric mucosa and are related to duodenal ulcer and gastric cancer. More specifically, Cag-A-positive strains are associated with the increased production of cytokines such as tumor necrosis

factor, interleukin-1, -6, and -8 that might alter the control of glycemia in DM patients. Thus, this correlation study is an important step in assessing the risk of the DMT2 patients with the complications of the *H. pylori* infection.

In the present study, prevalence of *H. pylori* infection in controls was found to be 67% while it was 88% in the DMT2 patients (Table 2). It could be seen that prevalence of *H. pylori* is more in the DMT2 patients than that of control population. Hence, it could be concluded that there is a strong association between *H. pylori* infection and DMT2, based on the chi square statistical test ($p < 0.05$) in the selected patient population.

In control population, it was found that 63.63 % males and 70.58 % females were found positive for *H. pylori*. Thus there was no significant difference for prevalence of *H. pylori* in terms of sex in controls. In case of the DMT2 patients, difference between the prevalence of *H. pylori* could be prominently seen (Table 4). Among the diabetics, 65.51% males have been found positive for *H. pylori*, as compared to the 34.48% of females. Review of literature did not reveal any such difference in the sex; however more number of patients can be studied to test the significance of these results.

This study showed that participants with DMT2 have a higher prevalence of H Pylori infection when compared with control participants. Our results are in consistence with previous reports of a higher prevalence of *H. pylori* infection with DM patients. In case of earlier studies, no specific references were found for DMT2 and *H. pylori* association. Thus this study is a source of important preliminary data for association of DMT2 and *H. pylori* association.

The association of the *H. pylori* infection with the DM patients is an important issue as this association was recently been found significant not only with the metabolic control of the DM but also with the variety of the cardiac complications. It has been recently reported that chronic infection with *H. pylori* may cause atherogenic modification of blood lipids.[29] This patho-mechanism might determine the risk for subsequent cardiovascular diseases in DMT2 patients, however, substantial evidence is not been documented.

Since DM affects both the cellular and humoral components of the immune system[30-32], colonization of *H. pylori* is not prevented efficiently. DM patients suffering from autonomic neuropathy, manifested as gastropathy, may be more prone to *H. pylori* infection. Another possible hypothesis states that presence of circulating antigens like sialic acid in DM patients[33], act as a specific receptor for *H. pylori* on the cell surface.[34]

Although the study outcome shows positive correlation between DMT2 and *H. pylori* association, study involves a small number of DMT2 patient population. For further affirmative confirmation of the hypothesis, population size has to be increased with more demographic locations.More specific correlation with biomarkers like Glycosylated Hb levels (HBA1C) and *H. pylori* specific antibodies such as IgM, IgG, IgM+IgG can reveal a true picture of the correlation based on immune response in DMT2 and *H. pylori* infection.

Based on these observations, more detailed and specific correlation study has been planned with more number of patients so as to confirm the various underlying mechanisms discussed above for association of *H. pylori* and DMT2 patients.

References:

1. Howden CW, Hunt RH. Guidelines for the management of *H. pylori* infection. The Am J Gastro 1998; 93: 2330-2333.

2. Jiang Q, Hiratsuka K, Taylor DE. Variability of gene order in different *Helicobacter pylori* strains contributes to genome diversity. Mol Microbiol 1996; 20: 833-842

3. Lindkvist P, Wadstrom T, Giesecke J. *Helicobacter pylori* infection and foreign travel. J Infec Dis 1995; 172: 1135-1136

4. Hulten K, Han SW, Enroth H. *Helicobacter pylori* in the drinking water in Peru. Gastroenterology 1996; 110: 1031-1035

5. Prasad S, Mathan M. Prevalence of *Helicobacter pylori*. J Gastroenterol Hepatol 1994; 120: 977-981

6. Gill HH, Majumdar P. Age-related prevalence of *Helicobacter pylori* antibodies in Indian subjects. Indian J Gastroenterol 1994; 13: 92-94

7. Khuroo MS, Mahajan R, Zargar SA, Javid G, Munshi S. Prevalence of peptic ulcer in India: an endoscopic and epidemiological study in urban Kashmir. Gut 1989; 30: 930-934

8. Katelaris PH, Tippett GHK, Norbu P, Lowe DG, Brenaan R, Farthing MJH. Dyspepsia, *Helicobacter pylori* and peptic ulcer in a randomly selected population in India. Gut 1992; 33: 1462-1466

9. de Luis, DA, de la Calle H, Roy G, Martin de Angila C, Valdezate S, CantonR, et al. *Helicobacter pylori* infection and insulin-dependent diabetes mellitus. Diab Res and Clin Prac 1998; 39: 143-146

10. Perdichizzi G, Bortari M, Pallio S, Fern MT, Carbone, Barresi G. Gastric infection by *Helicobacter pylori* and antral gastritis in hyperglycemic obese and diabetic subjects. New Microbiol 1996; 19: 149-152

11. Oldenburg B, Diepersloot RJ, Hoekstra JB. High seroprevalence of *Helicobacter pylori* in Diabetes Mellitus patients. Dig Dis Sci 1996; 41: 458-461

12. Ricci JA, Siddique R, Sterard WF, Sandler RS, Sloan S, Farup CE. Upper gastrointestinal symptoms in a US national sample of adults with diabetes. Scand J Gastroenterol 2000; 35: 152-159

13. Agrawal RP, Sharma A, Sharma RK, Kochar SK, Barjatya HC, Kothari RP. Correlation of *Helicobacter pylori* seropositivity with coronary artery disease and retinopathy in diabetics. Paper present at APICON-2000, New Delhi, India

14. Perisco M, Suozzo R, De Seta M, Montella F, Terolla R, Gentile S. Non-

ulcer dyspepsia and *Helicobacter pylori* in type 2 diabetes patients: association with autonomic neuropathy. Diab Res Clin Pract 1996; 31: 87-92

15. Cenerelli S, Bonazzi P, Galeazzi R, Testa I, Bonfigli AR, Sirolla C. et al. *Helicobacter pylori* masks differences in homocysteine plasma levels between controls and type 2 diabetic patients. Eur J Clin Invest 2002; 32:158-162

16. Zelenkova J, Souckova A, Kvapil M, Soucek A, Vejvalka J, Segethova J. *Helicobacter pylori* and diabetes mellitus. Cas Lek Cesk. 2002; 141:575-577

17. Xia HH, Talley NJ, Kam EP, Young LJ, Hammer J, Horowitz M, *Helicobacter pylori* infection is not associated with diabetes mellitus, nor with upper gastrointestinal symptoms in diabetes mellitus. Am J Gastroenterol. 2001; 96:1039-1046

18. Anastasios R, Goritsas C, Papamihail C, Trigidou R, Garzonis P, Ferti A., *Helicobacter pylori* infection in diabetic patients: prevalence and endoscopic findings. Eur J Intern Med. 2002; 13:376

19. Jones KL, Wishart JM, Berry M, Russo A, Xia HH, Talley NJ, Horowitz M, *Helicobacter pylori* infection is not associated with delayed gastric emptying or upper gastrointestinal symptoms in diabetes mellitus. Dig Dis Sci. 2002; 47:704-709

20. Maleki D, Locke GR III, Camilleri M. Gastrointestinal tract symptoms among persons with diabetes mellitus in the community. Arch Intern Med. 2000; 160: 2808-2816

21. Brown LM. *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiol Rev. 2000; 22:283-297

22. Gasbarrini A, Ojetti V, Pitocco D. *Helicobacter pylori* infection in patients affected by insulin-dependent diabetes mellitus. Eur J Gastrienterol Hepatol. 1998; 10: 469-472

23. Marrollo M, Latella G, Melideo D. Increased prevalence of *Helicobacter pylori* in patients with diabetes mellitus. Dig Liver Dis. 2001; 33: 21-29

24. Quatrini M, Boarino V, Ghidoni A, Baldassarri AR, Bianchi PA, Bardella MT. *Helicobacter pylori* prevalence in patients with diabetes and its relationship to dyspeptic symptoms. J Clin Gastroenterol. 2001; 32: 215-217

25. Berber abdulbari, Micallef R, Afifi M, Derbala M, Al-mulla H M, Usmani M A. Association between type 2 diabetes mellitus and *Helicobacter pylori* infection. Turk J Gastroenterol 2007;18(4):225-229

26. Sargyn M, Bayramicli O U, Sargyn H, Dabak R, Orbay E, Yavuzer D, Yayla A. Type 2 Diabetes mellitus affects eradication rate of *Helicobacter pylori*. World J gastroenterol 2003;9(5): 1126-1128

27. Demir M, Gokturk H S, Ozturk N A, Kulaksizoglu M, Serin E, Yilmaz U. *Helicobacter pylori* prevalence in diabetes mellitus patients with Dyspeptic symptoms and its relationship to glycemic control and late complications. Dig Dis Sci 2008;53: 2646-2649.

28. Peach HG, Barnett NE. *Helicobacter pylori* infection and fasting plasma glucose concentration. J Clin Pathol. 2001; 54:466-469

29. Hoffmeister A, Rothenbacher D, Bode G, Persson K, März W, Nauck MA, Brenner H, Hombach V, Koenig W. Chronic infections and lipoproteins in healthy subjects and patients with coronary heart disease – an atherogenic modification?, Aterioscler Thromb Vasc Biol. (In Press)

30. Hussein MJ, Alviggi L, Millwand BA, Lelle RDG, Pyke DA, Vergani D. Evidence that reduced number of natural killer cells in Type 1 (insulin dependent) diabetes may be generically determinated. Diabetologia 1987; 30: 907-11

31. Glass EJ, Steward J, Matthews DM, Collier A, Clarke BH, Weir DM. Impairment of monocyte "lectin-like" receptor activity in type 1 (insulin dependent) diabetes patients. Diabetologia 1987; 30: 228-31

32. Ohno Y, Aoki N, Nishimura A. In vitro production of interleukin-1, interleukin-6, and tumour necrosis factor alpha in insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1993; 77: 1072-77

33. Pickup JC, Day C, Bailey CJ, Samuel A, Oney GD, Garland HO et al. Sialic acid in animal models of diabetes mellitus. Life Sci 1995; 57: 1383-91

34. Valkonen KH, Ringner M, Ljungh A, Walker T. High affinity binding of laminin by *Helicobacter pylori* evidence for a lectin-like interaction. FEMS Intl Med Microbiol 1993; 7: 29-37