Original Article

Cytotoxin-Associated Gene (CAG) a of Helicobacter Pylori Seropositivity in Type 2 Diabetic Patients; Relation to Insulin Resistance, Beta Cell Function and Glycemic Control.

Eman Youssef Moursy[1], Azza Abdel Karim Ismael[1], Dalia Abdel Moaty El Neily[2], Marwa Ahmed Meheissen[3], Dalia Ibrahim Zakaria Ali[1].

Department of Internal Medicine.[1] Department of Clinical and Chemical Pathology.[2] Department of Microbiology and Immunology.[3] Faculty of Medicine University of Alexandria.

Abstract:

Introduction: diabetes mellitus is a worldwide problem and helicobacter pylori is a chronic infection occur in diabetic patients. **Objective:** to determine the relation between H.pylori Cag-A seropositivity and HOMA-IR, HOMA-B, and glycemic control in type 2 diabetic patients. **Methods:** 91 male diabetic patients (more than 40 years) were included in the study they were subjected to full history taking , clinical examination stressing on blood pressure, BMI, WHR, and laboratory investigations including: FBG, fasting insulin, HbA1c, HOMA-IR, HOMA-B, inflammatory markers (WBCs, ESR, hsCRP), lipid profile (TG,HDL-c,LDL-c, Cholesterol) and the seroprevalence of Cag-A IgG antibodies to H.pylori was performed for all patients. **Results:** 44 Cag-A seropositive were detected. They showed a significantly higher duration of diabetes (P=0.026*),BMI (P<0.001*), inflammatory markers (WBCs P<0.001*, hsCRP P=0.025*, ESR P<0.001*), HbA1c (P<0.001*), fasting insulin (P=0.039*), HOMA-B (P=0.013*), HOMA-IR (P=0.027*), serumTG (P=0.035*) and a significantly lower HDL-c (P<0.001*) than seronegative group. **Conclusion:** inflammatory mechanism of Cag-A infection may play a role in development of IR, ISD, obesity, and poor glycemic control in type 2 diabetic patients.

Keywords: CAG, H.Pylori, T_2DM

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs especially the eyes, kidneys, heart, nerves and blood vesseles.(1)

Type 2 diabetes mellitus (T2DM) continuous to be a major health problem worldwide. It is well known that T2DM is a metabolic disorder characterized by hyperglycemia, which arises from insufficient pancreatic insulin secretion, insulin resistance, and inadequate suppression of glucagon production.(1) This suppression in inadequate uptake, storage, and disposal of ingested glucose is accompanied by elevated hepatic production of glucose and profound hyperglycemia.(1)

H.pylori is a curved spiral shaped, gram-negative, bacteria about 0.5×3 µm in size and have up to 7 sheathed flagella that extend from one end and give the organism the mobility to withstand rhythmic gastric contractions and penetrate the gastric mucosa.(2) H. pylori produces a number of virulence factors, including vacuolating cytotoxin (vacA) and cytotoxin associated gene A (cagA) which causes cell injury and inflammation.(3)

The topic of extra-gastric manifestations of Helicobacter pylori infection continues to capture the attention of many researchers all over the wor ld. That it is the most common chronic infection in human and has been associated with a variety of extra-gastro-intestinal manifestations.

Infection with H.pylori causes the release of pro-inflammatory cytokines and vasoactive substances, such as (tumor necrosis factor α TNF- α, interferon-gamma, interleukin IL-1, 6, 8, 10, 12), eicosanoids as (leukotrienes, prostaglandins), and acute phase proteins (fibrinogen, C-reactive protein). It also increases inter cellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) , enhances platelet activation and platelet-leukocyte aggregation,[4] and was found to alter the apoptotic process.[5] The increased oxidative stress causes inflammation, accumulation of reactive oxygen species ROS, and oxidative DNA damage due to neutrophil infiltration.[6] This inflammatory mechanism was proved to be involved in the pathogenesis of IR.[7]

Furthermore the infection leads to reduction of vitamin B12 and folate acid concentrations, and the consequent increased of homocysteine (Hhcy) and lipid peroxide,[8] lower ghrelin [9] and increase leptin[10] levels, which are associated with impaired energy homeostasis, lipid metabolism, elevated fasting insulin levels and insulin sensitivity.[10]

Moreover, fetuin A, another acute-phase glycoprotein involved in mineralization and insulin signaling regulation.[11] Its dysregulation results in an excessive inhibition of insulin signaling in the liver and skeletal muscle.[12] H.pylori infection decreases the level fetuin A leading to defect in insulin signaling and development of insulin resistance.

Furthermore, fetuin-A levels appear significantly increased after successful H.pylori eradication treatment. The specific virulence factor cytotoxin associated gene A (CagA) has more strong relationship with the pathogenesis of type 2 diabetes that it implicated in insulin resistance and insulin secretion along with other traits that comprise the Metabolic Syndrome.[7]

Methods:

The study included 91 type 2 diabetic male patients above 40 years divided in to **Group I**: H.pylori (Cag-A) seropositive diabetic patients and **Group II**: H.pylori (Cag-A) seronegative of diabetic patients. They were selected from the outpatient clinic of the Diabetes and Metabolism Unit, Alexandria Main University Hospital. The study was approved by hospital ethics committee and written consents were obtained from all subjects after explaining the nature and the aim of the study.

Exclusion Criteria: Subjects who have history of peptic ulcer or previous H.pylori eradication, evidence of connective tissue or haematological diseases, severe cardiac decompensation, known history of myocardial infarction, cerebrovascular stroke, severe uncontrolled hypertension, end stage liver or renal diseases, inflammatory and neoplastic disorder, and who have urinary tract infection or any other infectious diseases are excluded from the study.

Clinical Examination: All patients were subjected to full history taking including (age in years, duration of diabetes in years and smoking habits), clinical examination, blood pressure measurement and anthropometric parameters were recorded as Body mass index **(BMI)** according to the **Quetelet equation: BMI=Body weight in (kg) / Height in (m^2)**. Waist hip ratio **(WHR)** was calculated according to the formula **WHR=Waist circumference / Hip circumference**.

Laboratory Investigation: done to each patient were **FBG** by hexokinase method, **HbA1c**, serum **TG**, serum **Cholesterol**, **HDL-c** by automated Hitach 912 autoanalyzer, [13,14] **LDL-c** was calculated by Friedewaled equation,[15] **hsCRP** by automated nephlometery,[16] **WBCs** by using System XT-1500, **ESR** by automated analyzer, Fasting insulin by IMMUNLITE immunoassay analyzer,[17] **HOMA-IR** was calculated according to the equation **HOMA-IR = (Fasting glucose in mg/dl×fasting insulin in mu/L)/405.**[17] **HOMA-B** was calculated by equation **HOMA-B= (Fasting insulin in mu/L×360)/(fasting glucose in mg/dl-63)**.[17]

Serological Diagnosis: Detection of specific **IgG** antibodies against H.pylori **(CagA)** was performed for all patients using ELISA (EUROIMMUN kits. UK).[18]

Statistical analysis:[19] Data were statistically analyzed using IBM SPSS (Statistical Package

for the Social Science) software package version 20.[20] Data were expressed as range, mean, standard deviation, and median. Chi-square test, t-test, and ANOVA tests were used for comparison between groups. Pearson correlation co-efficient used for comparison between variables.

Results:

The duration of diabetes was significantly longer in Cag-A seropositive diabetic patients than Cag-A seronegative diabetic patients (P=0.026*), BMI was also higher in Cag-A seropositive patients(P<0.001*), inflammatory markers including (WBCs, hsCRP, ESR) were higher in Cag-A seropositive diabetic patients (P<0.001*, P=0.025*, P<0.001*) than Cag-A seronegative diabetic patients. HbA1c, Fasting insulin, HOMA-IR, HOMA-B were significantly higher in Cag-A seropositive patients (P<0.001*, P=0.039*, P=0.027, P=0.013*) respectively. Regarding to lipid profile serum TG was higher in Cag-A seropositive patients (P=0.035*) while than HDL-c was significantly lower (P<0.001*).

Table I: Comparison between 2 groups in all parameters:

Parameter	Seropositive Cag-A (48.4%)	Seronegative Cag-A(51.6%)	p-value
Age (Y) **Mean± SD** **Median**	42.0-76.0 57.31 ± 8.79 56.50	41.0 – 72.0 58.06 ± 8.26 59.0	0.673
Duration of DM (Y) **Mean± SD** **Median**	2.0 – 21.0 6.97 ± 4.99 5.75	0.50 – 15.0 4.97 ± 3.61 4.0	0.026*
Smoking **No** **Yes** **Ex-smoker**	21 (47.7%) 19 (43.2%) 4 (9.1%)	20 (42.6%) 19 (40.4%) 8 (17.0%)	0.533
BMI Kg/m^2 **Mean± SD** **Median**	34.02 – 60.10 41.18 ± 5.42 40.35	22.80 – 44.20 30.67 ± 4.15 30.40	<0.001*
WHR **Mean± SD** **Median**	1.10 – 1.93 1.39 ± 0.18 1.36	1.04 – 1.90 1.38 ± 0.19 1.33	0.757
Systolic Bp(mmHg) **Mean± SD** **Median**	90.0 – 160.0 128.64 ± 18.75 130.0	100.0 – 160.0 128.51 ± 14.74 130.0	0.972
Diastolic BP(mmHg) **Mean± SD** **Median**	60.0 – 100.0 82.05 ± 10.47 80.0	60.0 – 100.0 78.94 ± 12.20 80.0	0.197
WBCs×10^3/µl **Mean± SD** **Median**	4.0 – 19.90 14.48 ± 3.37 14.05	2.10 – 12.0 6.70 ± 2.64 6.30	<0.001*
hsCRP mg/dl **Mean± SD** **Median**	1.10 – 39.40 7.35 ± 6.98 5.20	1.0 – 17.20 4.56 ± 3.80 3.20	0.025*
ESR1 **Mean± SD** **Median**	4.0 – 65.0 17.45 ± 15.59 11.50	2.0 – 65.0 13.62 ± 17.80 7.0	<0.001*
ESR2 **Mean± SD** **Median**	6.0 – 117.0 35.66 ± 25.37 27.0	5.0 – 110.0 28.43 ± 30.43 17.0	<0.001*
FBG (mg/dl) **Mean± SD** **Median**	70.0 – 315.0 179.30 ± 66.52 184.0	70.0 – 300.0 177.60 ± 61.99 172.0	0.900

HbA1c(%) **Mean± SD** **Median**	3.80 – 12.60 9.87 ± 2.38 10.30	3.90 – 12.50 8.01 ± 2.18 8.0	<0.001*
Fasting insulin(µu/ml) **Mean± SD** **Median**	4.06 – 57.50 19.96 ± 13.03 14.60	2.90 – 57.50 15.60 ± 11.92 11.50	0.039*
$HOMA_2$-IR **Mean± SD** **Median**	0.50 – 8.10 2.95 ± 1.88 2.15	0.40 – 10.20 2.40 ± 2.05 1.60	0.027*
$HOMA_2$-B **Mean± SD** **Median**	10.20 – 283.80 74.33 ± 63.32 51.55	5.10 – 236.10 48.57 ± 48.83 33.80	0.013*
Serum TG (mg/dl) **Mean± SD** **Median**	145.0 – 358.0 222.91 ± 53.0 224.0	120.0 – 265.0 202.62 ± 34.66 212.0	0.035*
Serum cholest (mg/dl) **Mean± SD** **Median**	210.0 – 400.0 296.98 ± 45.02 301.50	180.0 – 370.0 296.19 ± 39.56 302.0	0.930
HDL-c (mg/dl) **Mean± SD** **Median**	10.0 – 35.0 21.09 ± 8.04 23.0	14.0-48.0 30.45 ± 8.51 33.0	< 0.001*
LDL-c (mg/dl) **Mean± SD** **Median**	115.0 – 441.0 199.18 ± 53.13 198.0	90.0 – 255.0 193.28 ± 34.30 198.0	0.733

P-values ≤ 0.05 was significant and ≤ 0.01 highly significant.

Discussion:

In the present study, the duration of diabetes was significantly longer in seropositive Cag-A patients which show agreement with Roga et al.[21] and disagreement with Pietroiusti et al.[22] and Christie et al.[23]

In our study, BMI was significantly higher in seropositive Cag-A patients which is in agreement with Chen et al,[24] Arslan et al,[25] and Isomoto et al. The gastric colonization with Cag-A positive strains reduces gastric motility which would explain increase BMI in those patients.[26,27] However the studies of Jamshid et al,[28] Pietroiusti et al,[22] and Christie et al,[23] not report this difference in BMI.

In our study, inflammatory markers were significantly higher in seropositive Cag-A patients which is in accordance with Siddiqui et al,[29] Diomedi M et al,[30] Roga et al.[21] This would be attributed to production of hsCRP which is a hepatic protein produced in acute phase of inflammation, and its synthesis is regulated by various cytokines, predominantly IL-6.[31] which is released during H.pylori Cag-A infection.

In the present study, HbA1c and Fasting insulin were higher in seropositive Cag-A patients in agreement with Fernandini et al,[32] and Bener A et al.[33] This association may be through Cag-A strains containing type 4 secretory apparatus which allows translocation of Cag-A protein in to the cells and induces pro-inflammatory cytokines release and proliferation of the cells. This mechanism is considered an important contributor in metabolic syndrome.[34] However Jamshid et al,[28] Tanriverdi et al,[35] and Pietroiusti et al,[22] did not report this any significant difference in HbA1c and Fasting insulin.

In our study, HOMA-IR and HOMA-B were significantly higher in seropositive Cag-A patients which shows agreement with Wong F et al,[36] Franceschi F et al,[37] Afzalur et al.[38] This association between seropositivity and HOMA-IR and HOMA-B is due to that Cag-A infection stimulate the release of insulin counter regulatory hormones also induce hyperinsulinemia by decrease serum concentration of somatostatin which has inhibitory effect on insulin secretion which

lead to IR and ISD.[39] In contrast, Gillum et al, Park et al,[40,41] Naja et al, and Lu et al. [42,43] did not report any significant difference in HOMA indices (IR and B).

In our study, serum TG was higher and HDL-c was lower significantly in seropositive Cag-A pa tients. This show agreement with Hoffmeister A et al, and Roga et al. studies which report that significant difference in TG and HDL-c.[44,21] In contrast a meta-analyses of 18 studies involving 10000 seropositive Cag-A diabetic patients found no strong correlation between the infection and serum concentrations of TG and HDL-c.[45]

Conclusion: Cag-A seropositivity is associated with development of Obesity through increase body mass index also the inflammatory mechanism of the Cag-A infection may play a role in development of IR, and ISD, the infection also increase HbA1c leading to poor glycemic control.

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