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Journal

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Contents

Editorial Article

Original Articles

Letter from The Editor

Dear Colleague,

In this volume, we have included a very important editorial article which deals with an important issue of health care for persons with diabetes i.e. Peripheral Neuropathy. Two colleagues from Democritus University of Thrace and from the University Hospital of Alexandroupolis, Athens; Greece Nikolaos Papanas and Prof. Theano Penlioglou are sharing their experience with an article entitled: The role of alpha-lipoic acid in the treatment of diabetic peripheral neuropathy. Once again, we hope to meet your expectations, and until we meet in our next issue, deepest regards and best wishes.

The Editor

Prof. Samir Helmy Assaad Khalil

Editorial Article

The Role of Alpha-Lipoic Acid in The Treatment of Diabetic Peripheral Neuropathy

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Diabetic neuropathy (DN) is one of the major complications of diabetes mellitus (DM), affecting approximately one third of DM patients, while recent evidence indicates that it could be diagnosed even in the stage of prediabetes [1-3]. A variety of factors have been implicated, including DM duration, poor glycaemic control, vascular risk factors and others [1, 2, 4, 5].

The commonest form of DN is distal symmetrical polyneuropathy, also called diabetic peripheral neuropathy (DPN) [4, 5]. DPN affects both small and large nerve fibres, with a stocking-and-glove distribution. It represents a cardinal risk factor for diabetic foot ulcerations [1, 4, 5]. Additionally, some patients complain of neuropathic pain, described as burning, pins and needles, sharp, cramping, tingling, cold, or allodynia [1, 4, 5].

Diagnosis of DPN and alleviation of pain have greatly improved [1, 4, 5]. However, improvement is still needed in pathogenesis-oriented treatment [2, 4, 5]. Alpha-lipoic acid (ALA) is very useful in this context [4, 6]. It is an anti-oxidant, which is of particular relevance, given that oxidative stress is implicated in the pathogenesis of DPN [7-9]. ALA plays an important role in mitochondrial bioenergetic reactions. It contains two thiol groups capable of being oxidised or reduced. Its reduced form is dihydrolipoic acid and its oxidised form as ALA [6-9]. It crosses the blood-brain barrier and regenerates other antioxidants, such as vitamin C, vitamin E, and glutathione [7-9]. Acute ALA infusion appears to improve nitric oxide-mediated endothelium-dependent vasodilation, thus improving microcirculation in patients with DN [7-9]. Moreover, there is evidence that ALA reduces lipid peroxidation of nerve membranes, further protecting from the development of DPN [9].

ALA was recognised as an anti-oxidant in the 80s [10]. Normally, it is produced by both animals and human and can be detected in liver and skeletal muscles [7-10]. Today, except for natural sources, such as spinach, broccoli, tomatoes, garden peas and Brussels sprouts, there are also nutritional supplements of ALA, which are typically comprised either of R-alpha-lipoic acid alone or a racemic mixture of R-alpha-lipoic acid and S-alpha-lipoic acid [7-10].

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In DPN, the usual oral daily therapeutic dose is 600 mg [6-13]. It has also been used intravenously [13]. A meta-analysis by Ziegler et al. [14] has shown significant improvements in pain, burning, and numbness, as well as pin-prick and touch-pressure sensation and ankle reflexes after 3 weeks. Adverse events were not increased [13] .

The 4-year NATHAN (Neurological Assessment of Thioctic Acid in Diabetic Neuropathy) 1 trial [15] is the largest and longest trial with ALA. Overall, 460 DM patients were randomised to ALA or placebo. ALA achieved significant improvements in some clinical signs, especially muscular weakness of lower limbs [15]. Among ALA-treated subjects, improvement was more frequent and deterioration was rarer in comparison with placebo. However, no improvements were seen with ALA in nerve conduction attributes and quantitative sensory testing [15]. Of note, male gender, normal body-mass index and blood pressure, history of cardiovascular disease along with DM and DPN duration and DPN severity were identified as predictors of more favourable response to ALA treatment [16].

In the recent years, favourable experience with ALA has accumulated further through studies and pooled analyses [4, 6, 17]. Impressively, a more recent trial has demonstrated reduced neuropathic symptoms and triglycerides, as well as improved quality of life after 40 treatment days [18].

Furthermore, ALA may exert some beneficial metabolic effects, notably reductions of serum glucose and body weight, as well as improvements in insulin action and sensitivity [6] These actions represent added benefits for DM patients. Generally, ALA is very well tolerated [6] .

In conclusion, ALA has repeatedly demonstrated meaningful efficacy and acceptable safety in the treatment of DPN [4, 6] . It represents a useful pathogenesis-oriented treatment that deserves to be considered by the everyday clinician.

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Original Article

Study of the Prevalence of Helicobacter Pylori Infection Among a Cohort of Diabetic Individuals in Alexandria

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Abstract

The aim of our study was to estimate the prevalence of H. pylori infection in diabetics and to elucidate the effect of infection on HbA1C% control; Also, to investigate the correlation between H. pylori infection and duration & complications of DM and other parameters. **Patients & methods:** In this case control study, 100 type 1 diabetic patients, 100 type 2 diabetic patients and 100 control subjects were included. H. pylori were tested by stool antigen test. Also, we estimated HbA1c level. **Results:** We found no association between H.pylori infection and diabetes mellitus; The adjusted odds ratio estimates of H. pylori infection among type 2 diabetics and type1 diabetics compared to non-diabetic subjects were 0.89 (95 % CI: 0.43- 1.85) and 1.33 (95 % CI: 0.24- 1.05) respectively; i.e. non -significant, also H. pylori had no effect on the control of HbA1c level. No correlation was found between H. pylori infection and duration of DM& the incidence of diabetic complications. No association was found between H. pylori infection and the incidence of upper gastrointestinal symptoms among the three groups. No correlation was found between H. pylori infection and age, sex, BMI and social standard in the three groups.

Key words: Helicobacter pylori; Type 2 diabetes; Type 1 diabetes; Insulin resistance; Inflammation; Cytokines; BMI (body mass index); HbA1c (glycosylated haemoglobin).

Introduction

Helicobacter pylori is a gram-negative, spiral shaped pathogenic bacterium that specifically colonizes the gastric epithelium causing chronic gastritis, peptic ulcer disease, and/or gastric malignancy.(1) H. pylori is mainly acquired in childhood by the fecal-oral, oral- oral or gastro-oral route, and has been recognized as a worldwide public health problem that is more prevalent in developing countries.(2) Recently, there is a rapidly growing body of literature concerning a possible association between H. pylori infection and extra-gastric disorders. The extra-digestive diseases have been reported in those with evidence of H. Pylori infections in recent years include ischemic heart disease (3), autoimmune thyroid diseases (4), sideropenic anemia,(5) idiopathic thrombocytopenic purpura,(6) neurologic diseases,(7) and hepatobiliary diseases.(8) Indeed, The postulated role of H. pylori in the pathogenesis of extra gastric disorders is based on the facts; this bacterium produces a chronic low grade inflammatory state that lasts for several decades which is able to induce lesions both locally and remote to the primary site of infection, induces molecular mimicry mechanisms, and interferes with the absorbance of nutrients and drugs, possibly influencing the occurrence and/or evolution of many diseases.(9) Emerging data now indicate a strong relationship between H. pylori infection and the incidence of DM especially type2;(10) Nevertheless, there are other studies reported lack of such associations. (11).

Methods

This study was conducted from May 2015 to May 2017. We enrolled 300 subjects; diabetic patients were from the endocrinology clinic and non-diabetic control patients were from the general internal medicine clinic; the young subjects were from Students health insurance hospital (Sporting) and the older ones were from Alexandria main University hospital (AMUH); they were classified as group I: 100 patients with type 2 diabetes, group II: 100 patients with type 1 diabetes, group III: 100 non diabetics of matched age, sex and socioeconomic standard. We excluded patients who received proton pump inhibitors or antibiotics for the last three months. H. pylori were tested by stool antigen test "On Site H. Pylori Ag Rapid Test-cassette". HbA1c values were determined with High performance liquid chromatography (HPLC) and HbA1c below 6.54 % was considered normal. [12] All subjects gave informed consent to participate in the study. The study was approved by the Ethics Committee of Alexandria University; faculty of Medicine. Chi square and t-test were used for assessing the differences between the nominal and numerical variables respectively. Furthermore, logistic regression analysis was performed to measure the association between diabetes mellitus and H. Pylori infection. All analyses were performed at the 95 % significance level ($P < 0.05$) using statistical software IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp).

Results

There was no statistically significant difference between type 1 DM and type 2. Also there was no statistically significant difference between type 1 DM and the control group. The only statistically significant difference ($p<0.05$) was between type 2DM and the control group where infection was more in the control group than in type 2 DM; Table (I); as the prevalence of H .Pylori is highest in the control group (45%), then 38% in type 1 DM and lowest in type 2DM (31%) but after adjustment for age; Table (II), there was no significant correlation between the prevalence of H. pylori infection and diabetes. As regards the HbA1c control among the H .Pylori infected and the non-infected patients, no statistically significant association was found between the increase in HbA1c level and the presence of H .Pylori infection in both type 1 and type 2 DM; Tables (III, IV). The correlation between H. pylori positivity and the duration of DM in type 1 and type 2 showed that there was no statistically significant association between H. pylori infection and duration of DM; Tables (III,IV). In addition, the correlation between H. pylori positivity and the complications of DM in type 1 and type 2 showed no significant association between H. pylori infection and the incidence of diabetic complications; Tables (III,IV). As regards the correlation between the occurrence of upper GIT symptoms and H. pylori infection in the three groups, there was no statistically significant association between H. pylori infection and upper GIT symptoms in type1 DM& the controls, But there was statistically significant association between H. pylori infection and upper GIT symptoms in type 2 DM; as the percentage of occurrence of upper GIT symptoms was higher (69.6%) in H. Pylori negative than in the H. Pylori positive (32.3%); Tables (III,IV&V). No statistically significant correlation was found between H. pylori infection and age, sex, BMI and social standard in the 3 groups; Tables (III,IV&V). No statistically significant correlation was found between the increase in HbA1c level (≥ 6.5) and H. pylori infection in patients with high BMI (≥ 25 kg/m²) in group I and group II ;Table (VI). We noticed a higher prevalence of the infection in Hadara, Asafra, Sidi bishr and Fectoria.

Discussion

Although there is no concrete evidence demonstrating that H. pylori plays a role in diabetes, the possibility for a causal relationship is an intriguing issue deserving discussion. There to infection in diabetic patients. Firstly, a diabetes-induced impairment of cellular and humoral immunity may enhance an individual's sensitivity to H. pylori infection. [14] Secondly, diabetes-induced reduction of gastrointestinal motility and acid secretion may promote pathogen colonization and infection rate in the gut. [15] Thirdly, altered glucose metabolism may produce chemical changes in the gastric mucosa that promote H. pylori colonization. [16] Finally, individuals with diabetes are more frequently exposed to pathogens than their healthy counterparts as they regularly attend hospital settings. [17] However, there are also indications that H. pylori infection may contribute to the development of diabetes. DM especially type 2 and its complications have a complex pathophysiology including insulin resistance (IR), glucotoxicity, lipotoxicity, B-cell dysfunction, chronic inflammation, and genetic and epigenetic factors; [18] with risk factors related to lifestyle (e.g., diet, obesity& physical activity) and socioeconomic factors; [12] One from its risk factors are the gastrointestinal infections and the

composition of intestinal microbiota. [19] Recent evidence implicates the pathological involvement of inflammation in T2DM, which is an important process induced by H. pylori infection. [18] Recently, there is a growing evidence concerning an association between H. pylori infection and insulin resistance, [20] chronic inflammation,[21] the secretion of gastric-related hormones such as leptin and ghrelin, as well as gastrin and somatostatin which may influence a predisposition to diabetes [22]and insulin secretion deficiency.[23]Furthermore, the presence of Gram-negative bacteria, such as H. pylori, in the gut microbiota leads to increased production of lipopolysaccharide, which also activates innate inflammatory processes. [24] An alternative hypothesis is that gastro duodenal conditions resulting from H. pylori infection could delay gastric emptying [25] which has been postulated to cause poor glucose control in insulin-dependent children with diabetes.[26].The relationship between H. pylori and diabetes mellitus was first explored in 1989 by Simon, et al. [27] who found that the prevalence of H. pylori infection in patients with diabetes mellitus was significantly higher than in asymptomatic controls (62% vs. 21%).%). However, the test used for detecting H. pylori was only a rapid urease test, and their comparison did not adjust for age, which is a major confounding factor. In the present study, there was no statistically significant difference in the prevalence of H. pylori fecal positivity between diabetic patients either type 1(38%) or type 2 (31%) and the controls (45%); it even was higher in the controls than diabetics. i.e. we found no relation between H. pylori infection and diabetes mellitus. The study of Keramat, et al. was from the studies supporting our results where 79 diabetic patients and 84 control subjects with upper gastrointestinal symptoms; the serology test for H. pylori was positive in 54.4 % of diabetics and 61.4 % of non-diabetic patients (P = 0.689, non-significant); the prevalence of H. pylori increased with HbA1c level but there was no statistically significant association (P =0.565). No association was found between H. pylori infection and upper gastrointestinal symptoms among diabetics, prediabetics and controls. [28] Similar findings were observed by Sotuneh, et al. who revealed that in a large population of elderly in northern part of Iran, HP infection is not associated with BMI, serum glucose and lipid profile as well as blood pressure even after adjustment for other risk factors. [29] There are other studies which have not found a higher prevalence of H. pylori in diabetic patients and have not supported any correlation between metabolic control and infection. [30- 32] Therefore, DM might not be a risk factor for acquisition of H. pylori infection; the presence of microangiopathy in patients with DM may be a negative factor for colonization by H. pylori, because microvascular changes in the gastric mucosa may create an unfavorable environment for the establishment or survival of H. pylori. [33] In these cases the results may be also explained by the higher number of antibiotics taken by diabetics and, thus, a more frequent occasional clearance of the infection. [19] Contrary to our findings, Han, et al. [34] suggested that H. pylori infection was associated with the risk of type 2 diabetes in a middle-age and old-age Chinese population; as individuals with H. pylori infection" diagnosed by urea breath test" had a higher prevalence of type 2 diabetes (21.3% versus 20.2%, p = 0.026) . H. pylori infection was associated with higher risk of type 2 diabetes (odds ratio, 1.08 (95% confidence interval: 1.02–1.14); p = 0.008) after adjustment for other confounders. The association was significant among women, those who were above 65 years old, not overweight or obese, and those who did not smoke, did not consume alcohol and without family history of diabetes. Subjects with H. pylori infection had higher levels of HbA1c and fasting blood glucose (p < 0.0001) than those who did not. Similarly, other studies [10, 35- 38] reported a higher prevalence of infection in diabetic patients. Despite the evidence implicating a link between H. pylori infection and inflammation that predisposes individuals to T2DM, there are some contradictory data. Park, et al. [39] reported that metabolic and inflammatory parameters, including blood sugar, lipid profiles, IR, white blood cell count, and C-reactive protein (CRP) levels, were not changed after H. pylori eradication. A study by Jeon, et al. [15] failed to find any significant association between levels of inflammatory mediators (CRP and IL-6) and H. pylori infection or T2DM. A study by Brown, et al. [40] indicated that leptin has a protective role on pancreatic B cell function, showing that leptin (which increases in H. Pylori infection) could prevent apoptosis of pancreatic B cells through modulation of the Bcl protein (anti apoptotic protein) family. Several meta-analyses were conducted to estimate diabetes risk with H. pylori infection; Jun-Zhen Li, et al. [41] conducted a meta-analysis which included 79studies involved 57,397 individuals. The prevalence of H. pylori infection in DM group (54.9%) was significantly higher than that (47.5%) in non-DM group (OR = 1.69, P < 0 001). The difference was significant in

comparison between type 2 DM group and non-DM group (OR = 2.05), but not in that between type 1 DM group and non-DM group (OR = 1.23, 95% CI: 0.77–1.96, P = 0 .38). Their meta-analysis suggested that there is significantly higher prevalence of H. pylori infection in DM patients as compared to non-DM individuals. And the difference is associated with type 2 DM but not type 1 DM. The above conflicting results may be explained by considering that most previous studies attempting to clarify the association between H. pylori infection and DM were limited by cross-sectional analyses. Variables like age, sex, race, economic status, DM prevalence, and strains of H. pylori infection in the included studies varied. For the lack of enough detailed data, subgroup analysis stratified by age, sex, different stages of DM, and strains of H. pylori, which might bring up heterogeneity, could not be carried out. More well-designed and prospective cohort studies are needed for clarifying the association between H. pylori infection and DM to overcome methodological limitations of previous cross-sectional studies. To date, there are only two prospective studies to examine the impact of H. pylori infection on development of diabetes. Jeon, et al. [15] conducted a study in Community dwelling elderly Latinos followed up for 10 years and showed that individuals who were seropositive for H. pylori at enrollment were 2.7 times more likely at any given time to develop DM than seronegative individuals (HR = 2.69; 95%CI: 1.10-6.60), after adjustment for multiple factors, including age, gender, ethnicity, education, and cardio- metabolic risk factors. Thus, that study was able to establish the relative timing of seropositivity and development of DM, giving more credence to a potential causal relationship. However, several issues must be considered in interpreting these results. First, similar studies need to be repeated in other populations to ensure that the findings are related to the presence of infection itself and are not a peculiarity of the H. pylori-infected subjects in their community (i.e., due to particular dietary or living habits that may be linked to vulnerability to infection and diabetes). Second, findings in elderly individuals may not be generalizable to younger individuals considering that a younger population has a shorter history of infection. Third, only a small percentage of the population was seronegative for H. pylori (7%), which limited the power of the study. Finally, evaluation of the H. pylori infection status depended solely on the detection of H. pylori IgG antibody without further laboratory assessment such as urease breath testing. The presence of the H. pylori antibody does not distinguish recent vs. historic H. pylori infection. Candelli et al [42] evaluated the reinfection rate of H. pylori three years after a standard eradicating treatment and the late effect of eradication upon metabolic control in young diabetic patients. 75 diabetic patients and 99 controls re-evaluated for the presence of H. pylori by means of 13C-Urea Breath Test, metabolic control and the prevalence of gastrointestinal symptoms. The prevalence of H. pylori infection was higher in diabetic patients than in dyspeptic controls of similar age, gender and socioeconomical status after three years of follow-up; i.e. young patients with diabetes present a higher risk of H. pylori gastric reinfection than controls. Our study showed there was no statistically significant association between the increase in HbA1c level and the presence of H .Pylori infection in both type 1 and type 2 DM& no statistically significant correlation between the increase in Hb A1C level (≥ 6.5) and H. pylori infection in patients with high BMI (≥ 25 kg/m²). Keramat, et al, [28] Chabot, et al. [31] and others [44] reported no association. Vafaeimanesh, et al. [46] found that in patients with T2DM, the mean decrease in HbA1c and fasting plasma glucose levels in eradicated cases was similar to non-eradicated subjects three and six months after treatment. There are similar reports showing no effect of H. pylori eradication on HbA1c levels. [43, 45] On the other hand, Some authors have found an influence of H .Pylori infection on metabolic control like Han, et al. [34] and Bajaj, et al. [47] .Chen and Blaser [48] in two large national surveys: the National Health and Nutrition Examination Survey (NHANES) III and the NHANES 1999-2000. Their report showed that H. pylori seropositivity, and H. pylori cag A (cytotoxin associated gene A) positivity in particular, was associated with higher mean HbA1c levels, an association that persisted after excluding individuals with a history of diabetes mellitus and controlling for potential confounders. The association was evident mainly in adults over 18 years of age. They also showed a synergistic effect of H. pylori and BMI on increased levels of HbA1c, indicating a role of H. pylori in impaired glucose tolerance in adults that may be potentiated by a higher BMI level. Zojaji, et al. [49] showed that H. pylori treatment can improve the mean HbA1c and the metabolic abnormalities in patients with T2 DM. The data of present study didn't support any significant correlation between H. pylori infection and BMI. Sotuneh, et al. [29] supported our results in this correlation; they

reported that H. pylori infection is not associated with BMI. Furthermore, there was an inverse relationship between morbid obesity and H. pylori seropositivity in the study of Wu MS, et al. [50]Also, there are data demonstrating that H. pylori eradication significantly increases the incidence of obesity in patients with peptic ulcer disease, as it increases BMI [51], and/or enhances the appetite of asymptomatic patients by elevating plasma ghrelin and reducing leptin levels. [52] Han, et al. [34] reported a significant association among those who were not overweight or obese. Contrary to our findings, a study by Cohen, et al. [53] demonstrated that adults infected with H. pylori had higher BMI levels, even if asymptomatic, and further suggested that H. pylori therapy may lead to weight loss and improve diabetic control. In the present study, there was no statistically significant association between H. pylori infection and duration of DM. Similar findings were observed by a study carried out by Chabot, et al., [31] Zikri [36]and others. [44,45,55] In contrast, Arslan, et al. [37] stated that the rate of infection increases with IDDM duration. Sohair B, et al. [54] concluded that the infection by virulent strain is associated with older age of patients, larger BMI, Higher HbA1c and lower age of onset of diabetes; i.e. it is correlated with longer duration. Our study showed no significant association between H. pylori infection and the incidence of diabetic complications; This agree with Zafar, et al.; [55] who found that prevalence of Helicobacter pylori infection had no significant correlation with duration of diabetes, type of diabetes, glycaemia levels of diabetics and complications of diabetics Similarly, Zikry [36] found no significant relation between H. pylori seropositivity and presence of micro albuminuria. On the other hand, Zhou, et al. [32] supported an association between H. pylori infection and diabetic nephropathy. Kayar, et al. [56] reported a significant relationship between H. pylori infections and diabetic complications. In the present study, there was no statistically significant association between H. pylori infection and upper GIT symptoms in T1DM patients and in the controls. But there was statistically significant association in T2DM patients; symptoms were more frequent in H. pylori negative patients (69.6%) than H. pylori positive patients (32.3%). This is consistent with the results of Keramat, et al. [28] and others. [57] Others maintain that H. pylori infection does not affect the rate of gastric emptying in diabetic patients. [58] These findings are inconsistent with those of Arslan [37] and Senturk. [38] Gastroduodenal conditions resulting from H. pylori infection could delay gastric emptying, which has been postulated to cause mismatch between the onset of insulin action and the absorption of carbohydrates in insulin-dependent children with diabetes. [25, 26] However, it has also been suggested that delayed gastric emptying is a potential advantage, rather than a disadvantage, in relation to glycemic control in T2DM patients not treated with insulin. [59] We didn't found any correlation between H. pylori infection and age; Although there have been indications that T2DM may predispose an individual to H. pylori infection, [14-17] this seems unlikely considering the age at which the disease is typically acquired.[29] On the other hand, Han, et al. [34] reported a significant association with those who were above 65 years old. Our study didn't found any correlation between H. pylori infection and sex; a result consistent with Keramat, et al. [28] In contrast; Quadri [33] found higher prevalence of H. Pylori infection in diabetic than in control women (80% vs 37.5%; $p < 0.05$), whereas there was no difference between males. Han, et al. [60] reported a significant association among women. We didn't found any correlation between H. pylori infection and social standard; A strange finding that differs from a settled fact or concept that H. pylori is a poor man's gut pathogen;[60] a fact supported by a study, conducted over 10 years, showed that improved standards of living in Russia have substantially reduced H. pylori transmission. [61] But our finding is probably due to Changing climate, changing demography and changing economy which consequently leads to redrawing the global map of H. pylori epidemiology. Obviously, socioeconomic status is not restricted to income and social class but takes in consideration other factors, including living standards, sanitation, urbanization, and educational level.[62] Educational level, in particular, has been used as a marker of socioeconomic status and has been considered as one of the important determinants of H. pylori prevalence in both developed [63] and developing countries. [64] Rosenstock, et al. found that the short duration of schooling beside low socioeconomic status increases the likelihood of H. pylori infection in Denmark. [63] We found few cases of intra-familial H. pylori infections in the controls and T1DM patients; like two sisters and a boy and his mother; a finding supporting the concept of intra-familial clustering of H. pylori infection [65, 66]. As regards the geographical distribution of the studied cases of the three groups, We noticed a higher prevalence in Hadara, Asafra,Sidi bishrand Fectoria; for future

more accurate epidemiological surveys to verify such distributions, to quantify the risk ratio between diabetics and non- diabetics in these places and to discover contributing factors like hygiene ,clean water availability, living standards, sanitation, urbanization, and educational level if any is present.

Conclusion:

This study showed that there was no statistical association between H. pylori infection and diabetes mellitus. In addition, there was no association between H. pylori infection and HbA1c control, duration of DM, diabetic complications, upper GI symptoms frequency, age, sex, BMI& social standard. Though there is an important literature on some extra-gastric disorders of H. pylori infection, additional studies are needed to examine the strength of the evidence linking these disorders to H. pylori, and to better understand mechanisms on how H. pylori affects them. Although no current data provide concrete evidence that H. pylori plays a role in diabetes mellitus, the possibility cannot be ruled out. Larger prospective studies investigating the impact of H. pylori infection on diabetes and corresponding mediating factors are warranted. Meanwhile, large interventional studies are urgently needed to evaluate the long-term benefit of H. pylori eradication for prevention and progression of diabetes. Evidence supporting an etiological role of H. pylori in the development of T2DM would indicate that preventive measures, such as increased hygiene and treatments using antibiotics and proton pump inhibitor combinations, should be explored as targets of intervention in high-risk communities.

Table (I): Prevalence of H. Pylori Infection in the 3 studied groups

	Group I (n = 100)		**Group II (n = 100)**		**Group III (n = 100)**	
	No.	**%**	**No.**	**%**	**No.**	**%**
H.pylori antigen in stools						
Negative	69	69.0	62	62.0	55	55.0
Positive	31	31.0	38	38.0	45	45.0
Sig. bet. Grps	$p_1 = 0.298$, $\mathbf{p_2 = 0.041^*}$, $p_3 = 0.315$					

p1: p value for comparing between groups I and groups II
p2: p value for comparing between groups I and groups III
p3: p value for comparing between groups II and groups III
*: Statistically significant at p ≤ 0.05

Table (II): Univariate and multivariate analysis for the parameters affecting H. pylori fecal positivity; after adjustment for age

	H.pylori antigen in stool		Unadjusted OR			Adjusted OR		
	Negative	Positive	OR	C.I	p	OR	C.I	P
Group I	69	31	0.55	0.31 – 0.98	0.042*	0.89	0.43 – 1.85	0.756
Group II	62	38	0.75	0.43 – 1.32	0.316	0.50	0.24 – 1.05	0.066
Group III	55	45	1.00	-	-	1.00	-	-

Table (III): Correlation between Pylori fecal positivity and different parameters in group I

	H.pylori antigen in stool				Test of Sig.	P
	Negative (n = 69)		Positive (n = 31)			
	No.	%	No.	%		
Sex						
Male	45	65.2	15	48.4	χ^2= 2.525	0.112
Female	24	34.8	16	51.6		
Age (years)						
31 – 40	6	8.7	5	16.1	χ^2= 4.769	0.190
41 – 50	33	47.8	10	32.3		
51 – 60	16	23.2	12	38.7		
> 60	14	20.3	4	12.9		
BMI (kg/m²)						
Min. – Max.	19.77 – 34.89		22.03 – 34.46		t= 0.886	0.378
Mean ± SD.	27.17 ± 3.30		27.78 ± 2.84			
Median	27.44		27.70			
Social standard						
Low	1	1.4	4	12.9	χ^2= 6.864	^{MC}p= 0.064
Moderate	24	34.8	13	41.9		
Good	37	53.6	13	41.9		
High	7	10.1	1	3.2		
Complications of DM						
No	51	73.9	28	90.3	χ^2= 3.472	0.062
Nephropathty	4	5.8	1	3.2	χ^2= 0.298	^{FE}p= 1.000
Retinopathy	10	14.5	1	3.2	χ^2= 2.774	^{FE}p= 0.165
Diabetic foot ulcer	5	7.2	1	3.2	χ^2= 0.613	^{FE}p= 0.663
Duration of DM (years)						
< 5	20	29.0	9	29.0	χ^2= 2.043	^{MC}p= 0.870
5 – 9	23	33.3	11	35.5		
10 – 14	12	17.4	8	25.8		
15 – 19	8	11.6	2	6.5		
20 – 24	4	5.8	1	3.2		
25 – 30	2	2.9	0	0.0		
Min. – Max.	0.08 – 30.0		1.0 – 20.0		U= 1020.000	0.711
Mean ± SD.	8.51 ± 6.39		7.45 ± 4.54			
Median	7.0		6.0			
HbA1c %						
< 6.5	11	15.9	4	12.9	χ^2= 0.155	^{FE}p= 0.772
≥ 6.5	58	84.1	27	87.1		
GIT symptoms						
Absent	21	30.4	21	67.7	χ^2=12.221*	**<0.001***
Present	48	69.6	10	32.3		

χ^2: **Chi square test** FE: **Fisher Exact** MC**: Monte Carlo**

U**: Mann Whitney test** t**: Student t-test**

p: p value for comparing between the two categories *: Statistically significant at $p \leq 0.05$

Table (IV): Correlation between H.pylori fecal positivity and different parameters in group II

	H.pylori antigen in stool				Test of Sig.	P
	Negative (n = 62)		Positive (n = 38)			
	No.	%	No.	%		
Sex						
Male	36	58.1	25	65.8	χ^2= 0.591	0.442
Female	26	41.9	13	34.2		
Age (years)						
< 10	17	27.4	8	21.1		
10 – 20	45	72.6	29	76.3	χ^2= 1.936	^{MC}p= 0.397
21 – 30	0	0.0	1	2.6		
BMI (kg/m²)						
Min. – Max.	10.58 – 31.50		12.48 – 33.29			
Mean ± SD.	18.90 ± 4.60		19.80 ± 6.46		τ= 0.745	0.459
Median	18.73		18.36			
Social standard						
Low	3	4.8	4	10.5		
Moderate	16	25.8	13	34.2	χ^2= 2.569	^{MC}p= 0.471
Good	35	56.5	18	47.4		
High	8	12.9	3	7.9		
Complications of DM						
No	58	93.5	38	100.0	χ^2= 2.554	^{FE}p= 0.294
Nephropathty	3	4.8	0	0.0	χ^2= 1.896	^{FE}p= 0.286
Retinopathy	1	1.6	0	0.0	χ^2= 0.619	^{FE}p= 1.000
Diabetic foot ulcer	0	0.0	0	0.0	-	-
Duration of DM (years)						
< 5	39	62.9	19	50.0		
5 – 9	15	24.2	17	44.7		
10 – 14	7	11.3	2	5.3	5.071	^{MC}p= 0.116
15 – 19	1	1.6	0	0.0		
20 – 24	0	0.0	0	0.0		
25 – 30	0	0.0	0	0.0		
Min. – Max.	1.0 – 15.0		1.0 – 12.0			
Mean ± SD.	5.09 ± 3.40		4.53 ± 2.26		U= 1165.000	0.925
Median	4.0		4.50			
Hb A1C %						
< 6.5	2	3.2	0	0.0	χ^2= 1.251	^{FE}p= 0.524
≥ 6.5	60	96.8	38	100.0		
GIT symptoms						
Absent	32	51.6	27	71.1	χ^2= 3.681	0.055
Present	30	48.4	11	28.9		

χ^2: **Chi square test** FE: **Fisher Exact** MC: **Monte Carlo**
U: **Mann Whitney test** t: **Student t-test**p: p value for comparing between the two categories

Table (V): Correlation between H.pylori fecal positivity and different parameters in group III

	H.pylori antigen in stool				Test of Sig.	P
	Negative (n = 55)		Positive (n = 45)			
	No.	%	No.	%		
Sex						
Male	26	47.3	23	51.1	χ^2= 0.146	0.702
Female	29	52.7	22	48.9		
Age (years)						
< 10	4	7.3	4	8.9	χ^2= 8.575	^{MC}p= 0.195
10 – 20	7	12.7	8	17.8		
21 – 30	12	21.8	15	33.3		
31 – 40	8	14.5	10	22.2		
41 – 50	17	30.9	7	15.6		
51 – 60	4	7.3	0	0.0		
> 60	3	5.5	1	2.2		
BMI (kg/m²)						
Min. – Max.	10.74 – 36.73		12.62 – 33.20		t= 1.058	0.293
Mean ± SD.	24.64 ± 5.37		23.53 ± 4.99			
Median	25.71		23.04			
Social standard						
Low	3	5.5	2	4.4	χ^2= 1.406	^{MC}p= 0.755
Moderate	17	30.9	15	33.3		
Good	32	58.2	23	51.1		
High	3	5.5	5	11.1		
GIT symptoms						
Absent	29	52.7	15	33.3	χ^2= 3.778	0.052
Present	26	47.3	30	66.7		

χ^2: **Chi square test** MC: **Monte Carlo**
t: **Student t-test**
p: p value for comparing between the two categories

Table (VI):Correlation between H.pylori fecal positivity and HbA1c % in high body mass index (BMI) (≥ 25 kg/m²)

Hb A1C %	H.pylori antigen in stool				Test of Sig.	P
	Negative		Positive			
	No.	%	No.	%		
Group I(77)	(n = 51)		(n = 26)			
< 6.5	9	17.6	4	15.4	χ^2= 0.063	^{FE}p= 1.000
≥ 6.5	42	82.4	22	84.6		
Min. – Max.	5.0 – 15.80		4.90 – 10.90		t= 1.717	0.090
Mean ± SD.	8.69 ± 2.37		7.80 ± 1.65			
Median	**8.40**		**7.45**			
Group II(14)	(n = 6)		(n = 8)			
< 6.5	2	33.3	0	0.0	χ^2= 3.111	^{FE}p= 0.165
≥ 6.5	4	66.7	8	100.0		
Min. – Max.	6.40 – 9.50		7.50 – 10.0		t= 0.076	0.942
Mean ± SD.	8.15 ± 1.46		8.10 ± 0.80			
Median	**8.65**		**8.0**			
Group I & Group II	(n = 57)		(n = 34)			
< 6.5	11	19.3	4	11.8	χ^2= 0.878	0.349
≥ 6.5	46	80.7	30	88.2		
Min. – Max.	5.0 – 15.80		4.90 – 10.90		t= 1.928	0.057
Mean ± SD.	8.63 ± 2.29		7.87 ± 1.49			
Median	**8.40**		**7.70**			

χ^2: **Chi square test** FE: **Fisher Exact**
t: **Student t-test**
p:p value for comparing between the two categories

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Original Article

Study of Urinary Transferrin as a Marker of Diabetic Kidney Disease

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Abstract:

Objectives: The aim of the present study is to assess urinary transferrin as a marker of DKD and its correlation with different clinical predictive factors of DKD progression. **Patients and Methods:** 60 T2DM patients recruited from the internal medicine wards and outpatient clinic of Alexandria Main University Hospital divided in to 3 groups. Group A, consisted of 20 T2DM patients with normal albumin excretion (>30mg/24h), group B, 20 T2DM patients with moderately increased albumin excretion (30-299mg/24h), group C, 20 T2DM patients with severely increased albumin excretion (<300mg/24h). And 20 sex & age matched healthy subjects were included as control.Urinary transferrin was measured using quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique. Urinary creatinine was measured by buffered jaffé reaction. Urinary transferrin to creatinine ratio (TFCR) was calculated and expressed in terms of µg/gm. Results The mean urinary TFCR was significantly higher in the 3 studied patient groups (3.40 ± 2.08, 8.63 ± 4.44, 13.91 ± 9.35 µg/gm respectively) in comparison to the control group (0.64 ± 0.58) with p value (0.003, < 0.001, >0.001 respectively). Urinary TFCR was significantly higher in group B than in group A (p1=0.011) and in group C than in group A (p2>0.001), while there was no statistically significant difference in urinary TFCR between group B and C (p3=0.234).14 patients (70%) in group A had the level of urinary TFCR exceeding the upper limit in the control group (2.01 µg/gm).

There was statistically significant positive correlation between TFCR and urinary albumin to creatinine ratio (ACR) in group C and total sample of the 4 studied groups with a p value >0.001, also there was statistically significant negative correlation between TFCR and estimated GFR (eGFR) in group C and total sample of the 4 studied groups with a p value >0.001.m Conclusion Urinary transferrin could be considered as early marker of DKD progression.

Key words: Diabetic Kidney Disease, End Stage Renal Disease, Urinary TFCR.

Introduction :

Diabetes mellitus (DM) is a complex, chronic disorder that occurs when there are raised blood glucose levels as the body cannot produce any or enough insulin or use insulin effectively.[1] The prevalence of DM is increasing globally. It affects about 8.8 %of total world population by 2017 with higher prevalence in urban areas versus rural areas.[2]

Generally, chronic complications of diabetes are divided into microvascular complications and macrovascular complications.[3]

Diabetic kidney disease (DKD) is one of the most serious microvascular diabetic complications with significant impact on morbidity and mortality.[4]

Diabetic kidney disease was previously known as diabetic nephropathy (DN) and is defined as DM with albuminuria (ratio of urine albumin to creatinine ≥ 30 mg/ gm), impaired glomerular filtration rate (GFR) (< 60 mL / min/ 1.73 m2), or both. Today, DKD includes not only DN but also other renal insults that occurs as a direct result of DM such as, atheroembolic disease, ischemic nephropathy and interstitial fibrosis.[5]

DKD is diagnosed by the persistent presence of elevated urinary albumin excretion (albuminuria), low eGFR, or other manifestations of kidney damage.[6]

Elevated urine albumin excretion (UAE) is considered as a well-established glomerular marker of diabetic nephropathy. It is considered normal to mildly increased when it is less than 30 mg/day (formerly known as normoalbuminuria), between 30and 300 mg/day, moderately increased albuminuria (formerly known as microalbuminuria), and above 300 mg/day, severely increased albuminuria (formerly known as macroalbuminuria).[7] The rate of progression from micro to macroalbuminuria in type 2 diabetic patients is 2-3% annually. Screening for DKD should include measurements of serum creatinine, estimation of GFR and screening for microalbuminuria.[8]

Although microalbuminuria is considered to be the best predictor of renal and cardiovascular complications of DM, many earlier, more sensitive and specific markers of kidney damage have been studied for better diagnosis and treatment of DKD at an earlier stage to prevent the progression to ESRD.[9]

Urinary transferrin (TF) has been considered to be a sensitive marker of DKD progression. Transferrin is iron-binding plasma glycoprotein that control the level of free iron in biological fluids. The liver is the main site of transferrin synthesis but other tissues and organs, including the brain, also produce transferrin.[10] Transferrin is very similar in weight to albumin but it is less anionic and has an isoelectric point (pI) one unit higher than albumin, therefore, it is expected to be filtered through the glomerular barrier more readily.[11] Among type 2 diabetic patients, urinary transferrin significantly increases with the progression of glomerular damage proven by biopsy and it has been shown that some type 2 diabetic patients with diffuse glomerular lesions despite normal UAE, had microtransferrinuria.[12] Furthermore, increased urinary transferrin excretion could predict the development of microalbuminuria in normoalbuminuric type 2 diabetic patients.[13]

Moreover, recent advances in the same issue have been introduced to assess newly studied risk markers for DKD depending on certain clinical and biochemical characteristics associated with progressive kidney damage in both T1DM and T2DM.[14] Elevated baseline haemoglobin A1c (HbA1c), elevated systolic blood pressure, albuminuria grade, early GFR decline, serum uric acid, presence of concomitant microvascular complications (most especially retinopathy), sex category, age and duration of diabetes have been considered as established risk markers in prediction of DKD.[15] While dyslipidemia, elevated body mass index (BMI), smoking status, hyperfiltration, decrease in vitamin D and haemoglobin level, have been proposed as potential risk markers for DKD with further research required to assess the nature of any relationship. Though clinical use of these clinical predictive factors remains restricted.[16]

Patients and methods:

After obtaining the approval of the ethics committee of the Faculty of Medicine of Alexandria University, 60 T2DM patients with different levels of UAE from the internal medicine wards and outpatient clinics of Alexandria Main University hospital divided into 3 group each includes 20 patients and 20 healthy controls were recruited. An informed consent from the participant subjects was taken before conducting the study. Patients with advanced kidney diseases (defined as eGFR < 30 ml/min), kidney diseases other than DKD, liver insufficiency and patients on corticosteroid therapy were excluded.

Data including name, age, sex, duration of DM, past medical history, and data obtained from clinical examination including fundus examination were recorded at enrollment. Laboratory investigations included complete blood picture, lipid profile, renal function test, liver function test, fasting blood glucose, HbA1c, serum uric acid, urinary ACR were done and eGFR were calculated using CKD-EPI formula.[17]

Urinary transferrin level was estimated using quantitative sandwich ELISA technique and urinary transferrin to creatinine ratio (TFCR) was calculated and expressed in terms of µg/gm.

Results:

Table (I): Comparison between the studied groups regarding gender, age and BMI

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Sex					
Male	6 (30%)	8 (40%)	11 (55%)	9 (45%)	**0.447**
Female	14 (70%)	12 (60%)	9 (45%)	11 (55%)	
Age (years)	50.45[a] ± 8.13	53.15[a] ± 11.10	57.75[a] ± 7.48	39.80 ± 11.63	**<0.001***
BMI (kg/m²)	29.5(21 - 43)	28.5 (22 – 52)	29 (22 – 42)	26 (24 – 30)	**0.053**

Qualitative data were described using number and percentage. Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group

b: Significant with Group A

c: Significant with Group B

Table (II): Comparison between the studied groups according to history and clinical data.

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Duration of DM (years)	7 (0.5 - 25)	10 (0.5 - 23)	11.5 (2 - 20)		**0.467**
HTN	12[a] (60%)	13[a] (65%)	18[ab] (90%)	3 (15%)	**<0.001***
Systolic BP	127.5 ± 15.52	135.5[a] ± 21.64	148.5[a] ± 20.59	117.0 ± 14.55	**<0.001***
Diastolic BP	80.0 ± 6.49	86.0[a] ± 13.14	93.0[ab] ± 13.80	75.50 ± 7.59	**<0.001***
Smoking status					
No	17 (85%)	13 (65%)	11 (55%)	17 (85%)	
Smoker	2 (10%)	6 (30%)	6 (30%)	3 (15%)	**0.187**
X-Smoker	1 (5%)	1 (5%)	3 (15%)	0 (0%)	
Retinopathy	a	a	ab		
No	14 (70%)	9 (45%)	5 (25%)	20 (100%)	
Non-proliferative	4 (20%)	4 (20%)	4 (20%)	0 (0%)	**<0.001***
Proliferative	2 (10%)	7 (35%)	11 (55%)	0 (0%)	

Qualitative data were described using number and percentage. Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group
b: Significant with Group A
c: Significant with Group B

Table (III): Comparison between the studied groups according to different laboratory investigations

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Urea (mg/dl)	23.8 (15.8 – 38.8)	29[ab](13 - 49)	31[ab](13 - 83)	22.5 (15 - 42)	**0.008***
Cr (mg/dl)	0.72 (0.5 – 1.1)	0.8[a](0.4 – 1.5)	0.85[ab](0.44 – 1.96)	0.62 (0.42 - 1)	**0.014***
S .uric acid (mg/dl)	3.65 (2.7 – 5.8)	4.2 (2 - 8)	5.45 (2.8 - 13)	4.05 (2.4 – 7.8)	**0.080**
FBG (mg/dl)	176.5[a](98 - 360)	188.5[a](98 - 305)	190[a](93 - 302)	89.5(68-121)	**<0.001***
HbA1c %	8.71[a] ± 1.59	9.01[a] ± 2.34	9.29[a] ± 1.47	4.98 ± 0.69	**<0.001***
Cholesterol (mg/dl)	215[a](110 - 413)	201 (103 - 269)	195(111 - 426)	166.5(115 - 265)	**0.024***
TG (mg/dl)	118 (64 - 320)	138(38 - 431)	160.5[a](66 -723)	94.5 (58 -203)	**0.040***
LDL (mg/dl)	130[a](81 - 331)	114[a](56 - 211)	118.5[a](76 -194)	85[a](56 - 131)	**<0.001***
HDL (mg/dl)	48.90 ± 9.63	46.80 ± 11.57	42.30 ± 10.80	50.05 ± 10.12	**0.108**
eGFR (ml/min/1.73m^2)	99.7(76.9 – 143.6)	93.4[a](51.60 -234)	80.2[a](37.4-156.1)	139.4(59.5-233.7)	**0.002***
ACR (mg/gm)	5.9(2.9 – 18.1)	38.85[ab](32.5 - 105)	531.5[abc](332-3399)	12.65(2.7-35)	**<0.001***
Urinary.TF(ng/ml)	2.45[a](0.76 – 5.3)	6.15[ab](0.7 – 10.6)	12.2[ab](0.8 – 23.4)	0.45(0 - 1)	**<0.001***
TFCR (µg/gm)	2.97[a](0.69 – 8.43)	9.08[ab](0.57 – 16.61)	13.29[ab](0.88 – 39.79)	0.57(0 – 2.01)	**<0.001***

Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group; b: Significant with Group A; c: Significant with Group B

Table (IV): Correlation between TFCR with different parameters in each group and total sample

		TFCR (µg/gm)				
		Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	Total sample (n = 80)
Age (years)	r_s	0.372	0.325	0.244	-0.196	0.559*
	p	0.106	0.163	0.299	0.407	<0.001*
BMI (kg/m²)	r_s	-0.286	-0.391	-0.115	0.069	0.115
	p	0.222	0.089	0.630	0.773	0.309
Duration of DM(years)	r_s	0.442	0.004	0.030		0.183
	p	0.051	0.987	0.899		0.162
Systolic	r_s	-0.010	-0.197	0.195	0.209	0.460*
	p	0.967	0.405	0.411	0.376	<0.001*
Diastolic	r_s	-0.315	-0.263	0.249	0.198	0.463*
	p	0.176	0.263	0.289	0.403	<0.001*
HB (g/dl)	r_s	0.066	0.145	-0.387	0.198	-0.244*
	p	0.782	0.541	0.092	0.402	0.029*
S .uric acid(mg/dl)	r_s	0.004	0.086	0.239	-0.153	0.216
	p	0.987	0.719	0.311	0.520	0.054
HbA1c %	r_s	0.158	0.062	0.133	-0.013	0.606*
	p	0.505	0.796	0.578	0.956	<0.001*
Cholesterol (mg/dl)	r_s	-0.146	-0.283	-0.409	-0.209	-0.014
	p	0.539	0.227	0.073	0.377	0.903
TG (mg/dl)	r_s	-0.170	-0.258	-0.349	-0.256	0.122
	p	0.474	0.272	0.132	0.276	0.282
LDL (mg/dl)	r_s	-0.132	-0.020	-0.141	-0.107	0.184
	p	0.578	0.932	0.552	0.655	0.103
HDL (mg/dl)	r_s	0.082	-0.076	-0.118	0.016	-0.236*
	p	0.731	0.750	0.620	0.947	0.035*
Retinopathy	r_s	0.073	0.045	0.143		0.507*
	p	0.760	0.849	0.548		<0.001*
ACR	r_s	-0.272	-0.026	0.859*	0.030	0.670*
	p	0.246	0.912	<0.001*	0.899	<0.001*
eGFR	r_s	0.047	0.174	-0.732*	0.248	-0.465*
	p	0.845	0.462	<0.001*	0.293	<0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Discussion:

Diabetic Kidney Disease represents a major component of the health burden associated with DM. In many regions, diabetes is considered the main leading cause of ESRD with significant impact on morbidity and mortality specially in DM patients with proteinuria.[18]

A large number of studies support that the risk for rapid progression of DKD and consequently the development of ESRD is strongly associated with the progression of albuminuria in diabetic patients. So that, albuminuria has often been considered as surrogate for hard renal endpoints.[19] However, earlier, more sensitive and specific markers of kidney damage are needed for better diagnosis and treatment of DKD at an earlier stage to prevent the progression to ESRD.[20]

Transferrin is an iron-binding plasma glycoprotein that is very similar in weight to albumin, therefore, it is expected to be filtered more readily through the glomerular barrier. Urinary transferrin is considered to be a more sensitive marker of glomerular damage in diabetic patients.[21]

In this study, the mean urinary TFCR was significantly higher in the 3 studied patient groups in comparison to the control group, the mean urinary TFCR was significantly higher in group B than in group A and in group C than in group A, while there was no statistically significant difference in urinary TFCR between group B and C. Moreover, 70% of patients in group A had the level of urinary TFCR exceeding the upper limit in the control group.

There was statistically significant positive correlation between TFCR and ACR in group C and total sample of the 4 studied groups, while, there was no statistically significant correlation between TFCR and ACR in group A, group B or in control group.

We can conclude from the previous results that high levels of urinary TF is a marker of progression of DKD either with or without albuminuria and this relationship is more established in patients with severely increased albuminuria. Moreover, transferrinuria frequently occurs before the development of microalbuminuria, so it could be considered as a sensitive marker for early detection of DKD in T2DM patients with normal albumin excretion. These results are in agreement with 3 studies that investigated the role of urinary TFCR in assessment of progression of DKD in T2DM.

The first one is by Randy et al[22] who observed higher level of TFCR in patients with T2DM than in control group and also found a highly significant correlation between urinary albumin and transferrin excretion in patient groups. They concluded that urinary transferrin excretion is increasing with the degree of albuminuria with highly significant correlation to urinary albumin excretion in T2DM with overt proteinuria.

The second study is by Cheung et al[23] who found that, 61% of normoalbuminuric diabetic patients had high TFCR (higher than the 95th percentile value seen in healthy subjects). TFCR was significantly higher in the patient groups than in control group. Moreover, they observed that TFCR was significantly higher in patients with clinical retinopathy and it correlated with arterial BP. They concluded that transferrinuria is very common in T2DM patients and frequently occurs before microalbuminuria and suggested that it could be a sensitive marker of incipient nephropathy.

The third study is more recent by Narita et al[24] who studied the parallel increase in urinary excretion rates of four markers including transferrin in normoalbuminuric T2DM patients and found that TF excretion rate was significantly increased in the normoalbuminuric patient group than in control group, so they suggested that increased urinary TF excretion could predict a future development of microalbuminuria in normoalbuminuric T2DM patients.

The results of the previous studies and our study were nearly similar. The possible interpretations of these findings are that proteinuria in DN has been shown to result from a disturbance in glomerular size selectivity to different proteins. Therefore, increased urinary TF excretion, which is somewhat larger, that often precede the onset of microalbuminuria in diabetic subjects could be explained by altered glomerular size selectivity to these two proteins , while in patients who already developed albuminuria, the urinary transferrin excretion has a linear relationship with UAE.[25]

Moreover, in T2DM, transferrinuria precedes biopsy proven tubulointerstitial changes found in diabetic kidney as tubular reabsorption of TF results in release of reactive iron which produce oxidative stress on the tubular epithelium. Transferrin excretion was also associated with some markers of proximal tubule damage such as alpha-1-microglobulin and urinary N-Acetyl-β-D glucosaminidase (NAG) in diabetic patients but it is not yet clear if TF is the cause of tubular damage or transferrinuria is secondary to decreased tubular reabsorption.[26]

In our study, there was also statistically significant negative correlation between TFCR and eGFR in group C and total sample of the 4 studied groups,

while there was no statistically significant correlation between TFCR and eGFR in group A, group B or in control group.

Jiang et al[27] found that, urinary levels of glomerular, including transferrin, and tubular damage markers of DKD were higher in T2DM patients with slightly decreased eGFR , interestingly, they also found that some of these markers including transferrin, were already elevated in diabetic patients with relatively normal eGFR (≥90 mL/min/1.73 m2) suggesting that these markers are potential sensitive markers for early diabetic kidney damage.

They also demonstrated that all damage markers were significantly negatively associated with eGFR and positively associated with albuminuria but only glomerular not tubular markers lose their significant negative correlation with eGFR after adjustment of albuminuria and other confounding factors like HbA1c.[27]

In the current study, the prevalence of hypertension was significantly higher in the 3 studied patient groups than in control group. the prevalence of hypertension was also significantly higher in group C than in group A. This is coinciding with the results of Bakris et al[28], who found that the prevalence of hypertension in DN increases at each stage of CKD, approaching 90% for ESRD patients. This could be explained by interaction of multiple mediators that result in renal sodium reabsorption and peripheral vasoconstriction such as activation of the renin-angiotensin-aldosteron system (RAAS), upregulation of endothelin (ET-1), upregulation of reactive oxygen species (ROS), and downregulation of nitric oxide (NO) which are important factors that result in development of hypertension and progression of diabetic renal and cardiovascular events.[28]

As regards diabetic retinopathy (DR), the prevalence of DR in our study was significantly higher in the 3 studied patient groups than in control group and also it was significantly higher in group C than in group A. This implies that DR is strongly associated with the progression of albuminuria. These results coincide with the results of Zhang et al[29] who found that patients with DKD and DR were more likely to have higher levels of serum creatinine and proteinuria, and decreased eGFR and he concluded that there was a strong associations between the presence of DR and the renal outcomes in T2DM patients. These findings suggested a potential common pathway between the DR and DKD, as the presence of retinopathy may represent diabetic related systemic microvascular damage that lead to both breakdown of the blood vessel–retinal tissue barrier and progressive renal dysfunction. Furthermore, the presence of DR may identify individuals who may be at increased risk for DN or more serious renal microvascular damage.[30]

As regards the association of HbA1c with DKD, we found that the level of HbA1c was statistically significant higher in the 3 studied patient groups than in control group. It was also higher in group C than in group A and B but without a statistically significant difference.

In a study by Kaminska et al[31], urinary albumin excretion was higher in T2DM patients with poor glycemic control (HbA1c ranged between 6.6 and 10%) than in T2DM patients with good glycemic control (HbA1c ranged between 6.1 and 6.5%) suggesting that the urinary albumin excretion rises with increased glycemia and percentage of glycosylated haemoglobin.

Hyperglycemia is essential for development of DKD, and glycemic control is the primary determinant of the onset of nephropathy. The UKPDS (United Kingdom Prospective Diabetes Study) in type 2 diabetes have revealed a strong relationship between glycemic control and risk of the development of diabetic microvascular complications, although there is no easily defined HbA1c threshold. However, there has been only limited evidence suggesting that glycemic control slows the rate of GFR decline and retards progression to ESRD.[32]

As regards lipid profile, we found no statistically significant difference between the 3 studied patient groups as regards total serum cholesterol, serum triglycerides (TG) and serum low density lipoproteins (LDL). However, total serum cholesterol was statistically significant higher in group A than in control group, serum TG was

statistically significant higher in group C than in control group, serum LDL was statistically significant higher in the 3 studied patient groups than in control group while there was no statistically significant difference between the 4 studied groups as regards serum high density lipoproteins (HDL).

These results coincides partially with Shoji et al[33] who reported no differences between diabetic patients with normoalbuminuria and those with microalbuminuria as regards total serum cholesterol, serum TG and serum LDL, but that not for those with macroalbuminuria. However, in another study by Al-Jamei et al[34], they found significant increase in the serum levels of total serum cholesterol and serum LDL in diabetic patients with macroalbuminuria than those with microalbuminuria and normoalbuminuria. Moreover, they found significant increase in serum TG and decrease in serum HDL in patients with microalbuminuria and macroalbuminuria than those with normoalbuminuria, but they found no significant difference between microalbuminuric and macroalbuminuric patient groups as regards serum TG and HDL.

Dyslipidemia in DM results from insulin resistance and defective insulin action on lipoprotein metabolism. Thus, there is increased lipolysis with increased production of very low density lipoproteins (VLDL), TG, LDL, and rapid breakdown of HDL. dyslipidemia also have been found to facilitate glomerulosclerosis under hyperglycemic conditions.[35]

The current study also demonstrated a statistically significant positive correlation between TFCR and both systolic and diastolic BP, retinopathy and HbA1c. These results are in agreement as regards BP and retinopathy with the study mentioned above that is by Cheung et al(23) who reported a significant positive correlation between TFCR and both arterial BP and clinical retinopathy supporting that TF may be a sensitive marker for detecting diabetic complications. However, the same study was against our study as regards HbA1c, as they found no significant correlation between TFCR and HbA1c, suggesting that excess transferrinuria is not explained by short or medium term fluctuations in glycemic control but more probably by an intrinsic abnormality of glomerular function. However, more studies with larger sample sizes are needed to prove relationship between urinary TFCR and different clinical predictive markers of DKD.

Conclusion:

Urinary transferrin is considered as early and sensitive marker of DKD progression and can predict the occurrence of albuminuria and other diabetic complications.

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Original Article

Incidence of Helicobacter Pylori infection in Obese & Non-Obese Subjects

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Abstract

Background: One of the most popular health problems that have become a worldwide epidemic is obesity. Many complications have been raised as a sequence of obesity such as, diabetes mellitus, sleep apnea syndrome, hypertension and many other cardiovascular diseases. (1) Helicobacter pylori (H pylori) is a Gram-negative, flagellated bacterium that is widely distributed all over the world especially in developing countries. (2) How often H pylori infection is found in obese patients is a debate , some reports showed low prevalence of H pylori in obese patients if compared to non-obese patients (3), other reports showed a higher prevalence in obese patients (4), while some studies found an increase in body mass index (BMI) after H pylori has been eradicated. (5) **Objective:** Incidence of H pylori infection in obese patients in comparison to non-obese one. **Method:** fifty obese and fifty non obese patients were included in the study, H pylori infection was tested using stool antigen test. **Results:** There was high incidence of H pylori infection in obese patients as compared to non- obese one. **Conclusion:** H pylori infection is more common in obese patients than non-obese one.
Keywords: H.Pylori, Obesity

Introduction

Many factors are encountered as a cause of obesity; these factors may be genetic, behavioral, metabolic, or environmental. The most important of which are the behavioral and environmental factors. (1)

Obesity has a social and psychological impact on the individual in addition to its impact on the health, as many diseases are associated with obesity such as diabetes mellitus, dyslipidemia, hypertension, and ischemic heart disease. Moreover high body mass index (BMI ⩾35 kg/m2) is associated with high mortality rates. (1)

H. Pylori is a microaerophilic , gram negative organism, considered as the leading cause of many diseases such as chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. H. pylori can affect other systems in our body such as cardiovascular and immune systems, recently it was found to be involved in insulin resistance and, obesity. (2)

How often H pylori infection is found in obese patients is a debate, some reports showed low prevalence of H pylori in obese patients if compared to non-obese patients, (3) other reports showed a higher prevalence in obese patients, (4) while some studies found an increase in body mass index (BMI) after H pylori has been eradicated. (5) More studies are needed to determine the incidence of H pylori infection in obese subjects in comparison to non-obese one.

Methods

The study will be conducted on 50 obese patients, and 50 non-obese patients attended the outpatient clinic or admitted to Medical research institute inpatient wards for any medical reason (without overt gastrointestinal manifestations), after taking their informed consent in a case control study.

Inclusion criteria:

Obese patients with body mass index (BMI) ≥ 30kg/m2 and non-obese patients with 18.5>BMI < 25 kg/m2 according to WHO criteria. (6)

Exclusion criteria:

Individuals with any of the following will be excluded: chronic liver or renal failure, malignancy, those who having history of eradication therapy and those with history of significant upper GI symptoms.

- Data will be collected from the hospital medical records.
- Thorough clinical examination including weight and height.
- Routine laboratory investigations including: liver function tests, renal function tests, electrolytes, complete blood picture, fasting plasma glucose level, and lipid profile. (7)
- Ultrasound abdomen. (8)
- Stool antigen test for diagnosis of H.pylori infection. (9)

Results

The mean age for H pylori +ve patients was (36.2±12.1yrs) and in H pylori –ve patients it was (34.1±12.3yrs), P= 0.39.

The mean BMI in obese patients was (46.1±7.3), and in non-obese patients it was (24.4±1.9).

Male and female distribution among H pylori + ve obese patients was found to be 30%, and 70% respectively, while in H pylori + ve non-obese patients it was 35%, and 65% respectively, P= 0.041.

The prevalence of H pylori in Obese patients, was found to be 64%, while in non-obese patients it was 31%, P= 0.05.

The incidence of fatty liver in H pylori + ve obese patients, was 55.1%, and in H pylori – ve obese patients it was 30.1%, P= 0.05.

However, the incidence of fatty liver in H pylori + ve non-obese patients was 41.1%, and in H pylori –ve non-obese patients was 21.2%, P =0.06.

Discussion

Finding a relation between obesity and H pylori infection is still under investigation, it is known that H pylori infection is more prevalent in older age group and confirmed through multiple studies, one of them is the study of Marusic, (10) who found that patients with H pylori are around 45-64 years, in this study the peak incidence was between 24-48 years, this is because most of the obese patients who were involved in the study were in the middle age group.

H pylori distribution between male and female showed high prevalence of infection in obese females as it was found to be 70% versus 30% in obese males, and 65% in non- obese females versus 35% in non-obese males, this found to be matching with the study of Ghadimi who did his study in Babol, (11) and Marusic study who found the infection is predominant in females with male to female ratio about 0.92.(10)

In this study fatty liver was more common in H pylori + ve patients specially the obese one as *H. pylori* infection is found to be a risk factor for the development of nonalcoholic fatty liver disease (NAFLD), as it is linked to insulin resistance which is closely related to NAFLD. (12)

This study confirmed the high prevalence of H pylori in obese patients more than non-obese one as it was found in 64 % of obese patients while it was found only in 31% of non-obese patients and these results are matching the results of Arslan (13) who found prevalence of H pylori in obese patients to be 66.2%, and in non-obese patients to be 35.2%. This high prevalence of H pylori in obese patients is attributed to alter both innate and adaptive immunity that found in obese subjects, this is attributed to presence of less

mature monocytes with lower number transformed into macrophages with reduced number of polymorphnuclear bactericidal ability. [14]

In addition, there is decreased activity of natural killer cells in morbidly obese subjects if compared to normal individuals matched for age and gender. [15] Another study found suppression of peripheral blood mononuclear cells chemokine and cytokine production, leading to impaired immune response to infection which is corrected by weight loss. [16]

On the other hand some studies found that H pylori infection decreases ghrelin secretion through its effect on gastric mucosa and consequently reduce appetite, leading to increased weight after H pylori eradication with increased plasma ghrelin levels. [17, 18]

The different results obtained from previous studies may be due to using different diagnostic tools in detecting H pylori infection, as some studies used serologic testing which is less sensitive and specific (85% and 79%) if compared to histologic testing (>95% for both), [19] it is also inferior to rapid urease test and antigen stool test for the diagnosis of H pylori, as antibodies remain in the blood of the patient even after eradication of infection. [9, 20]

Conclusion

There is high prevalence of H pylori infection in obese patients, and this study raised the issue of whether obese patients should be screened for H pylori infection even if not symptomatic.

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Original Article

Potential Role of Circulating Adipocyte Fatty Acid Binding Protein 4 (A-FABP /FABP-4) Level in Chronic Hepatitis C - Associated Insulin Resistance: a Case - Control Study

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Abstract

The Aim of the Work : was to evaluate the role of A-FABP in development of IR in CHC patients with early stage liver disease **Patients & Methods:** The study included 85 Egyptian males categorized as 70 chronic hepatitis C (CHC) patients and 15 healthy controls. Oxidized low density lipoproteins (oxLDL) and A-FABP were determined in serum using enzyme-linked immunosorbent assay (ELISA).**Results:** According to HOMA-IR results,49 CHC patients (70%) were found to be insulin resistant while 21 patients (30%) were non- insulin resistant. A-FABP showed a significant increase in CHC with IR. It showed significant positive correlation with the duration of HCV infection and viral load in CHC with IR. At cutoff value of 9.4ng/ml, A-FABP had higher sensitivity compared to serum insulin in CHC with an area under curve (AUC) of 0.998 versus 0.966 respectively.

Conclusion: A direct relation between HCV infection and increased circulating A-FABP level, through elevation of oxLDL level, may induce insulin resistance in CHC. Serum A-FABP surpassed fasting serum insulin in determination of insulin resistance and it could be a more sensitive biomarker in CHC patients with early stage liver disease.

Key words: Chronic hepatitis C, Insulin resistance, A-FABP, oxLDL

Introduction

Chronic HCV infection is considered a systemic disease as it affects not only the liver, but other organs as well leading to several extrahepatic complications.[1] Insulin resistance (IR) is a well known complication associated with chronic HCV infection [2] It plays the main role in the development of Type 2 diabetes mellitus (T2DM).[3] HCV associated IR increases the risk of hepatocellular carcinoma [4], cardiovascular events.[5] and associated with low response rates to antiviral treatment. [6] Thus, HCV associated IR is a therapeutic target at any stage of HCV infection. It has been reported that IR occurs in early stages during HCV infection and is not exclusively related to cirrhosis. [7] HCV associated IR has been found to be genotype-dependent (1 and 4), [8] to be related to HCV viral load [9] and to be improved in patients with HCV clearance following antiviral therapy. [10] Therefore, it was found that the underlying mechanism of HCV associated IR is not completely understood [11]

Adipocyte fatty acid binding protein (A-FABP) also called FABP-4 or adipocyte P2 (aP2), which belongs to fatty acid binding proteins (FABP) family of intracellular chaperones, is highly expressed in adipose tissue and activated macrophages. [12] It has a low molecular mass (15 kDa) and it is the major cytosolic protein of mature adipocytes, accounting for about 6% of the total cellular protein. [12] The expression of A-FABP is regulated by insulin and fatty acids, [13] with higher circulating levels in females than males owing to the higher subcutaneous fat content.[14] Several proinflammatory stimuli have been reported to upregulate FABP-4 expression in macrophages, including peroxisome proliferator–activated receptor- (PPARγ) agonists, oxidized low-density lipoproteins (oxLDL) and toll-like receptor (TLR) agonists [15, 16] Clinical studies showed that A-FABP is associated with metabolic disorders including IR, diabetes, obesity, non-alcoholic liver

disease (NAFLD) and cardiovascular diseases [13, 17] Studies on A-FABP revolve around the hypothesis that inhibition or eradication of this protein could help the treatment and prevention of diseases like diabetes, atherosclerosis and other problems in association with adipose tissue. [18, 19]The aim of this work was to evaluate the potential role of circulating AFABP level in the development of chronic hepatitis C (CHC)-associated IR.

Subjects and methods:

Eighty five Egyptian males were selected for the study. They were categorized as 70 patients diagnosed with chronic HCV infection (CHC group) recruited from the Experimental and Internal Medicine Department of the Medical Research Institute during the period from February 2015 to May 2016 and they were not receiving any antiviral treatment. The second category was 15 age matched apparently healthy male controls (group I). Informed oral and written consents were taken from all studied subjects and after the approval of the Ethical Committee of the Medical Research Institute, the study was started. Patients with diabetes, obesity (BMI ≥30), hypertension, cardiovascular or renal disease were excluded. Liver diseases other than CHC or advanced liver cirrhosis (Chid-Pugh class more than A) were ruled out.

To all the studied subjects, full history, clinical examination and abdominal ultrasound were done. Anthropometric measures were done and the body mass index (BMI) was calculated using the following formula: (weight/height2 in kg/m2).

Fatty liver condition was evaluated by abdominal ultrasonography and confirmed by calculating the validated Fatty Liver Index (FLI) to quantify the degree of liver steatosis using the following formula : e y / (1 + e y) × 100, Where y = 0.953 × log (triglycerides, mg/dL) + 0.139 × BMI (Body mass index), kg/m2 + 0.718 × log (gamma gluatmyl transferase activity, U/L) + 0.053 × waist circumference, cm – 15.745[20]

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. Logistic regression analysis was done for the statistically significant factors where anxiety and depression were the dependant variables. The used tests were: Mann Whitney test which was used to compare between two studied groups for abnormally quantitative variables, and Spearman coefficient to correlate between two abnormally quantitative variables .

Laboratory Workup:

To all studied subjects, 10 ml venous whole blood samples were withdrawn after 12 hours fasting and the following laboratory investigations were done:

1. Routine biochemical laboratory investigations including liver function tests and glucose parameters were performed on the auto-analyzer OLYMPUS AU400 Germany.

2. Non-estrified Free Fatty acids (NEFA) were determined in serum by titration (Dole's method) [21]

3. Fasting serum insulin was measured using chemiluminescent enzyme immunometric assay (CLIA) on Immulite 1000 Automated Analyzer (Diagnostic Products Corporation) then Homeostasis Model for Assessment of insulin resistance (HOMA-IR = fasting insulin (μIU/ml) * fasting glucose (mmol/L)/22.5) [22] was calculated and any patient with a result of ≥ 2.5 was considered to be insulin resistant.

4. Hepatitis viral markers; HBsAg and anti-HCV Ab were measured by enzyme-linked immunosorbent assay (ELISA) while HCV RNA was detected by quantitative PCR.

5. Serum oxLDL concentration was determined using a commercial ELISA kit (Mercodia, Uppsala, Sweden)

6. Serum level of adipocyte fatty acid binding protein (A-FABP) was determined by ELISA technique from commercially available kit, (BioVendor Human Adipocyte FABP (FABP4), Laboratorni medicina a.s.).

Statistical method:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov test was used to verify the normality of distribution of variables. Student t-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing between more than two groups and followed by Post Hoc test (Tukey) for pairwise comparison. Mann Whitney test was used to compare between two groups for not-normally distributed quantitative variables, while Kruskal Wallis test was used to compare different groups and followed by Post Hoc test (Dunn's) for pairwise comparison. Spearman coefficient was calculated to assess the association between A-FABP and other risk factors for IR. Univariate and multivariate logistic regression was assessed for the parameters found to be significantly associated with HOMA-IR. Receiver operator characteristic (ROC) curves for A-FABP and insulin against HOMA-IR were constructed. Youden's indices were calculated to get their best cut off values. Significance of the obtained results was judged at the 5% level..

Results:

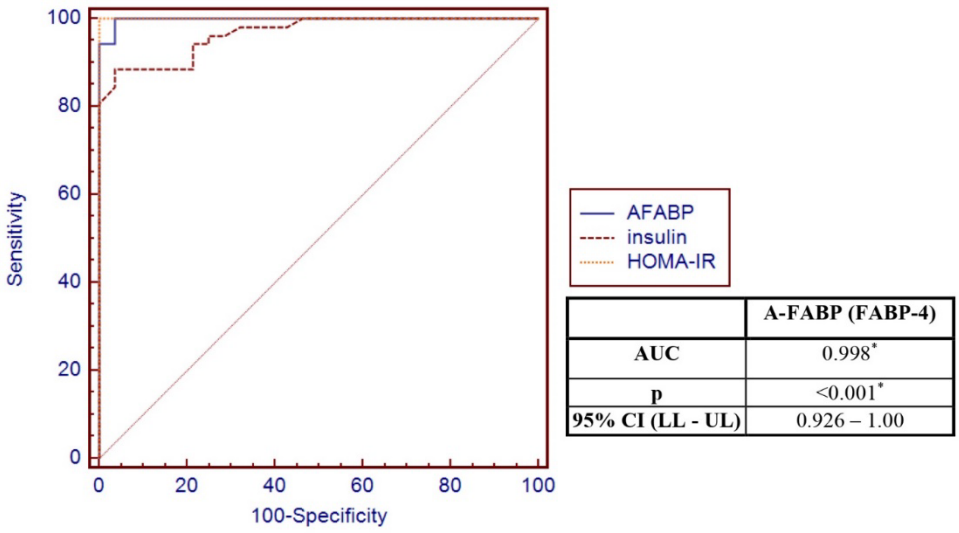
HOMA-IR was found to be high in most CHC (70%) patients. Accordingly, CHC were classified into insulin resistant group (group II-b) (n=49, 70%) (HOMA-IR ≥ 2.5) and non- insulin resistant group (group II-a), (n=21, 30%) (HOMA-IR <2.5) as shown in Table (I).The median serum A-FABP values showed a significant increase in group II-b (with IR) more than group II-a (without IR) as well as group I (controls), (39.25, 12.1 and 8.5 ng/ml, respectively), (p <0.001), but A-FABP level in group (II-a) was not significantly different from group (I). Regarding the fatty acid status and steatosis markers including FLI, serum oxLDL as well as NEFA in the three studied groups are shown in Table (I). Significant correlations of A-FABP are shown in Table (II).

Diagnostic performance of A-FABP (FABP-4):

Figure (1) shows the characteristics of Receiver operator characteristic (ROC) curves for A-FABP and insulin against HOMA-IR, the most widely used indicator of insulin sensitivity to compare their diagnostic performance.

Determinants of insulin resistance in CHC patients:

Univariate logistic regression analysis revealed that duration of HCV infection, viral load (HCV-PCR) and serum A-FABP were positively associated with the occurrence of IR in CHC patients, whereas multivariate regression analysis showed that serum A-FABP level remained the independent determinant of IR in CHC patients after adjusting for all confounding factors, (standardized β = 0.146, P =0.009), Table (III).



	A-FABP (FABP-4)
AUC	0.998*
p	<0.001*
95% CI (LL - UL)	0.926 – 1.00

Figure (1): ROC curves of A-FABP and insulin to determine insulin resistance in CHC.

Table (I): Comparison between the studied groups according to different clinical and biochemical parameters

	Group I (n=15)	Group II-a(n=21)	Group II-b (n=49)	Test of sig.	P
Age (years)	49.4±5.99	46.15±5.72	45.85±7.25	F=1.499	0.233
BMI (Kg/m2)	25.53±1.22	26.47±1.54	26.75±1.83	F=2.795	0.070
Waist circumference (cm)	92.5± 6.6	96.5 ± 6.1	94.5±7.6	F=1.133	0.330
Duration of HCV(years)	-	1(1–1.5)	3(2–5)	U=0.0*	<0.001*
Liver function tests:					
AST (U/L)	19b(15–30)	33a(19–123)	40.5a(18–329)	H=21.282*	<0.001*
ALT(U/L)	14b(10 – 27)	42a(10 – 97)	27a(10 – 124)	H=18.290*	<0.001*
GGT (U/L)	14b(13–20)	17b(15–23)	29ab(22–40)	H=9.262*	0.010*
Total bilirubin (mg/dl)	0.7b(0.3–0.9)	0.9a(0.4–3.1)	0.85a(0.4–4.9)	H=12.108*	0.002*
Direct bilirubin (mg/dl)	0.2b(0.1–0.3)	0.3a(0.1–1.7)	0.3a(0.1–3.4)	H=9.995*	0.007*
Indirect bilirubin (mg/dl)	0.4b(0.2–0.6)	0.5a(0.3–1.4)	0.5a(0.3–2.3)	H=9.377*	0.009*
HCV RNA (IU/ml)	-	113490±69901	671062±344426	t=7.934*	<0.001*
NEFA (mmol/L)	0.6b(0.4–0.9)	1a(0.5–1.4)	0.85a(0.5–1.8)	H=11.179*	0.004*
Cholesterol (mg/dl)	155±12.87	167.77±33.36	159.04±23.64	F=1.018	0.368
Triglycerides (mg/dl)	80(61–100)	97(37–155)	90(47–274)	H=0.946	0.623
HDL-cholesterol (mg/dl)	49.6a±6.3	33.46b±11.05	37.38ab±10.09	F=11.812*	<0.001*
LDL-cholesterol (mg/dl)	88.85b±9.55	115.12a±31.62	101.83ab±23.11	F=4.609*	0.014*
FSG (mg/dl)	83.6b ± 6.4	86.4b± 7.2	106.1a ± 12.3	F=8.062*	0.001*
PPG2h (mg/dl)	95.93b±17.42	102.62b±13.2	121.96a±12.75	F=18.138*	<0.001*
Fasting insulin (µIU/mL)	2.52b(2.0–6.25)	6.51b(3.37–10.0)	14.0a(9.29–35.30)	H=42.671*	<0.001*
HOMA-IR	0.56b(0.35–1.37)	1.39b(0.76–1.97)	3.16a(2.6–7.66)	H=43.470*	<0.001*
FLI	22.3b±6.4	47.8a±13.3	56.7ab±15.1	F=34.496*	<0.001*
A-FABP (ng/ml)	8.5b(5.0–14.5)	12.1b(10–22.5)	39.3a(19.8–104.9)	H=42.317*	<0.001*
oxLDL(U/L)	11.4b±2.4	24.9a±4.3	31.5ab±11.5	F=27.349*	<0.001*

F: **F for ANOVA test**, Pairwise comparison between each 2 groups was done using **Post Hoc Test (Tukey)**

H: H for **Kruskal Wallis test,** Pairwise comparison between each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the studied groups Group I:controls, group II-a: CHC without IR

Group II-b: CHC patients with IR *: Statistically significant at $p \leq 0.05$

Common letters are not significant (i.e. **Different letters** are significant)

BMI: body mass index.

FLI: fatty liver index.

OxLDL: oxidized low density lipoproteins.

NEFA: non-estrified fatty acids.

FSG: fasting serum glucose.

PPG2hr: two hours postprandial glucose.

A-FABP (FABP-4): adipocyte fatty acid binding protein-4.

HOMA-IR: homeostasis model of assessment for insulin resistance.

HDL-cholesterol: high density lipoprotein-cholesterol.

LDL-cholesterol: low density lipoprotein-cholesterol.

AST: aspartate transaminase.

ALT: alanine transaminase.

GGT: gamma gluatmyl transferase.

HCV-RNA: ribonucleic acid of hepatitis C virus

Table (II): Correlations between A-FABP and other parameters in each studied group

Groups	Parameter	A-FABP (FABP-4)	
		r_s	p-value
Group I (n=15)	Insulin(µIU/mL)	0.567	0.028*
	FLI	0.060	0.832
	oxLDL (U/L)	0.055	0.847
Group II-a (n=21)	Age (years)	0.694	0.008*
	AST(U/L)	0.694	0.008*
	FLI	0.070	0.821
	D.bilirubin (mg/dl)	0.655	0.015*
Group II-b (n=49)	HCV –RNA	0.800	<0.001*
	FPG (mg/dl)	0.426	0.030*
	Insulin(µIU/mL)	0.620	0.001*
	HOMA-IR	0.526	0.006*
	FLI	0.327	0.103
	Duration of HCV (years)	0.747	<0.001*
	oxLDL (U/L)	0.526	0.006*
	NEFA (mmol/L)	0.381	0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Table (III): Univariate and multivariate analysis showing factors independently associated with HOMA-IR (n = 70)

	Univariate				#Multivariate			
	B	P	95% CI.		B	P	95% CI.	
			LL	UL			LL	UL
Age (years)	0.018	0.646	-0.062	0.099				
BMI (Kg/m2)	0.141	0.365	-0.170	0.452				
Waist circumference	0.004	0.913	-0.072	0.080				
Duration of HCV (years)	0.791	<0.001*	0.456	1.125	0.105	0.528	0.229	0.439
NEFA(mmol/L)	-0.880	0.296	-2.563	0.802				
CHOL (mg/dl)	0.0	0.980	-0.020	0.020				
TG (mg/dl)	-0.002	0.787	-0.013	0.010				
HDL-C (mg/dl)	0.018	0.491	-0.034	0.069				
LDL-C (mg/dl)	-0.002	0.839	-0.022	0.018				
HCV–RNA (IU/ml)	0.267	<0.001*	0.0	0.0	0.128	0.645	0.237	0.259
A-FABP (ng/ml)	0.112	<0.001*	0.036	0.069	0.146	0.009*	1.003	4.175
Fatty liver by US	1.069	0.042	0.041	2.097	0.033	0.922	0.706	0.641
Fatty liver index (FLI)	0.034	0.057	-0.001	0.068				
oxLDL (U/L)	0.039	0.160	-0.016	0.094				

B: Unstandardized coefficient LL: Lower limit UL: Upper Limit # All variables with $p \leq 0.05$ were included in the multivariate analysis CI: Confidence interval *: Statistically significant at $p \leq 0.05$

Discussion

The prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatic disorders. [23] In accordance to these findings, the present work revealed that 70%of the studied CHC patients were insulin resistant, even after exclusion of obesity, diabetes and advanced cirrhosis. This was in agreement with Ahmed et al [24] who found that (69%) of the studied Egyptian CHC patients had become insulin resistant according to their HOMA-IR.

Since both CHC infection and A-FABP are related to the development of insulin resistance and NAFLD, [17, 25] so this study aimed to investigate whether there is an association between A-FABP and CHC associated insulin resistance.

In the present study, the difference in A-FABP level found between CHC with IR (group II-b) and CHC without IR (group II-a) compared to controls could be attributed to HCV infection itself, as CHC patients with metabolic diseases that could contribute to IR were excluded. This link between A-FABP level and HCV infection is also supported by the significant high correlation found between A-FABP and the viral load (HCV-RNA), (r=0.8, $p<0.001$), Table (II).The later correlation also explains the absence of significant difference in A-FABP level between CHC patients without IR (group II-a) and the control group, as group (II-a) had lower viral load than group (II-b),($p<0.001$) Table (I). Up to our knowledge in the present work it was the first time to measure A-FABP in CHC patients.

The observed significant elevation of serum A-FABP levels in CHC patients with IR, may be explained as HCV is known to cause NAFLD [25]. Moreover, NAFLD was found to increase A-FABP expression in macrophages.[17]Similar increase in serum A-FABP level due to NAFLD was described by Jeon et al.[26] This finding could be confirmed in our study by the higher value of FLI found in CHC patients with IR compared to those without IR as well as the controls, confirming possible A-FABP role in lipid accumulation in the liver of CHC patients adding to the risk of development insulin resistance in such patients

In addition, the significant positive correlation found between A-FABP and serum NEFA in CHC patients with IR may be attributed to previous finding that A-FABP enhances the hydrolytic activity of hormone-sensitive lipase (HSL) and could increase the risk of IR. [27]

Furthermore, elevation of serum A-FABP could be explained by the previous finding that HCV core protein induces elevation of PPARγ transcriptional activity, [28] which in turn increases the expression of A-FABP leading to insulin resistance [15] This suggestion is supported by the significant high positive correlation between A-FABP with HOMA-IR, insulin, FBS, PP2h in CHC patients, Table (II).

Another possible mechanism for elevated A-FABP concentration in CHC patients that we suggest in our study is the elevated oxLDL found in CHC patients compared to control group, Table (I). Solbach et al [29] showed that serum oxLDL level was increased in HCV patients to inhibit HCV cell entry and inhibit its infectivity.

Remarkably, our results showed significant elevation of serum oxLDL level in CHC patients especially in group II-b with IR more than group II-a and controls, Table (I). On the other hand, as previously mentioned, oxLDL increases A-FABP expression in activated macrophages.[16] From these findings, it became evident that elevated oxLDL added to the risk of IR development in CHC through stimulation of A-FABP expression.

Moreover, the present study revealed that serum A-FABP levels were independently and positively correlated with insulin resistance after adjusting for confounding factors among CHC patients, Table (III). In addition, our results showed that at the best cut off value of 19.4 ng/ml, serum A-FABP (FABP-4) had higher diagnostic sensitivity for determination of IR compared to serum insulin, (100% versus 88.46%; respectively) with similar specificity of 92.86% for both and an AUC of (0.998 versus 0.966, respectively) Figure (1).

From our previously mentioned results and as the role of A-FABP in the development of insulin resistance in CHC has been illuminated, it is hoped that this relation will allow the internist to use the available A-FABP blockers to protect CHC patients from the serious complications of insulin resistance. However, larger scale studies including female subjects are warranted.

In conclusion, insulin resistance was highly prevalent in early stage CHC even after all other possible causes were excluded. CHC male patients with IR had higher serum A-FABP levels than those without IR and the control group. A-FABP level showed high positive correlation with HCV-RNA. In addition, elevation of serum oxLDL level in CHC could be the possible explanation of the direct relation between HCV infection and increased A-FABP level leading to development of insulin resistance. Serum A-FABP is an

independent determinant of IR in CHC and its sensitivity surpassed serum insulin in detection of insulin resistance at early stage CHC.

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Original Article

Correlation of Plasma Osteopontin Level with Disease Severity in Knee Osteoarthritis Patients Graded By Conventional Radiological Images.

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Abstract

Introduction: Osteoarthritis (OA) is a common degenerative joint disease that is characterized by progressive destruction of the articular cartilage. Osteopontin (OPN) is a highly phosphorylated and sulfated glycoprotein with cell binding properties. Over-expression and high level of its presence in the synovial fluid and articular cartilage were found to be associated with osteoarthritis progression. Osteopontin is one of the major non- collagenous bone matrix proteins produced by various cell types, including activated T cells , macrophages , osteoblasts and chondrocytes . Recent studies, have taken attempt to use this protein as a diagnostic marker of osteoarthritis and as a target for the drug development against osteoarthritis. **Aim of the Work:** To measure plasma and synovial fluid osteopontin in patients with primary knee osteoarthritis, and evaluate the possible correlations with severity of the disease. **Subjects :** The study includes : Sixty patients with primary knee osteoarthritis fulfilling the American College of Rheumatology Criteria for Knee osteoarthritis and twenty healthy persons with matched age and sex as a control. **Methods:** 1-Full medical history taking and thorough clinical examination with specific stress on local examination of the knee joint. 2- CBC, FBS, 2hrs PP, blood urea, serum creatinine, ESR 1st hr, CRP mg/dl 3-Measuring of plasma Osteopontin (OPN) levels using ELISA technique. 4-knee Radiographs assessed using Kellgren and Lawrence (K/L) radiological score of severity. 5- Synovial fluid aspiration for osteopontin level from osteoarthritis patients presented with knee effusion. **Results:** Statistically significant elevated levels of plasma OPN was found in patients with primary knee osteoarthritis when compared to controls ($P<0.001$). Statistically significant elevated levels of plasma and synovial OPN was found with radiological grades of severity of knee osteoarthritis ($P<0.001$, $P<0.05$ respectively). **Conclusion :** Measurements of both plasma and/or synovial fluid levels of Osteopontin could possibly serve as a specific biochemical parameter for determining disease severity , joint destruction and predicting the progression of osteoarthritic disease process as regarding radiological grading .

Key Words: : Osteopontin, Knee Osteoarthritis, Cartilage Degradation, Synovial Fluid.

Introduction

Osteoarthritis is the most common form of arthritis, which is mainly characterized by a group of mechanical abnormalities including articular cartilage and subchondral bone degradation, and remodeling of adjacent bone with new bone (osteophyte) formation.[1, 2] The clinical manifestations of OA include joint pain, stiffness, tenderness, reduced motion, locking of joints and sometimes an effusion. [3, 4, 5] OA is supposed to be the fourth leading cause of disability by the year 2020 due to the increasing life expectancy and aging populations. [6, 7] The genetic factors (although the responsible genes are largely unknown), constitutional factors (age, obesity, gender, high bone density) and biomechanical risk factors (joint injury, occupational usage, loss of muscle strength,

impairment of peripheral nerves leading to decreased movements, joint laxity and misalignments of bones) have major contribution on OA development and progression. [2, 8] Osteopontin (OPN) is a multifunctional phosphoprotein which comprises 300 amino acids. OPN, also known as bone sialoprotein I (BSP-1 or BNSP), is a 44~75 kDa non-collagenous extracellular matrix (ECM) protein. [9,10] This protein is secreted by a variety of tissues and cells including osteoblasts, osteoclasts, chondrocytes, synoviocytes, macrophages, smooth muscle cells, tendon fibroblasts and activated T cells. [11] OPN is also a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family and known as early T cell activation 1 protein (Eta-1). It is originally isolated from bone extracellular matrix, where it mediates important cell–matrix and cell–cell interactions. [12,13,14] OPN is an acidic protein, highly phosphorylated and glycosylated and has an Arg-Gly-Asp (RGD) sequence as well as two heparin-binding sites, one thrombin cleavage site and a putative calcium-binding site. [9,15] Full length OPN (OPN-FL) is cleaved by thrombin, resulting in thrombin-cleaved OPN (OPN-R) with two functional fragments. The N-terminal part contains a RGD sequence that interacts with integrin receptors α4β1, α9β1, α9β4, αv (β1, β3, β5) and α5β1 and activates various molecules involved in MAPK and NFkB signaling pathways. [9, 11] OPN is thought to be involved in the process of inflammation, immune response and bone metabolism. [16] Both the cleaved and native OPN can interact with integrin and play an important role in regulating inflammation, biomineralization, bone remodeling, immune functions, chemotaxis, cell activation and apoptosis. [17] Osteopontin is a critical intrinsic regulator that plays an important role in OA progression. [18] The increased expression of OPN has been observed in the joints of patients that were reported to be correlated with the severity of joint lesion and inflammatory status in the OA patients. [11, 13, 19]

AIM OF THE WORK :

The aim of the study was to measure plasma and synovial fluid osteopontin in patients with primary knee osteoarthritis and to assess its relation to disease severity .

SUBJECTS:

The study include: Sixty patients with primary knee O.A fulfilling the American College of Rheumatology Criteria for the classification of Knee osteoarthritis. Twenty persons with matched age and sex as a control group. Exclusion Criteria : Patients with secondary osteoarthritis as post-traumatic , rheumatoid arthritis , diabetes mellitus, malignancy , patients with systemic diseases as hepatic , renal , and patients with generalized osteoarthritis including concomitant knee and hip osteoarthritis .

METHODS:

All patients will be subjected to : 1- Full medical history taking and thorough clinical examination , and gait examination with specific stress on local examination of the knee joint , also ; examination of small joints of hands , feet , shoulders , hip joints, and ankle joints as a screening for primary generalized osteoarthritis . 2- Routine laboratory Investigations : Complete blood count using coulter counter [20] , Fasting and Post- Prandial blood sugar [21] , Renal function tests [22] , Liver function tests [23] , ESR 1st hr (mm hr) [24] , CRP mg/dl [25] using ELISA . 3- Measuring of full length Osteopontin (OPN) levels in plasma using ELISA technique [26]. 4- knee Radiographs: Standardized Plain X - ray weight bearing antero-posterior and lateral radiographs of the knee will be performed and disease severity was assessed using Kellgren and Lawrence, 1957 (K/L) radiological score of severity and classified into: Stage 1: Incipient osteoarthritis, beginning of osteophyte formation on eminences. Stage 2: Definite osteophyte and possible narrowing of joint space. Stage 3: Multiple osteophytes, definite narrowing of joint space, and some sclerosis and possible deformity of bone ends. Stage 4: Osteophytes, marked narrowing of joint space, subchondral bone sclerosis and definite deformity of bone ends. 5- Synovial fluid aspiration for Osteopontin level under Ultrasound Guidance.

Statistical Analysis of the Data:

Data shown are the means ± the standard error of the mean (SEM). All statistical analyses for data were performed using SPSS software. Data were analyzed between two groups using student's t - test, while among more than two groups data were analyzed by the one-way ANOVA method. Differences of $P < 0.05$ were considered significant.

RESULTS:

The study was conducted on 60 patients with primary knee OA; 15 were males, 45 patients were females and 20 healthy persons with matched age and sex as a control group. The patients' age ranged from 40.0 - 72.0 with a mean of 56.74 ±5.20, while in the control group the age ranged from 35.0 - 64.0 with a mean age of 52.2 ± 9.51. Patients with bilateral knee OA were 48 (68.5%), and those presented with unilateral knee OA were 22 (31.4%), 40 patients (57.1%) were with right knee OA, and 30 (42.8%) were with left knee OA. The duration of inactivity stiffness ranged from 10 minutes in 25 patients (62.5%), up to 15 minutes in 12 patients (30.0%), and up to 20 minutes only 3 patients (7.5%). According to Kellgren and Lawrence, 1957 (K /L) radiological score of measuring severity of knee OA patients ; 10 patients (14.2%) were grade (1) , 25 patients (35.7%) were grade (2) , 23 patients (32.85%) were grade (3) , and 12 patients (17.1%) were grade (4) . Regarding of plasma OPN level (ng/ml) among knee OA patients and control; there was highly significant difference between the OA patients and controls ($P < 0.001$). Also, a significant elevated level of plasma OPN in O.A patients with knee swelling and deformities than those without knee swelling or deformities ($P < 0.001$). There was a significant positive correlation between plasma and synovial OPN levels among OA patients. ($r = 0.609$, $P < 0.05$). Comparing the radiological grades of O.A as regards the mean synovial OPN levels (ng/ml) ; there was a statistically significant difference between grade 3 and grade 2 ($P<0.05$) , highly significant difference between grade 4 and grade 2 ($P<0.001$) , and a statistically significant difference between grade 4 and grade 3 ($P<0.05$) . Also , the plasma OPN levels among OA patients correlated with the clinical , radiological grading of disease severity and synovial OPN ; there was statistically significant positive correlation between mean OPN (ng/ml) and the age of patient, duration of pain , joint swelling , knee deformity, and limited range of motion in patients with primary knee OA ($P<0.05$).

Table (I): The Characteristics of pain in OA patients.

		No.	%
Onset	Gradual	63	90.0%
Course	Progressive	56	88.8%
	Stationary	7	11.1%
Increased By	Movement	33	52.3%
	Walking	23	36.5%
	Stair climbing	7	11.1%
Decreased By	Rest + analgesics	35	55.5%
	Analgesics	17	26.9%
	Rest	6	9.5%
	Non	5	7.9%

Table (II): Descriptive and comparative study of Clinical data of O.A patients

	+ve Signs		- ve Signs		
	No	%	No	%	χ^2
Cold swelling (effusion)	36	51.4 %	34	48.5%	p > 0.05
Deformity	7	10.0 %			
Valgus	2	2.8 %	63	90.0%	p < 0.05
Varus	5	7.1 %			
Tenderness	46	65.7 %	24	34.2%	p > 0.05
Painful range of motion	45	64.2 %	25	35.7%	p > 0.05
Limited range of motion	32	45.7 %			
1. In flexion	13	18.5 %	38	54.2%	p > 0.05
2. In extension	9	12.8 %			
3. In both	10	14.2 %			
Palpable Coarse Crepitus	30	42.8 %	40	57.1%	p > 0.05
Sign of acute inflammation	0	0 %	70	100%	

Table (III): Correlation of O.A patients with different demographic data and BMI as regards mean OPN (ng/ml) .

	OPN		
	Pearson Correlation	p	Sig.
Age	0.358	< 0.05	S
Sex	0.280	>0.05	NS
Duration of pain	0.382	<0.05	S
Body Mass Index (BMI)	0.281	>0.05	NS

Table (IV): Comparison between O.A patients with different Joint stiffness duration as regards mean OPN (ng/ml).

Inactivity	OPN (MEAN ± SD)	F	p	Sig.
Up to 10 minutes (n=25)	75.76 ±33.97			
Up to 15 minutes (n=12)	60.32 ± 58.64	1.012	> 0.05	NS
Up to 20 minutes (n=3)	93.75 ± 63.03			

Table (V): Comparison between O.A patients without and with different types of limited movement in knee as regard mean plasma OPN (ng/ml) levels.

	OPN (MEAN ± SD)	F	P	Sig.
No limitation (n=32)	69.42 ± 12.139			
Limitation in extension (n=9)	98.75 ± 39.76	10.233	< 0.05	S
Limitation in flexion (n=13)	129.3 ± 25.43			
Limitation in extension & flexion (n=10)	146.25 ± 33.99			

Table (VI): Plasma Osteropontin level at (K/L) Grading

	O.A grades				F	P	Sig.
	KL 1 (n=10)	KL II (n=25)	KL III (n=23)	KL IV (n=12)			
Plasma OPN (Mean ± SD)	82.76 ± 6.32	135.17± 5.98	175.92± 7.10	220.50± 8.01	78.42	< 0.001	HS

Table (VII): Synovial Osteropontin level at (K/L) Grading

	O.A grades			F	P	Sig.
	KL II (n=6)	KL III (n=7)	KL IV (n=7)			
Synovial OPN (Mean ± SD)	250.26 ± 9.88	365.95± 10.14	470.00 ± 6.08	28.25	< 0.05	S

Table (VIII): Comparison between Grades of O.A as regards the mean synovial OPN levels (ng/ml) .

Grade of O.A	Grade O.A	Z	P	Sig.
Grade 3	Grade 2	23.45075	< 0.05	HS
Grade 4	Grade 2	58.12434	< 0.001	HS
	Grade 3	27.7854	< 0.05	S

Table (IX): Comparison between plasma OPN levels patients Clinical, Radiological Grading and Synovial OPN.

	Plasma OPN	
	P	Sig.
Age	< 0.05	S
Sex	> 0.05	NS
Duration of pain	< 0.05	S
Body Mass Index (BMI)	> 0.05	NS
Inactivity stiffness	> 0.05	NS
Cold swelling (effusion)	< 0.05	S
Limited range of motion	< 0.05	S
Knee deformities	< 0.001	HS
X-ray grades	< 0.001	HS
Synovial OPN	< 0.05	S

Discussion

Degeneration of articular cartilage is the main pathological change in OA [27]. Chondrocytes maintain the cartilage homeostasis stimulated by appropriate joint load. Abnormal mechanical load to chondrocytes alters the composition and metabolism of articular cartilage [28], which may induce the release of OPN and enhanced level of OPN in cartilage led to the induction of matrix metalloproteinase 13 (MMP-13) [29] . Elevated levels of OPN in cartilage also activate the transcription factor NF-κB pathway that is involved in the production of many inflammatory factors, including chemokines and cytokines (IL-1, IL-6 , IL-8 , TNF- alpha) in cartilage, which leads to the spontaneous production of NO , PGE 2 , IL-1b , IL-6 and IL-8 . The over-production of these cytokines and mediators exerts injurious effects on chondrocyte functions which leads to an imbalance of cartilage homeostasis resulting in progressive articular degeneration [13]. Our results showed a statistically significant elevated levels of plasma OPN was found in patients with primary knee osteoarthritis when compared to controls ($P < 0.001$). A statistically significant elevated levels of plasma and synovial OPN was found with radiological grades of severity of knee osteoarthritis ($P < 0.001$, $P < 0.05$ respectively). Pullig et al. [31] analyzed the expression level of OPN in osteoarthritic human cartilage and found increased OPN protein deposition and mRNA expression mostly in deep zone of articular cartilage of patients with OA. Martínez-Calleja et al. [32] also found an increased expression of OPN in the superficial zone of articular cartilage of patients with early OA. Zhang et al [33], found increased level of OPN in fibroblast-like synoviocytes (FLS) of patients with OA. Some contradictory findings also exist, as few studies reported that decreased level of OPN is involved in the progression of spontaneous OA lesions [18,34]. Honsawek et al. [35] analyzed OPN levels in both plasma and synovial fluid of OA patients and healthy controls. They found significantly higher OPN levels in both plasma and synovial fluid in patients with OA compared to healthy individuals. Mohammed et al. [36] found significantly higher OPN level in both plasma and synovial fluid compared to the healthy individuals. This increased level of OPN was suggested to be related with the progressive joint damage in OA. Gao et al. [37] investigated OPN levels in both synovial fluid and articular cartilage in patients with OA and healtjy controls to evaluate the found the higher OPN concentration in both synovial fluid and articular cartilage compared to healthy controls. Xu et al. [38] analyzed the phosphorylation level of OPN in OA cartilage and normal cartilage by immunoprecipitation and found a higher phosphorylation level of OPN in OA cartilage than in normal cartilage.

Fan et al. [39] investigate the effects of anti-OPN mAb on the clinical severity of the disease and pathologic changes in the joints. There is a lack of enough evidence, which supports that targeting and reducing the expression of Osteopontin are successful in treating patients with OA.

Conclusion:

As the high level of OPN is frequently found in plasma and synovial fluid of OA patients. , OPN can serve as a promising biochemical marker for diagnosis of OA. Osteopontin is a protein responsible for OA progression. OPN promotes expression of MMP-13 through activation of NF-KB pathway in OA .The discovery of this study has great potentials for understanding the pathogenesis and treatment of OA through manipulation of the NF-KB.

Recommendations:

Further research studies, should focus on the possibility of general use of this protein as biochemical parameter for determining disease severity and may be predictive of prognosis with respect to the disease progression.

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Original Article

Helicobacter Pylori Infection and Its Impact on the Severity of Rheumatoid Arthritis.

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Abstract

Abstract

Background: Autoimmune diseases have unclear etiology, but environmental factors such as viral and bacterial infection or chemical exposure in genetically susceptible individuals may play a role. (1) Helicobacter pylori (H. pylori) is a Gram-negative, flagellated bacterium, which has increased incidence in developing countries (80%), as well as in western countries (50%). (2) H.pylori is able to induce chronic immune response in the host, that's why it was thought that it may have a role in aggravation of autoimmune diseases. (3)

Objective: Evaluate the effect of H. pylori infection on the severity of rheumatoid arthritis.
Method: Fifty rheumatoid arthritis patients were tested for the presence of H. pylori infection using H pylori antigen in stool, and evaluated as regarding the severity of rheumatoid arthritis using DAS 28/ESR as well as estimating the levels of ESR and CRP **Results:** No effect of H pylori infection on the severity of rheumatoid arthritis has been found. Conclusion: Severity of Rheumatoid arthritis is not affected by presence of H pylori infection.
Keywords: H.Pyloris RA

Introduction:

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder characterized by bilateral and symmetric affection of small, medium and large joints, environmental factors in genetically susceptible patients contribute to disease course and severity. (4) Moreover, some viral and bacterial pathogens such as, Epstein-Barr virus, parvovirus B19, Hepatitis C virus, Proteus mirabilis, and mycobacterium tuberculosis, may play a role in triggering some of these diseases. (5) H pylori, is a gram-negative, flagellated bacterium that has been associated with some autoimmune diseases, as being a chronic infection it serves as a source of antigenic stimulation that can induce an inflammatory response. (6) The continuous exposure to the bacterium allows the host immune mechanisms to trigger autoimmune response. Molecular mimicry of H. pylori antigens was found to activate cross- reactive T cells which may lead to autoimmune gastritis. (7) Chronic stimulation of B cells by ureas produced by H. pylori has the ability to stimulate production of autoantibodies including IgM rheumatoid factor, Moreover, other autoantibodies such as anti-single stranded DNA antibody and anti-phosphatidyl choline antibodies, were produced by B cells after their activation by H. pylori antigens. (8) Another possible mechanism of autoimmunity is the sequence homology between microbial heat shock protein (HSP) and human HSP. (9)
Estimation of the severity of RA in patients with and without H. pylori infection has to be evaluated to detect any relation between the bacterium and the disease severity.

Methods:

The study was conducted on 50 patients with rheumatoid arthritis attended the outpatient clinic or admitted to Medical research institute inpatient wards. All patients gave informed consent to participate in the study.

Inclusion criteria

patients with seropositive rheumatoid arthritis fulfilling the American College of Rheumatology and European League Against Rheumatism criteria for rheumatoid arthritis. (10)

Exclusion criteria: Patients with elevated ESR or CRP due to any other causes such as anemia, active infection, other autoimmune disease or malignancy. (11)

- Data will be collected from the hospital medical records.
- Thorough clinical examination.
- Routine laboratory investigations including: liver function tests, renal function tests, electrolytes, and complete blood count. (12)
- ESR, CRP as a marker of inflammation. (11)
- Clinical activity of the disease will be assessed by 28-joint disease activity score using erythrocyte sedimentation rate (DAS28-ESR), which uses tender joint count (TJC), swollen joint count (SJC), patient global assessment-visual analogue scale (PGA-VAS) and ESR. The cutoff value representing remission was defined as < 2.6. (13)
- Stool antigen test for diagnosis of H.pylori infection. (14)

Results:

The two groups of H pylori-positive and H pylori- negative RA patients in this study were well matched as regarding treatment protocol. The prevalence of H pylori in RA patients was 45%, (28.6%) males and (71.4%) females. The mean age for H pylori +ve patients was (46.4±2.4yrs) and in H pylori –ve patients it was (58.7±3.2yrs).

As regards CRP it was elevated in H pylori +ve more than H pylori – ve patients, the mean value in H pylori +ve patient was (12.1±3.6 mg/dl), and in H pylori – ve patients it was (9.9±3.5 mg/dl), P=0.1. As regards ESR the mean value in H pylori +ve patients was (35.8±16.7 mm/hr), and in H .pylori – ve patients it was (33.8±20.1 mm/hr), P=0.8. The clinical activity of RA was assessed by DAS28/ESR, the mean value in H pylori + ve patients was (3.5±1.2), and in H pylori – ve patients it was (3.8±1.3), P=0.4.

Discussion:

In the present study, the prevalence of H pylori infection in RA patient was 45%, matching the results of Zentilin who found its prevalence to be 48 %,(15) Tanaka also evaluated the prevalence of H pylori infection in Japanese patients with RA and he found the prevalence of H pylori in patients with and without NSAID use were 47.5% and 54.7%, respectively. (16)

In some studies, H. pylori infection rate detected by urea breath test was found to be 78% in men and 82% in women. (17) In another study of Marusic, (18) there was also female predominance with male to female ratio about 0.92. In the present study H pylori infection detected by stool antigen test was also predominant in female patients (71.4%) as autoimmune diseases are known to be more common in females.

Marusic, (18) found that the peak age for H pylori infected patients was between 45-64 years, in this study the mean age of H pylori infected patients was 46 years.

Zentilin, (15) when he assessed RA activity, he found that H pylori infected patients had more severe disease than non-infected patients; this was indicated by increased number of painful joints and mobility dysfunction. Moreover, the activity indices including ESR and CRP were higher in infected patients.

This may be attributed to the presence of continuous stimulation with H pylori antigens producing various cytokines. (19) On the other hand, Nakamura (20) found that rheumatoid factor was inversely related to the presence of H pylori infection and rheumatoid factor value was lower in infected patients, and he stated that H pylori infection may not be the cause of gastrointestinal disorders in rheumatoid arthritis, and that rheumatoid factor significantly reduces the incidence of the infection.

In one of the studies of Matsukawa, (21) he found that RA patients may have increased activity following eradication of H pylori which may be due to disruption of oral tolerance against stress protein such as mycobacterial heat shock protein 65, this was suspected because there were increased ESR, CRP, and development of joint pain after infection eradication.

Another study done by Moriyama, (22) who found that H pylori infection didn't affect the activity of rheumatoid arthritis, but the infection contributes to mucosal atrophy, as there was no difference in clinical or laboratory parameters between infected and non-infected rheumatoid arthritis patients in a period 26 to 49 months of follow up.

In the present study, CRP was higher in patients with H pylori infection but without statistical significance, and there was no difference in ESR or DAS28-ESR between H pylori- Positive and H pylori – negative RA patients, as treatment of those patients with immunomodulators was controlling the cytokines released by the chronic infection with H pylori. Moreover, as the most common cytokine released in response to H pylori infection is interlukin -8 which in turn activate polymorphic cells and monocytes that produce other cytokines, such as interleukin-1, -6, -7 and -10 and tumor necrosis factor –α. [15]

And because patients with RA are usually treated with methotrexate that inhibit interleukin-1 and interleukin-8, [23] the inflammatory response to H pylori infection doesn't increase cytokines production and doesn't lead to increased RA activity.

Conclusion :

Severity of Rheumatoid arthritis is not affected by presence of H pylori infection

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