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Determination of *Helicobacter pylori IgG* and Smoking as a risk factor for Atherosclerosis in Iraqi patients

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Abstract:-

The present work aims to determine the role of *H. pylori* IgG on the clinical expression of Atherosclerosis among Iraqi patients, and to provide a new evidence about the relationship between Smoking and *H. pylori* IgG .

This study included 80 male patients, with Atherosclerosis ,Who were divided into two sub groups smokers (51) and non smokers (29) , the age ranged between 40-58 years. A control group of 20 Healthy volunteers ,with age ranged between 36 -58 years .A control group matched with patients for sex and ethnic. *H. pylori* infection was diagnosed by detection of IgG antibodies against *H. pylori* by ELISA. Present study shows a significant increasing in concentration of *H. pylori* IgG antibodies in serum of both patient groups compared to control, furthermore smoker patients showed non-significant increasing compared to non smoker patients ,present result provides evidence that *H. pylori* infection is a risk factor for atherosclerosis , Which may be associated with smoking .

Introduction :-

Atherosclerosis is one of the most common disease with pathological process could lead to cardiovascular disease, the systemic immune system as well as the local vascular immune system is involved in the development and progression of atherosclerosis (1) Many factors like hypertension, increased blood lipids, smoking and diabetes mellitus consider as classical risk factors [2], fail to account for all the epidemiological variants of the progression of atherosclerosis , therefore the investigators continued to investigate other possible risk factors and their association with classical risk factors [3] , recent studies indicate that the number of microbial infections (the pathogen burden) critically determine the development and progression of atherosclerotic disease. [4] ,therefore the infection with pathogen may contribute with classical risk factors in the pathogenesis of atherosclerosis, since microbial motifs and amplify cytolytic T-cell function by inducing a local or systemic inflammatory response [2].Chlamydia, herpes simplex and cytomegalovirus have been detected in atherosclerosis plaques (1)these pathogens may contribute to the initial vascular injury via the induction of autoimmune responses that may cause activation Lymphocyte (4), Which play an important role in the production of antibodies by B-cells in a T-cell-dependent or independent manner .Furthermore some of these antibodies have the ability to block the import of modified Lipoproteins via macrophage scavenger receptors (1).

Helicobacter pylori (*H. pylori*) is an organism first identified in 1983 by Warren and Marshall and called *Campylobacter pylori* until 1989, which is gram –negative ,multiflagellate, ,with ability to adhere to gastric epithelium, it is armory of urease and mucolytic enzymes, since all these factors allow it to survive and proliferate in the dangerous gastric milieu [5] .Moreover , It is equipped with specific receptors that mediate adhesion to the gastric surface epithelium [6],and can stimulate an intense inflammatory and immune response that enhance antigen presentation and facilitates immune stimulation [1]. .Consequently

,pro inflammatory cytokines are secreted by immune cells ,such as interleukin -1 (IL-1), IL -8 , IL-6 , IL-12 , IL-10, tumor necrosis factor alpha (TNFa), interferon-γ and platelet-derived growth factor [1,5] .

H. pylori colonization of the gastric mucosa can remain asymptomatic or give rise to severe clinical manifestations including chronic gastritis, duodenal or gastric ulcers, mucosa-associated lymphoid tissue, lymphoma and gastric cancer [7]. Furthermore chronic bacterial infections are involved in the genesis of many illnesses like atherosclerosis [5].Serological study illustrated no correlation between evidence of *H. pylori* infection and classical risk factors like blood pressure, leukocyte count, serum total cholesterol, triglycerides, fibrinogen and C-reactive protein [8].

There is many studies focused on the presences of antibodies to *H. pylori* and atherosclerosis ,Particularly in countries with high prevalence of *H. pylori* [9].The prevalence of *H. pylori* among adult population in the middle east was about 71 to 92 % [10] .Moreover, *H. pylori* may be endemic among Arab patients dyspepsia [11] , Furthermore, other factors that may increase the risk of *H.pylori* infection in Saudi Arabia are smoking lower educational level, and older age [12].Iraqi smoker individuals with no gastrointestinal symptoms recorded a positive result for *H. pylori* IgG antibodies ,the incidence ranged from (58.9%) to (30.3%) for heavy to mild smokers, while it was (7.1%) for control [13].A study about Iraqi patients with coronary heart disease who recorded significant increasing level of H.pylori IgG antibody considered *H.pylori* infection as a risk factor for coronary heart disease [14].Most infections of *H.pylori* are cured during childhood and in 90% of population in Western countries [2].The hypothesis of an infectious etiology to atherosclerosis is suggested a link between *H.pylori* antibody and the progression of disease ,so the aim of this study was to evaluate the effects of *H.pylori* on atherosclerosis as a risk factor with classical risk factor like smoking.

Materials and methods:-

A total of 80 male patients with atherosclerosis who were admitted to Ibn Al-Nafees Teaching hospital in Baghdad were studied and they were divided into two sub groups smokers(group A) and non smokers (group B) .Diagnosis was carried according to the clinical examination by physicians and laboratory investigation.

Control group consist of 20 healthy individuals (non smokers),it was age , sex and ethnic matching with patients group .Female were excluded from the study because of a low proportion of female smokers .From each subject, 5 ml of blood were aspirated , then serum was separated by centrifugation at 3000 rpm for two minutes , the serum was divided into small 1 ml aliquot and immediately frozen at $(-20)C^{o}$ until used thawing of each frozen sample was allowed only once at a time of the test [15] .

IgG antibodies to *H. pylori* was carried by *ELISA* technique . The micro plates are sensitized with a semi-purified antigen extract that containing non flagella antigens are likely to cause cross-reactions. [15]. IT is provided by Bio- RAD company, France .Statistical analysis was done using SPSS by using Duncan and Chi-square.

Results:-

The comparison among study groups was carried by statistical analysis as it shows in table 1 .Both patients groups illustrated significant increasing compared to control ,with no significant differences between patient groups (P≤0.05) ,but the patient group A has the highest mean (3.13 ±1.776) among them.

Table (1) Statistical analysis among the study groups according to the concentration of H.pylori IgG antibodies

Study Groups	NO.	Mean ± SD	Duncan*
Patient group A	51	3.136 ± 1.776	b
Patient group B	29	2.747 ± 1.936	b
control	20	1.692±0.988	a

*** The similar letters means that there are no Significant Differences (P≥0.05)**

Table (2) shows the distribution of study groups ,this distribution is according to the status of *H.pylori* infection. Results are considered positive when concentration is more than 1.1 U/L and negative as concentration less than or equaling 1.1 U/L. Positive and negative results are calculated as a percentage from the total number of each study group . Positive results represent a high percentage for both groups of patients ,moreover the *group A* shows the highest percentage for positive results among groups ,while control group showes the lowest percentage . At the same time, positive percentage for *H.pylori* with control group is lower than negative percentage ,while negative control was higher than other negative results. The differences were not significant among all study groups ($\acute{\alpha}^2$=29.723) by Chi-square as total comparison .On the other hands the differences were significant between patient groups A and B($\acute{\alpha}^2$=2.331), although the percentage of positive test for group A(smoker patients) was higher than positive percentage of group B (non smoker patients).

Table (2) Distribution of study groups according to the status of *H.pylori* infection

Groups	NO.	*H.pylori* IgG antibodies level			
		positive		negative	
		NO	%	NO.	%
Group A	**51**	**44**	**86.275**	**7**	**13.725**
Group B	**29**	**21**	**72.414**	**8**	**27.586**
control	**20**	**4**	**20**	**16**	**80**

X^2=29.722 df=2 P≥0.05 (NS) (total comparison).

X^2=2.331 df=1 P≤0.05 (S) (between group A and group B).

Discussion

The current data is considered a high IgG antibodies level as a marker of a previous infections, this fact was taken from recent study ,this recent study reached to interesting feature ,that *H.pylori* in addition to acute infections cause chronic or latent infection [5] . The results revealed that all patients are showed a significant higher concentration of *H.pylori* IgG antibodies (U/L) compared to control . These results agree with the concept that *H.pylori* could be a candidate trigger for atherosclerosis [10],at the same time ,the mean level of *H.pylori* IgG antibodies for smoker patients was higher than the mean of non smoker patients, these finding agree with previous finding that the progression of atherosclerosis influences by classical risk factors that promote vascular inflammation and plaque rupture [3, 16].Viral and bacterial infections contribute to the pathogen of atherosclerosis [7] so even that infectious may be the first or recurrent infection in present patients ,acute infections might play a more important pathogenic role in the early phase of atherogenesis [16].Furthermore ,chronic infection has been found to be significantly associated with the development of atherosclerosis [10],because the obligate intracellular pathogens having a latent state may contribute to the atherogenic process where resistance is observed in the cellular response or by humeral response [2].Atherosclerotic plaque sense microbial motifs and amplify cytolytic T-cell functions ,thus providing a link between host-infectious episodes and acute immune-mediated complication of atherosclerosis [17] ,moreover unstable atherosclerotic plaque is characterized by infiltrate of inflammatory cells. Both macrophage and T-cells have been implicated in mediating the tissue injury releasing cytokines leading to plaque rupture [18],besides it is facilitating the developing of other factors like atherogenesis because antibodies of any pathogens have the ability to block the import of modified Lipoproteins via macrophage scavenger receptors [1,19].

Results of the present study disagree with a previous data , which suggested that prior infection with *H. pylori* is not a major factor determining the risk of atherosclerosis, because the mechanisms of how *H. pylori* infection results in the Coronary atherosclerosis and the relationship between *H. pylori* infection and clinical with classical risk factors have not been fully understood [8]. In addition measuring IgA antibodies for *H.pylori* and others pathogens might reflect more recent and repeated infectious episodes [3].

The seropositive rate for *H.pylori* infection in Iraqi patients with atherosclerosis is agreement with the prevalence of *H.pylori* in other Arab population with positive result for *H.pylori* test even with asymptomatic infection [10], at the same time ,the rate of positively of *H.pylori* infection among Korean adults is reported to reach as much as 70% [20] ,furthermore present data agree with previous finding that the prevalence of disease among Asians not be much different from that Caucasians in spite of the high rate of *H.pylori* infection for both races[21],besides the present positive ratio is agreement with previous study about Middle east [7] . Furthermore, present data shows a synergistic effect between smoking as classical risk factor and *H.pylori* since both of them recorded high positive percentage because smoking may be a risk factor for *H.pylori* infection [13] .

All pervious information demonstrates the importance of the type of immune response mounted by the host to the infectious agents in determining whether or not the infection predisposed to vascular disease , medical diagnosis and treatment need more prospective studies for evaluating the relationship between *H.pylori* with progression the clinical features and severity of disease. present problem needs Further insight by using specific methods like polymerase chain reaction PCR technique to detect the strains of bacteria .Further studies most conducting to obtain more clarification about the relationship among classical risk factors as hyperlipidemia and *H.pylori*.

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دراسة عن الاجسام الضدية صنف *IgG* لبكتريا *Helicobacter pylori* مع التدخين كعامل خطورة لتصلب الشرايين في مرضى عراقيين

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الخلاصة:-

أجريت الدراسة الحالية لايجاد المزيد من الايضاحات حول دور عوامل مناعية في التطور المرضي لتصلب الشرايين بين المرضى العراقيين ولالقاء المزيد من الضـــوء على عوامل الخطورة التقليدية وعوامل جديدة ممكن قد يكون بالامكان اعتبارها عوامل خطورة تستخدم في الكشف المبكر والتشخيص للمرض وتوضيح دور التدخين كاحد عوامل الخطورة التقليدية مع الاصابة الكائنات الممرضة.

الدراسة الحالية شملت 80 مريضا بتصلب الشرايين وقسم المرضى الى مدخنين وغير مدخنين وقورنت النتائج ب 20 فردا من الاصحاء وتراوح العمر للمرضى بين (40 -58)سنة و (36 -58) سنة للسيطرة و شخصت الاصابة سريريا من قبل الكادر الطبي الاستشاري لمستشفى ابن النفيس وتم التشخيص بواسطة الدلائل المختبرية في مصل المرضى واتضح زيادة في تركيز الاضداد صنف IgG الخاصة ببكتريا *Helicobacter pylori* في مجموعتي المرضى المدخنين وغير المدخنين مقارنة بالسيطرة وبهذا يمكن ان تعتبر عامل خطورة لتصلب الشرايين عند المرضى والذي قد يرتبط بالتدخين لديهم .