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Effect of *Helicobacter Pylori* Infection on Interleukin-4 and Interleukin-8 Levels in Smoker Males

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Abstract

Background: Smoking is a risk factor for various chronic diseases (including various infections, cancer, heart disease and respiratory diseases), and is characterized by neutrophil inflammation and the release of pro-inflammatory mediators (such as IL-8 and IL-4). Smoking has been found to be associated with higher incidence of *Helicobacter pylori* and gastroduodenal diseases caused by this bacterium are a consequence of an inappropriate host's immune response.

Materials& Methods: This study aimed to study the effect of smoking on certain immunological parameters, and it is a risk factor for *H. pylori* infection in Erbil city. The study was carried out on 71 smoker males and 20 nonsmokers. *H. pylori* infection and the concentration of IL-4 and IL-8 were done by ELISA Technique. *P values* <0.05 were considered statistically significant.

Results: Of the 91males participants, 71 (78%) were smokers and 20 (22%) were non-smoker. The mean age of smoker men was 24.37±6.52 and 23±4.67 for non-smoker men. The prevalence rate of *H. pylori* infection was significantly higher (*P value:*0.02*)* in smoker males 65(9.5%) than non-smoker males12 (6.9%). Levels of IL-4 and IL-8 were significantly higher in smoker males when compared with nonsmoker males (*P*< 0.01& *P*< 0.03 respectively), and their level were significantly higher in smoker males infected with *H. pylori* (*P*<0.001).

Conclusions: This study showed that the rate of *H. pylori* infection among smoker is higher than that of non-smokers and cigarette smoking was an important risk factor for *H. pylori* infection as well, smokers have higher levels of IL-4 and IL-8 than never-smokers.

Keywords:
Smoker males, IL-4, IL-8, *Helicobacter pylori*.

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تأثير عدوى هيلكوباكتر بيلوري على انترلوكين -4 ومستويات انترلوكين-8 في الذكور المدخنين

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الخلاصة

المقدمة: يعد التدخين عامل خطر للإصابة بأمراض مختلفة (بما في ذلك إصابات بكتريه مختلفة، السرطان، أمراض القلب وأمراض الجهاز التنفسي) ويتصف بالالتهابات العدلية ويتميز بإنتاج خلايا مناعيه مساعده قبل الالتهابية منها IL-8, IL-4. ولوحظ أن التدخين له علاقة بارتفاع معدل الإصابة بالبكتيريا الملوية البوابية وأمراض المعدة والاثني عشر التي تسببها هذه البكتيريا والتي بالنتيجة استجابة المناعية للمضيف تكون غير ملائمه.

طرق العمل: هدفت هذه الدراسة إلى دراسة تأثير التدخين على بعض المقاييس للخلايا المناعية، واعتبره كعامل خطر اوضار للإصابة بالبكتيريا الملوية البوابية في مدينه أربيل. اجريت الدراسة على 91 ذ كر منها 7 مدخنين و20 غير مدخنين. وتم قياس تركيز كل من IL-4،IL-8 باستخدام تقنية مناعي مرتبط بالإنزيم (ELISA) . واعتبرت قيمه إحصائية $P<0.05$.

النتائج: جمعت عينه الدم من 91 ذكرًا، منها71 (78٪) مدخنين و20 (22٪) غير مدخنين كان متوسط عمر الرجال المدخنين 24.37 ± 6.52 و23 ± 4.67 للرجال غير المدخنين .ومعدل انتشار عدوى الملوية البوابية أعلى بشكل ملحوظ (*P value*=0.02) في الذكور المدخنين 65 (9.5٪) مقارنه لغير المدخنين (6.9٪) .وكانت مستوى التركيز كل من IL-4و IL-8 أعلى بشكل ملحوظ في الذكور المدخنين مقارنة بالذكور غير المدخنين (P <0.03, P <0.01) ، وتبين بالخص ان مستوى تركيزها أعلى بشكل ملحوظ في الذكور المدخنين المصابين بالبكتيريا الحلزونية(*P <0.001*) .

الاستنتاج: اظهرت هذه الدراسة أن معدل الإصابة بالبكتيريا الملوية البوابية بين المدخنين أعلى مقارنه لغير المدخنين وأن تدخين السجائر يعتبر عامل خطر مهم للإصابة بالبكتيريا الملوية البوابية ، كما تبين أن المدخنين لديهم مستويات تركيز أعلى لكل من IL-4 و IL-8 .

الكلمات المفتاحية:

ذكور مدخنين، IL-8 ,IL-4، البكتيريا الملوية البوابية (Helicobacter pylori)

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Introduction

The mixture of cigarettes has numerous chemical's components are added to the tobacco content, paper, and filter during the manufacturing process that have toxic, mutagenic and carcinogenic properties [1]. Tobacco use is considered an immunomodulator in the oral cavity, which is a risk factor associated with chronic diseases [2]. Many types of cells and inflammatory mediators that produce tissue injury initiates the pulmonary inflammatory cascade by an inhalation of tobacco and lead to increase in inflammatory markers, such as IL-8, IL-6, IL-1, IL-10, and IL-12 [3].

A bacterium that parasitizes the gastric mucous layer and the epithelial lining of the stomach called Helicobacter pylori, and itis causative agent of many diseases in the gastrointestinal (GI) tract, gastritis, peptic ulcer disease, and gastric carcinoma, and thought that the infected individuals develop overt clinical disease around 10% while 90% remain subclinical, and the infection can persist throughout life if untreated and cigarette smoking is a major risk factor in H. pylori acquisition. Noted that this transmission is mainly through the oral–oral or fecal–oral route [4].

Previous study found that cytokine polymorphisms are associated with H. pylori infection namely IL-4 and IL-6, which play a role in gastric inflammation processes [5]. It is found that IL-4 has an inhibitory effect on the expression, production, and release of inflammatory cytokines [6].

A study observed that serum level of IL-8 increased and he indicated that the patients, which had gastric carcinoma were smoker, and he confirmed that the smoking and drinking were the two most famous factors correlated to gastric carcinoma risk throughout the world [7]. The aim of present study was investigated the effect of Helicobacter Pylori on Interleukin-4 and Interleukin-8 in smoker males.

Materials and Methods

The study is a case-control study and was conducted from August to December 2019. At the beginning of the study, detailed information about male age, smoking, drinking habits, education level, diet type, daily smoking, body mass index, and stomach symptoms were obtained from questionnaires filled out by all subjects. Informed written consent was obtained from all individuals using approved protocols by the Research Ethics Committee of Erbil Polytechnic University.

Blood samples will collect from 71 smoker males and 20 from nonsmoker males, all samples will centrifuge to obtain the serum. The serum will test for anti *H. pylori* Ab (IgG U/ml., IgA U/ml., IgM U/ml.) by (enzyme-linked immunosorbent assay (ELISA) kit from Monobind Inc. (Accubind ELISA

microwells), Also serum IL-4 and IL-8 levels will measure with the (enzyme-linked immunosorbent assay (ELISA) kit from KOMABIOTECH. Standard range IL-4: 32-2000 pg/ml. and standard range IL-8: 16-1000 pg/ml.

Statistical analysis

GraphPad Prism version 6 (GraphPad Software, SanDiego,C A) and SPSS (IBM. version 25, 2017). Data of study were analyzed by using Chi Square, Paired T test. A *P-value* of < 0.05 was considered statistically significant. The nonparametric data were reported as Mean+/-Standard Deviation.

Results

A total of 91 males 71(78%) were smokers and 20(22%) were nonsmokers. Between the ages of 14 and 50, the average of mean & SD of smokers (24.27±6.52) and the average of mean &SD of non-smokers (23±4.67), the education level of smokers, the number of cigarettes, and *Helicobacter pylori* infection were significantly greater High (*p value* = 0.02, 0.001, 0.001 respectively) than nonsmokers. The distribution of demographic variables among 91 smoker and non- smoker males were shown in table (1).

Table (1) Distribution of demographic variables among 91 smoker and non- smoker males (No.,%, Mean±SD)

Variables	**Smoker status (n= 71) 78%**	**Non-smoker status (n=20)22%**	***P value***
Age/ years	24.27±6.52	23±4.67	0.41(NS)
Level of education	1.08±0.28	1.15±0.082	0.02 (S)
Educate	65 (91.5%)	17(23.9%)	
Non-educate	6(8.5%)	3(4.2%)	
Type of dietary	2.27±0.93	2.25±0.97	0.9 (NS)
Vegetarian	23(32.4%)	7(9.9%)	
Meat	6(8.5%)	1(1.4%)	
Both	42(59.2%)	12(16.9%)	
Alcohol Drink	1.97±0.167	2±0.0	0.44(NS)
Yes	2(2.8%)	0(0%)	
No	69(97.2%)	20(100%)	
No. of Cigarette/day	1.22±0.499		0.001(S)
3 cigarette/day	19(26.8)		
>3 cigarette / day	52(73.2%)		
Gastric symptoms	1.56±0.49	1.7±0.47	0.27(NS)
Yes	31(43.7%)	6(8.5%)	
No	40(56.3)	14(19.7)	
H. pylori infection	(1.13±0.34) (83.5%)	(1.67±0.49)(16.5%)	0.001(S)
Yes	66(92.9%)	10(50%)	
No	5(7.1%)	10(50%)	
BMI (body mass index) (Kg/M2)	1.38±0.49	1.40±0.5	0.5(NS)
Normal weight	44(62%)	12(16.9%)	
Over weight	27(38%)	8(11.3%)	
IL-8 (pg/ml)	0.9329±0.344	0.649±0.151	0.03 (S)
IL-4(pg/ml)	0.886±0.341	0.216±0.089	0.01 (S)
IgM (U/ml)	1.34±0.467	1.96±0.308	0.001(S)
IgG (U/ml)	1.18±0.390	1.55±0.510	0.001(S)
IgA (U/ml)	1.38±0.489	1.8±0410	0.001(S)
S: significant , NS : non significant			

The serum level of IL-4 and IL-8 in smoker and nonsmoker samples according their infection by *H. pylori* was reported in table (2) and found that the level of both interleukins (0.984±0.323, 0.914±0.33 respectively) were significantly (*p value* =0.001) higher in smoker samples with *H. pylori* positive compared to nonsmokers.

Table (2) Mean& SD of IL-8 & IL-4 according *H. pylori* infection among smoker and non-smoker males

***H. pylori* infection**	**IL-8**	**IL-4**
Yes	0.9913±0.3136	0.9197±0.32545
No	0.3673±0.1494	0.402±0.21
Total Mean ± SD	0.89±0.374	0.834±0.363
Paired T test sample	*p value* =0.001 (S)	

Compared with non-smokers, the levels of all three immunoglobulins (IgA, IgG, IgM) in smoking men were statistically significantly different (p <0.001). Serum IgM (95.9%) and IgA (91.7%) levels were higher in smokers than serum IgG (86.6%), (Table 3).

Table (3) Comparison between Smokers and nonsmokers among *H. pylori* positivity

***H. pylori* infection (positive)antibody**	**Smokers (No.,%,(M±SD)**	**Nonsmokers (No.,%,(M±SD)**	**Total**	***P value***
IgM	(47) 95.9% (1.34±0.48)	(2) 4.1% (1.96±0.31)	49(53.8%)	0.001(S)
IgG	(58)86.6% (1.18±0.32)	(9) 13.4% (1.55±0.51)	67(73.6%)	0.001(S)
IgA	(44) 91.7% (1.38±0.49)	(4)8.3% (1.80 ±0.41)	48(52.7%)	0.001(S)

As shown in the Figure (1) and Figure (2) the levels of IL-8 and IL-4 in the smoker group were significantly higher (*P*= 0.03 & *P*= 0.01respectively) than those of non-smokers .

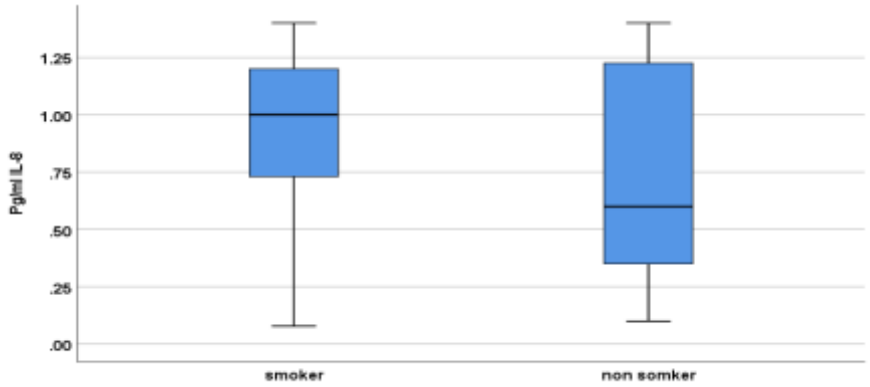

Figure (1): shows the concentration of IL-8 in smoker and non-smoker

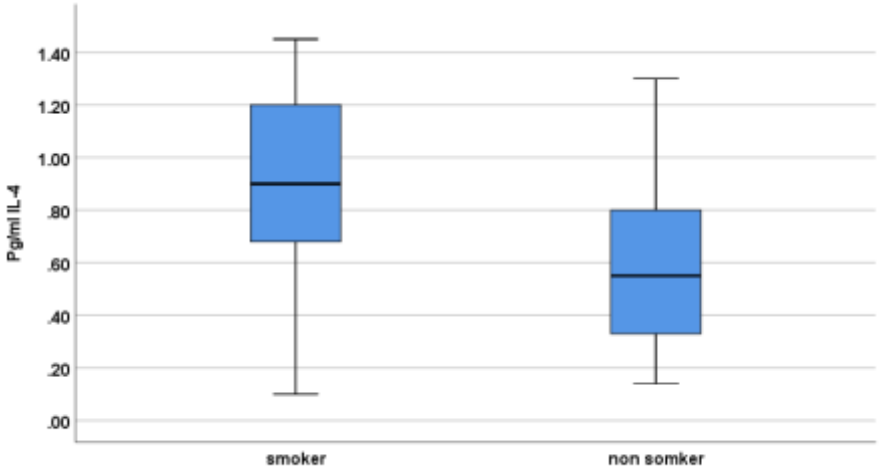

Figure (2): shows the concentration of IL-4 in smoker and non-smoker

Discussion

In many studies, smoking has been identified as a risk factor for many diseases. In this study, the effect of smoking on the expression of cytokines and the acquisition of certain bacterial infections was studied. As shown in Table (1), confirming these findings, among 91 men, smokers accounted for 78% and non-smokers accounted for 22%. Demographic data showed that the age, alcohol use, type of dietary, BMI and gastric symptoms for individuals in the smoker and nonsmoker groups were statistically not significant differences, and the majority of the participants in the smoker group (91.5%) as well as in the nonsmoker group (23.9%) were educated, these results agree with other study [2].

From the data of socio-demographic, found that smoking was significantly increased the chance for *H. pylori* infection and it is a major risk factor in *H. pylori* acquisition. Notably, data in the table (1) shows most of *H. pylori* positivity in smokers (92.9%) compared in nonsmokers (50%) (*p value: 0.001*). This result is consistent with the results of previous study who reported that the prevalence of *H. pylori* among smokers were 79.4%. *Helicobacter pylori* (*H. pylori*) infection in both developed and developing countries is a major public health problem [12]. Epidemiologic studies have been indicated that about 90% of adults in developing countries and 50% of adults in developed countries are positive to *H. pylori* [12].

Based on their results, some studies have stated that the high prevalence of *H. pylori* in Kano, and they clarified that unclean water sources, low socioeconomic status and cigarette smoking were significant risk factors for *H. pylori* infection [13]. Although the possible way to obtain *H. pylori* may be direct ingestion of bacteria through the ingestion of fecal-contaminated bacteria in drinking water, deemed that socioeconomic, environmental, sociocultural practice's factors may all contribute toward the acquisition of *H. pylori* infection· In facts, other factors including immunological response and lifestyle factors developed during adulthood such as smoking[12].

In a recent study have been published in 2017 established that cigarette smoking has a significant effect on the microbiota and cytokines expression of the buccal mucosa [10]. Following to the results in the table (2) showed that there were significant differences in the serum levels of cytokines IL-4 and IL-8 when comparing *H. pylori* (+) and *H. pylori* (-) ($p<0.001$), this results in a good agreement with a number of studies have estimated that IL-4, was higher In *H. pylori* (+) patients when compared with *H. pylori* (-) subjects [11]. Previous studies reported that *H. pylori* infection can activate inflammatory cytokines and inflammatory cells [6].

A study done in 2015 proved that high levels of IL-4 production are related to the occurrence of chronic gastritis [5]. The Gastric mucosa when exposed to *H. pylori* the expressed of IL-8 is increased and the expression of this interleukin directly correlates with a poor prognosis in gastric cancer [14].

It is worth noting that the anti-*H. pylori* IgM, IgA, and IgG of smokers was significantly higher than that of non-smokers (*p value*= 0.001), while the levels of IgM and IgA in smokers were higher than IgG (Table 3). A previous study reported that *H. pylori* infection was more in smokers and ex-smokers than in those who had never smoked [4]. Prevalence of *H. pylori* infection were strongly associated with cigarette smoking ($P < 0.0001$) [13].

It is also desirable to determine markers of immune response within the smoker group. So that the results in the figure (1) showed that the serum level of IL-4 and IL-8 were elevated in the serum of smoker compared to nonsmoker. A study reported that the smoker had 4-fold serum level of IL-8 than in nonsmoker [8]. Previous study in them data found that IL-4 were significantly (p = 0.007) higher in smoker samples [2]. Furthermore, in a study done in 2019 found that increased serum IL-8 level can precede the diagnosis of certain cancer this due to that IL-8 could induce angiogenesis and promotes tumor growth, so that results indicated that IL-8 be closely related to smokers with gastric carcinoma [7]. A study concluded that the smoking had acute effect and has been shown to increase the levels of IL-8 [9]. In the blood, level of IL-8 positively correlated with the degree of cigarette smoking and it is a potent neutrophil chemoattractant and activator [8].

Conclusions:

Current study investigated the effects of smoking on the two markers of inflammation IL-4, IL-8 and the incidence of *H. pylori* infection which was statistically significant difference between smokers, non-smokers..

Conflict of interests.

There are non-conflicts of interest.

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