

Estimate levels of some cytokine in patient infection with *Helicobacter pylori*.....

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ABSTRACT

This study includes a collection of 140 samples of blood which have been collected from patient infected with *H. pylori* ,these samples were collected from AL – Kindy Teaching Hospital in a period from (November- 2015 to March - 2016) with age ranging from 15- 70 years .

The results showed that from 140 blood samples diagnosis infection with *H.pylori* by rapid test and make confirmatory for the infection by ELISA technique, make ELISA IgG test technique in this test give 60 patients. Measure the serum level cytokine in the patient infected with *H.pylori* explain that anti-inflammatory IL-6 chronic phase: (391±5.4 pg/ml)and acute phase : (318.6±7.7 pg/ml) . In the cytokine pro inflammatory TNF-α chronic phase : (391.8±8.6 pg/ml) and acute phase :(290±11.3 pg/ml) and in the proinflammatory MIF cytokine chronic phase : (45.05±2.5 pg/ml) and acute phase : (29.08±2.1 pg/ml) .

INTRODUCTION

Helicobacter pylori previously *Campylobacter pylori*, is a gram-negative, microaerophilic bacterium found usually in the stomach. It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren (1) . Up to 85% of people infected with *H. pylori* never experience symptoms or complications (2). Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea (3) . Where this develops into

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chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea, bloating, belching, and sometimes vomiting or black stool (4). Its spiral shape and flagella allow it to corkscrew through the gastric mucus gel , and numerous adhesions enable selective adherence to the epithelium . *H.pylori* has multiple mechanisms for protection against gastric acid ; notably , 15%of its protein content comprises preformed cytoplasmic urease . When the external PH is less than 6.5 , a specific channel opens in the bacterial cytoplasmic membrane , allowing ingress of urea (5) . Oral-to-oral, fecal-to-oral, and gastric-to-oral transmission account for most infections (6). Accordingly, improvements in hygiene and living conditions are important factors in decreasing the prevalence of infection (7). There have also been association studies with maternal infection and socioeconomic status being an important risk factor for pediatric infection (8, 9) . *H.pylori* bacteria have several virulence factor such as urease ,flagella , and Vaculating cytotoxin A (VacA) (10) . The aim of this is to study detect the level of the cytokine in the serum of the patient.

MATERIAL AND METHOD

One hundred and forty specimens of blood took from patient infected with of *H.pylori* with age ranging 15 -70 years ,diagnosis the specimens of blood by Rapid Anti *H.pylori* Test in this test patient infection with *H.pylori* positive give two pink lines in the test line and control line if *H.pylori* negative in the control line only give pink and also make immunological test as confirmation test by ELISA test IgG (DIAsource) *H.pylori* give sixty from eighty four total patient (11).This research studied the level of the cytokine in the patient infection with *H.pylori*.

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Table (1) comparison between levels of TNF- α in serum of the patient infected with *H.pylori* and control

| Patient`s phase | TNF- α (pg/ml) Mean \pm SD |
|------------------------|--|
| Chronic No. | 391.8 \pm 8.6 20 |
| Acute No . | 290 \pm 11.3 40 |
| Control Healthy No. | 7.25 \pm 0.0* 20 |
| Positive Control | 241 |
| (P<0.05) | |

P: Probability Compared to Control P< 0.05.

*: Significant differences between patient and control .

Table (2) comparison between levels of IL-6 in the serum patient infected with *H.pylori* and control

| Patient`s phase | IL-6(pg/ml) Mean \pm SD |
|------------------------|------------------------------|
| Chronic No. | 391 \pm 5.4 20 |
| Acute No. | 318.6 \pm 7.7 40 |
| Control Healthy No. | 36.25 \pm 3.5* 20 |
| positive Control | 285 |
| (P<0.05) | |

P: Probability Compared to Control P< 0.05.

*: Significant differences between patient and control

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Table (3) serum level of MIF in acute and chronic phase of dyspeptic ulcer patient

| Patient phase | MIF (pg/ml) Mean ±SD |
|------------------------|-------------------------|
| Chronic No. | 45.05±2.5 20 |
| Acute No. | 29.08±2.1 40 |
| Healthy control No. | 17.5±2.1* 20 |
| (P<0.05) | |

P: Probability Compared to Control P< 0.05.

*: Significant differences between patients and control.

Table (4) explain serum levels of all cytokine in this study and compared between them

| Type of cytokines | Chronic | Acute | Control |
|-------------------|-----------|-----------|-------------|
| IL-6(pg/ml) | 391 ±5.4 | 318.6±7.7 | 36.25 ±3.5* |
| TNF-α(pg/ml) | 391.8±8.6 | 290±11.3 | 7.25±0.0* |
| MIF (pg/ml) | 45.05±2.5 | 29.08±2.1 | 17.55±2.1* |
| (P<0.05) | | | |

P: Probability Compared to Control P< 0.05.

*: Significant differences between patients and control

DISCUSSION

Anti inflammatory cytokine TNF-α is released in response to infection and inflammation produced by activated macrophage and lymphocytes which are found over expression TNF -α with the advance lymph nodal metastasis (12). In this study the serum levels of patient group was high compared with control group ,and statistical analysis give significant differences between patients and control group at P< 0.05 . Fan *et al* ,1993 showed that anti inflammatory cytokine (TNF-α)

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has higher levels of production by antral mucosa cells in *H.pylori* infection may reflect the mucosal infiltration by macrophages and T lymphocytes (13).

Proinflammatory IL-6 is being regarded as a main cytokine in progression pathway of chronic inflammatory process . It is multi functional protein produced by monocytes , endothelial cells ,the cell fibroblast , and mast cells (12) .Level of the pro inflammatory cytokine IL-6 is high in the patient infected with *H.pylori* bacteria the plasma cells differentiation in the acute phase to stimulate antibody secretion IL-6 . In the chronic phase the concentration of IL-6 is also elevated in compared with control depression. In this study results show a high level of IL-6 in patient group as compared with control group , and statistical analysis give significant differences between patients and control group at $p < 0.05$.

MIF is a proinflammatory cytokine with several actions(14).This cytokine an initial inflammatory mediator stimulates expression of other cytokines such as TNF- α and IL-1(15). Infection of *H.pylori* is also associated with increased expression of proinflammatory MIF in macrophages and T cells and in the lamina propria. In this study the results show high level of proinflammatory cytokine MIF in patient group as compared with control group , and statistical analysis give significant differences between patient and control groups at $p < 0.05$.

In the begging the interaction between *H.pylori* and innate immune response for the host is mediated through surface receptors expressed on gastric epithelial cells and antigen – presenting cells (APCs –DCs and macrophages) (16) .

Gastric of human activation DCs via *H.pylori* directs naive CD4+ T cells to Th1 differentiation through IL-12 production and can these cells to secrete cytokines like TNF- α ,IL-1,IL-6 and IFN- γ via activation of the Stt4 (signal transducer and activation of transcription factor 4) and transcription factors T – bet (T box expressed in T cell) (17).The conclusion of this study

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is that :Rapid test of *H.pylori* is an important in primary diagnosis infection of dyspeptic ulcer *H. pylori* in addition to that of ELISA IgG is more sensitive and specific for confirmation *H.pylori* infection as a serological test. Concentrations of serum level Tumor necrotic factor alpha (TNF $-\alpha$) , Interleukin -6 (IL-6) and Migration inhibition factor (MIF) increased significantly compared to control group during *H.pylori* infection specially at chronic phase of disease so these serum factors can be use as immunological markers for detection infection of *H. pylori* if it is acute or chronic .

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تقدير مستويات بعض السيتوكينات لدى المرضى المصابين الـ

Helicobacter pylori

الخلاصه

تم جمع 140 عينة من الدم من مرضى مصابين ببكتيريا *H. Pylori* هذه العينات تم جمعها من مستشفى الكندي التعليمي في الفترة من (تشرين الثاني 2015 اذار 2016) تتراوح اعمارهم بين 15-70 عاما.

وأظهرت النتائج 140 عينة دم تم تشخيصها بالاصابة ببكتيريا *H. Pylori* بواسطة اختبار سريع وتم تأكيد الإصابة بواسطة تقنية ELIAS IgG في هذا الاختبار اعطى 60 شخص مصاب . قياس مستوى المصل الخلوي في المريض المصاب ببكتيريا *H. Pylori* يوضح ان عوامل ضد الالتهاب IL-6 في المريض ذو الاصابة طويلة الامد: (391±5.4 pg/ml) و الاصابة الحادة : (pg/ml) (318±7.7) المحلول المصلي الخلوي ما قبل للالتهابي TNF-α ذو الاصابة طويلة الامد : (391.8±8.6 pg/ml) و الاصابة الحادة : (290±11.3 pg/ml) والاصابة ما قبل للالتهاب ذات المحلول المصلي الخلوي MIF في المريض طويل الاصابة: (45.05±2.5pg/ml) والاصابة الحادة: (29.08 ± 2.1pg/ml) .