



# DETERMINATION OF INTERLEUKIN-6 LEVELS IN HUMAN INDUCED BY *HELICOBACTER PYLORI* INFECTION DIAGNOSED BY USING OF RECOMBINANT ANTIGEN COATED LATEX BEADS IN MICROPLATE AGGLUTINATION TEST (MAT)

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## Abstract

In this study 50 adult patients with dyspepsia, gastritis, duodenal ulcer, and gastric cancer symptoms have been exposed to collecting samples of biopsy and blood during the period of November 2017 to May 2018 at Medical City Hospital in Baghdad. Also twenty healthy persons as control group are involved in this study, from them only blood samples were collected. *Helicobacter pylori* bacteria were harvested through the culturing of biopsies which obtained from gastroduodenal endoscopy under medical consultations. Murphy method was used to extract the proteins of outer membrane of *H. pylori*, furthermore and by using the spectrophotometer, the protein concentration was determined. These proteins were electrophoresed for the purpose of detection and molecular weight estimation. The concentration of such proteins was found to be (2.76028) mg/mL. The obtained proteins are used as an antigen by coating on latex beads of agglutination test for the antibody detection. Enzyme-Linked Immunosorbent Assay (ELISA) was used for detection of IL-6 in serum samples which high level of IL-6 that significantly different in comparison to IL-6 level in group of control.. Interleukin-6 was found to be high in 42 patient's serum samples with a mean of  $0.6167 \pm 0.05$  pg/ml when compared to a mean of  $0.346 \pm 0.06$  pg/ml in control group, and it was significantly different ( $P < 0.05$ ). Latex microplate agglutination test showed that 38 serum samples were positive to OMP out of 50 collected serum samples.

**Key words:** *Helicobacter pylori*, outer membrane protein, Latex agglutination, immunological tests.

## Introduction

It is well known that peptic ulcer and gastritis in human was attributed in part to *H. pylori*. Many data have mentioned from epidemiological point of view that gastric cancer in human was associated with infections with *H. pylori* (Mar *et al.*, 2000).

The initiation of inflammatory cascade events were known and pointed when *H. pylori* was discovered as the etiological agent for such events. It was found that over several decades the spans of gastric cancer was developed and started with infections by *H. pylori* that also led to chronic gastritis (Gatti *et al.*, 2007).

Although, *H. pylori* was carried by populations worldwide but development of gastric cancer and ulcer were noticed in few of them. Presence of flagella and

the spiral shape of the bacterium facilitated its corkscrew movement through the mucus layer gastric lining and enable the selective adhesion of the bacterium with other factors or adhesion mechanisms. Presence of urease in the cytoplasm of the bacteria and the high level of bacterial protein (15%), all aided in production of urea by the bacterium that allowed the protection of *H. pylori* from gastric acid when it was reduced less than 6.5 by pumping the urea through specific channel in cytoplasmic membrane of bacteria (Atherton *et al.*, 2009).

Adaptation of *H. pylori* to acidic environment of stomach was attributed to unusual outer membrane protein (OMP). It was present in large set and under the control of 4% of bacterial genome that encoded for such proteins. These genes were arranged in five families of paralogous genes (Mónica *et al.*, 2013 and Alm *et al.*, 2000). World Health Organization (WHO) advised the

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use of latex agglutination test as alternative method for serotyping, diagnosis and identification of many microbes. Many laboratories around the world are used the test because of its rapidity and simplicity (Barbara *et al.*, 2014).

Many cell types are produced the multifunctional IL-6 (Hiroko *et al.*, 2013). In gastric infection with *H. pylori* the levels of IL-8, IL-6 and IL-1 were elevated as cytokines of inflammatory process (Odenbreit *et al.*, 2006).

The present study focused on the use of purified OMP for coating of beads of latex and showed the successful use of latex agglutination test the diagnosis of *H. pylori* serologically and assessment the role of *H. pylori* infection on the levels of IL-6.

## Materials and methods

### Specimens' Collection

Biopsies were collected from 50 adult patients with dyspepsia, gastritis, duodenal ulcer, and gastric cancer symptoms by endoscopies which were performed by experienced endoscopists during the period lasted from November 2017 to May 2018 at Medical City hospital. Blood samples were collected from 50 patients plus 20 control, the sera were obtained, centrifuged at 3000 rpm for 10 minutes using cold centrifuge and kept in freezer (-20°C) until be used.

Bacterial growth media and serology were used as indicators for diagnosis of *H. pylori*.

### Outer membrane proteins (OMP) Extraction and Partial Purification :

Extraction of outer membrane proteins from *H. pylori* outer surface was carried out according to (Murphy *et al.*, 1983).

### Estimation of Outer membrane proteins concentration:

Proteins concentration was determined according to (Kalcker *et al.*, 1947) by using the equation:

$$\text{Concentration of} = 280\text{nm absorption} \times 1.55 - 260\text{nm absorption} \\ \times 0.76 \text{ protein (mg/cm}^3\text{)}.$$

### Latex agglutination test

#### Latex beads coating with antigens

According to the method of (Kalcker *et al.*, 1947) an equal amount (v/v) of OMP solution in concentration of (2.76028 mg/ml) was mixed with the beads of latex in ratio of (2.5%, 1  $\mu\text{m}$ ). The mixture was stirred at room temperature for 18 hours. This was followed by addition of blocking buffer that contained 0.25 M ethanolamine

and 0.2 M borate. The blocking of latex beads was left at room temperature for 30 minutes. Washing of blocked latex beads was performed by 1% bovine serum albumin in 0.2 M borate buffer for three times. The final concentration of the beads was adjusted to (w/v) 2.5% by using of storage buffer and kept at 4°C (Yussaira *et al.*, 2014).

### Microplate Agglutination Test (MAT)

A microtiter plate of 96 wells (Nunc, Roskilde, Denmark) was used in this test. The patient serum sample was diluted 50 folds in sterile phosphate buffer saline. This was followed by adding of 50  $\mu\text{L}$  of the solution of latex beads and 50  $\mu\text{L}$  the diluted patient serum in each of round bottom well of the plate. Then the plate containing the mixture was incubated for 18 hours at room temperature. Positive result was pointed by the agglutination of the mixture in well (Kimura *et al.*, 2008).

### Interlukin-6 (IL-6) Determination

The level of IL-6 in patients was determined according to (Mayr *et al.*, 2018 and Yang *et al.*, 2018). Accordingly, an enzyme linked sorbent assay (ELISA) kit (Abcam's IL-6) was used. This test is highly sensitive and used to measure quantitatively the IL-6 plasma, serum, buffered solution, supernatant and other fluids of the body. Serum samples those were collected from study groups were subjected to this test following the instruction manual of the manufacturer (Abcam's IL-6 [Interleukin-6] Human High Sensitivity in vitro ELISA, England). This kit will recognize both endogenous and recombinant Human IL-6.

### Statistical Analysis

The effects of difference factors in this study were statistically analyzed by SAS 2010. The comparative between means and chi-square of the percentages of present study were analyzed by LSD (least significant differences).

## Results and discussion

### Outer Membrane Protein (OMP) Extraction

After culturing the obtained biopsies by endoscopy, one isolate was selected for outer membrane proteins extraction which was made by using sonication that refract most of the cell according to (Murphy *et al.*, 1983).

### Evaluation of Protein Concentration in the OMP Extraction

According to (Kalcker *et al.*, 1947) the solution absorption evaluated at 260nm and 280nm before and after dialysis. The final protein concentration was (2.76028) mg/mL.

## Detection of Antibodies for *H. pylori* OMP

### Microplate Agglutination Test (MAT)

The results of this study using MAT found this test can be performed easily, very fast and accurate. The results can be read clearly in first line of loaded wells. The sensitivity of the test came consistently with other workers (Yussaira *et al.*, 2014).

The present study showed that the OMP of *H. pylori* as an antigen is strongly recommended to be used for the diagnosis of such bacterial infection with *H. pylori* when 32 serum samples were positive to antibodies to OMP antigens out of 50 serum samples.

### Interleukin-6 (IL-6) Detection

It was reported that mucosal levels of IL-6 and numerous other cytokines, are increased in the stomachs of *H. pylori*-infected persons compared to uninfected persons (Lobo *et al.*, 2005). Study of (Hiroko *et al.*, 2013) showed that elevated levels of IgG antibodies against *H. pylori* always associated with high level of IL-6. Another study (Lobbess *et al.*, 2006) reported high level of IL-6 was related disease of cardiovascular system. In present study 42 human serum samples out of 50 collected from *H. pylori* infected patients were positive to IL-6 with mean level of  $0.6167 \pm 0.05$  pg/ml in comparison to a mean of  $0.346 \pm 0.06$  pg/ml of control group. The comparison between such levels of patients and control group was appeared significantly ( $P < 0.05$ ) different (Table 1).

**Table 1:** The mean concentration of IL-6 in serum samples of patients and control groups.

Group	Mean $\pm$ SE
	IL-6 (pg/ml)
Patients	$0.6167 \pm 0.05$
Control	$0.346 \pm 0.06$
LSD Value	0.267 *

\* ( $P < 0.05$ ).

This study concluded that the outer membrane proteins are useful to be used as antigens for diagnostic procedure for *H. pylori*, the microbe that induced high level of IL-6 in sera of patients with peptic ulcer.

## Conclusion

- 1- The OMP of *H. pylori* as an antigen is strongly recommended to be used for the diagnosis of such bacterial infection with *H. pylori*.
- 2- Outer membrane protein extract antigen gave a good result in microplate agglutination test to detect spscific

*H. pylori* antibodies in patients serum.

- 3- Interleukin-6 (IL-6) increased significantly compared to control group during *H. pylori* infection.

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