Original Research Article
doi: http://dx.doi.org/10.20546/ijcmas.2016.501.001

Evaluation of Novel Immunological Mediator in Patients With Helicobacter pylori in Baghdad City, Iraq

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ABSTRACT

Helicobacter pylori (H. pylori) infection is endemic in Iraq and important cause of gastrointestinal disorders, as well as an increase in blood levels of certain inflammatory markers. Sixty patients infected with H. pylori was inserted in the current study with age (31.4±3.96) randomly selected from Al Yarmok of Teaching Hospital in Baghdad during April 2015. Patients were diagnosed by using stool antigen and CagAIgG. The medical history was taken, body weight and height were measured and body mass index (BMI) was calculated. Serum Monocyte chemoattractant protein 1 (MCP1) was determined, as well as Interleukin 6 (IL-6) and Fetuin A levels. For comparison, thirty apparently healthy subjects which were matched with patients group for age, weight and BMI (n=30, age=30.5±3.77 years; BMI= 27.13± 2.13 kg/m2; mean±SD). The prevalence of anti H. pylori Cag A-IgG antibodies in patient group (78.3±9.8 U/ml) significantly higher(<0.0001) than healthy subjects group (4.2±2.8 U/ml). H. pylori was capable with a significant rise in the inflammatory mediators (fetuin A, MCP1 and IL-6). Fetuin A levels were very highly significant lower(p<0.0001) in patient, group when compared to healthy subjects(29.53±5.25 vs 53. 45±8.37 respectively). The MCP1 levels which significantly increased(p<0.0001)in patient group(48.79±6.03) when compared to control group (37.2±6.85). The mean of IL-6 also shown highly significant difference in patient group when compared to healthy control. The current study also shown there was a positive correlation between MCP1 and IL-6.

Keywords
H. pylori, Cag A-IgG, Fetuin A, IL-6, MCP1

Article Info
Accepted: 07 December 2015
Available Online: 10 January 2016

Introduction

Helicobacter pylori (H. pylori) is a highly adapted gastric pathogen that chronically infects more half of the world population (AminTalibi, 2014). H. pylori are gram negative, microaerophilic that colonize the stomach gastritis adenocarcinoma and peptic ulcer (Davand et al., 2013,Fischer et al., 2009).The Cag A gene, which is the marker for the presence of pathogenicity is land has been shown to be involved induction of proinflammatory cytokine release (Nader Baghert et al., 2015).
part of the host defence against invading pathogen, however, they may also contribute to pathogenesis of disease by promoting mucosal damage and epithelial dysfunction for example, intestinal metaplasia may be induced by cytokines for promoting persistent epithelial cell activation and intracellular signaling (Crabter 1998). Monocyte Chemoattractant Protein-1 (MCP-1) may induce release of oxygen-free radical and proteases from inflammatory cell causing organ damage and failure (Klier et al., 2001). Biological factors that affect clinical outcome in H. pylori infection virulence determinant in H. pylori strains, immunological factors in the host are likely to play crucial role, clinical expression mucosa is related to increased production of proinflammatory cytokines, including IL-6 which are believed to contribute to maintaining the gastric inflammation and causing epithelial cell damage (Zandi et al., 2013). IL-6 is a cytokine with a wide variety of biological functions. This is a potent inducer of the acute phase response. IL-6 plays an essential role in find differentiation of B cell into IgG-secreting cells involved in lymphocyte and monocyte differentiation. H. pylori infection secreted various cytokines, including MCP1 and thus induced T cell cox-2 expression and activity. Fetuin A inhibit insulin receptor tyrosine activity by blocking autophosphorelation of tyrosinnase and IRS-1 induce lower grade inflammation (A.M.Hennige et al., 2008). Many studies have shown that H. pylori infection elevated production of proinflammatory cytokines, regulators of immune and some peptide chemokines such as interleukin 6 (Arabi 2010). The aim of the present study to investigate the relationship of mediator CagA-IgG, MCP-1, IL-6 and Fetuin A among H. pylori infection.

Material and Methods

Sixty patients infected with H. pylori was inserted in the current study with age (31.4±3.96) randomly selected from those attending Al-Yarmook Teaching Hospital in Baghdad during April in 2015. Patients were diagnosed by using stool Ag test (Coris-Bio, BELGIUM). Another test also done -Cag-A IgG (Biocompare, USA) to support the diagnosis. The medical history was taken, body weight and height were measured and body mass index (BMI) was calculated. Serum MCP-1 was determine by using ELISA technique (Elabscience Biotechnology Co. Japan). As well as, human Fetuin -A and IL-6 determine by ELISA kit (RayBiotech, USA).

For comparison, thirty apparently healthy subjects who were matched for age, weight, and BMI [n=30; age=30.5± 3.77 (years); BMI = 27.13± 2.13 (kg/m2); mean ± SD] the control subjects do not suffer from any disease and not taking any medication.

Statistical Analysis

Statistical analysis was performed using SPSS-21 (Statistical Packages for Social Sciences- version 21). Unpaired t-test was used to assess significant difference between means. P < 0.05 was considered statistically significant. Receiver operation characteristic method (ROC curve) was performed by MedCalc -12 program (IBM corp 2012).

Results and Discussion

The prevalence of anti-H. pylori CagA IgG antibodies in patients group (78.3±9.8 U/ml) significantly higher (<0.0001) than healthy subjects group (4.2±2.8 U/ml), while there were no significant differences between patients and healthy groups in the anthropometric measurements [weight (83.3±6, 82.2±6.4 respectively) and height (172.3±4.6, 174.1±5.27 respectively)], table (1).

H. pylori was capable with a significant
differences in the inflammatory mediators (fetuin-A, MCP-1 and IL-6), as shown in table 2.

Fetuin A levels were very highly significant lower (P<0.0001) in patients group when compared to healthy subjects group (29.53±5.25 vs. 53.45±8.37)

This was contrary to the MCP-1 levels which significantly increased (P<0.0001) in patients group (48.79±6.03 compared to control group (37.02±6.85), figure (2).

The mean of IL-6 also shown highly significant difference (P<0.0001) in patients group when compared to healthy control

To find the sensitivity and specificity for each mediator, the receiver operation characteristic was done but it cannot be applied only in the MCP-1 because of the lack in overlap for the results except for the results of MCP-1.

In the current study, there was a positive correlation between MCP-1 and IL-6, as shown in table (3).

The effect of body weight also studied in the present study by divided the patients group to subgroups according to the values of BMI Prime (as Patient/optimal ≤1 and Patient/overweight>1) as shown in table (4)

BMI Prime, a simple modification of the BMI system, is the ratio of actual BMI to upper limit BMI (currently defined at BMI 25). As defined, BMI Prime is also the ratio of body weight to upper body-weight limit, calculated at BMI 25. Since it is the ratio of two separate BMI values, BMI Prime is a dimensionless number without associated units. Individuals with BMI Prime less than 0.74 are underweight; those with between 0.74 and 1.00 have optimal weight; and those at 1.00 or greater are overweight. BMI Prime is useful clinically because individuals can tell, at a glance, by what percentage they deviate from their upper weight limits. For instance, a person with BMI 34 has a BMI Prime of 34/25 = 1.36, and is 36% over his or her upper mass limit.

Table above shows the multiple comparisons among three groups.1 vs 2, 1 vs 3 and 2 vs 3. In this analysis we found that the two groups (Patient/optimal weight and overweight) did not differ in all parameters. In other words, it means that the BbodiesMIP has no effect on these parameters.

The prevalence of anti- H.pylori CagA Ig G antibodies in patient group significantly higher(<0.0001) than healthy subjects groups, a proportion similar to that reported also in Africa(Sanz-Pelaez et al., 2008, Smithsi et al2002). There is no significantly significant association with CagAIg positivity and age agreement with (Alsharipours et al., 2014). The current study found increase significantly IL-6 levels (Table2) agreed with (Hiroke Nakagawa et al.,2015) who found that serum IL-6 level was significantly among H.pylori infected in adult Japanese. Other study shown that H.pylori infection is associated with increased IL-6 and TNF-α production within the gastric mucosa (Jamshid Vafaieimanesh et al., 2014).

Fetuin A is anti inflammatory mediator that participate in macrophage deactivation specially fetuin A, enhance the cellular uptake of cationic inhibitors of proinflammatory cytokines synthesis by macrophage, thus preventing the morbid sequelleae of infection that result from over production of pro-inflammatory cytokines (Ombrellion M et al., 2001).
**Table 1** The Clinical Characteristics of *H. pylori* Infected Patients Compared to Control Group

|          |     |     |            |            |      |      |
|----------|-----|-----|------------|------------|------|------|
|          | N   | Mean| Std. Deviation| Std. Error Mean| P    |
| Age      |     |     |            |            |      |      |
| Control  | 30  | 30.53| 3.77       | 0.68       | 0.36 |
| Patient  | 60  | 31.44| 3.96       | 0.73       |      |
| Weight   |     |     |            |            |      |      |
| Control  | 30  | 82.20| 6.46       | 1.17       | 0.49 |
| Patient  | 60  | 83.31| 6.03       | 1.11       |      |
| Height   |     |     |            |            |      |      |
| Control  | 30  | 174.13| 5.27      | 0.96       | 0.17 |
| Patient  | 60  | 172.34| 4.63      | 0.86       |      |
| CagAIgG  |     |     |            |            |      |      |
| Control  | 30  | 4.28 | 2.80       | 0.51       | <0.0001 |
| Patient  | 60  | 78.36| 9.82       | 1.82       |      |

**Table 2** The Immunological Markers in Patients vs. Control Group

|          | N   | Mean| Std. Deviation| Std. Error Mean| P    |
|----------|-----|-----|------------|---------------|------|
|          | N   |     |            |            |      |      |
| Fetuin   | 30  | 53.45| 8.37       | 1.52         | <0.0001 |
| Patient  | 60  | 29.53| 5.25       | 0.97         |      |
| MCP1     | 30  | 37.02| 6.85       | 1.25         | <0.0001 |
| Patient  | 60  | 48.79| 6.03       | 1.12         |      |
| IL6      | 30  | 4.35 | 1.16       | 0.21         | <0.0001 |
| Patient  | 60  | 9.25 | 1.96       | 0.36         |      |

**Table 3** The Correlations Between Studied Parameters in Patients Group

|          | Wt   | Heigh | Cag-A-IgG | Fetuin-A | MCP-1 | IL-6 |
|----------|------|-------|-----------|----------|-------|------|
| Age      | .703**| .438* | -.252-    | .010     | -.079-| -.015-|
| Sig. (2-tailed) | .000 | .017   | .187      | .960     | .684  | .938 |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | Pearson Correlation | 1    | .756**    | .134     | .145  | -.071-| -.017-|
| Sig. (2-tailed) | .000 | .489   | .453      | .714     | .931  |      |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | Pearson Correlation | 1    | .212      | .029     | .131  | -.052-|
| Sig. (2-tailed) | 1    | .271   | .881      | .498     | .790  |      |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | Cag-A-IgG |     |           |          |       |      |
| Sig. (2-tailed) | 1    | .098   | .084      | .075     |      |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | MCP-1 |     |           |          |       |      |
| Sig. (2-tailed) | .614 | .614   | .666      | .697     |      |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | Fetuin-A |     |           |          |       |      |
| Sig. (2-tailed) | 1    | -.152- | -.067    | .758**   |      |
| N        | 60   | 60    | 60        | 60       | 60    | 60   |
|          | IL-6 |     |           |          |       |      |
| Sig. (2-tailed) | 1    | .431   | .731      | .000     |      |
| N        | 60   | 60    | 60        | 60       | 1     |      |
Table 4 Studied Parameters in the Subgroups

| Descriptives | N  | Mean  | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | Min. | Max. |
|--------------|----|-------|----------------|------------|---------------------------------|------|------|
|              |    |       |                |            | Lower Bound                      |      |      |
|              |    |       |                |            | Upper Bound                      |      |      |
| Control      | 30 | 30.53 | 3.77           | .6893      | 29.12                              | 31.94| 25.00| 41.00|
| Patient/optimal | 14 | 28.14 | 2.61           | .9863      | 25.72                              | 30.56| 25.00| 32.00|
| Patient/obesity | 46 | 32.50 | 3.76           | .8024      | 30.83                              | 34.17| 25.00| 41.00|
| Total        | 90 | 30.98 | 3.86           | .5027      | 29.97                              | 31.99| 25.00| 41.00|
| Control      | 30 | 4.29  | 2.81           | .5117      | 3.24                               | 5.33 | 1.50 | 12.10|
| Patient/obesity | 14 | 76.60 | 6.24           | 2.36       | 70.83                              | 82.37| 67.00| 86.00|
| Patient/obesity | 46 | 78.93 | 10.79          | 2.29       | 74.14                              | 83.70| 43.00| 94.00|
| Total        | 90 | 40.69 | 38.02          | 4.95       | 30.78                              | 50.61| 1.50 | 94.00|
| Control      | 30 | 53.45 | 8.37           | 1.52       | 50.32                              | 56.58| 39.20| 76.80|
| Patient/obesity | 14 | 28.43 | 6.14           | 2.32       | 22.74                              | 34.11| 19.60| 37.40|
| Patient/obesity | 46 | 29.90 | 5.05           | 1.08       | 27.65                              | 32.13| 21.00| 39.00|
| Total        | 90 | 41.69 | 13.92          | 1.82       | 38.07                              | 45.32| 19.60| 76.80|
| Control      | 30 | 37.02 | 6.85           | 1.25       | 34.46                              | 39.58| 22.50| 48.20|
| Patient/obesity | 7  | 50.04 | 5.86           | 2.22       | 44.63                              | 55.46| 39.60| 55.40|
| Patient/obesity | 22 | 48.40 | 6.17           | 1.32       | 45.67                              | 51.14| 41.20| 66.30|
| Total        | 59 | 42.81 | 8.73           | 1.14       | 40.53                              | 45.08| 22.50| 66.30|
| Control      | 30 | 4.35  | 1.16           | .21        | 3.91                               | 4.88 | .70  | 6.20 |
| Patient/obesity | 7  | 8.71  | 2.21           | .83       | 6.70                               | 10.76| 6.30 | 11.40|
| Patient/obesity | 22 | 9.42  | 1.90           | .41       | 8.58                               | 10.33| 7.20 | 13.30|
| Total        | 59 | 6.76  | 2.94           | .38       | 5.99                               | 7.51 | .70  | 13.30|
| Control      | 30 | 27.13 | 2.13           | .39       | 26.34                              | 27.97| 22.98| 31.16|
| Patient/obesity | 7  | 26.36 | .82            | .31       | 25.59                              | 27.12| 24.91| 26.96|
| Patient/obesity | 22 | 28.56 | 1.01           | .21       | 28.13                              | 29.01| 27.36| 31.10|
Table 5 The Multiple Comparisons among Three Groups

| Dependent Variable | (I) trt | (J) trt | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|--------------------|---------|---------|-----------------------|------------|------|------------------------|-------------|-------------|
| Age                | 1       | 2       | 2.39048               | 1.53800    | .126 | -6.905-                | -5.4715     | 1.1719-     |
|                    | 1       | 3       | -1.96667              | 1.02847    | .061 | -4.026-                | -5.4715     | .0936       |
|                    | 2       | 1       | 2.39048               | 1.53800    | .126 | -5.4715                | -6.905-     | 1.1719-     |
|                    | 2       | 3       | -4.35714*             | 1.59002    | .008 | -7.542-                | -3.1719     | 7.5423      |
|                    | 3       | 1       | 1.96667               | 1.02847    | .061 | -1.093-                | 1.1719      | 4.0269      |
|                    | 3       | 2       | 4.35714*              | 1.59002    | .008 | 1.1719                 | 7.5423      |             |
| CagAIgG            | 2       | 1       | 72.31333*             | 3.02302    | .000 | -78.3692-              | -70.5865    | 78.3692     |
|                    | 2       | 3       | -2.32273              | 3.12528    | .460 | -8.583-                | 3.9380      |             |
|                    | 3       | 1       | 74.63666*             | 2.02153    | .000 | 70.5865                | 80.6857     |             |
|                    | 3       | 2       | -74.63666*            | 2.02153    | .000 | -80.6857               | 80.5865     |             |
| Fetuin             | 2       | 1       | -2.32273              | 3.12528    | .460 | -3.938-                | 8.5834      |             |
|                    | 2       | 3       | 25.02143*             | 2.96486    | .000 | 19.082-                | 30.9608     |             |
|                    | 3       | 1       | 23.55909*             | 1.98264    | .000 | 19.587-                | 27.5308     |             |
|                    | 3       | 2       | 1.46234               | 3.06515    | .635 | -7.602-                | 4.6779      |             |
| MCP1               | 2       | 1       | 13.02286              | 2.73025    | .000 | 7.553-                 | 18.4922     |             |
|                    | 2       | 3       | 1.64286               | 2.82260    | .563 | -4.011-                | 7.2972      |             |
|                    | 3       | 1       | 11.38000*             | 1.82575    | .000 | 7.722-                 | 15.0374     |             |
|                    | 3       | 2       | -1.64286-             | 2.82260    | .563 | -7.297-                | 4.0115      |             |
|                    | 1       | 3       | -13.02286-            | 2.73025    | .000 | -18.492-               | -7.553-     |             |
|                    | 3       | 1       | -11.38000-            | 1.82575    | .000 | -15.037-               | -7.722-     |             |
| IL6                | 2       | 1       | 4.36095*              | .67492     | .000 | 3.009-                 | 5.7130      |             |
|                    | 2       | 3       | -0.70844              | .69775     | .314 | -2.106-                | 2.1062      |             |
|                    | 3       | 1       | 5.06939*              | .45133     | .000 | 4.165-                 | 5.9735      |             |
|                    | 2       | 2       | 7.7752                | .70339     | .274 | -6.315-                | 2.1866      |             |
|                    | 1       | 3       | -1.42533-             | .47036     | .004 | -2.367-                | -1.483-     |             |
|                    | 2       | 1       | -7.7752               | .70339     | .274 | -2.186-                | -4.6315     |             |
|                    | 2       | 3       | -2.20286-             | .72718     | .004 | -3.659-                | -0.746-     |             |
|                    | 1       | 3       | 1.42533*              | .47036     | .004 | .483-                  | 2.3676      |             |
|                    | 2       | 3       | 2.20286*              | .72718     | .004 | .7461-                 | 3.6596      |             |

* The mean difference is significant at the 0.05 level
Figure.1 Mean of Fetuin A in Patient and Control Groups

![Graph showing mean of Fetuin A in Patient and Control Groups]

Figure.2 Mean of MCP-1 in Patients and Controls Groups

![Graph showing mean of MCP-1 in Patients and Controls Groups]

Figure.3 Mean of MCP-1 in Patients and Controls Groups

![Graph showing mean of MCP-1 in Patients and Controls Groups]
**Figure.4** Receiver Operator Curve Analysis for the Investigated Parameters

*H.pylori* Patients and Controls

| Area under the ROC curve (AUC) | 0.898 |
|-------------------------------|-------|
| Standard Error^a               | 0.0383|
| 95% Confidence interval^b      | 0.792 to 0.962|
| z statistic                   | 10.406|
| Significance level P (Area=0.5)| <0.0001|

Fetuin A increase significantly in the current study (Fig1,2) and this finding in agreed with (Kebapiclar 2010) Who reported significant increase in anti-inflammatory markers such as Fetuin A. MCP1 to be higher in *H.pylori* positive versa agreed with (Nomura et al., 2004). In the current study, there was appositive correlation between MCP1 and,as shown in (Table 3). To our knowledge, there have been no reports for the correlation between MCP1, fetuin A and IL6 in Iraq. To evaluate infurther studies the molecular epidemiology of *H.pylori* infection in the general population.

In conclusion, MCP1 was found to be significantly elevated in patients group versus the control group, also there was a significant difference in IL-6 level in *H.pylori* +ve versus healthy group. Fetuin A was found to be high in pateints group when compared to healthy subjects and there positive correlation between IL-6 and MCP-1.

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How to cite this article:

Thamer Mutlag Jasim. 2016. Evaluation of Novel Immunological Mediator in Patients With Helicobacter pylori in Baghdad City, Iraq. Int. J. Curr. Microbiol. App. Sci. 5(1): 1-9. doi: http://dx.doi.org/10.20546/ijcmas.2016.51001