

Role of adhesion molecules & IL 1 β in peptic ulcer disease

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Abstract: Background: a peptic ulcer is an excavated defect in the gastric or duodenal mucosa that extends through the muscularis mucosa into the deeper layers of the wall and can lead to hematemesis and perforation. Cytokines and chemokines are small peptide molecules, synthesized and released by nearly all cell types present in the human body. They play a key role as communicators between cells; modulating a wide variety of functions. Cytokines are known to induce, amplify, perpetuate and terminate inflammation. **Aim-** of this study is to detect if there a role in of the cytokines (specifically interleukin 1 beta) & the adhesion molecules (specifically ICAM-1 & VCAM-1) in cases of peptic ulcer disease along with the presence and absence of H. pylori and the intake of NSAIDs. **Patients and methods;** This study was conducted during the period from December 2011 to December 2015; on 60 subjects divided as follow; 40 patients who had a history suspected of gastro-duodentis and who attended in the outpatient clinic and inpatient section of internal medicine department in El-Hussein University hospital and Farwaniahospital and were documented to have a peptic ulcer disease. Where 20 patients had a recent attack of peptic ulcer and another 20 patients had a history of recurrent attacks of peptic ulcer disease with detailed history of aspirin intake. Those two groups along with another group of 20 healthy subjects were tested for the presence or absence of H.pylori, serum IL 1B, I-CAM, V-CAM, complete blood count, kidney and liver functions. **Results;** Showing the highly significance of IL1B, I-CAM and V-CAM levels in patients with peptic ulcer disease. **Conclusion;** 1) The presence of adhesion molecules (ICAM-1, VCAM-1) and IL-1 beta is significantly correlated with the presence of peptic ulcer disease. 2) The increase the values of adhesion molecules (ICAM-1, VCAM-1) and interleukin 1 beta is correlated with the increase the suspicion of peptic ulcers recurrence.

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Keywords: Role; adhesion; molecule; IL 1 β ; peptic ulcer disease

1. Introduction

A peptic ulcer is a defect in the gastric or duodenal mucosa that extends through the muscularis mucosa into the deeper layers of the wall (*Soybel DL 2005*).

Peptic ulcer may present with dyspeptic or other gastrointestinal symptoms, or may be asymptomatic. Also, it may be presented with complications such as hemorrhage or perforation (*Dempsey D.T. 2007*). As already been held long time ago; that the acidic gastric environment is deadly to microorganisms, and that a stomach with normal acid secretion is sterile. Thus, gastric microbiology had unfortunately been neglected until Warren and Marshall cultured and identified *Helicobacter pylori* (*Warren et al. 2003*).

Helicobacter pylori infection and the intake of non-steroidal anti-inflammatory drugs (NSAIDs) are known to be the major causes of ulcers recurrence (*Taha et al. 2014*).

The key pathophysiological event in H pylori infection is initiation of an inflammatory response.

Bacteria or their products trigger this inflammatory process and the main mediators are cytokines. Cytokines, including interleukins, are soluble peptide molecules that mediate the interaction between immunocompetent and haematopoietic cells and between the immune and neuroendocrine systems (*Fridman WH & Tartour E 1997*).

Adhesion molecules are important in infiltration of leucocytes including neutrophils at the sites of inflammation. Inflammatory cytokines such as interleukins (ILs) and tumor necrosis factor (TNF)- α are increased in both H. pylori and NSAIDs associated gastric damages (*Mackay CR. 2008*).

Neutrophils accumulate in scarred mucosa and ulcers recur when there are many infiltrating neutrophils. Prostaglandin may reduce the number of neutrophil to control levels at scarred mucosa and brings the lower rate of ulcer recurrence (*Watanabe et al. 2001*).

Cytokines and chemokines are small peptide molecules synthesized and released by nearly all cell

types present in the human body. They play a key role as communicators between cells modulating a wide variety of functions. Cytokines are known to induce, amplify, perpetuate and terminate inflammation (*Miossec P, et al. 2012*).

The wide spectrum of biological effects derives mainly from the ability of IL-1B to induce expression of many other genes by either initiating their transcription or stabilizing their mRNA (*El-Omar EM et. Al., 1997*).

IL-1B also induces expression of genes for adhesion molecules such as ICAM-1, VCAM-1, and ELAM, and for some oncogenesis. Inflammation is a crucial process in the normal defense mechanisms against various pathogens, and leukocytes are the principal cellular mediators of inflammation. Inflammation is characterized histologically by the accumulation of leukocytes in the affected tissue due to migration of circulating leukocytes out of the vasculature, a process which is actively mediated and precisely controlled by leukocytes, the cytokines they produce, and the vascular endothelium (*Zarbock A. & Ley K. 2008*).

Interactions between leukocytes and the endothelium are mediated by several families of adhesion molecules, each of which participates in a different phase of the process. The surface expression and activation of these molecules during an inflammatory response is tightly controlled under normal conditions (*Butcher EC. 1991*).

Aim of this study

Our study performed to evaluate the role of cytokines (specifically interleukin 1 beta) & the adhesion molecules (specifically ICAM-1 & VCAM-1) levels in cases of peptic ulcer disease; either in recent and recurrent attacks along with H.pylori and NSAIDs intake.

Patients and methods: patients

The present study was conducted between December 2011 and December 2015 upon 40 patients with peptic ulcers and 20 healthy volunteers included as a control group (group III) and they were (10) males and (10) females with their age ranging between 29 & 50 years and a mean age 39.95 years.

The patients were selected from outpatient clinic and inpatient section of internal medicine department, Al-Hussein university hospital and Farwania hospital. They were (28) females and (12) males with a mean age 42.5 ± 10.5 years.

Inclusion criteria; the selected patients presented with a long history of heart burn with or without hematemesis or melena.

All cases detected to have a peptic ulcer then, we divided them into 2 groups; 20 cases had recently diagnosed peptic ulcer (gastric or duodenal) (group I)

and they were (8) males and (12) females with their age ranging between 32 & 70 years and an average age 48 years, 20 cases of having a previous history of peptic ulcer (group II) and they were (4) males and (16) females with their age ranging between 33 & 81 years and an average age 53.4 years.

From all the selected subjects; we took a history of NSAID intake, history of co morbidities, constitutional symptoms, and clinical examination which includes (signs suggestive of chronic illness as: weight loss, iron and vitamin deficiencies, general and abdominal examination).

Exclusion criteria: Patients who refused to be enrolled in the study. Patients with organic failure such as heart failure, renal failure and liver cell failure to rule out congested causes of peptic ulcers. Also, Patients with fever or apparently infectious diseases to rule out the false elevation of inflammatory cytokines.

Methods

Laboratory investigations: for kidney and liver functions, CBC and coagulation profile. Also, venous blood samples about 5 ml. to detect IL-1B, I-CAM and V- CAM levels; Using a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature then centrifugation done for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C.

Requirements:

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm. • Pipettes and pipette tips. • Deionized or distilled water. • Squirt bottle, manifold dispenser, or automated microplate washer. • 100 mL and 500 mL graduated cylinders. • Test tubes for dilution of standards and samples.

H. pylori test:

Stool antigen tests for H. pylori provide an alternative to the urea breath test. H. pylori Antigen (Cat #1506-11). The sensitivity is 89 to 98 percent and a specificity of over 90 percent.

RUT or CLO test at the time of doing Upper GI endoscope (Simple and rapidest done once biopsy specimen has been obtained); sensitivity of 80 to 95%, specificity of 95 to 100%. With red colour in case of positive H. pylori and yellow colour in case of no H.pylori detected (discussed in the review of literature).

Cytokine Assay:

The serum concentrations of IL-1beta were quantified by sandwich ELISA using a commercial kit (Recombinant Human IL-1 β /IL-1F2) Catalog Number (201-LB/CF). The analyses were performed in duplicates and according to the manufacturer's procedure.

Adhesion molecules:

Measurement according to Human Immunoassay Quantikine sandwich ELISA Catalog Number (R & D Systems, Catalog # PDVC00) by Pharm Pak technique for the quantitative determination of human soluble Cell Adhesion Molecule (CAM). The color develops in proportion to the amount of (CAM) bound. The color development is stopped and the intensity of the color is measured.

Upper gastroduodenoscopy:

Was done for all patients groups, using Olympus(GIF XP 160) – (GIF XO 260/290) to detect; marks for peptic ulcers, gastritis and taking a biopsy to detect the presence or absence of H.pylori and also, to detect the presence or absence of malignant transformation.

Results:

As regards the results of the present study, they were statistically analyzed using; IBM SPSS statistics (V. 23.0, IBM Corp., USA, 2015).

Data were expressed as Mean±SD for quantitative parametric measures in addition to Median Percentiles for quantitative non-parametric measures and both number and percentage for categorized data.

The following tests were done:

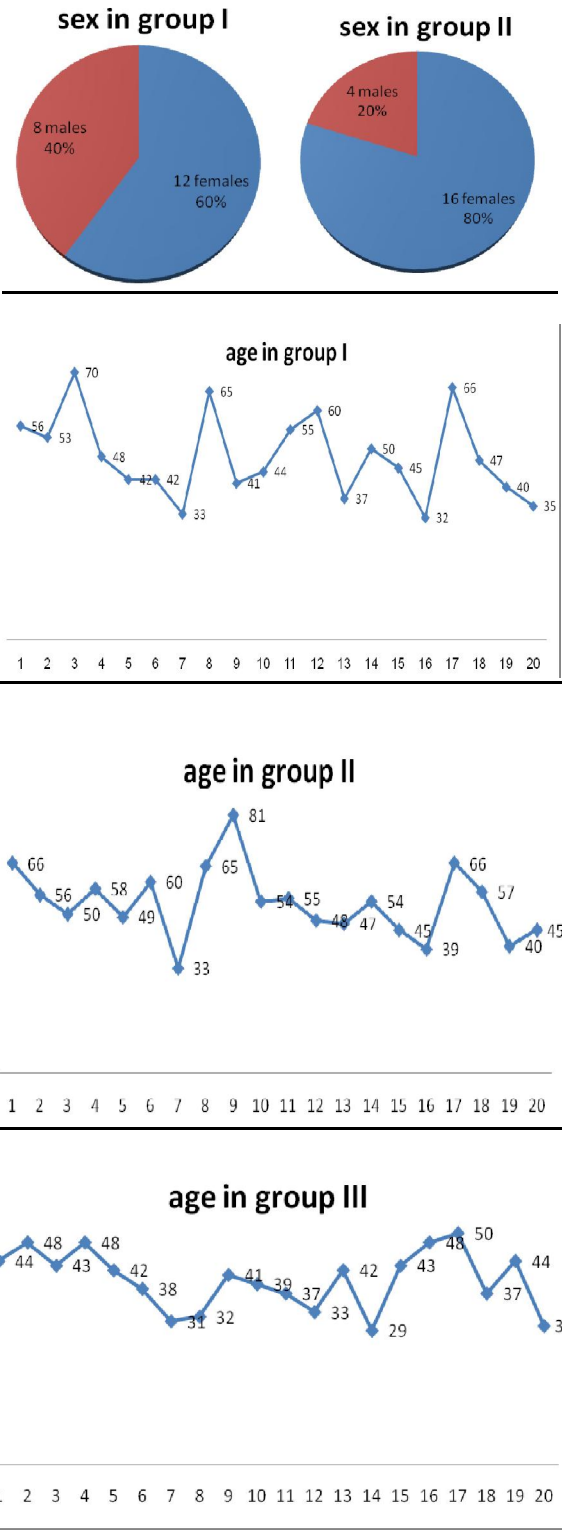
1. Comparison between two independent mean for parametric data using Student t test.
2. Pearson correlation test to study the possible association between each two variables among each group for parameteric data.
3. Chi-square test to study the association between each 2 variables or comparison between 2 independent as regards the categorized data.

The probability of error at 0.05 was considered sig., while at 0.01 and 0.001 are highly sig.

4. Diagnostic validity test: It includes:

- a. The diagnostic sensitivity: It is the percentage of diseased cases truly diagnosed (TP) among total diseased cases (TP+FN).
- b. The diagnostic specificity: It is the percentage of non-diseased truly excluded by the test (TN) among total non-diseased cases (TN+FP).
- c. The predictive value for a +ve test: It is the percentage of cases truly diagnosed among total positive cases.
- d. The predictive value for a -ve test: It is the percentage of cases truly negative among total negative cases.
- e. The efficacy or the diagnostic accuracy of the test: It is the percentage of cases truly diseased plus truly non-diseased among total cases.

The ROC was constructed to obtain the most sensitive and specific cutoff for each technique. To evaluate the most discriminating markers between the compared, AUC can also be calculated.



Demography of the studied groups

Detailed number and percentages of presence and absence of H.pylori in groups (I & II) with chi-square NS effect as regard presence of H.pylori

				Total	
		Recent.P.U.	Recurrent.P.U.		
H.Pylori	-	Count	9	7	16
		%	45.0%	35.0%	40.0%
	+	Count	11	13	24
		%	55.0%	65.0%	60.0%
Total		Count	20	20	40
		%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	P
Pearson Chi-Square	.417 ^a	.519

Detailed number and regard the endoscopic

i-square HS effect as

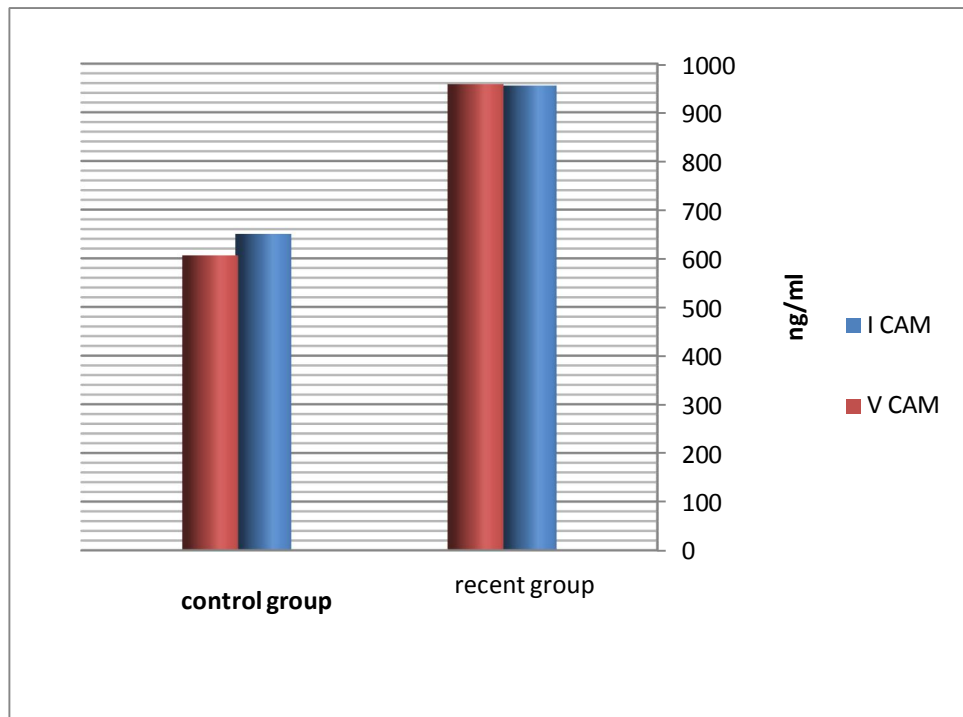
				Total		
		Recent.P.U.	Recurrent.P.U.			
Endoscopic.Finds	none	Count	15	0	15	
		%	75.0%	0.0%	37.5%	
	Atrophic gastritis	Count	0	1	1	
		%	0.0%	5.0%	2.5%	
	gastritis	Count	5	18	23	
		%	25.0%	90.0%	57.5%	
	Hypertrophic gastritis	Count	0	1	1	
		%	0.0%	5.0%	2.5%	
	Total		Count	20	20	40
			%	100.0%	100.0%	100.0%

Detailed number and percentages of those used to take aspirin in groups (I & II) with chi-square NS effect as regard aspirin intake

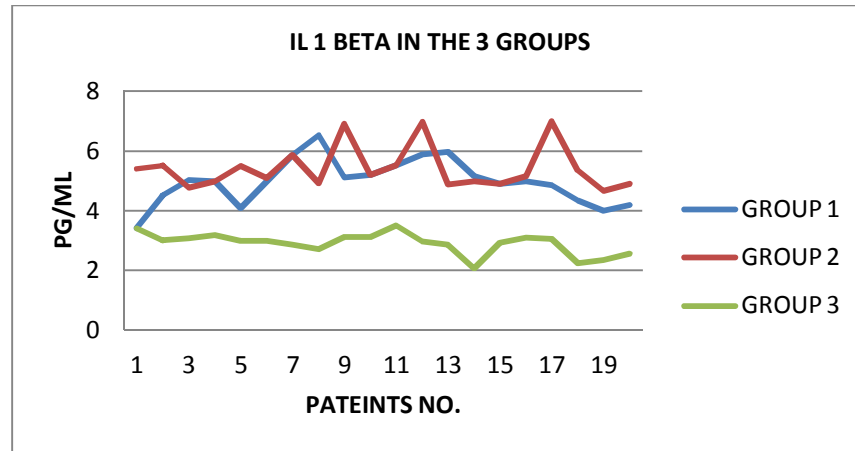
				Total	
		Recent.P.U.	Recurrent.P.U.		
Aspirin	-	Count	14	16	30
		%	70.0%	80.0%	75.0%
	+	Count	6	4	10
		%	30.0%	20.0%	25.0%
Total		Count	20	20	40
		%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	P
Pearson Chi-Square	.533*	.465



Showing the lowest I CAM & V CAM values in group I in compare to the highest values in group III (self discrimination between diseased and control group)



Best value for IL-1B in compare diseased versus control groups at 3.5 pg/ml.

Discussion:

Among the studied groups; the history of aspirin intake and other nonsteroidal anti-inflammatory drugs were significant in the diseased (peptic ulcer groups) rather than the control group (p -value = 0.025). This matched well with previous studies which showed that the use of aspirin and the intake of NSAIDs increase the risk of the development of peptic ulcer disease (*Hawkey CJ. 2000*) and (*Taha et al. 2014*).

But in our current study; we could not find the significance of aspirin and NSAIDs intake to increase the rate of recurrences of peptic ulcers or differentiate between group I and group II (p -value = 0.519 as in table 14) as comparing our results with the results done by (*Hirschowitz BI & Lanan A. 2002*) that mentioned that; continuing NSAID use is a leading cause of refractory and recurrent peptic ulceration.

This discrepancy may be due to the little number of patients used to take NSAIDs in group II than in group I.

Thorough using the upper gastroduodenoscope; the feature of gastric mucosa as regard the feature of gastritis which either atrophic, hypertrophic; was highly significant in cases of group II in comparison with group I (p -value = 0.000 as in table 17) which matches with many studies that found the features of the mucosa correlate well with the chronicity and recurrence of ulcers (*Hatz et al., 1997*).

The ulcer sites had found to be in the duodenum in 28% of total case numbers and in the stomach in 12% in total case numbers, which matches with the study done by (*Iqbal Siddique et al. 2014*) that pointed to the higher incidence rate of duodenal ulcers 61.6% than gastric ulcers 13.1%. But no statistical significance in comparing the group I and group II (p -value = 0.490 as in table 15).

Among the studied groups; the H.pylori testing was highly significantly positive in comparing the peptic ulcer groups and the group III. These results

were matched with many studies about the role of H.pylori in peptic ulcer disease like that prescribed by (*Malnick SD, et al, 2014*) which mentioned that; Helicobacter pylori (H. pylori) colonizes the mucosa of the human stomach and establishes a long-term infection that leads to the development of chronic gastritis and peptic ulcer disease (PUD).

Also in a study done by (*Lanan A, et al., 2000*) that mentioned that; persistent H. pylori can cause refractory or recurrent peptic ulceration. But, in our current study we could not find a statistical significance of H. pylori in between the group I and group II (p -value = 0.519 as in table 14).

This result might be more or less not matched the previous studies, which pointed to the role of H.pylori in peptic ulcer recurrences (*Taha et al. 2014*). This difference may be due to the near numbers of infected individuals by the H. pylori in both groups 11 in group I and 13 in group II.

Histopathological examination after doing upper gastroduodenoscope and biopsies had been taken, then, no evidence of malignant transformations or lymphoma was found.

In our current study; it was found that the mean IL- 1B was highly significant (p -value < 0.01) in comparing the group I and group II in one side with group III in another side. This results were matched with a study done by (*Toshio Watanabe et al., 2001*) which concluded that; IL-1B can cause recurrences of experimental gastric ulcers in rats, and neutrophilic infiltration into scarred mucosa may be responsible for this recurrence.

By analysis of our results; it was found a cut off value that can exclude the presence of peptic ulcer disease by values less than (**3.5 pg/ml**) at which SP=100%, Sn=95%, P-ve=95.2%, P+ve=100% & efficacy=97.5%.

It has been found that the (I-CAM) levels were highly significant and self discriminating between the

diseased groups and group III, as the lowest values for I CAM levels (955 ng/ml was detected in group I) was higher than the highest level of the group III (650 ng/ml).which means that; I-CAM level below (650 ng/ml) denies the presence peptic ulcers, while the level of I-CAM above (955 ng/ml) is pointing to the high susceptibility of the presence of peptic ulcer disease.

And this result of significant increase of ICAM level in peptic ulcer disease was matched with the study done by (Bevilacqua et al., 1987) which concluded that; ICAM-1 is an important factor in leukocyte adherence and emigration and is constitutively expressed on endothelial cells. The activation of endothelial cells with lipopolysaccharide (LPS) causes upregulation of ICAM-1 expression. Also, the maximum levels of leukocyte adherence were noticed to be associated with ICAM-1 expression (Farhood et al., 1995).

As regard the VCAM results in our study: it has been found that the (V-CAM) levels were highly significant and self discriminating between the diseased groups and group III, as the lowest values for V-CAM levels were noticed at (958 ng/ml in group I) and the highest value in the group III was (606 ng/ml). Which means that; V-CAM level below 606 ng/ml means no suspicion of peptic ulcers, while the level above 958 ng/ml is highly suspicious of peptic ulcer disease.

Also, there were no statistical significant differences in between the recent and recurrent groups as regard the presence of bleeding (p-value = 0.212 as in table 16). But, Apparent significance in relation to group I.

Conclusions and a look to the future

The presence of adhesion molecules (ICAM-1, VCAM-1) and IL-1 beta is significantly correlated with the presence of peptic ulcer disease.

The increase the values of adhesion molecules (ICAM-1, VCAM-1) and interleukin 1 beta is correlated with the increase the suspicion of peptic ulcers recurrence.

IL-1B is an important proinflammatory cytokine with profound effects on gastric physiology.

Recommendations:

Future research should focus on identifying the molecular pathways that mediate some individuals infected with H pylori to develop gastric cancer while others do not.

Early eradication and management of H. pylori.

Upper gastrointestinal diseases that may alter gastric physiology should be targeted in the future studies.

The studies for bacterial factors and host genetic factors that contribute to the pathogenesis of the peptic ulcer disease should continue.

Multicenter studies on much larger subjects are needed in the future to confirm our findings.

However, our study was not designed to determine the virulence factors, and this could be considered a limitation of this study.

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