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# Relation Of Infection With *Helicobacter Pylori* and Vacuoles Formation Inside The Infected Cells.

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**ABSTRACT**

One hundred fifty patients who were included in this study visited the endoscopy unit these complaining from epigastric pain, dyspepsia, acidity, vomiting, abdominal pain, flatulance, heart bum and melena, All underwent esophagogastroduodenoscopy (OGD) and biopsies were taken, on which bacteriological and histopathological tests were done, aiming, first, isolation of *Helicobacter pylori* and second, looking for documents regarding the role of this bacteria in pathogenesis of peptic ulcer. Direct smear of biopsies were examined and then cultured on selected media of *H.pylori.* Histopathological examination was also done, using paraffin and plastic sections. When detection of *H. pylori* was considered, the highest sensitivity rate was found 74.1% when it was cultured on [Brucella agar + (5-7% )sheep blood + skirrow's supplement] while direct smear examination from biopsies was the second and it was 70%, the third position was obtained by histopathological examination of paraffin section and it was 67.9 %, on the contrary plastic section cames in the fourth place 43.5 %. Vacuole formation (in-vivo) was documented on histopathological examination and it was the most important finding as we think that may explain the role of *H. pylori* in peptic disease. The vacuoles then enlarge leading to cell ruptures and cell death knowing this fact we think peptic ulcer is caused by *H. pylori*.

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# علاقة جرثومة الملوية البوابية بتكوين الفجوات بداخل الخلايا المخموجة

**الملخص**

تضمنت عينة الدراسة 150 مراجعاً لوحدة الناظور يشكون من الالم في الشرسوف وعسر الهـضم والحموضة والقيء والم في البطن وانتفاخ البطن وحرقة الفؤاد وتغوط اسود. اذ تـم استئـصال الخـزع النسيجية من الاشخاص ضمن عينة الدراسة وذلك لاجـراء الاختبـارات التشخيـصية: البكتريولوجيـة والنسيجية المرضية وذلك بهدف، اولاً: عزل بكتري *Helicobacter pylori*، وثانيـاً : لتحديـد الـدور الامراضي الذي تقوم به البكتريا في احداث القرحة الهضمية. تضمنت الاختبارات البكتريولوجية، اجـراء الفحص المجهري المباشر للمزيج الخزعي النسيجي ثم زرعه على الاوساط الاختيارية لبكتريـا لبكتريـا *H.pylori*. اجريت الاختبارات النسيجية المرضية باستخدام نوعين مـن المقـاطع النـسيجية الـشمعية

(البارامضية) البلاستيكية. حيث اظهرت النتائج ان اعلى نسبة حساسية كانت 74.1% في تشخيص وعزل البكتريا عند زراعة المزيج الخزعي النسيجي على وسط [اكار البروسيلا + (5-7%) دم اغنام + مغذي النمو Skirrows]، اما حساسية التشخيص عند اجراء الفحص المجهري المباشر فكانت بالمرتبة الثانية وبلغت 70% والمرتبة الثالثة في حساسية تشخيص البكتريا كانت للاختبارات النسيجية المرضية باستخدام المقاطع النسيجية حيث بلغت 67.9%، اما المقاطع البلاستيكية فاحتلت حساسيتها المرتبة الرابعة في تشخيص البكتريا وبلغت 43.5%. ان توثيق تشكيل الفجوات بداخل الخلايا المصابة (في الحي in-vivo) بالاختبارات النسيجية المرضية يعتبر من اهم النتائج في ايجاد دور بكتريا *H.pylori* في احداث القرحة الهضمية. وذلك بان الفجوات تتشكل ثم تكبر بالحجم وبالنتيجة تؤدي الى تمزق وموت الخلية المصابة وبهذه الحقيقة يمكن اثبات ان القرحة الهضمية ناتجة عن الاصابة ببكتريا *H.pylori*.

## INTRODUCTION

The relation of *H. pylori* and the pathogenesis of gastroduodenal diseases have came to the view 16 years ago. *H.pylori*, is a spirally- shaped, gram negative, bacterium with a very powerful urease activity and apolar flagella. These Features allow *H.pylori* 10 survive in the lumen of stomach (Gupte, 1995; Horniek, 1987).

Urease activity buffers the pH at the cell surface allowing bacterium survival until it enters the mucus layer, a protective barrier against the high proton concentration (Owen el al., 1985). Although the pathogenesis of gastroduodenal diseases caused by *H.pylori* have not been shown yet. *H.pylori* infection is the major acquired factor in the pathogenesis of a wide spectrum of gastroduodenal diseases including gastritis, duodenal ulcer, gastric ulcer, gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT)- type lymphoma (Park et al., 1998; Wang et al., 1997). The putative pathogenic determinant factors of *H. pylori* can be divided into two major groups : virulence factors, which contribute to the pathogenic effects of the bacterium and maintenance factors, which allow the bacterium to colonize and remain within the host. Virulence factors contribute to three major pathogenic effects of *H.pylori* : gastric inflammation, disruption of the gastric mucosal barrier and alteration of gastric physiology. It is likely that many of them function as both virulence and maintenance factor (Dunn et al., 1997). One of the most important virulence factor is vacuolating cytotoxin (VCT). The culture supernatant of about half of the *H. pylori* isolate induce large cytoplasmic vacuoles in eukaryotic cells (in-vitro) study. Despite this fact still there is no direct evidence regarding the virulence role of *H.pylori* VCT on( in -vivo) pathogenesis (Err-cheng et al., 1996).

### Aim of this study

Identification of the role of VCT in pathogenesis of gastroduodenal diseases caused by *H.pylori* infection.

### Material and methods

One hundred fifty patients referred for esophagogastroduodenoscopy in Ibn-sena teaching hospital were complaining from symptoms complexes including : epigastric pain, dyspepsia, acidity, vomiting, abdominal pain, flatulance, heart burn and melena.

Two antral biopsies were taken from each patient. These biopsies were then studied microbiologically, and histologically.

**1- Microbiology :-**

One antral biopsy placed in Stuart's transport media and transported to the laboratory for processing within two hours from sampling . Each of the specimen was plead in one (ml) of a sterile normal saline (0.9 %) and homogenized by stomaker (SEWARD MEDICAL UAC HOUSE) for five minutes. One drop of homogenate was smeared on slide for direct gram staining and 100µl. smeared on the surface of Brucella agar enriched with (5-7 %) sheep blood, and skirrow's supplement. Vancomycin 10mg/L., Thrimethoprim 5mg/L.and polymyxin B 2500 IU/L. The plates then incubated at 37°C in microaerophilic atmosphere (5 % $O_2$ ,10 % $CO_2$), for 5-7 days (Clyne and Drumm, 1996; Xia et al., 1994). Bacterial colonies were identified as *H.pylori*, on the basis of growth characteristics colonial morphology, grain stain examination, catalase, oxidase reactions and urease production (Lee and Megraud, 1996). According to these findings patients were classified as *H.pylon* positive, when H.pylori either cultured from biopsies or they were detected in microscopic slides, stained by gram method.

**2- Histology :-**

one antral biopsy specimen from the first hundred patients were fixed in 10% buffered formalin , then processed routinely and embedded in paraffin wax; (Whitehead et al., 1972), the sections then cut and stained by Harris-Haematoxylin-Eosin. The remaining fifty biopsies were fixed in FGA buffered solution (Formal- Glutar Aldehyde fixation ), then processed routinely to prepare plastic sections which were stained with Tollidine-blue (Lewis and Knight, 1977; Richards et al., 1993). Histopathological examination of both paraffin and plastic sections were made to evaluate the relationship between the presnce and distribution of *H.pylon* and its ability to form vacuoles which lead to damage of the infected cells and finally to ulcer.

## RESULTS

*H.pylori* was isolated from 115 (76.7 %) out of 150 patients examined, depending on Histological and bacteriological examinations ( culture and direct microscopy ) as shown in ( Fig: 1,2,3,4,5 ).

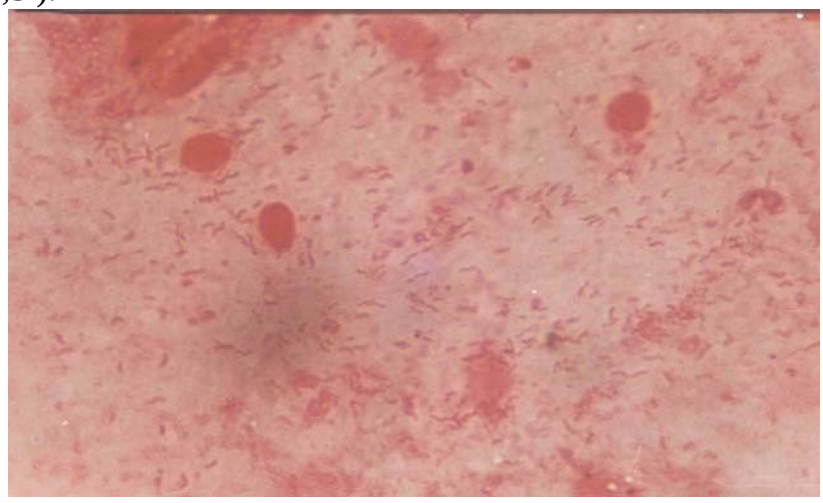

Fig. 1: Gram stain of fresh clinical isolate of *H.pvlor* : X1250

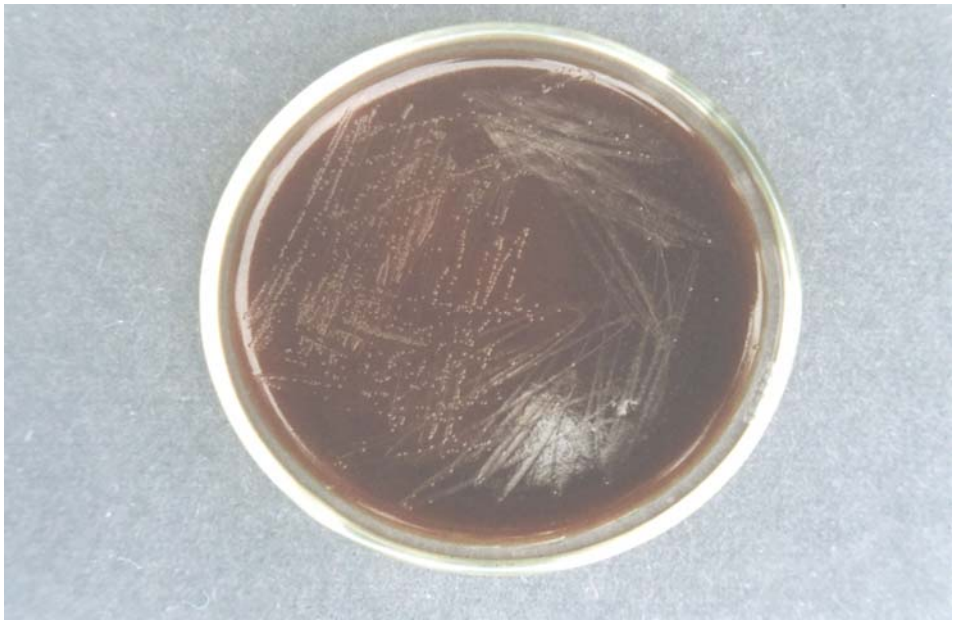

Fig. 2: The typical appearance of selective primary isolate of *H.pylori* from biopsy.

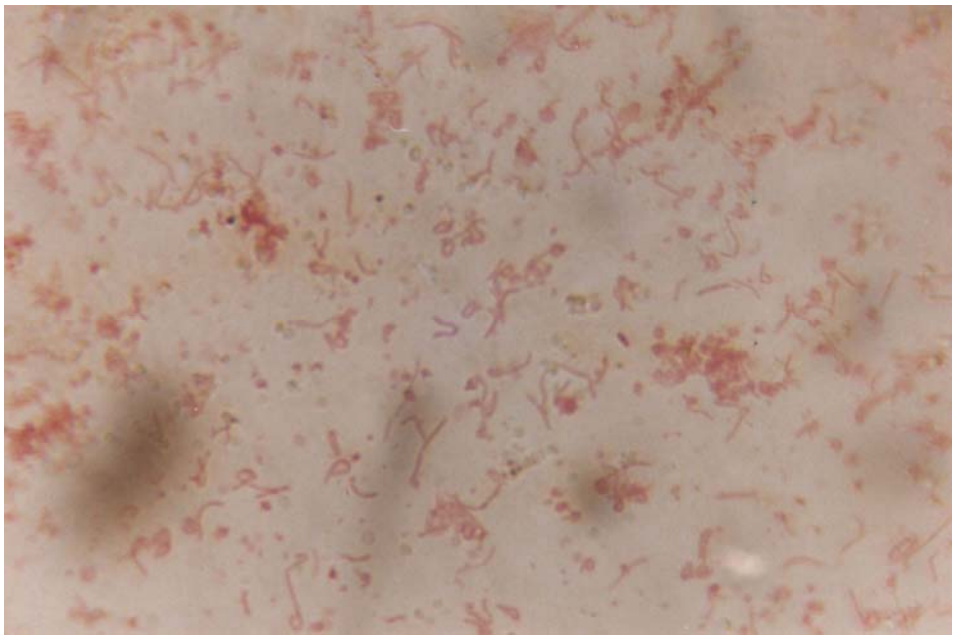

Fig. 3: Gram stain of *H.pylori* from culture XI 250.

Fig. 4: Numerous bacteria are attached to the surface epithelium of gastric antral mucosa XI 250 ( paraffin section stained with H&E)



Fig. 5: Numerous bacteria are attached to the surface epithelial cells of gastric antral mucosa XI 250. (plastic section stained with Toluidine-blue)

The sensitivity and specificity rates, positive and negative predictive values were different using variables modes of testing as in Table: 1.

Table 1: sensitivity and specificity results.

| Method for diagnosis | | Sensitivity % | Specificity % | Positive predicted% | Negative predicted % |
|---|---|---|---|---|---|
| Culture | | 74.1 | 95.1 | 98.8 | 39.8 |
| Direct gram stain | | 70 | 100 | 100 | 35.7 |
| Histology | Paraffin section with H&E | 67.9 | 100 | 100 | 32.5 |
| | Plastic section with Toluidine blue | 43.5 | 75 | 90.9 | 18.8 |

Figure 6: shows vacuoles formation in epithelial cells of 20% positive *H.pylori* specimens, which lead to damage and destruction of the affected epithelial cells, by their ability to produce VCT which will result in the formation of vacuoles, which will later enlarge and evantually rupture with their containing cells or they cause cell death directly as shown in (fig:7).

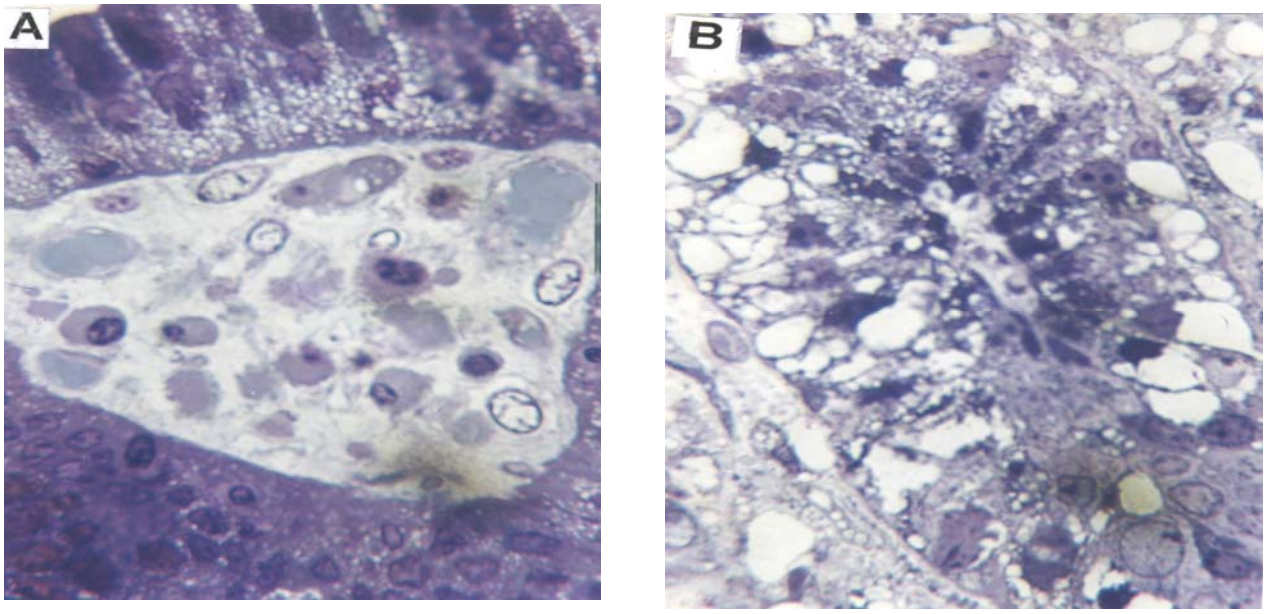


Fig. 6: Plastic sections stained with Toluidine- blue XI 250
A-Vacuole formation inside the epithelial cells.
B-Enlargment of the vacuoles.

Fig. 7: Rupture and death of the infected cells by H.pylori XI 250.
A- Plastic section stained with Touidine- blue.
B-Parafine section stained with H&E.One of the most important result in this study is the confirmation of the relationship between infection with VCT producing strains and gastroduodenal diseases, especially ulcers. This relation confirmed by the finding ofH.pylori inside abig vacuole within an infected epithelial cell as shown in (fig:8).

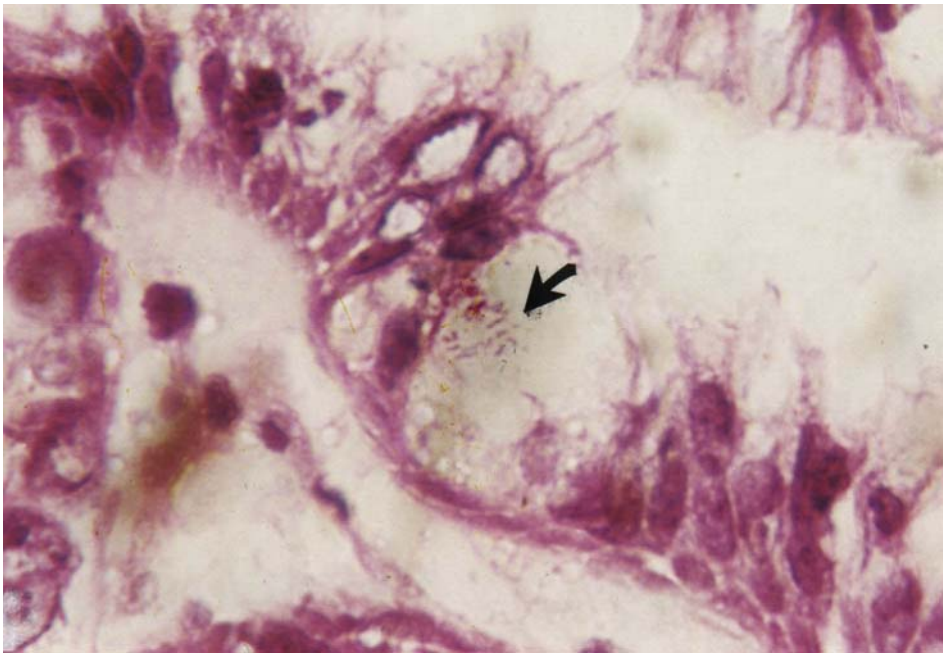

Figure 8: Bacteria inside abig vacuole within an infected epithelial cell XI 250 (paraffine section stained with H&E)

## DISCUSSION

The present results clearly showed that VCT can be produced in an active form in the cytosol of human cells. This finding provides a strong evidence that VCT is a toxin that act in the infected cell cytosol with *H.pylori*. According to the case of toxigenic strain of *H.pylori* cause progressive vacuolation in mammalian cells such as Hela cells (Bernard et al., 1997). It has been speculated that the phenomenon of vacuole formation is associated with gastric pathologies.such as gastric ulcer and gastritis induced by *H.pylori* infection. *H.pylori* produces ammonia by a potent urease enzyme and thus increases the pH of the surrounding mucus, facilitating colonization by H.pylori. This product (ammonia) induces damage to the gastric mucosa. Moreover, vacuolating activity may be potentiated by urease mediated ammonia production (Wang et al., 1997). The hypothetical model of the cell intoxication by VCT: VCT is produced by *H.pylori* as 140 KDa precursor, is cleaved at the C-terminal domain and released into the extracellular medium as 95 KDa mature protein that oligomerizes into heptamers and hexamers. VCT shows the unique property of bieng a protein activated by, and resistant to pH values as low as 1.5 that may be reached in the stomach lumen (Bernard et al., 1998). In the stomach by bacterial proteinases VCT will cleave to generate two fragments of(p37) 37 KDa and (p58) 58 KDa, which will remain associated to each other by non covalent interactions (Bernard el al., 1997). The toxin is proposed to bind to the apical portion of the epithelial cells via its carboxy-terminal domain (p58) to a receptor (R)on the epithelial cells.whose nature is still unidentified (Zoratti et al., 1999). Active monomeric toxin, but not oligomeric inactive complexes, insert into the plasma membrane vi^ hydrophobic protein-lipid interactions. The insertion step, which requires and involves the amino-terminal domain (p37), results in the formation of anion-selectivej channals of a low conductance. These toxin channals are liky to result fromre-j assembling of asingle toxin molecule into H new oligomeric structure. Endocytosiq and transport to the endosomes of the toxin, a step which again requires (p37), is proposed to increase the anionic permeability of these compartments, which in turn would enhance the vacuolar ATP proton pumping activity.In the presence of weak; bases in particular ammonia generated by the *H.pylori* urease, the ndosomal accumulation of osmotically active acidotrophic ions ($NH_4^+$) is predicted to increase^ this leads to water intlux and vesicle swelling, an essential step in vacuole formation, Changes in' the para cellular route of permeabitlity of polarized epithelial cell mooolayers might result from a still unknown secondary mechanism, triggered by plasma membrane-associated channals. Alternatively, cytosol-delivered active portions of VCT, formed by (p37) plus ammo-terminal region of (p58), would act on cell-cell junction, to modify the trans-epithelial electrical resistance. Such a putative eyiosolic activity of the toxin could be of catalytic nature and may play a role iq vacuole formation as well (Bernard et al ., 1998 ; Kanghinis , 1995). This study proved the speculated hypothesis regarding the effect of VCT in pathogenesis of gastroduodenal diseases , in addition, *H.pylori* is able to enter inside vacuoles formed by the effect of VCT within infected cells.

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