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Histopathological Study Post *Helicobacter Pylori* Infection in Mice

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Abstract:

*Helicobacter pylori*isthe causesulcers in gastrointestinal tract such asgastric and pepticulcer besides the malignancy of gastric carcinoma, lymphoma andnon-gastricdiseases.

The histopathologicalchanges that occurred indifferent organs of mice post*H. pylori* infectionwere investigated. The bacterial isolate was taken from a patient with duodenal ulcer. The result tissue section revealed polymorphonuclear cells(PMNs) and lymphocytes infiltrated the layers of kidney, liver, and heart with degeneration.

H. pylori has a significant sever role in the incidence the inflammatory reaction in stomach.

Keyword: Helicobacter pylori, Rapid urease test, infection, diagnosis.

Introduction:

Most disorders of *Helicobacter pylori* infection is gastric or duodenal ulcers, which occurs where their tissues lost the protection (Wang *et al*., 2014), mechanism to disposethe acids&pepsin enzymes. Gastriculcer occurs when the mucosa of the stomach or duodenum was very weak for the protection.

Gastric ulcer means inflammation of stomach lining (gastritis)&the perforation occurs when the ulcer untreated or treated weak(Naito& Yoshikawa, 2002).

H. pylori produce urease enzyme which cause the release of free radicals that cause damage the epitheliumneither invading the cells of surrounding tissues &nordeveloping the immunity for repeated infection (Martin,2005).*H. pylori*represented as major etiological factor of gastritis&duodenal ulcer(NIH Consensus conference,1994;Franceschi,2002). The association between *H. pylori*&causes of cancer was fixed as a carcogenic by the international Agency for Research on Cancer (IARC,1994).

H. pylori colonizes epithelial cells of gastric mucosa and the infectionwas limited in stomach, duodenum and esophagus (Graham,2014).

Experimental procedure:

1- Bacterial isolation& identification:

A-The special media for isolation of *H. pylori* is skirrow agar with the antibiotics (polymyxin B, Vancomycin, Trimethprim B, &Amphotericin) (Dent *et al*.1988).

B- Bacterial isolate is taken from biopsies of duodenal ulcer patient in the Educational Baghdad hospital. Tow histological biopsies were taken , one used for bacterial culture with skirrow media then transfer to microaerophilic condition with gas generating kit from oxide for 3-7 days at 37˚C ; the second biopsy used for rapid urease test by liquid urea medium prepared by Marshall (Marshall *et al*. 1987).

C- *H. pylori* diagnosed according to Bergey's classification (Holt *et al*.1994); as following:

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i. Microorganism examination: the smear of bacterial colonies stained with gram stain& examined by light microscope.
ii. The motility of bacteria was examined by suspension drop with light microscope.
iii. Biochemical tests: used catalase & oxidase test.
iv. Sensitivity test for *cephalothin*&naldixic acid.

D- *H. pylori*Diagnosed in the biopsies was done by:

i. Direct smear of biopsies according to (Montgomery *et al*.1988).
ii. Rapid urease test: the color of media was change from yellow to red through 5-10 min due to highly activity of urease enzyme.

2- Bacterial suspension for inoculation; prepared to contain 10^9bacterial cells of *H. pylori* per 1ml of physiological phosphate buffer (Wang *et al*.1997).

3-histopathological examination:

i. Thirty of white male mice BALB/CMusMusculus, aged between 8-14 weeks & weight about 250-350 gm, healthy and good management from light to temperature available for them.
ii. All animals were infected orally with 0.1ml of bacterial suspension which contains10^9 bacterial cells/ml for 3 times, dose for each two days, and thenthe animals were monitored along periods of 3&4 weeks of experiment.
iii. At 4^{th} week post infection the histological samples were taken from the animals after killing, the samples are the liver, kidney & heart preserved in 10% formalin as fixative to prepare histopathological sections was done according to Guyer instructions (Guyer,1953).

Results:

H. pylori isolated from laboratory Animals match the patient isolates.

Histopathological examined of the infected organs(kidney,liver,&heart)through the first three weeks demonstrated degenerative changes in the epithelial cells with progression of inflammatory response& infiltration of lymphocytes in all the examined tissues .In fourth week, viewed lesions characterized by intercellular swelling clearly in the renal tubular epithelium cells(Figure1)&hepatocytes(figure2,3)to vacuolar degeneration of the cardiac muscles(Figure4)&perivascular mononuclear cells cuffing, also few infiltration in the parenchyma , congestion & dilatation of blood vessels(Figure5).

Discussion:

The *H. pylori*bacteria can causes histopathological changes in non-stomach tissues especially in the kidney, liver & heart, although their main role in infection of the stomach by causing gastric, peptic, duodenal ulcer& gastric cancer.These changes may attribute to virulence of two factors, Vaculatingcytotoxin A (VacA) and Cytotoxin-associated gene (CagA) produced by *H. pylori*.

Vaculatingcytotoxin A is pore-forming toxin, secreted by *H. pylori* caused increased in plasma membranepermeability, changes in the structureof endosome, mitochondrial membrane permeability and then cell death (Jones *et al*.,2010), while CagA (Cytotoxin-associated gene A) is the second protein produced by H. pylori transfer from it into host cells by type IV secretion system (Terradot and Waksman, 2011). These two proteins are determined the pathogenesis of *H. pylori*(Figueiredo*et al*.,2005).

In kidney tissue, show vacuolar degeneration (star shaped) with infiltration of lymphocytes within glomerulus, some studies showed correlation between chronic kidney disease and *H. pylori*prevalence (Ganji and Rafieian, 2017), there's one study has not supported the correlationbetween*H. pylori*presence and the kidney infection(Wijarnpreecha*et al*.,2017), but our study agreement with Ganji and Rafieian study when showed agreement between *H. pylori* and kidney infection.

In the liver tissue, show vacuolar degeneration & leukocyte cuffing, vasculardilatation, enlargement of hepatocytes,depression of the sinusoids. *H. pylori* inoculated orally may reached the hepatocytes and cause inflammation as etiological factor (Huang *et al*.,2009).

Rapid urease test is the most useful tool has used in our study for *H. pylori* diagnosis faster than other tools, rapidurease test had specificity and sensitivity reached 90%, expensive, andisthe only test was performedwithin few minutesdirectly after collect biopsy specimen by endoscopy(Atkinson& Braden, 2016; Al-Rubai, A., 2006).One study reportsthe lowering levels of fasting blood ammonia in *H. pylori* infection than without *H. pylori* infection in cirrhosis case of the liver organ(Kini*et al*. 2001).Some studiesdepend on rapid urease test for evaluated the status of*H. pylori*(Al-Rubai, A., 2006; Vasconez*et al*. 1999).

In the heart tissue, show thrombosis, perivascular leukocyte cuffing &edema with vacuolar degeneration of cardiac muscle fibers.

Diseasesof the heart like: ischemic heart disease, myocardial infarction, atherosclerosis, coronary artery disease,in addition to *H. pylori*hasrepresentsone of etiological factor of heart disease by releasingtwo main toxigenic nutrients, vaculating associated gene (Vac A) and cytotoxic-associated gene A (Cag A), Cytotoxin-associated gene A (Cag A) is the most virulence factorparticipating in formation of cholesterol patches in the arteriescauses autoimmune disorder, and started the immune response (Jamkhande*et al.* 2016). *H. pylori* adhesionto the host cell causedinflammation, cellular damage,and increases the release of most important virulence factors for *H. pylori*: Cag A andVac Aandfound that *H. pylori* strains positive toCag A are associated with heart disease morethan strainsnegative to Cag A. *H. pylori*strains positive to Cag A increases the activities of cycloxygenase-1 and 2 in the endothelial cells of the blood vessels. Inflammation caused by Cag A stimulate release many cytokines like IL-1 to IL-12, monocytes, macrophages (like tumor necrosis factor α (TNF-α)), T and B lymphocytes, then causes heart disease or shock, and autoimmune reaction among anti-Cag A antibodies and the vascular wall antigens, this suggested that antibodies participated in activation the inflammatory cells within atherosclerosis lesions (Lee *et al*., 2018; Al-Quarashi&Hodhod, 2012).

Burden of *H. pylori* is to contain protein similar to the heat shock protein-60 that present on the surface of endothelial cells in the artery. Immune cross reaction occurs between human and bacterial heat shock protein-60 due to the immune response to *H. pylori* which in turns causes autoimmune reaction and local inflammation in the artery(Sulewska, 2004). Another speculation about*H. pylori* role in development of atherosclerotic plaque, has found that bacterial deoxyribonucleic acid in the arterywall forms patches of infection, which results in heart disease(Sulewska , 2004).The role of *H. pylori* in many diseases depends on the basis of rapid urease test.

Infection of *H. pylori*play role in progression of vascular disease (Lee *et al*.,2018; Elkind&cole, 2006). Seroepidemiologicaland eradication studies demonstratethe relationship between atherosclerosis andthe infection with *H. pylori*(Ando *et al*.,2006).

Reached *H. pylori*andtheir biochemical contain from the mucosa of the stomach to the circulation (Guo*et al*., 2007) are in agreement with the exposure ofendothelium to virulence factorssecreted by*H. pylori*. In atherosclerosis plaque sites, these factors reaches to high levels in the microenvironment of the artery wall (systemic circulation) and cause endothelial dysfunction & lesion development(this may attributed to Vac A,that altering intercellular vesicularcausingformation of vacuole (Reyrat*et al*.,1999).

Conclusion:

The significant pathologic infection of isolated *H. pylori* in mice.Anti-*H.pylori* antibodies cross react with antigens of erythrocyte membrane in the human,which probablya guide for the relationship between infection with *H. pylori*and vascular disorders . Successful *H. pylori* eradication led to a reduction in the platelet activation.

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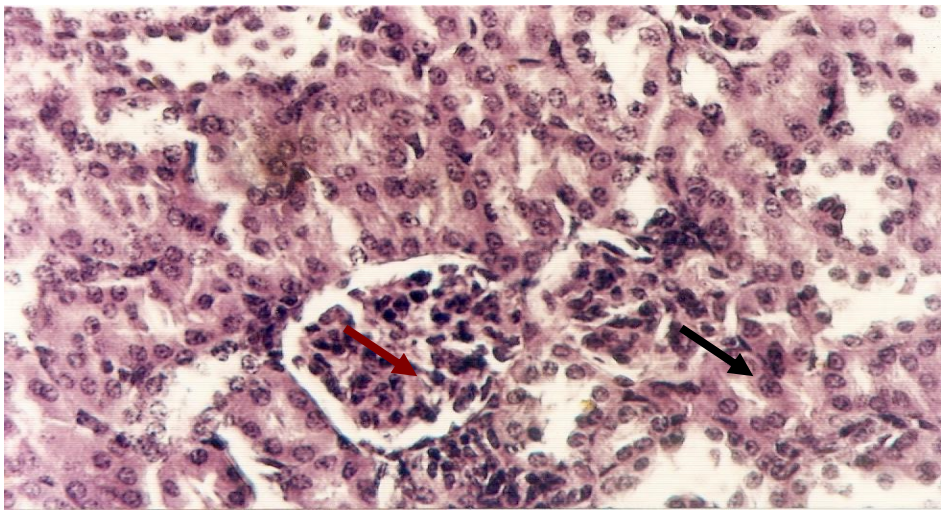

Figure(1):Kidney in mice, vacular degeneration (star shaped)( )with infiltration within glomerulus.( )(H&E) (X40).

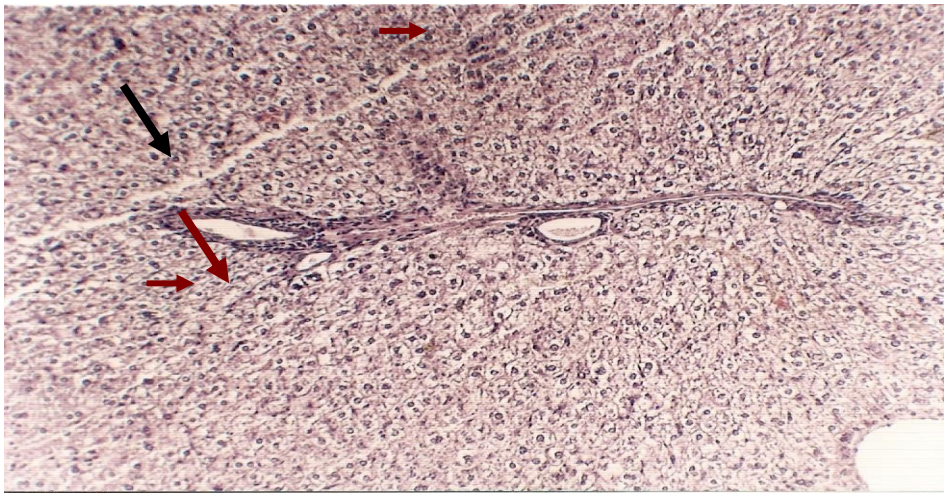

Figure (2):Liver in mice, Vaculardegeneration( ) & perivascular leukocyte cuffing( ).(H&E) (X10).

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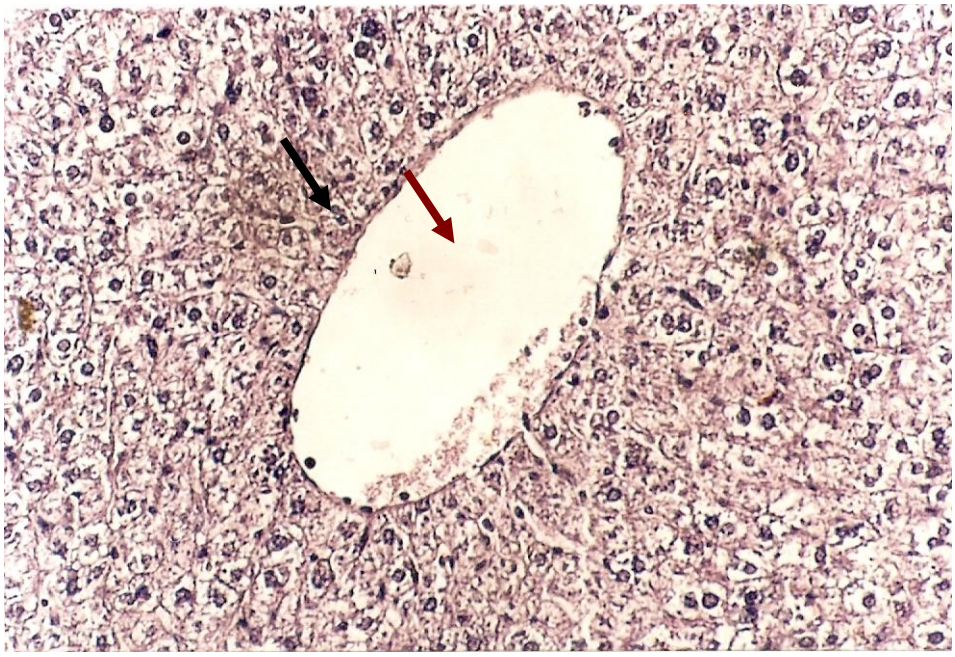
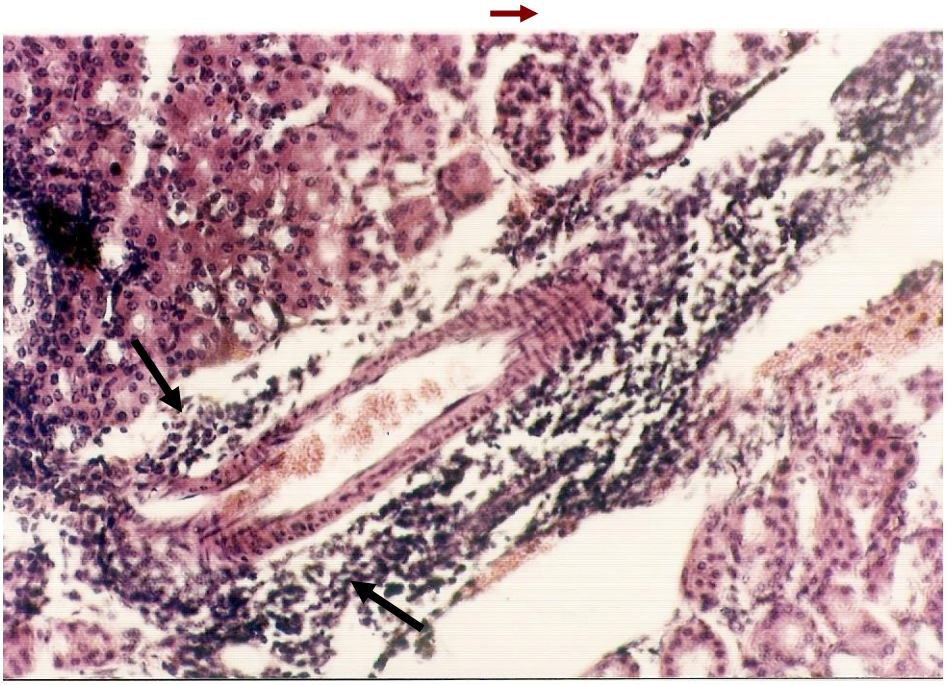


Figure (3): Liver,diltation of the central vein ( ) ,enlargement of hepatocytes (vacuolar degeneration)( ) ,depletion of the sinusoids.



Figure(4):perivascular leukocyte cuffing(lymphocytes)( ) &vacuolar degeneration.(H&E) (X20).

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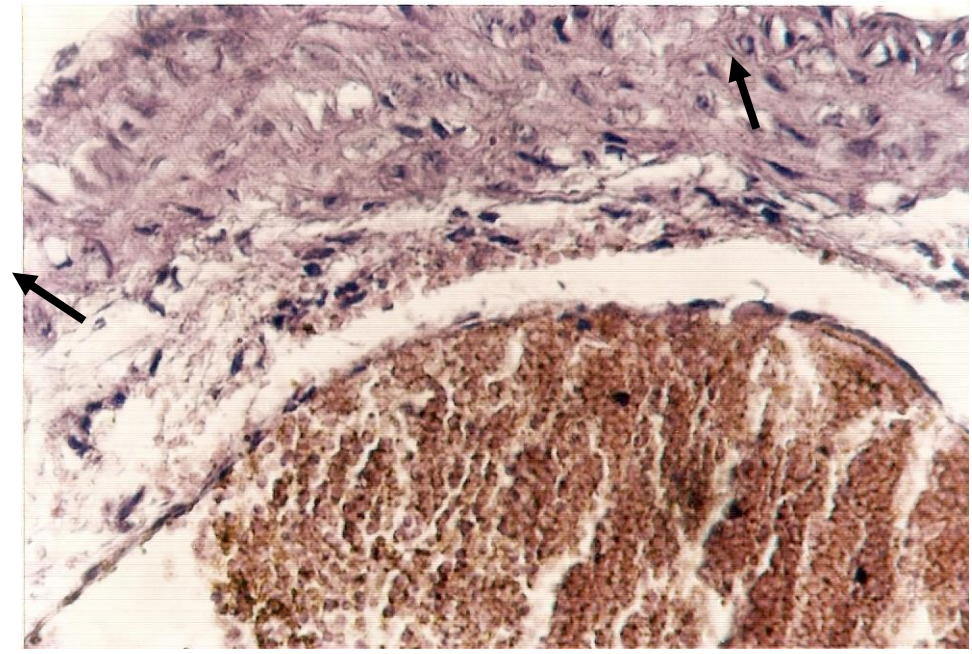

Figure (5): Heart in mice, Show thrombosis, perivascular leukocyte cuffing & edema with vaculardegeneration( ) of cardiac muscle fibers.(H&E) (X40).