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Laboratory diagnosis of *H. pylori* among dyspeptic patients using Culture and Rapid urease test

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Abstract

Globally Helicobacter pylori has been defined is the prime reason of stomach ulcer and gastric cancer. Medical laboratory analysis of H. pylori infection is done by two ways invasive and non- invasive methods. Invasive techniques frequently disapproved by patients because it is inconvenient but still remain reliable methods. Among invasive methods, culture is considered as gold standard method from which other methods are compered. A sum of eighty six persons with average of ages between 18-77 years old with mean 37.58 years ± (forty three males , forty three females) who visited to endoscopic center / Azadi Teaching Hospital for endoscopic examination from June to Oct. 2013.From everyone , two antral biopsies, one for urease test, the other for culture were collected. Biographies from each case were taken in a questionnaire form after approval by Research Ethics Committee of the college of medicine / Duhok university. H. pylorus was found in 37.2% and 68% by Culture and Rapid urease test respectively. The biggest value percentage of H. pylori positive cases was detected by rapid urease test while the fewest value percentage was by Culture. Combination of both tests it did not approve as a diagnostic test for detection of this pathogenic bacteria. The study did not detect any statistical correlation on the impact of age factor on H. pylori infection by both methods. In this research appear males were less positive for H. pylori than females by urease test and no such statistical association was

noticed count on the sex and Helicobacter pylori pathogenicity via culture. Research never noticed any considerable correlation was found between smoking status and patient's residence with H. pylori positive cases by both methods. This study was performed to estimate the appropriate and better diagnostic tests for diagnosis of H. pylori among various types of samples. Due to the increasing incidence of treatment failure (caused in part by antibiotic resistance), post-treatment testing is recommended to confirm H. pylori eradication. Knowledge of the epidemic and the Routes of transmission of this pathogen are important points to avoid from spreading and may be useful in identifying high-risk populations, especially in areas that have high rates of gastric lymphoma, gastric cancer, and gastric ulcer. The current study conclude (RUT) was superlative than culture for the detection of Helicobacter pylori.

Key words: Rapid urease test , Culture, *Helicobacter pylori*

1. INTRODUCTION

Helicobacter pylori is a Gram negative, motility, spiral – curved and microaerophilic bacterium that is present in the human stomach of approximately 50% of the world´s population. In 1983, Warren and Marshall were the first to describe and isolate this bacterium microorganism and associate to gastritis, a fundamental change occurred toward the causes and remedy plans of peptic ulcer. Prior this historical event nobody was believed among microbiologists that bacteria can survive and flourish under unfavorable stomach surrounding. Over production of acid cause gastritis it has been believed by Clinicians therefor their remedies were towards suppressing of acid only. Now it becomes facts that this bacterium is main cause of peptic ulcer and has a strong relationship with gastric carcinoma. Therefore, the treatment was changed from anti acid drugs to both antibacterial and anti-acid drugs [1].The main characteristics of this bacterium are negative for Gram stain, curved rods with sheathed lophotrichous, slow growing requires 5-7 days and fastidious requires special media enriched with antibacterial drugs and microaerobic conditions [2]. The bacterium was first called Campylobacter like organism but later moved to the new genus and renamed *Helicobacter pylori* [3]. This bacterium is classified as rapid urea splitting bacteria which splits urea into ammonia gas creating an alkaline cloud to protect themselves from gastric acidity [4]. This bacterium is cosmopolitan in distribution and a bout 70-90% of individuals in non-developed countries are Occupied by this bacterium, while fifty percentages among individuals living in advanced countries [5]. Routes of transfer of this bacteria from person to person has been shown to occur via in different ways , between seventeen to thirty seven peptic ulcer patients participated in acidity secretion study was possibly result from cross infection with H pylori via pH probes distinctly , nevertheless direct contact with infected person is not normal .

Three methods is available for passing this microorganism from a person infected by this bacteria to a person uninfected without intervention of an external source have been suggested: by faeces, saliva and vomitus [6]. Various methods have been used for screening and determination of *H.pylori* some of them require endoscopy to take biopsy like cultivation on medium, Smear histological examination and Urease test, while other do not require biopsy and named non- invasive. Although invasive methods are non-convenient for patients but still more reliable for diagnosis. Therefore two of the popular invasive methods namely Culture and (RUT) were used to show the effect of these tests for identification of

Helicobacter pylori among peptic ulcer cases [7]. The aim of the present research to study the effect of gender, age, residency and smoking on the prevalence of *H.pylori* of studied patients and to assess the efficacy of bacteriological method (Culture) and rapid urease test for screening H. pylori in dyspeptic patients who underwent endoscopy examination .

2. LITERATURE REVIEW

In 1983 this microorganism was discovered since that time several methods have been used for diagnosing *Helicobacter pylori* bacteria. Which include both invasive methods and non-invasive methods [8 and 9].Invasive methods require endoscopy to obtain gastric biopsies for analysis by histopathology, culture, or urease tests as well as by molecular technique [10]. Non-invasive methods include Urea Breath Test (UBT), Serology and Stool antigen test [11and 12]. [13] isolated *H. pylori* from 45% of gastric biopsies using (BHI) agar with seven percentage horse red blood cells plus (Oxoid, Basingstoke, England) antimicrobial combination and thioglycolate broth as a transport media. This bacterium was screened about 64% of cases using blood agar plus Campylosel (Bio Merioux, France) by [14]. *H. pylori* was isolated from 120 (54.54%) of 220 patients with endoscopic ulcers in which 38 (31.66%) from patients with duodenitis and 13 (10.83%) from patients with gastritis using thioglycolate broth as a transport media and Campylobacter selective media with antibiotics [15]. In another study by [16] who used Columbia blood agar medium plus 7% lysed horse red blood cells supplemented with *H. pylori* selective (DENT) antibiotics (Oxoid, Basingstoke, UK) and Campy Gen bags (Oxoid, Basingstoke, England) for isolation of *H. pylori* from antral biopsies. They isolated *H. pylori* from 163 (55%) antral biopsies and confirmed their diagnosis by Gram stain morphology and enzymatic tests such as urease, oxidase, and catalase. [17] isolated *H. pylori* from 97 (48%) out 201 patients using Stuart's medium as a transport media. They grinded biopsies in 250-300 µL of normal saline using sterile mortar and pestle and 100 µL were seeded onto a selective agar incubated in a microaerophilic condition for a period of seven days. *H. pylori* was isolated from 55.2% of gastric biopsies using Columbia blood agar (Oxoid) supplemented with polymyxine B, vancomycin, and trimethoprim as selective medium [18], while [19] isolated *H. pylori* from 38 (36.5%) out of 104 gastric biopsies using selective medium and incubated at 37°C for one week under microaerophilic. Out of 82 patients, 53 (64.6%) were positive for *H. pylori* by Culturing on medium using two biopsies, one from the antral and the other from fundus [20]. *H. pylori* was detected in 76 (82.60%) out of 92 Gastric aspirations gathered from last part of the stomach using CLO test [21]. [22] Used rapid urease test under the name Pronto Dry Kit and found that 70 (68.62%) from 102 gastric biopsies were positive. While the diagnostic test is read with 1 min and it was requires high colony of this microbes and the specificity of test was typical result, but reduces with the period of the incubation. [23] Used gastric aspiration samples for the detection of *H. pylori* by RUT, They found 94% from 300 samples were positive.

3. MATERIALS AND METHODS

3.1. Gastric biopsy

Two antral biopsies from different sites were obtained from the pyloric region of 86 dyspeptic patients using a GIFXQ 40 endoscope (Olympus Optical Company, Tokyo, Japan). Biopsy from everey patient was tested via (RUT) where it is called *Helicotec*UT® plus (Strong biotech corporation, Taipei, Taiwan) which was done immediately after collection at the same center while other biopsies were transported to the Microbiological Laboratory/ Dept. Biology/ College of Science, Univ. of Duhok, using thioglycolate broth (Himidia, India) as transport media under cool temperatures.

3.2. Isoltion

Biopsies were processed in less than two hours from collection under aseptic condition. Each biopsy was homogenized then inoculated into Columbia blood agar (SR0147E, Oxoid, UK)

enriched with 5% Sheep blood and antimicrobial susceptibility (Oxoid, UK). Inoculated fresh and pour plates were incubated in microaerobic conditions using Campy gas packs (Bio Merieux, France) at 37°C for one week. Plates showed growth of small, transparent, grey and non-hemolytic colonies were suspected for *H. pylori* and processed for identification**.** Diagnosis of *H. pylori* was made according to the bacterial and biochemical tests like catalase, oxidase and urease tests.

3.3.Rapid Urease test

HelicotecUT® plus (Strong biotech corporation, Taipei, Taiwan) was used as a rapid urease test. One antral biopsy from each studied patient was immediately tested and the test was considered positive when the color of the test paper changed from yellow/orange to pink/purple within 15 min at 25 °C but if it remained yellow in color after an hour, then the test was negative.

4. RESULTS

This microaerophilic organism was identified in 68% and 37.2% by Rapid-urease test and Culture respectively as illustrated in Table 1.Efficacy of two diagnostic tests (Culture and Rapid Urease Test) against *Helicobacter pylori*.

Table 1. Efficacy of two diagnostic tests against *Helicobacter pylori*

Methods	**With *H.pylori* Number of patients / %**	**Without *H.pylori* Number of patients / %**
RUT	59 / 68	27 / 32
Culture	32 / 37.2	54 / 62.8

(100%) of study population depending on age categories were positive and their ages were more than 60 years old patients by Rapid-Urease Test, while Culture and Performance both of them showed variable results in other age groups as depict in figure. 1 and no association was detected between ages and RUT, Culture.

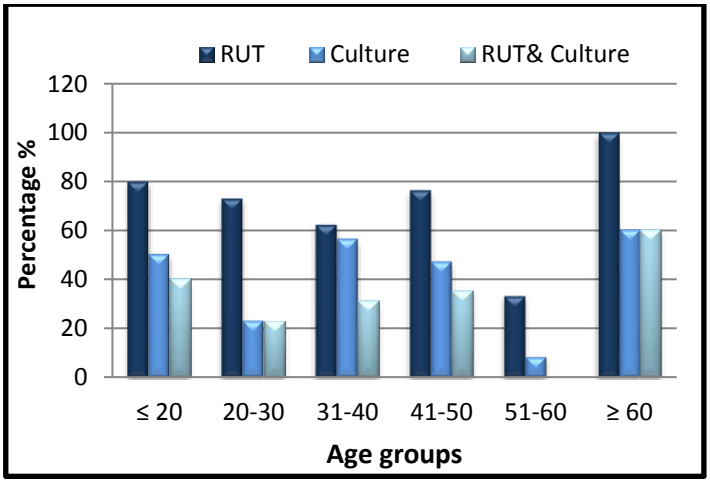

Fig.1**.** Age distribution in cases with positive tests

A high percentage (79.06%) of this bacterium was in female patients by rapid urease test compared to 55.81% in male patients. 41.86% of positive samples were recorded in males using culture method compared to 32.55% in females, while 32.55% of females were positive

Using both rapid urease test and culture methods compared to 23.25% in male patients, as shown in figure 2.
Statistical analysis showed that the sex (female) affected significantly (p=0.0238) on the rapid urease test, while no significant association was recorded between sex and culture, sex and culture with rapid urease test.

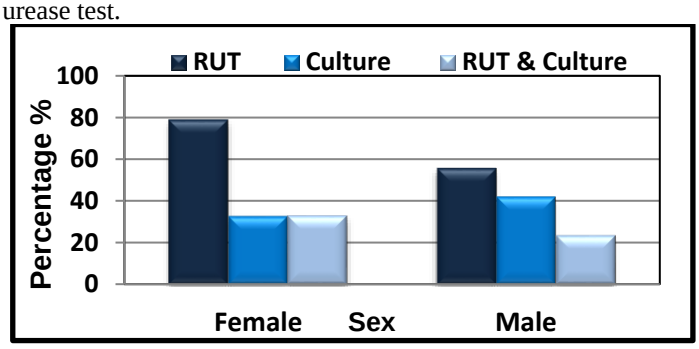

Fig.2. Screening and Determination of *H. pylori* in sex groups by RUT and Culture

Among smokers, 60% gave positive compared to 52.17% in non-smoker patients by rapid urease test, while By culture, 30% of smokers and 52.17% non-smoker patients gave positive using culture. By both culture method and rapid urease test, *H. pylori* was indicated in 15% in smoker persons compared to 26.08% in non-smoker patients as illustrated in Fig 3 No evidence of smoking status correlation was detected by these tests.

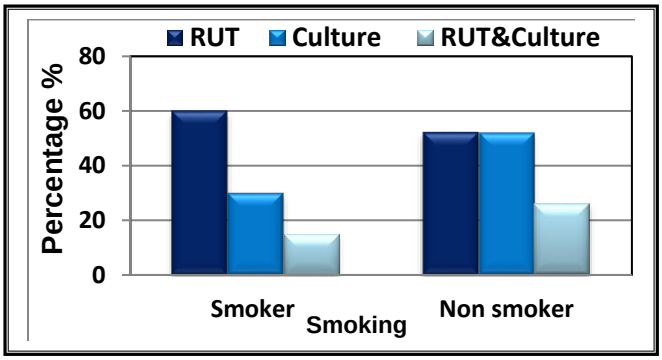

Fig.3. Frequencies of *Helicobacter pylorus* infection with (RUT and Cult.)
in correlation to smoking

This anaerobic microorganism was detected in 72%, 38% and 30% by Rapid Urease Test, Culture & both culture plus rapid urease test among the 50 patients who lived in the urban areas compared to low percentages of *H. pylori* among individual who lived in villages in which 63.88%, 36.11% and 25% of patients were positive for *H. pylori* by rapid urease test, culture and both culture with rapid urease test alternately, as depict in Fig 4.Evidence association was no detected based on residency impact and these tests.

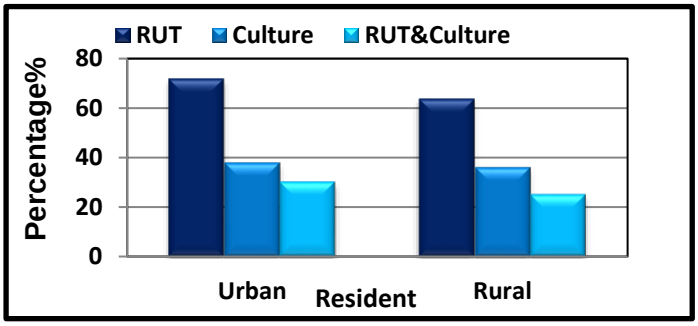

Fig.4.Detection of *Helicobacter pylori* based on residency factor by RUT and Culture.

5. DISCUSSION

Helicobacter pylori is the commonest microbe it cause dyspepsia chronic infection around the world and The majority of patients suffered from this infection stay asymptomatic in a period of time. Various methods used for Laboratory confirmation of *Helicobacter pylori*, which are divided into biopsy-based methods called invasive methods like culture, RUT and histopathology. Non-invasive methods include UBT and serological tests for detection of immune responses and stool antigen. The goal of the current research was to assess the efficacy of two commonly used invasive methods namely (RUT) and culture for identification of *Helicobacter pylori* from gastric biopsies. The study included 86 patients who were referred to endoscopically examination center. Culture of the biopsy specimens inappropriate to be used routinely as it is time consuming and is very difficult to maintain the strict anaerobic measures . The maximum percentage (68.0%) of positive cases was registered by RUT while the minimum percentage (32.7%) was by Culture. The Results of the culture method were agreeing to those recorded via [24] who diagnosed that 36% of cases were positive by culture method. The results of this study were in disagreement with those found by several researchers who recorded high percentages of *H. pylori* from gastric biopsies, such [25] isolated 60% .The results of the study were different from other researchers who recorded lower percentages of *H. pylori* from gastric biopsies such as [26] isolated 19.4%,.These discrepancies in the results of isolated *H. pylori* from gastric biopsies can be attributed to many causes such as low number of studied samples, pathogenicity and the antral biopsy may not to be containing this bacteria. Therefore, the distribution of *H.pylori* is patchy or coccoid forms are difficult to detect and hence this microorganism cannot be diagnosed in such condition .requirements of multiple biopsy (at least three) from several sites of the stomach , should be taken so as to increase the diagnostic yield, taking of antibiotic , ethical considerations, genetic characteristics, preclude performing endoscopy on suffering patients from gastritis and methodology. The results of rapid urease test (RUT) in this study were identical to those recorded by [27] 61.7%. On the other hand, the results were dissimilar to those found by [28] 51.4%. These differences in the findings of RUT may be due to many factors such as the kind of test, small size of biopsy, patchy distributed *H. pylori* in the stomach, presences of contaminant urea splitting bacteria, such as *Proteus sp* and *Klebsiella sp*. receiving of antimicrobial drugs, the activity of urease enzyme and rubbing the biopsy specimen very well over a dry glass slide of RUT. High percentages (68.60%) of *H. pylori* infections were detected by rapid urease test in all studied age categories and age groups did not show any significant effect on the rapid urease test for detecting *H. pylori*. Our findings were dissimilar to those of most studies who found that *H. pylori* positivity was becomes more with the increasing of age. [29] Detected that positive cases of this bacterium were increased by aging, revealed that the age has a significant influence on the prevalence of H. pylori and found a high percentage in the age group among the 25-34 years old, while a low percentage was recorded above 50 years old patients. The reasons of these differences may be due to several factors, such as the socio-economic level, the number of enrolled samples, seasonal diversity, Distribution of immunoreactive bands according to ethnic origin, individual susceptibility to infection, Epidemiology varies widely by geographical areas of the study, mode of transmission, educational & nutritional level and medical services.

H. pylorus was detected in 37.2% patients by the culture method and no any significant correlation was noticed between ages and culture method. The outcomes of current study were in contrast to the results found by other researchers who found impact of age on the result of culture. [30] Found effect of culture on variable range of age between <15-50> years old and the prevalence of H. pylori infection was increased with the age when considered the culture report. These dissimilarities between the present results and other results can be due to the same interpretation highlighted previously, like the socio-economic level of studied population, size of sampling, type of patients, location of the study, methodology and, the irregular distribution of the H. pylori in the gastric mucosa could influence on the results obtained. A high percentage (79.06%) of positivity was recorded

among females compared to 55.81% in males by the rapid urease test and a considerable correlation was registered between females and positive cases. The results were comparable to those found by [31] while different with the results of other researchers like [32] concluded that males to be more infected (68.5%%) than females In current study no considerable linkage was detected between gender and *H. pylori* infection by the culture method. The results were agreed to those found by [33] Detected females patients were more infected than males. The differences in the results can be attributed to the socio-economic status, place of living, hygiene, physiological and hormonal factors, number analyzed samples and methodology. Any considerable correlation was not detected between smoking status and *H. pylori* positivity by rapid urease test and culture method. The findings were different from those reported by [34] who found a significant correlation between smoking and *H. pylori* positivity via the Rapid urease test and culture method. The discrepancies of these results can be attributed to the small number of studied specimens as well as to individual variation.

6. CONCLUSION

1. The most superior test it was Rapid-urease test in comparison to Culture for detection this microaerobic organism

2. The study concluded that smoking and residence were not found related to this pathogenic bacteria.

3. It was observed the statistical correlation between gender and *Helicobacter pylorus* pathogenic bacteria by rapid urease test as it is females were more infected than males, but culture did not find such correlation

4. No obvious influence of age was registered on *Helicobacter pylorus* infection by these of two tests.

5. The detection of *H. pylori* in endoscopic biopsies by performs of both of culture and rapid urease test was not necessary in medical diagnosis as it did not improve the diagnostic rate over a combination of culture and rapid urease test as it was time consuming and expensive.

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