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Evaluation of Endoscopy Based *H. pylori* Diagnostic Techniques in Iraqi Patients with upper Gastrointestinal Disorders

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Abstract

Objectives: The aim is to detxermine the accuracy and degree of agreement of invasive methods. **Methods:** Gastroduodenal biopsies examined by Homemade Rapid Urease Test (HM-RUT), culture, Gram staining, Hematoxylin and Eosin staining (H&E) and *insitu* hybridization (ISH). **Results:** A total of (106) patients with mean age (44.70) years, male to female ratio was 1.25/1. *H. pylori* associated disorders diagnosed in (33-41), (69-77) and (24-32) years, mainly gastritis(37.7%), gastropathy (27.4%), gastric ulcer (15.1%), duodenal ulcer (12.3%), duodenitis (5.66%) and Pre pyloric ulcer (1.89%). Positivity of tests were HM-RUT (82.08%), HE (61.32%), Gram stain (60.4%), culture (21.7%) CagA-ISH (45.28%). Accuracy measures were HM-RUT, H&E staining, Gram staining, CagA-ISH and Culture respectively. Good agreement (82.08%) was recorded between H&E and Gram stain. Moderate agreement (79.25%, 78.30%) between HM-RUT, H&E and Gram stain respectively. Fair agreement (55.66%) between H&E and culture, Gram stain and culture, Gram stain and Cag A-ISH, Culture and Cag A-ISH (61.32%, 59.43%, and 68.87%) respectively. Poor agreement between HM-RUT - culture and CagA-ISH, H&E and Cag A-ISH (39.62%, 55.66% and 55.66%) respectively. **Conclusions:** HM-RUT was the most accurate and can be used as gold standard alternative for H&E and Gram stain for *H. pylori* diagnosis. Culture alone cannot be used as gold standard due to sensitivity, diagnostic accuracy, agreement with other tests was low. Cag A-ISH is not favora-ble for routine diagnosis but for tissue localization of *H. pylori* Cag A.

Keywords: *H.pylori*, Invasive Tests, Accuracy

1. Introduction

Helicobacter pylori is a gram-negative bacterium which has been involved in the pathogenesis of peptic ulcer, gastritis, low grade gastric mucosa-associated lymphoidt issue lymphomas and gastric carcinomas[1-3]. Numerous methods have been mentioned for the diagnosis of *H. pylori* and usually classified as invasive and noninvasive which have been continually developed and extended over time[4,5].The invasive tests including histology, urease tests and culture, require upper gastrointestinal endoscopy for obtaining the diagnostic sample. Non-invasive methods include the urea breath test, serology and stool antigen test[6]. Both invasive and noninvasive methods have both merits and demerits. A comparison with a gold standard test must be used to define the usefulness of each diagnostic test yet, according to accuracy and reliability, there has been no single beneficial test that can be used as a gold standard for*H. pylori*detection[7].

1.2 The Aims of this Study

This study aims to determine the accuracy of invasive methods (rapid urease test, home –made, HM-RUT, culture, Gram staining, Hematoxylin and Eosin (H&E) staining and CagA insitu hybridization-ISH) for *H. pylori* diagnosis in various upper gastrointestinal disorders. Determine a possible relationship between test sensitivity and specificity and type of digestive disorders; determine the degree of agreement between the different tests, finally according to accuracy and agreement this study try to suggest a set of invasive tests that can be used for routine diagnosis.

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2. Materials and Methods

2.1 Patients

In this cross sectional study, (106) patients, age range 15-80 years with clinical indications for upper gastrointestinal tract endoscopy during February 2013 to April 2014 were studied. This study was conducted according to the principles of Helsinki declaration. Dully filled consent form was obtained from all patients participating in the study before endoscopy in gastroenterology department of Baqubah teaching hospital in Diyala province-Iraq. Approval of ethical review Committee of College of medicine –Diyala University-Iraq, was taken prior to initiation of the work.

2.2 Exclusion Criteria

Patients were excluded in the following circumstances : having a history of previous gastric surgery, recent or active gastrointestinal bleeding, taking aspirin or non-steroidal anti-inflammatory drugs in the past 4 weeks, or are on proton pump inhibitors, patients with previous *H. pylori* eradication therapy, if the informed consent was not obtained.

2.3 Collection and Transportation of Specimens

Upper gastrointestinal endoscopy: After topical pharyngealanesthesia and midazolam sedation (3-5 mg intravenously) for overnight fasted Patients, Asterile flexible endoscope was introduced for full investigation of Stomach and duodenum. Six endoscopic biopsie sobtained from congested, inflamed orerosive lesions via sterile biopsy forceps. The samples for microbiological culture and staining procedures were retired from the biopsy forceps by using a sterile needle, and placed in an eppendorf tube containing 0.5 mL of sterile saline as a means of transportation[8]. Samples for rapid urease test placed in separate vial, previously identified, containing the appropriate medium for test.

2.4 Definition of *H. pylori* Status

True positive results considered if a combination of at least two invasive tests, three or more gives positive results for a single biopsy specimen. This combination of positive results for a single biopsy used as gold standard test for judgment of diagnostic accuracy. A negative *H. pylori* status was considered if all invasive tests performed gave concordant negative results or in case of only one invasive test gave positive results to prevent statistical bias[9].

2.5 Rapid Urease Test (RUT)

The RUT performed using homemade test (HM-RUT). This test was performed with a homemade solution with 1 ml distilled water, one drop of 1% phenol red(pH 6.5), and 100 mg urea, prepared just before endoscopy. One antral sample placed in the solution and tube then incubated at 37°C. The test was considered positive when the color changed from yellow, pink to red within 24 hours and time taken was noted and classified in to three grades: Grade 0 (no color change), 1 (624 hr.), 2 (16 hr.) and 3 (< 1 hr.)[10].

2.6 Gram Staining

Biopsy sample placed in sterile glass slide with a drop of normal saline and teased with sterile scalpel to make smaller fragments of tissue then another sterile glass slide placed over the teased first tissue and the tissue crushed between the two glasses then stain by Gram's staining. Existence of Gram negative spiral bacteria embedded in the tissue cells was diagnostic for *H. Pylori*[11]. *H. pylori* was designated as(1) negative Grade(0) (2) Grade(1) mild, more than 1-less than 50 *H. pylori/high power field* (HPF), Grade(2) moderate 50-100 *H. pylori*/ HPF or heavy Grade(3) > 100 *H. pylori*/ HPF[12].

2.7. Culture

In less than 4 hours after collection, samples transferred to the laboratory. A sterile surgical blade was used for mincing of tissue on a sterile glass slide then placed in a Brucella agar base supplemented with sheep blood 5% and 10 mg/l vancomycin, 5 mg/l trimethoprim,2 mg/l amphotericin B were added. Media were incubated in a moist microaerobic atmosphere (5% O2, 10% CO2 and 85% N2) using anaerobic jars and gas generating envelopes, anaerocult C® (Merck, Darmstadt, Germany), at 35oC.Cultures were monitored at 3, 5 and 7 days. Positive cultures were identified by-Colonial and Gram stain morphology, Positive catalase and oxidase tests and Strong urease activity[8].

2.8 Histopathological Evaluation

A biopsies were mixed in a 10%formaldehyde solution and were processed by the usual steps then proceeding to make serial sections of up to 5 µm, and stained with H&E. All specimens were evaluated by two pathologists unknowledgeable of the clinical features and the results of other tests. Discussion and consensus were used for any results difference. When *H. pylori* identified it was designated as(1) negative Grade(0) (2) Grade(1) mild, more than 1-less than 50 *H. pylori/high power field*(HPF), Grade(2) moderate50-100 *H. pylori/* HPF or heavy Grade(3) >100 *H. pylori/* HPF [12].

2.9 Insitu Bybridization (ISH)

H. pylori Cag A gene expression was detected by ISH procedure in 5µm thickness serial gastric mucosal sections fixed on positively charged slides using biotinylated long DNA probe for *H. pylori/* Cag A Gene, Cat. No.: IH-60061(HPY-6001-B) (Maxim biotech-USA) and the DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech-USA), according to Maxim biotech instruction manual[13]. The examination and scoring were done under light microscope by pathologists at powerX400 according to the scoring system[14].

3. Statistical Analysis

Demography and cross tabulation were calculated by Statistical analysis using SPSS for windows TM version 17.0.Sensitivity,specificity, positive, negative predictive values, the positive and negative likelihood ratios, the accuracy and the corresponding 95% confidence intervals for all tests were calculated using MedCalc statistical software, Version 13.1.1, Belgium. The concordance of the RUT, HE, Gram stain, culture, and ISH was studied using the Cohen's kappa index of agreement. The level of confidence limits was 0.095 and Here is one possible interpretation of Kappa value[15].

- Poor agreement = Kappa value Less than 0.20 (b)Fair agreement = Kappa value 0.20 to 0.40
- Moderate agreement = Kappa value 0.40 to 0.60 (d) Good agreement = Kappa value 0.60 to 0.80
- Very good agreement = Kappa value 0.80 to 1.00

4. Results

Demography of (106) patients shown in Table 1. Mean age (44.70) years, male / female ratio was 1.25/1.The majority of patients age groups (33-41), (69-77) and (24-32)years. Frequency of Gastritis (37.7%), gastropathy (27.4%), gastric ulcer (GU) (15.1%), Duodenal Ulcer (DU) (12.3%), duodenitis (5.66%) and Pre pyloric ulcer(PPU) (1.89%). Using HM-RUT, *H. pylori* was diagnosed within (16 hr.) in (32.1%), hour in (29.2%), within 6-24hr in (20.8%) of cases (Table2). In HM-RUT positive cases, Gastritis represent (29.25%), gastropathy (20.75%), GU (13.21%) and (11.32% DU, Duodenitis and PPU cases were at the bottom (Table 3).

Table 1. Demographic characters of patients

	Parameter	
Age (years)	Minimum	15
	Maximum	80
	Mean± Std. Deviation	44.70±18.26
age group(years)	*H.pylori* positive	*H.pylori* negative
15-23	3(2.83%)	5(4.72%)
24-32	16(15.09%)	7(6.60%)
33-41	24(22.64%)	6(5.66%)
42-50	8(7.55%)	2(1.89%)
51-59	7(6.60%)	3(2.83%)
60-68	4(3.77%)	1(0.94%)
69-77	17(16.038%)	2(1.89%)
78-86	3(2.83%)	0(0%)
Total	**80 (75.47%)**	**26(24.53%)**
Gender	male	59(55.7%)
	female	47(44.3%)
	Male/female ratio	1.25/1
Endoscopic diagnosis	No. (%)	
gastric ulcer	16(15.1%)	
Duodenal ulcer	13(12.3%)	
gastropathy	29(27.4)	
gastritis	40(37.7%)	
Duodenitis	6(5.7%)	
Prepyloric ulcer	2(1.9%)	

H. pylori was detected via H&E stain in (61.32%). *H. pylori* heavy colonization was detected in (22.64%), moderate (23.58%) and (15.09%) mild colonization (Table 2 and Figure 3). H&E positive *H. pylori* Gastritis

Table 2. Description of *H. pylori* diagnostic tests used in present study

Diagnostic test	Score		No.%	
HM-RUT	negative		19 (17.92%)	
	Positive	Grade 1 (624 hr.)	22(20.8%)	87(82.08%)
		Grade 2 (16 hr.)	34(32.1%)	
		Grade 3 (< 1 hr.)	31(29.2%)	
Hematoxylin–Eosin stain	negative		41(38.7%)	
	positive	Mild	16(15.09%)	65(61.32%)
		moderate	25(23.58%)	
		heavy	24(22.64%)	
Culture on Selective medium	negative		83 (78.3%)	
	positive		23 (21.7%)	
Gram stain	negative		42(39.6%)	
	positive	Mild	16(15.09%)	64 (60.4%)
		moderate	25(23.58%)	
		heavy	23(21.70%)	
Cag A- ISH	negative		58(54.7%)	
	Positive	low	18(17%)	45.28%
		intermediate	11(10.4%)	
		High	19(17.9%)	

Table 3. Description of *H. pylori* Diagnostic tests according to Endoscopic Diagnosis

Diagnostic test		Endoscopic diagnosis						Total
		gastric ulcer	Duodenal ulcer	Gastropathy	gastritis	Duodenitis	Prepyloric ulcer	
HM-RUT	negative	2(1.89%)	1(0.94%)	7(6.60%)	9(8.49%)	0(0%)	0(0%)	19 (17.92%)
	positive	14(13.21%)	12(11.32%)	22(20.75%)	31(29.25%)	6(5.66%)	2(1.89%)	87 (82.08%)
Total		16(15.09%)	13(12.26%)	29(27.36%)	40(37.74%)	6(5.66%)	2(1.89%)	106(100%)
H&E	negative	6(5.66%)	4(3.77%)	12(11.32%)	19(17.92%)	0(0%)	0(0%)	41(38.68%)
	positive	10(9.43%)	9(8.49%)	17(16.04%)	21(19.81%)	6(5.66%)	2(1.89%)	65(61.32%)
Total		16(15.09%)	13(12.26%)	29(27.36%)	40(37.74%)	6(5.66%)	2(1.89%)	106(100%)
culture	negative	9(8.49%)	9(8.49%)	25(23.58%)	33(31.13%)	6(5.66%)	1(0.94%)	83(78.30%)
	positive	7(6.60%)	4(3.77%)	4(3.77%)	7(6.60%)	0(0%)	1(0.94%)	23(21.70%)
Total		16(15.09%)	13(12.26%)	29(27.36%)	40(37.74%)	6(5.66%)	2(1.89%)	106(100%)
Gram stain	negative	8(7.55%)	3(2.83%)	11(10.378%)	19(17.92%)	1(0.94%)	0(0%)	42(39.62%)
	positive	8(7.55%)	10(9.43%)	18(16.98%)	21(19.81%)	5(4.77%)	2(1.89%)	64(60.38%)
Total		16(15.09%)	13(12.26%)	29(27.36%)	40(37.74%)	6(5.66%)	2(1.89%)	106(100%)
Ca-gA-ISH	negative	1(0.94%)	6(5.66%)	16(15.09%)	31(29.25%)	4(3.77%)	0(0%)	58(54.72%)
	positive	15(14.15%)	7(6.60%)	13(12.26%)	9(8.49%)	2(1.89%)	2(1.89%)	48(45.28%)
Total		16(15.09%)	13(12.26%)	29(27.36%)	40(37.74%)	6(5.66%)	2(1.89%)	106(100%)

and gastropathy were (19.81%) and (16.04%) respectively, GU (9.43%) and DU (8.49%), while Duodenitis and PPU cases were at the bottom (Table 3). Using culture on selective media, *H. pylori* was detected in (21.7%) of total cases (Table 2), in which gastritis and GU represent (6.60%),Gastropathy and DU detected in (3.77%) cases while PPU detected in one case only (Table 3). *H. pylori* was detected by Gram stain in (60.4%) of cases, a heavy colonization detected in (21.70%), (23.58%) for moderate and (15.09%) for mild colonization (Table 2 and Figure 2).Gram stain positive *H. pylori* gastritis and gastropathy detected in (19.81%) and (16.98%) respectively (Table 3). GU with positive Gram stain represents (7.55%) and (9.43%) for DU. Duodenitis and PPU cases with positive Gram stain were at the bottom. Using ISH, Cag A positive *H. pylori* was detected in (45.28%) of cases in which a high positive score detected in (17.9%), intermediate positive score in (10.4%) and low positive score in (17%) (Table

2 and Figure 1). Cag A positive GU and gastropathy detected in (14.15%) and (12.26%) respectively. Gastritis and DU come in the second line (8.49%) and (6.60%) respectively, while Cag A positive duodenitis and PPU cases were at the bottom (Table 3).

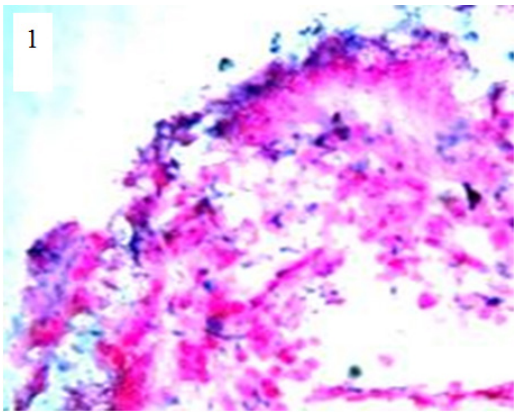

Figure 1. H.pylori CagA positive insitu hybridization in gastric tissue section stained by BCIP / NBT (bluish purple) counter stained by nuclear fast red. Bar size 50µm.

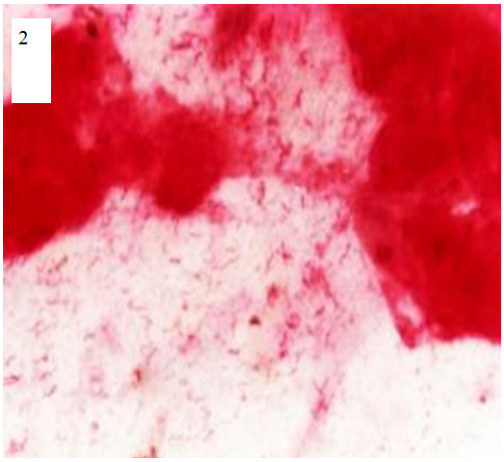

Figure 2. Gram staining of antral biopsy from patient with gastritis. Notice a numerous H. pylori. Bar size 50µm.

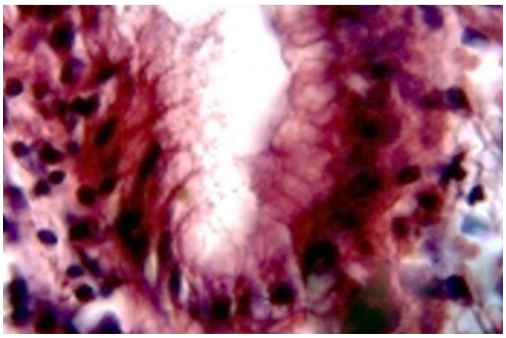

Figure 3. Antral mucosal section stained with H&E. Notice a numerous H. pylori. Bar size 50µm.

H. pylori prevalence at (95%CI) was (75.47%) in selected patients. Sensitivity, specificity, and other diagnostic accuracy measures of all invasive tests in (106) patients presented in Table 5. HM-RUT comes in the top ranking of diagnostic tests according to the outstanding results followed by H&E staining,Gram staining at third level and CagA –ISH comes in the fourth level. Diagnosis of *H. pylori* by culture on selective media comes in the bottom of the list in its diagnostic accuracy (Table 4).

As shown in Table 5, HM-RUT, H&E and Gram stain give interesting results regarding reliabilty. In HM-RUT when compared with gold standard tests, Kappa = 0.804 (p <.0.001), 95% CI (86.40 - 97.08%) indicate perfect agreement (overall agreement93.40%). In H&E, The Kappa = 0.680 (p <.0.001), 95% CI (77.42 - 91.6%) which indicate good agreement with overall agreement (85.85%).In case of Gram stain, The Kappa = 0.662 (p <.0.001), 95% CI (76.34 – 90.86%) indicate good or Substantial agreement (overall agreement 84.91%). Culture method give poor agreement with other gold standard tests, Kappa = 0.165 (p <.0.005), 95% CI (36.59 – 56.14%),with overall agreement (46.23%). CagA-ISH give also poor agreement with other gold standard tests, Kappa = 0.280 (p <.0.001), 95% CI (35.92 – 59.08%), overall agreement (62.26%).

The reliability for HE compared with HM-RUT as a gold standard test, Kappa = 0.514 (p <.0.001), 95% CI (70.05-86.27%) indicate moderate agreement, overall agreement (79.25%). In case of Gram stain, The Kappa = 0.499 (p<0.001), 95% CI (69.03-85.48%) indicate moderate agreement between HM-RUT and Gram stain, overall agreement (78.30%). In culture compared with HM-RUT, Kappa = 0.114 (p <.0.05), 95% CI (30.39-49.61%) indicate poor agreement, overall agreement (39.62%). The reliability for the CagA-ISH compared with HM-RUT, Kappa = 0.164 (p <.0.05), 95% CI (45.71-65.20%) indicate poor agreement, overall agreement (55.66%).

The reliability for the Culture compared with H&E as a gold standard test, Kappa = 0.231 (p <.0.005), 95% CI (46.64-66.09%) indicate Fair agreement, with overall agreement (55.66%). The reliability for the Gram stain compared with H&E, Kappa = 0.624 (p <.0.001), 95% CI (73.17-88.60%) indicate good agreement, overall agreement (82.08%). The reliability for the CagA-ISH compared with H&E, Kappa = 0.132 (p <.0.05), 95% CI (45.71-65.20%) indicate poor agreement, overall agreement (55.66%).The reliability for Gram stain

Table 4. Comparison of *H. pylori* invasive diagnostic tests by gold standard

Diagnostic method			Gold standard		Sensitivity (95%CI)	Specificity (95%CI)	PLR (95%CI)	NLR (95%CI)	PPV (95%CI)	NPV (95%CI)	Diagnostic Accuracy (95%CI)	Disease prevalence (95% CI)
			-ve	+ve								
HM-RUT	-ve		19	0	100% (95.45 to 100%)	73.08% (52.21 to 88.38%)	3.71 (1.97 to 7%)	0.00	91.95% (84.12% to 96.69%)	100% (82.20 to 100%)	93.40%	75.47% (66.16 - 83.31%)
	+ve	Grade1	7	15								
		Grade 2	0	34								
		Grade 3	0	31								
		Total	26	80								
H&E	-ve		26	15	81.25% (70.96 to 89.10%)	100% (86.65% to 100%)	Infinity* (NaN to infinity)	0.19 (0.12 to 0.30)	100% (94.43% to 100%)	63.41% (46.94% -77.87%)	85.85%	
	+ve	Mild	0	16								
		moderate	0	25								
		heavy	0	24								
		Total	26	80								
Culture		-ve	26	57	28.75% (19.18 to 39.96%)	100% (86.65 to 100%)	Infinity* (NaN to infinity)	0.71 (0.62 to 0.8%)	100% (85.05% to 100%)	31.33% (21.59% to 42.44%)	46.23%	
		+ve	0	23								
		Total	26	80								
Gram stain	-ve		26	16	80% (69.56 to 88.11%)	100% (86.65 to 100%)	Infinity* (NaN to infinity)	0.20 (0.13 to 0.31)	100% (94.34 to 100.00%)	61.90% (45.64% to 76.42%)	84.91%	
	+ve	Mild	0	16								
		moderate	0	25								
		heavy	0	23								
		Total	26	80								
ISH	-ve		22	36	55% (43.47 to 66.15%)	84.62% (65.11 to 95.55%)	3.58 (1.42 to 9)	0.53 (0.40 to 0.7)	91.67% (80% to 97.63%)	37.93% (25.52 to 51.63%)	62.26%	
	+ve	low	4	14								
		intermediate	0	11								
		high	0	19								
		Total	26	80								

* (The entry 'NaN' in any of the above cells means that the calculation cannot be performed because the values entered includes one or more instances of zero. PLR: Positive Likelihood Ratio; NLR: Negative Likelihood Ratio; PPV: Positive Predictive Value; NPV: Negative Predictive Value)

Table 5. Inter-tests agreement and Kappa statistics for all staining techniques utilized current study

Parameter	Measure of Agreement Kappa value	Asymptotic Std. Error (a)	Approximate T value (b)	Approximate Significant	Overall Agreement (%)	Overall Disagreement (%)	95% confidence interval of observed value
Gold standard							
HM-RUT	0.804	0.070	8.440	0.000	93.40%	6.6%	86.40 - 97.08%
H&E	0.680	0.073	7.390	0.000	85.85%	14.15%	77.42 - 91.6%
Culture	0.165	0.041	3.090	0.002	46.23%	53.77%	36.59 – 56.14%
Gram stain	0.662	0.073	7.245	0.000	84.91%	15.09%	76.34 – 90.86%
Cag A -ISH	0.280	0.074	3.525	0.000	62.26%	37.74%	35.92 – 59.08%
HM-RUT							
H&E	0.514	0.081	6.058	0.000	79.25%	20.75%	70.05-86.27%
Culture	0.114	0.032	2.533	0.011	39.62%	60.38%	30.39-49.61%
Gram stain	0.499	0.080	5.939	0.000	78.30 %	21.7%	69.03-85.48%
CagA-ISH	0.164	0.067	2.342	0.019	55.66%	44.34%	45.71-65.20%
H&E							
Culture	0.231	0.062	3.337	0.001	56.60 %	43.4%	46.64-66.09%
Gram stain	0.624	0.078	6.424	0.000	82.08%	17.92%	73.17-88.60%
Cag A -ISH	0.132	0.091	1.429	0.153	55.66%	44.34%	45.71-65.20%
Culture							
Gram stain	0.308	0.061	4.390	0.000	61.32%	38.68%	51.33-70.48%
Cag A -ISH	0.342	0.082	4.064	0.000	68.87%	31.13%	59.03-77.31%
Gram stain							
Cag A -ISH	0.260	0.089	2.800	0.005	59.43%	40.57%	52.28-71.34%

a. Not assuming the null hypothesis. b. Using the asymptotic standard error assuming the null hypothesis.

compared with Culture as a gold standard test, Kappa = 0.308 (p <.0.001), 95% CI (51.33-70.48%) indicate fair agreement, overall agreement (61.32%).The reliability for the CagA-ISH compared with Culture as a gold standard test, Kappa = 0.342 (p <.0.001), 95% CI (59.03-77.31%) indicate fair agreement, overall agreement (68.87%).The reliability for the CagA-ISH compared with Gram stain as a gold standard test, Kappa = 0.260 (p <0.01), 95% CI (52.28-71.34%) indicate fair agreement, overall agreement (59.43%) (Table5).

5. Discussion

Three peaks of age groups infected with H. pylori determined,(33-41),(69-77) and (24-32)years with mean age of 44.7 years which come in agreements with other studies[1,16]. Age distribution of H. pylori infection did not show any trend towards increase or decrease in infection. The maximum H. pylori positivity (16.03%)in (69-77) years, this can be attributed to much less number of individuals under investigation. The prevalence of *H. pylori* infection was higher in male than in female that come in concordance of other studies[17,18].

The majority of cases have gastritis (37.7%) followed by gastric or DU, gastropathy (27.4%) and PPU (1.9%) and this agree with reports from India[19] and Brazil[20]. *H. pylori* associated gastritis, gastropathy, GU, DU; duodenitis and PPU were ranked according to diagnostic techniques which revealed that (82.08%) were positive by HM-RUT, (61.32%) by H&E, (60.4%) by Gram stain imprint, (45.28%) by Cag A- ISH and (21.7%) by culture. Similar ranking was reported in previous Iraqi study[21]. In the present study *H. pylori* positive gastritis detected in (29.25%) compared with reports from Bangladesh in which ranking of cases approximately similar in DU (34.7%), gastritis (33.7%) while GU represent only (10.8%)[22]. In contrast to present study, in Bangladesh the RUT was positive in (56.4%)of all disorders and H&E positive in (45.6%)[22]. in Indian study the endoscopy results come closely to the results of present study, gastritis accounted for (30%) in which RUT was positive in (31.82%) and Gram stain was positive in (38.89%), GU detected in (17%) of cases in which RUT was positive in (13.64%) and Gram stain was positive in (5.56%), DU accounted for (36%)in which RUT was positive in (45.45%) and Gram stain(44.44%), some limited disparity noticed and this may attributed to the small sample size under investigation in Indian study [19]. A Turkish study

come in line with current results stated that *H. pylori* detected in (64.4%) by H&E in dyspeptic patients, in contrast culture was positive in (42.4%), and ISH was positive in (61.4%)[23].

The results of this present study agree with Indian study[24], stated that age (15-90) years having mainly gastritis (49.77%) and in less extent duodenitis (5.5%) with *H. pylori* positive results in Gram stain, RUT and culture. Low number of positive cases by culture on selective media reported in present study (21.7%) with overall accuracy (46.23%), sensitivity (28.75%) and specificity(100%),supported by Brazilian and Iranian studies,. In this study the less number of culture positivity (21.7%) might be due to the fact that distribution of *H. pylori* in stomach may be patchy i.e. the tissue sample size in square millimeters is so small from 800 sq. cm and the nature of *H. pylori* colonization may be patchy beside the grade of *H. pylori* colonization in gastric tissue, which may leads to catch a biopsy that don't contain it, or may be due to effect of biopsy transportation and biopsy Processing on *H. pylori* viability or some patients took anti *H. pylori* drugs for a time prior endoscopy and do not clarify it during consent. The relatively short incubation time in this study (3-7)days might be other possible factor[25].

A great disparity was reported in the prevalence of *H. pylori* around the world. In this study the prevalence of *H. pylori* was (75.47%) with wide range of confidence interval (66.16 to 83.31%) which give a real evidence about the establishment of this pathogen among patients referred to gastroenterology unite in Baqubah teaching hospital and in Diyala province local community in general. In Iran the prevalence of *H. pylori* in patients attended to GIT center was (50.5%)[26] While in Brazil (33.3%)[27].These disparity in *H. pylori* prevalence in a centers based clinical studies reflected several possible things, general health surveillance by heath authorities, community have good Healthy habits that limits the spread of *H. pylori*, the type of diagnostic tests that used for judgments on truly infected cases which may be omit the actual numbers for infected individuals in general population.

In present study HM-RUT give (100%) sensitivity, (73.08%) specificity and (93.40%) diagnostic accuracy. The sensitivity of HM-RUT is concordance with others[22,25,26,28]. The specificity, NPV,PPV of RUT is rather the same as those reported by other workers, though we did not get any false positive result by RUT in contrast to other studies[28,29]. About one third of infected cases in present study give positive HM-RUT results during a time range from ≥1 hour to 6 hours that come closely to other studies[30,31.] The possible causes of moderate to good specificity of HM-RUT in present study may be due to the our criteria for true infected cases and more than one diagnostic method used as reference standard, that omit any patient with single positive test such as HM-RUT and negative by others, also the presence of blood may also adversely affect the performance of RUT leading to a false negative result. This is due to the buffering effect of serum albumin on the pH indicator, rather than by a direct inhibition of the urease activity. In present study (20.8%) of HM – RUT positive cases reaction delay for more than 6 hrs. to 24 hrs. that come in concordance with that recorded by[31,32].Theoretically the possible causes for differences or even delay in reaction time between studies might be due to difference in the prevalence of infection in local community under investigation that lead logically to variability in bacterial load in the biopsies which play vital role in the RUT sensitivity is affected by the amount of bacteria in the biopsy; at least 10000 cells are required for a positive result[4]. Other factors such as contamination of biopsy with other bacteria from the mouth or in case of excessive salivation of patients or patients have reflux alkaline bile into the stomach that could cause a weak Positive reaction because the liquid may contaminate a small gastric biopsy specimen such that the resulting surface pH is >6.0[33].

H&E sensitivity (81.25%), (100%) specificity and Diagnostic accuracy (85.85%) which closely to other reports[28], and come in contrast with other[26]. In Iranian study H&E give sensitivity (95.6%),specificity(77.8%) and Diagnostic accuracy (86.8%) which close to what this study reported[28]. In Bangladesh H&E sensitivity (77.6%) and specificity (97.7%)[22].In Taiwan study H&E give sensitivity, specificity and Diagnostic accuracy (95.12%)[34]. The difference in sensitivity might be associated with criteria of judgment on true positive cases and the gold standard test which is PCR test as in Iranian study beside the fact that if patients under investigation took antibiotic whether for treatment of *H. pylori* or any other infection results in the transformation of *H. pylori* bacillary form to a coccoid form that is the morphological manifestation of bacterial cell death without an infective capacity and this might be the cause false negative results with low sensitivity and specificity of histological examination that depends on the pathologist experience. Other factors

associated with low sensitivity such as sampling errors, insufficient bacterial load, bacterial clearance, and patchy bacterial distribution are common causes of false negative results[34].

Fluorescent In Situ Hybridization (FISH) uses a set of fluorescent protein-labeled oligonucleotide probes which target specific genes for detection of *H. pylori* specific virulence factors on tissue sections from infected subjects. It takes about 3 hours to perform this assay which adds value to the diagnosis of *H. pylori*. ISH has been used to detect the precise location of the bacteria in the gastric mucosa. Cag A- ISH was detected in (45.28%) of cases, sensitivity (55%,95%CI=43.47to 66.15%) and Specificity (84.62% ,95%CI =65.11 to 95.55%) and Diagnostic accuracy (62.26%). There was no previous study used CagA –ISH for H.pylori diagnosis compared with other methods, all previous studies used 16S rRNA or 23S rRNA probe FISH to detect *H. pylori*. Low sensitivity in present study reflect the fact that the used probe customized for detection of CagA gene in gastric biopsy, which present globally in 60-70% of *H. pylori* strains beside exclusion of four single positive Cag A- ISH cases according to study exclusion criteria. Other possible factors responsible for low sensitivity and specificity of CagA –ISH such as probe degradation by proteases and nucleases present in the sample, poor permeability of the microbial cell wall for the probes, and low accessibility of the probe to the target region of the rRNA due to the ribosomal secondary structure[35].

The present study reported a moderate agreement (79.25%) between H&E and HM-RUT, poor agreement(39.62%) between culture and HM-RUT which disagree with other study stated a good agreement between the two tests[36]. In contrast to present study, Brazilian study[35], recorded best agreement between histology and RUT (91.7%), culture and histology (78.3%), culture and RUT (75%), Overall accuracy of histology (97.5%), RUT (94.2%) and culture (80.8%). The differences in accuracy measures related to choose of gold standard in which is missed in Brazilian study. The present study reported a good agreement between Gram stain and HE, (82.08%), moderate agreement (78.30%) between Gram stain and HM-RUT which come in concordance with other studies[2,47]. Fair agreement (61.32%) between Gram stain and Culture that come in contrast with[7], they reported a 90% agreement with accuracy (80%) for gram stain and (78%) for culture.

This study conclude that HM-RUT was the most accurate test that can be used as gold standard for comparison with others. According to the results of sensitivity, specificity, diagnostic accuracy and agreement test, HM-RUT can be used as alternative for H&E histopathology and Gram stain only for causes other than *H. pylori*. That saves resources for governments and time for patients and physicians. Culture method alone cannot be used as gold standard and not favorable for *H. pylori* diagnosis because of low sensitivity, low diagnostic accuracy and low agreement with other diagnostic tests unless for anti-microbial sensitivity. As Cag A-ISH give low sensitivity, moderate diagnostic accuracy and poor to fair agreement with other invasive diagnostic test so, it is not favorable for routine *H. pylori* diagnosis but for tissue localization of *H. pylori* Cag A+.

6. References

1. Al-Ezzy AIA. Molecular and Immunopathological Role of Nuclear Factor K B Detected By Insitu Hybridization In Pathogenesis of Chronic Atrophic Gastritis in Iraqi Patients. International Journal of Advanced Research. 2014; 2(1):67-78.
2. Al-Ezzy AIA. Molecular and Immunopathological role of Gastric versus lymphocytes Interleukin 8 Gene Expression in H.pylori induced Fas-Fasl apoptotic pathway in Gastroduodenal Ulcer in Iraqi patients. Journal of Biology, Agriculture and Healthcare. 2015; 5(5):141-53.
3. AL-Ezzy AIA. Evaluation of Clinicopathological and Risk Factors for Nonmalignant H. Pylori Associated Gastroduodenal Disorders in Iraqi Patients. OA Maced J. Med. Sci. 2015; 3(4):655-60.
4. Garza-Gonzalez E, Perez-Perez, Ignacio G, Maldonado-Garza, Jess H, Bosques-Padilla, et al. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World Journal of Gastroenterology. 2014; 20(6): 1438–49.
5. Hussen BM, Qader SS, Ahmed HF, Ahmed SH. The Prevalence of Helicobacter pylori among University Students in Iraq. Indian Journal of Science and Technology. 2013; 6(8): 5019-23.
6. Megraud F, Bessed E, Lehours P. Diagnosis of Helicobacter pylori infection. La Revue du Praticien. 2014; 64(2):201-6.
7. Ruparelia JR, Sodagar NR, Patel JS, Pandya HB, Singh NR, Naik KB, et al. Comparison of Conventional Diagnostic Modalities with PCR for Detection of Helicobacter Pylori Infection in Symptomatic Patients. Asian J. Med. Pharm. Res. 2013; 3(4):105-10.
8. Islam MSU, Nahar S, Sarker MN, Salimullah A, Rahman MA, Ahmed DS, et al. Efficacy of Different Laboratory Tests to Diagnose Helicobacter pylori Infection. Faridpur Medical College Journal. 2013; 8(1):11-14.
9. Tzeng J-E, Lin Y-L, Chung S-M, Chu Y-T. Comparison of

four diagnostic methods for Helicobacter pylori. Tzu. Chi. Med. J. 2005; 17(5):339-43.
10. Seo J-H, Park JS, Yeom JS, Lim J-Y, Park C-H, Woo H-O, et al. Correlation between Positive Rate and Number of Biopsy Samples on Urease Test in Childhood Helicobacter pylori Infection. Journal of Korean Medical Science. 2014; 29(1):106-9.
11. Subbukesavaraja V, Balan K. Comparative study of invasive methods for diagnosis of Helicobacter pylori in humans. Int. J. Curr. Microbiol. App. Sci. 2013; 2(7):63-68.
12. Sujatha R. Comparative study of helicobacter pylori infection with invasive (culture, histopathology, rapid urease test) and non invasive (serology) techniques. 2007.
13. Biotech M. Institute hybridization detection system San Francisco, California: http:// www.maximbiotech.com [cited 2014 20 October].
14. Intisar SP, Azad MB. Identification of helicobacter pylori in gastric biopsies of Patients with chronic gastritis: histopathological and Immunohistochemical study. Duhok Medical Journal. 2011; 5(1).
15. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam. Med. 2005; 37(5):360-63.
16. Chen H-L, Chen M-J, Shih S-C, Wang H-Y, Lin I, Bair M-J. The socioeconomic status, personal habits, and prevalence of Helicobacter pylori infection in inhabitants of Lanyu. Journal of the Formosan Medical Association. 2014; (113):278-82.
17. Li M, Lu B, Chu L, Zhou H, Chen M-Y. Prevalence and characteristics of dyspepsia among college students in Zhejiang Province. World Journal of Gastroenterology. 2014; 20(13): 3649–54.
18. AL-Ezzy AIA. Immunopathological Role of FAS-FASL Apoptotic Pathway in H. pylori CagA Positive Associated Chronic Atrophic Gastritis in Iraqi Patients. Journal of Biology, Agriculture and Healthcare. 2014; 4(23):67-74.
19. Subbukesavaraja V, Balan K. Comparative study of invasive methods for diagnosis of Helicobacter pylori in humans. Int. J. Curr. Microbiol. App. Sci. 2013; 2(7):63-68.
20. Faintuch JJ, Silva FM, Navarro-Rodriguez TS, Barbuti RC, Hashimoto CL, Rossini AR, et al. Endoscopic findings in uninvestigated dyspepsia. BMC Gastroenterology. 2014; 14(1):19p.
21. Baqir HI, Al-Aoubaidi MH, Fakri S, Al-Mousili MM, S. H. evaluation of serological test for diagnosis of H.pylori infection IJGE. 2003; 1(4):41-7.
22. Akanda M, Rahman AN. Comparative Study of Different Methods for Detection of Helicobacter Pylori in Gastric Biopsies. Dinajpur Med. Col. J. 2011 Jan; 4(1): 1-6.
23. Orhan Cem Aktepe, İhsan Hakkı Çiftçi, Birol Şafak, İhsan Uslan, Dilek FH. Five methods for detection of Helicobacter pylori in the Turkish population. World J. Gastroenterol. 2011; 17(47):5172-76.
24. Pandya HB, Patel JS, Agravat HH, Patel SB, Thakkar MC. Identification of Helicobacter pylori by different conventional staining techniques and its comparison with polymerase chain reaction. Saudi Medical Journal. 2013; 34(9):942-48.
25. Jemilohun A, Otegbayo J, Ola S, Oluwasola A, Akere A. Diagnostic accuracy of rapid urease test for the diagnosis of Helicobacter pylori gastic biopsies in Nigerian with dyspepsia. African Journal of Clinical and Experimental Microbiology. 2011; 12(2):62-66.
26. Khalifehgholi M, Shamsipour F, Ajhdarkosh H, Daryani N, Pourmand M, Hosseini M, et al. Comparison of five diagnostic methods for Helicobacter pylori. Iranian Journal of Microbiology. 2013; 5(4):396-401.
27. Ramis IB, Moraes EPd, Fernandes MrS, Mendoza-Sassi R, Rodrigues O, Juliano CRV, et al. Evaluation of diagnostic methods for the detection of Helicobacter pylori in gastric biopsy specimens of dyspeptic patients. Brazilian Journal of Microbiology. 2012; 43(3):903-08.
28. Choi J, Kim CH, Kim D, Chung SJ, Song JH, Kang JM, et al. Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, 13C urea breath test, and serology. Journal of Gastroenterology and Hepatology. 2011; 26(6):1053-59.
29. Al-Ali J, Al-Asfar F, Dhar R, Dhar P, Kapila K. Diagnostic performance of gastric imprint smear for determination of Helicobacter pylori infection. Canadian Journal of Gastroenterology. 2010; 24(10):603-06.
30. Dahorea C, Coman G, Ailiesei O. Diagnostic value of microscopic exam and urease test in helicobacter species infections. Analele Stiintifice ale Universitatii Alexandru Ioan Cuza" din Iasi Sec II a Genetica si Biologie Moleculara. 2004; 5(1):6p.
31. Andriani Koumi, Theodoros Filippidis, Vassilia Leontara, Loukia Makri, Panos MZ. Detection of Helicobacter pylori: A faster urease test can save resources. World J. Gastroenterol 2011; 17(3): 349-53.
32. Goh KL CP, Navaratnam P, Chin SC, Xiao SD. HUITAI rapid urease test: a new ultra-rapid biopsy urease test for the diagnosis of Helicobacter pylori infection. J. Dig. Dis. 2007; (8):139-42.
33. Va n Keeken N vHE, de Boer WA. Validation of a new, commercially available dry rapid urease test for the diagnosis of Helicobacter pylori infection in gastric biopsies. Neth. J. Med. 2006; (64):329-33.
34. Lee H-C, Huang T-C, Lin C-L, Chen K-Y, Wang C-K, Wu D-C. Performance of Routine Helicobacter pylori Invasive Tests in Patients with Dyspepsia. Gastroenterology Research and Practice. 2013, p. 5. http://dx.doi.org/10.1155/2013/184806.
35. Cerqueira L, Fernandes RM, Ferreira RM, Oleastro M, Carneiro F, Brando C, et al. Validation of a fluorescence in situ hybridization method using peptide nucleic acid probes for detection of Helicobacter pylori clarithromycin resistance in gastric biopsy specimens. Journal of Clinical Microbiology. 2013; 51(6):1887-93.
36. Reden S, Petersson F, Trnkrantz E, Levander H, Mrdh E, Borch K. Reliability of diagnostic tests for Helicobacter pylori infection. Gastroenterology Research and Practice. 2011, p. 6. http://dx.doi.org/10.1155/2011/940650.