Original Article

Comparison of Different Diagnostic Methods of Helicobacter Pylori Infection in Children.

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Abstract

Background: *Helicobacter Pylori Infection (HPI) is usually acquired during childhood, without specific treatment this infection rarely resolves spontaneously. Therefore, correct diagnosis is necessary for the effective eradication and there is no single test can be regarded as the gold standard for the identification of H. pylori.*

Objective: *To compare the sensitivity, specificity, and accuracy of the stool antigen test, serologic test, rapid urease test, and histology for the diagnosis of H. pylori infection.*

Materials and Methods: *This cross-sectional descriptive study was conducted at the Department of Pediatric Gastroenterology & Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from April 2019 to October 2021 on 54 children aged less than 18 years suspected to be H. pylori infection, who attended the outpatient as well as admitted. In a typical data sheet, a complete clinical history, exam results, and investigation reports were documented. Results were expressed as sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy.*

Results: *The gastro-duodenal biopsy and Rapid Urease Test (RUT) (100%) have the highest sensitivity, followed by serology (95%), and Saturation (SAT) (70%). Highest specificity was found in gastro-duodenal biopsy 88.2% followed by RUT 67.6% & SAT 41.2% and only 32.4% by serology. The gastro-duodenal biopsy had the highest diagnosis accuracy (92.6%), followed by RUT (79.6%), serology (55.6%) and SAT (51.9%).*

Conclusion: *In the study, the gastro-duodenal biopsy and rapid urease test both showed high sensitivity, specificity, and negative predictive value; as a result, the combination of these tests constituted the best alternative for making the diagnosis. Because of their high sensitivity, serological tests may be useful non-invasive screening methods.*

Keywords: *H. pylori infection, Children, Diagnostic methods, Abdominal pain.*

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Introduction

Helicobacter pylori is a spiral shaped gram-negative micro-aerophilic bacterium which colonizes the human gastric mucosa due to its special affinity to gastric mucosa.[1] Helicobacter pylori was discovered in 1983, and in 2005 the Nobel Prize for Medicine was awarded to an observant pathologist, Robin Warren, and an enterprising physician, Barry Marshall from Australia for the discovery of the organism and its role in peptic ulcer diseases and gastritis.[2]

The prevalence of H. pylori infection varies widely across both geographic regions and ethnic groups & it depends on socioeconomic status and overcrowding.[3] In developing countries more than 50 % of children are infected by the age of 10 years with prevalence of infection rising to more than 80 % in young adults.[4] H. pylori infection may be acquired at any age but usually most infection acquired in early childhood and persists throughout life & natural eradication of H. pylori is rare,

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infection is sustained a lifetime unless appropriate treatment for infection is applied.[5,6] H. pylori infection is clinically important because it is regarded as the major cause of gastro-intestinal diseases, including gastritis, gastric or duodenal ulcer, dyspepsia, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer throughout the patient's lifetime. Additionally, H. pylori infection is also associated with extra-intestinal problems such as subnormal growth, malnutrition, iron deficiency anemia and thrombocytopenic purpura in children.[7,8]

H. pylori infection can be detected by invasive (rapid urease test [RUT], histology, and bacterial culture [BC] from biopsy specimen) and non-invasive (stool antigen test [SAT], urea breath test [UBT], and serology) methods. Particularly in regions with low H. pylori prevalence in industrialized countries, serological testing is less accurate than UBT and SAT and is unable to distinguish between past and present infection.9 Serology is useful for initial screening & only tests which are not likely to give false negative results in patients receiving treatment (antibiotics, bismuth compounds or PPI) in the recent past or presenting acute bleeding.[10,11]

Stool antigen assay is a relatively new diagnostic procedure that uses an ELISA/ ICT to detect the presence of H. pylori antigen in the stool & because of bacterial antigen detection it can be used as a reliable means of diagnosing active & on-going infection and also useful for follow-up purposes.[12] SAT have the advantage of indicating current, on-going infection, but both are affected by several parameters, such as colonization density, nutrition, and co-medication.[13]

Invasive tests have been considerate the gold standard and most specific, but in biopsy-based methods there may be sampling error or may give false results because of the patchy nature of the infection[4], low concentration of bacteria in fragments, topographical changes in the stomach, low sensitivity for culture and it requires experienced pathologists and good quality biopsy specimens.[14] Histologic evaluation has traditionally been the gold-standard method & it allows for definitive diagnosis of infection, as well as of the degree of inflammation or metaplasia.[15] Measuring urease production from biopsy specimen can be accomplished by rapid urease test (RUT). This test has the advantages of being inexpensive, simple, and provides more rapid results and does not need highly experienced staff than either histology or culture.[16]

All the current diagnostic procedures have their own advantages, disadvantages and limitations. According to joint ESPGHAN/NASPGHAN guidelines, 2016 it has become clear that no single procedure is optimal.[17] Previous research revealed that children in Bangladesh have a high frequency of H. pylori infection. While many studies have been done on Bangladesh's adult population for diagnostic evaluation, there have been relatively few studies done on children. So the aim of this study is to compare between different H. pylori infection diagnostic methods that is histology, RUT, serology and stool antigen test.

Materials and Methods

This Cross sectional descriptive study was conducted in the department of Pediatric Gastroenterology and Nutrition with the collaboration of department of pathology, BSMMU, Dhaka, Bangladesh from April 2019 to October 2021. A total of 54 children who required endoscopy for suspected H. pylori infection (abdominal pain, recurrent vomiting, dyspepsia, poor growth, and unexplained anemia) were studied. Children who had previously been infected with H. pylori, who had taken antibiotics, proton pump inhibitors (PPI), or NSAIDs at least two weeks prior to the endoscopy, and who had a history of concurrent illness (such as inflammatory bowel disease, eosinophilic gastritis/enteropathy, or coagulopathy) were all excluded from the study. 5 ml venous blood was collected from the patient in the sample collection corner by a lab technician and transferred to the laboratory within 4 hrs. Anti-H.pylori IgG antibody titer was measured by chemiluminescent immunoassay system using IMMULITE 2000 XPi in the Department of Microbiology, BSMMU. The result was positive if titer > 20 U/ml, negative if < 20 U/ml.

Stool antigen test was done by rapid chromatographic immunoassay/ICT by using the H. pylori antigen Rapid Test Cassette kit (contains monoclonal anti- H. pylori antibodies coated particles and monoclonal anti-H. pylori antibodies coated on the membrane). Endoscopy was performed in department of Pediatric Gastroenterology & Nutrition and biopsies were taken from gastric antral mucosa. During the procedure, endoscopic findings (esophagitis, gastric erythema, gastric ulcer, antral nodularity, duodenal ulcer, pigmentation, mucosal friability, polyps, active bleeding etc.) were recorded. One antral biopsy specimens was taken for histopathology examination & one specimen for rapid urease test. The biopsy specimens were fixed in 10% formalin and sample were sent to the Department of Pathology, BSMMU. The specimens were stained with hematoxylin & eosin, and Giemsa stain. After that histology slides were evaluated by experienced pathologists blinded to the results of the other tests. The RUT was performed using a non-commercial validated test with a laboratory made RUT media supplied by the Department of Microbiology, BSMMU using 95 ml Urea agar base & 5 ml of 40% Urea solution. The test was considered positive when the color changed from clear to red, pink or purple color within 24 hours. Histologic presence of the H. pylori bacteria concordance with rapid urease test positive were considered positive for H. pylori infection.[13]

The detailed clinical history, physical examination findings and investigation reports were recorded in a pre-designed standard data sheet by the researcher himself. After collection of data, they were manually checked and analyzed by using Statistical Package for Social Science (SPSS 25.0 Chicago, Illinois) for Windows XP. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of anti H. pylori IgG, stool antigen test, endoscopic biopsy & histopathology, and rapid urease test were calculated for the diagnosis of H. pylori infection.

Results

The study was carried out at the department of Pediatric Gastroenterology and Nutrition and clinical characteristics, anthropometry, complete blood count, anti H. pylori IgG, stool antigen test, endoscopic findings, histopathology findings and rapid urease test of 54 suspected H. pylori infected child were included and studied.

Among the studied sample, 20 (37%) children were diagnosed as H. pylori gastritis and 34 (63%) as non-H. pylori gastritis (Figure-1).

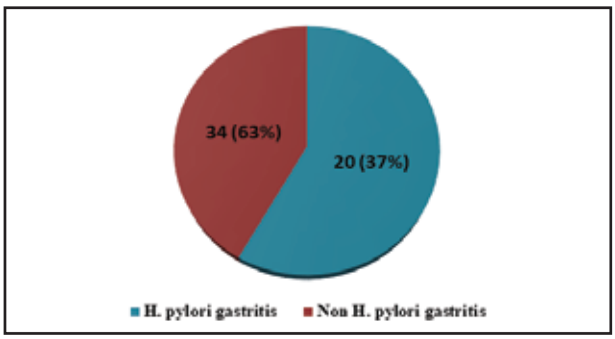

Figure 1: Distribution of studied sample by diagnosis (N=54)

Among the studied sample, the age ranged from 5-15 years with a mean age 10.6 ± 2.6 years. The highest frequency of studied participants was seen in >10 year age group (28, 51.9%), followed by between 6-10 years group (24, 44.4%) and only 2 (3.7%) were below 5 years of age (Figure-2).

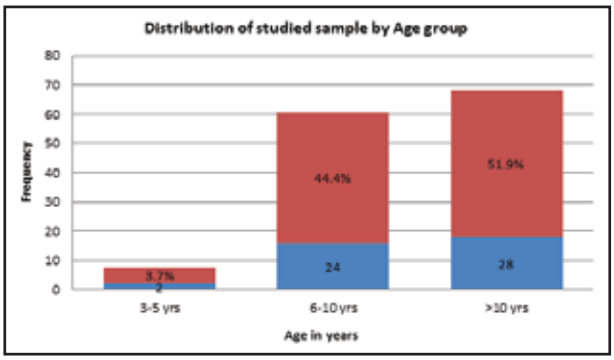

Figure-2: Distribution of studied sample by age group (N=54)

Among the 54 studied sample highest frequency was observed 12 (60%) in between 6-10 years and 20 (58.8%) in >10 years age group in H. pylori and non-H pylori gastritis group respectively and statistical significance between these two group was non-significant (p-0.176). In case of gender distribution in H pylori group male child were 12 (60%) slightly exceeds their female counterpart (8, 40%) and in non H pylori also group male children (18, 52.9%) were slightly exceeds than female child (16, 47.1%). Regarding socio-demographic status, highest participants belongs to middle socioeconomic background that is 15 (75%) & 29 (85.3%) and also 13 (65%) came from urban & 21 (61.8%) were from rural area in respective of H. pylori and non-H. pylori gastritis group. But, statistically significant difference was not found in between this two group in terms of sex distribution (p=0.778), residence (p=1.000) and socioeconomic condition (p=0.651) (Table-I)

Table-I: Comparison of age, sex, residence and socioeconomic status of studied children between H. pylori and non- H. pylori gastritis group (N=54)

	H. pylori gastritis (n=20)		Non H. pylori gastritis (n=34)		p value	
	n	%	n	%		
Age in group						
< 5 year	0	0	2	5.9		
6 - 10 year	12	60	12	35.3	a0.176	ns
> 10 year	8	40	20	58.8		
Gender						
Male	12	60	18	52.9	b0.778	ns
Female	8	40	16	47.1		
Residence						
Urban	7	35	13	38.2	b1.000	ns
Rural	13	65	21	61.8		
Socioeconomic condition						
High class	3	15	3	8.8		
Middle class	15	75	29	85.3	a0.651	ns
Lower class	2	10	2	5.9		

Table II showed that among the studied sample (N=54) mean age was 10.4 ± 2.9 years and it was statistically insignificantly lower in H. pylori gastritis group (10.2 ± 2.1) than in non-H. Pylori group (10.9 ± 2.9) (p=0.38). All the studied participants had history of abdominal pain and the median duration of pain was 3 months (IQR 2-5.3) and median duration between two groups 4 (3-6) and 3 (2-4.3) were statistically insignificant (p=0.06).

Table II: Comparison of age and duration of abdominal pain of H. pylori and non-H. pylori gastritis group (N=54)

	Total (N=54)	**H. pylori gastritis (n=20)**	**Non H. pylori gastritis (n=34)**	**p value**
	mean ± SD	**mean ± SD**	**mean ± SD**	
Age	10.4 ± 2.9	10.2 ± 2.1	10.9 ± 2.9	a0.38 ns
	median (IQR)	**median (IQR)**	**median (IQR)**	
Median duration of pain (months)	3 (2 - 5.3)	4 (3 - 6)	3 (2 - 4.3)	b0.06 ns

All the studied participants undergone endoscopic procedure and macroscopic findings were noted. Among them 7 (35%) & 29 (85.3%) were normal in H. pylori and non-H. pylori gastritis groups. Other important endoscopic findings were antral nodularity (6, 30%) followed by gastric erythema, duodenal nodularity, submucosal Hg (3, 15%), prolapse gastropathy, gastric erosion & duodenal erosion (1, 5%) in H. pylori group. Most of the macroscopic abnormalities were in H. pylori group and among them antral nodularity & submucosal Hg were statistically significant and p-value was 0.008 & 0.046 respectively. Regarding histopathology findings chronic gastritis were found 16 (80%) & 29 (85.3%) in H. pylori and non-H. pylori gastritis groups respectively. Other histological diagnosis was chronic active gastritis 6 (30%) & 4 (11.8%) followed by chronic duodenitis 2 (10%) & 2 (5.9%) in H. pylori and non-H. pylori gastritis groups respectively and 1 (5%) patient was diagnosed as chronic gastritis with intestinal metaplasia in H. pylori group. These histological findings were statistically non-significant between two groups (Table-III).

Table-III: Comparison of endoscopic findings of H. pylori and non-H. pylori gastritis group both macroscopic and histopathology findings (N=54)

	H. pylori gastritis (n=20)		**Non H. pylori gastritis (n=34)**		**p value**
	n	**%**	**n**	**%**	
Macroscopic					
Normal	7	35	29	85.3	a0.000 s
Gastric erythema	3	15	2	5.9	a0.347 ns
Gastric erosion	1	5	2	5.9	a1.000 ns
Antral nodularity	6	30	1	2.9	a0.008 s
Duodenal nodularity	3	15	1	2.9	a0.138 ns
Submucosal Hemorrhage	3	15	0	0	a0.046 s
Prolapse gastropathy	1	5	1	2.9	a1.000 ns
Duodenal erosion	1	5	0	0	a1.000 ns
Histopathology					
Chronic gastritis	11	55	29	85.3	a0.712 ns
Chronic active gastritis	6	30	4	11.8	a0.147 ns
Chronic gastritis with intestinal metaplasia	1	5	0	0	a1.000 ns
Chronic duodenitis	2	10	1	2 .9	a0.622 ns

Chronic gastritis: Infiltration of gastric mucosa with chronic inflammatory cells; Chronic active gastritis: Infiltration of gastric mucosa with both active and chronic inflammatory cells.

Applying ROC curves, the sensitivity and specificity of anti H. pylori IgG antibody were studied and the cut off values with best sensitivity and specificity were determined in figure 4. The area under curve was 0.645 (95% CI: 0.497-0.792) with P-value-0.078. Anti H. pylori IgG antibody could diagnose H. pylori gastritis at a cut off value of 22 with 95% sensitivity and 67.6% specificity (Figure-3).

Table-IV shows sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of macroscopic findings of endoscopy, gastro-duodenal biopsy, Rapid urease test (RUT), Serology (anti H. pylori IgG antibody) and stool antigen test (SAT). For calculation of sensitivity and specificity histological presence of H. pylori by Geimsa stain concordance with rapid urease test positive is considered gold standard as per operational definition. Only 13 (65%) and 5 (14.7%) participants had macroscopic endoscopy findings in H. pylori and non-H. pylori gastritis groups respectively and the sensitivity is around 35% but specificity, PPV, NPV & diagnostic accuracy was very low. Sensitivity was found very high 100% in gastro-duodenal biopsy & RUT followed by serology 95%, and SAT 70%. Highest specificity was found in gastro-duodenal biopsy 88.2% followed by RUT 67.6% & SAT 41.2% and only 32.4% by serology. Gastro-duodenal biopsy had high PPV 83.3% followed by RUT (64.5%) and RUT & Gastro-duodenal biopsy had high NPV 100% followed by serology 91.7%. Gastro-duodenal biopsy had high diagnostic accuracy of 92.6% followed by RUT, serology and SAT 79.6%, 55.6% & 51.9% respectively.

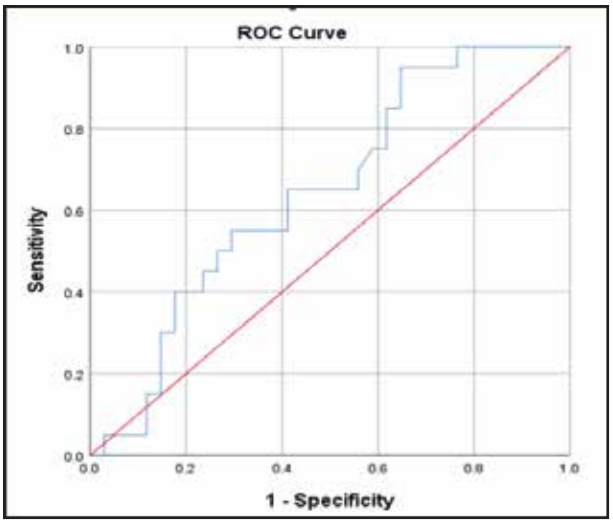

Figure-3: ROC curve of anti H. pylori IgG antibody for diagnosing H. pylori gastritis

Table IV: Comparison of different methods for diagnosis of H. pylori infections.

Methods	H. pylori infection		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
	Positive n=20	Negative n=34					
Endoscopy							
Normal	7	29	35	14.7	19.4	27.8	22.2
Abnormal	13	5					
⁎Gastro - duodenal biopsy							
Positive	20	4	100	88.2	83.3	100	92.6
Negative	0	30					
RUT							
Positive	20	11	100	67.6	64.5	100	79.6
Negative	0	23					
Serology							
Positive	19	23	95	32.4	45.2	91.7	55.6
Negative	1	11					
SAT							
Positive	14	20	70	41.2	41.2	70	51.9
Negative	6	14					

⁎Gastro-duodenal biopsy =Histological presence of H. pylori by Geimsa stain

Discussion

In this study, among 54 participants (20, 37%) children were diagnosed as H. pylori gastritis slightly less (49.8%) than study conducted among 303 children by Hasosah et.al[13] & quite similar to a previous study by Shady et. al.[18] Among the diagnosed H. pylori infected group, male children (60%) slightly surpassed the female children (40%) in this study which is contradiction to previous study by Hasosah et al. that showed 54.6% females were affected with H. pylori infection.[13] In a large meta-analysis, de Martel and Parsonnet observed that male gender was predominant in a group with H. pylori and they hypothesized that differences in exposure between genders might explain these differences in results. By contrast, this study detected no gender differences in the prevalence of H. pylori infection.[19]

In a previous study conducted on hundred sample by Shady et al.[18] found mean age at presentation was 7.23 ± 1.94 yrs, but in this study it was 10.4 ± 2.9 yrs. The age group between 6-10 years had a higher likelihood of H. pylori infection, 60% than > 10 years group but no one in below 5 years age group had H. pylori infection in this study. Several previous studies supported that with increasing age chance of H. pylori infection is more[13,20] because childhood is known to be a time of high risk for H. pylori acquisition due to more chance of environmental exposure like school going, more contact with other children, consumption of contaminated food & water etc.[20]

The risk factors predisposing to H. pylori infection in children include residence in a developing country, inadequate sanitation practices, poor socio-economic conditions, overcrowding or high density living conditions, ethnic and genetic predisposition.[3] Study participants of the current study showed that 65% were came from rural area and most of them 75% belonged to middle socioeconomic background family in H. pylori infected children. The association between H. pylori infection and poor deprived area was found in 34% compared to children from more affluent areas (16%) by Malcolm et al.[21] Another study by Chen et al. observed that prevalence of H. pylori infection was strongly associated with poor socioeconomic condition.3 Despite most of the study participants belong to middle socioeconomic background but no statistically significant association was found between residence (p-1.000) and socioeconomic condition (p-0.651) with H. pylori infection in this study. The vast majority of studies reported a fair agreement between the endoscopic and histological diagnosis of gastritis, mainly in children and support the routine collection of biopsy samples during endoscopy in children.[22] This study reported that almost entire (85.3%) H. pylori negative patients had normal endoscopic findings and only 2.9% child had antral nodularity, duodenal nodularity, prolapse gastropathy whereas 35% H. pylori positive patient had normal endoscopic findings. A study for Iranian children found 64.9% H. pylori negative & 22.2% H. pylori positive patient had normal endoscopy.[23] The other endoscopic findings in this study were gastric erosion (5%), submucosal hemorrhage (15%), and gastric erythema (15%) among H. pylori positive children but in H. pylori negative children 5.9% patient had gastric erythema and erosion. Aantral nodularity (30%) is the most common endoscopic findings in this study in H pylori positive child but none of H.pylori negative child had it, which is similar to the study by Luzza et al (2001), nodularity was not seen in any H. pylori negative patient.[24] On the other hand, several studies showed that antral nodularity was the most common symptom 40%, 47.4 % by Motamed et al.[23, 24] So, these findings can conclude that presence of nodularity in endoscopy has a strong association with H. pylori infection in children. Regarding histopathology findings none of H. pylori positive child had normal gastric pathology, among them 55 % had chronic gastritis, 30% had chronic active gastritis, 10% had chronic duodenitis & only one child (5%) had chronic gastritis with intestinal metaplasia in this study. However, the study by Cardenas-Mondragon et al (2013) had similar findings and showed that none of the H. pylori positive patients had normal gastric pathology findings, 49.9% of the total had gastritis.[25]

In this study microscopic evidence of H. pylori was found in 36.1% and the sensitivity, specificity, PPV, NPV & diagnostic accuracy was 100%, 88.2%, 83.3%, 100% & 92.6% respectively. Due to the H. pylori distribution being patchy, the quantity and dispersion of the biopsy materials taken will determine how well the H. pylori histological diagnosis is made. The low sensitivity and accuracy of this may be due to the single biopsy taken only from the antrum of the stomach. Kocsmár et al (2017) found H. pylori infection by Giemsa stain had lower sensitivity (83.3%) than IHC (98.8%) & the sensitivity markedly dropped to 33.6% in patients without active inflammation.[26] RUT is a rapid, simple and cheap but invasive method for H. pylori diagnosis. In addition the accuracy of this method can be affected by biopsy location, bacterial load, and recent use of antibiotics.[7] Hasosah et al, 2019 have found sensitivity and specificity of RUT were 92.6% and 100% and current study showed 100% sensitivity, 67.6% specificity & 64.5% PPV and diagnostic accuracy was 79.6%.[13]

Non-invasive serological H. pylori testing has been widely used for epidemiological studies, useful for detecting past or present infection and has advantages of not giving false negative results in patients receiving PPI, antibiotic and presenting acute bleeding.[12] This study showed relatively high sensitivity 95% and low specificity of 32.4% that differ from previous study done by Hasosah (2019) showed sensitivity & specificity of 50.9% & 77.9% respectively.[13] But the diagnostic accuracy was found to be 55.6% which is different to 73.6% reported by Khalifehgholi et al[14]. A sensitivity and specificity of 96.5% and 93% were detected using cut off level of IgG 10U/ml with AUC of 0.953 at the ROC curve reported by Shady et al.[18] But, using a cut off level 22 U/ml this study presented sensitivity of 95% and specificity of 67.6% with AUC of 0.645 with significance of 0.078 at the ROC curve. Many studies have claimed that the stool antigen test is beneficial for the primary diagnosis and post-treatment follow-up of H. pylori infection despite the variation in reported sensitivity and specificity rates. Most of them have acceptable results.[14] This study of monoclonal SAT had sensitivity of 70% and low specificity of 41.2%. Several previous studies showed same sensitivity like 69% by Hasosah et al (2019), and 73.9% by Khalifehgholi et al (2013) but they had found comparatively better specificity 73% and 86.7% respectively.[13,14]

The accuracy of the tests for diagnosing H. pylori in children can be ranked in the following order based on the findings of this study: Gastro-duodenal biopsy > RUT > serology > stool antigen test.

Conclusion

Gastro-duodenal biopsy and rapid urease test shown high sensitivity, specificity, and negative predictive value in the current investigation. Due to its excellent diagnostic accuracy, specificity, and sensitivity, the results of this study revealed that the combination of a gastro-duodenal biopsy and a fast urease test represented the best option for confirming the diagnosis among invasive procedures. Due to its high sensitivity, serological testing may be a significant non-invasive screening method.

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