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Study the Prevalence of *Helicobacter Pylori* among Children in Wasit Province

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Annotation: The bacterium H. pylori is commonly found in the stomachs of humans and other primates. It is a gram-negative, spiral-shaped bacterium that thrives in low-oxygen conditions that causes systemic infections in stomach. Given this fact, One hundred thirty four samples were collected from children (6-12 years old) suffering from weakness, emaciation, digestive disorders, stomach and colon problems, frequent vomiting, abdominal pain. The sample were collected by the specialists of the Department of Internal Medicine, the Section of Gastrointestinal from Disease and the outpatient center at Al-Karama Teaching Hospital, Al-zahraa Teaching Hospital during the period from 3rd March 2024 to 3rd November 2024. The current study showed, the Prevalence of H. pylori was 106 /134 (79.1 %) their age 6 to 12 years. Regarding of Diagnosis H. pylori the study indicated that 76.1% (102 out of 134) of the specimens tested positive for the Assure H. pylori Ab Rapid Test , The result of the stool antigen test (SAT) yielded positive results in 98 out of 134 specimens, indicating a positivity rate of (83.1%),while, The result of Urea Breath Test revealed that from 102

tested specimens (86.4%) were positive . and The results of PCR technique indicated that 88% (118 out of 134) of the specimens were positive to this test. Regarding the hematological parameters of the H. pylori test among participants who had positive test compared to participants who had negative test compared. The mean levels of hemoglobin (10.4±3.8vs. 13.1±2.5 g/dL), red blood cell count (RBC 3.9*109±0.9 vs.4.4*109±0.9/μL), white blood cell count (WBC 11.2*109±1.9/L vs.6.7*109±1.8/L) and hematocrit (Hct 34.1±6.2 vs. 39.4±6.0 %) were significantly lower among participant has positive test compared to participant has negative. The result this study showed many factors that increase the risk of H. pylori infection in children such as place of residence, source of drinking water, consumption of fast food and chips, regular hand washing before meals, tooth decay. the study Concluded there are high Prevalence of Helicobacter Pylori among children in in Wasit province.

Introduction

Helicobacter pylori, previously referred to as Campylobacter pylori, is a type of bacteria that is grame-negative, microaerophilic, and has a helical shape (1). It is commonly found in the stomachs of approximately half of the global population. H. pylori is transmitted from person to person and is typically contracted during early childhood. Those who are infected may develop chronic gastritis, which often remains symptomless in 85 percent of cases. However, in some instances, it can lead to various conditions, such as peptic ulcers or gastric adenocarcinoma. Sadly, gastric adenocarcinoma claims the lives of over 800,000 individuals globally annually(2) . H. pylori is widely recognized as the primary culprit behind stomach ulcers, duodenal ulcers, gastritis, and various forms of gastric cancer, including adenocarcinoma and mucosa-associated lymphoid tissue carcinoma (MALT) (3). Various factors, such as cigarette smoking, diets, and alcohol consumption, have been identified in epidemiological studies as having an impact on the risk of acquiring peptic ulcer disease and gastric cancer (4). This section will explore certain factors that increase the risk of H. pylori infection and its potential to cause cancer in infected individuals. Researchers have made numerous attempts to accurately detect H. pylori infection. These methods include invasive tests like culture and histology, which involve endoscopic surgery and biopsy specimens. PCR is another method that can

be both invasive and non-invasive, depending on the type of specimen. On the other hand, non-invasive tests like UBT, SAT, and serology tests have also been used (5). The primary rationale for opting for noninvasive tests is to circumvent the need for endoscopy. Numerous guidelines have advocated for noninvasive tests as the preferred initial option (6,7).

1. Materials and Methods

Sample collection

One hundred thirty four Samples were collected from children (6-12 years old) suffering from weakness, emaciation, digestive disorders, stomach and colon problems, frequent vomiting, abdominal pain. The sample were collected by the Specialists of the Department of Internal Medicine, the Section of Gastrointestinal from Disease and the outpatient center at Al-Karama Teaching Hospital, Al-zahraa Teaching Hospital during the period from 3rd March 2024 to 3rd November 2024.and from patients in out patenting unit encompassing both male and female patients.

1- Five ml of vein blood from the arm of the patients was collected and placed in an EDTA tubes and a gel tube. 2- Stool specimens were collected from the same patient and placed in a stool cap. 3- Saliva specimens were collected from the same patients using a cup.

$$\frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Using the level of significance of 5% (p=0.05), the Z1-α/2 value of A standard ordinary variate was 1.96, and with a level of significance of 1% (p=0.01), it was 2.58. The algorithm uses a p-value of 1.96 because, as is customary in research, p-values below 0.05 are assumed to be statistically significant. In this equation, p stood for the predicted population proportion according to earlier studies (7)

Diagnosis of H. pylori

1. Serum Antibody Test

utilizing a 5 mL syringe. The blood was carefully transferred into a gel tube, and placed at room temperature to naturally clot. The serum was carefully separated from the material through a centrifugation process at a speed of 5000 xg for a duration of 5 to 10 minutes. Following the completion of the necessary steps, the sample has been adequately prepared and is now in a suitable state for testing. It is recommended to open the inner package when you are prepared to utilize its contents (9).

2. Urea Breath Test (UBT)

Urea Breath Test (UBT) The patients willingly consumed a test capsule containing urea labelled with radioactive carbon-14 and water. This was potentially done either before having a meal or a couple of hours after eating and before receiving any medication. YHO4E was employed for detecting H. pylori (14C) labeled urea. In the event of an H. pylori infection, it is possible for the urea in the test to undergo a breakdown process, "leading to the generation of isotope-labelled carbon dioxide (10). The instrument detects the carbon dioxide that is exhaled by the patient The procedure was conducted following the guidelines provided by the manufacturer, Headway Company in Chin.

3. Stool Antigen Test

Stool Antigen Test for Helicobacter Pylori Detection Stool samples were taken from patients using a stool cup and were promptly examined. To collect a stool specimen from solid samples, unscrew the cap of the specimen tube. Then, carefully insert the specimen rod into the stool at three different locations, ensuring that approximately 50 mg of stool is collected. When handling fluid specimens,

hold the dropper vertically and extract two drops of stool specimens. Transfer these drops into the sample collection tube that contains the extraction solution to secure the cap onto the specimen's collection tube, and vigorously shake the tube to thoroughly blend the sample with the buffer solution contained within, the tube remained undisturbed for a period of two minutes (11).

4. DNA Extraction

A commercial kit (PrestoTM Mini gDNA Bacterial Kit, Geneaid, Thailand) was used to extract deoxyribonucleic acid (DNA) for use in polymerase chain reaction (PCR) experiments. We followed the manufacturer's instructions to extract DNA of the *Staphylococcus aureus* isolates. The electrophoresis tank was prepared for electrophoresis on agarose gel by adding 1x Tris-borate-EDT (TBE) buffer. The agarose tray was then submerged in the tank. The buffer was made to sit a few millilitres above the surface of the agarose. The tank was filled and sealed after 5µl of specimen and 2µl dye fluorescence were added to each well. A gel run under electrophoresis gradient of 70 volts/cm was used for the experiment. The agarose was removed off the tank and shown using gel paper.

Primers were optimised by mixing 2.5µl of master mix along with 5-6µl DNA molecules and 1µl of forward along with revers primers. Primers from different gene grades were selected, and the PCR annealing temperatures have been set at 55°C, 58°C, and 52°C, respectively, for the *16S rRNA* gen. Following the manufacturer's recommendations, a mixture of 12.5 ml master mix, 5-6 ml DNA, 1 ml reverse and forward primers, and 20 ml of nuclease-free deionised water was used to detect 16S rRNA gene. In order to identify the target genes, we recorded the PCR cycle program parameters (Tables. 1). (12).

Complete Blood Count (CBC)

Complete Blood Count (CBC) A complete blood count (CBC) is a laboratory test that analyses the components of blood. samples were collected from patients using a syringe with a capacity of 3-5 mL and then transferred into an EDTA tube containing exactly 2 mL, the samples were mixed thoroughly (13).

Statistical Analysis

The data was examined by means of SPSS 20. To examine the data, a chi-square test was employed. The significance level was deemed to be $P<0.001$.

Table (1) .the sequence and source of the gene primers used in the study

Target gene	Primer pair (5′-3′)	Size(bp)	References
16S rRNA	F::CTGGAGAGACTAAGCCCTCC R: AGGATCAAGGTTTAAGGATT	446	(**Mnena**, et al., 2017)

Result and Discussion

Prevalence of *Helicobacter Pylori* among children

Recently, *Helicobacter Pylori* considered a worldwide public health and a social problem in many countries, The cases of *H.pylori* infection showed a significant increase in children in the last years, so this study was conducted on 134 children patients with gastrointestinal disease symptoms after being diagnostic clinical by physician, the Prevalence was 106 /134 (79.1 %) their age 6 to 12 years (Table . 2).

Table (2). Prevalence of *Helicobacter Pylori* among children

Children age	Number	Percentage
6-8	38	35.8%
8-10	42	39.6%
10-12	26	24.5%

Total	106	100%
X2	47.7*	
P value	<0.01	

* Highly significant difference (P<0.01)

The current study's findings were consistent with a community-based investigation that found an infection rate of 40.3% in Iraq's Sulaimani province (14). The seropositivity rate among the recruited subjects in another study conducted in the Iraqi province of Duhok was 50%, which is slightly less than the rate identified in our investigation (15). The sample size, age groups, and diagnostic techniques used in different research can all affect the rates of H. pylori infection.

Diagnosis By Serum Antibody Test

result of this study indicated that 76.1% (102 out of 134) of the specimens tested positive for the Assure H. pylori Ab Rapid Test , the result were in agree with the result in Iraq by (16), the serum antibody test accurately diagnosed 81.95% of infected patients, which closely aligns with the findings of the present study.(17) conducted a study that reported a positive serological test result in 55.6% of patient specimens. These findings differed from the results of the current study. The discrepancy in findings may be attributed to interindividual variability in immune responses (18). The serum antibody test is a straightforward and effortless diagnostic procedure. Furthermore, this test is a rapid and qualitative method used to detect H. pylori antibodies in the blood serum of patients. The test can be utilised in both laboratory settings and field surveys, eliminating the need for equipment and devices (19).

Diagnosis Using Stool Antigen Test

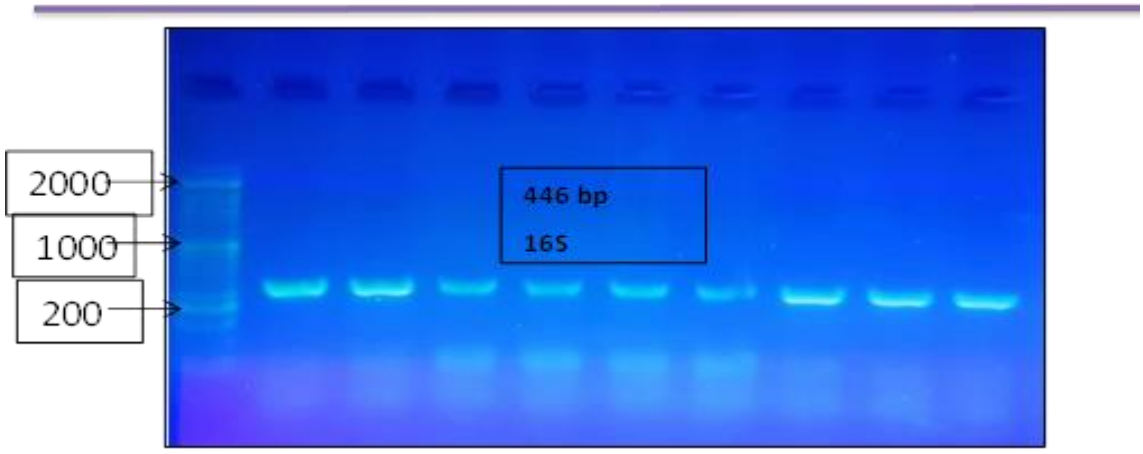
The result of this test in indicated that the stool antigen test (SAT) yielded positive results in 98 out of 134 specimens, indicating a positivity rate of (83.1%) A study carried out in Iraq by (20), the stool antigen test diagnosed positive results for 52.5% of infected patients, which is similar to the findings of the current study. In a study carried out by the investigator (21) found that 33.3% of participant's diagnosis with H. pylori through a stool antigen test..

Diagnosis Using Urea Breath Test

(Phenotypic Diagnosis) The Worldwide Consensus Report states that UBT and SAT research are recommended as the primary diagnostic methods due to their high specificity and sensitivity, both exceeding 90%. The patients ingested a test capsule containing radioactive carbon 14-labeled urea and water at a Private Medical Lab. Urea breath samples from 134 patients were analyzed using the HUBT-20P Helicobacter pylori detector, which measures the activity of the urease enzyme. The result of UBT revealed that from 102 tested specimens (86.4%) were possitive.

Detection by 16S rRNA Gene.

The 16S rRNA gene employed as a detected gene to verify the presence of *H. pylor*.i. The results of this study indicated that 88% (118 out of 134) of the specimens that tested by pcr technique to detect H.pylori were positive to this test, as shown in (figure 1).



(Figure .1) 1.5% Agarose gel for NOS3 PCR amplification 16S rRNA of H.polyri

to indicte if there are any indel (insertion, deletion) nucleotide or any changes in 16S rRNA gene, the peripheral genomic DNA amplified with specific primers targeting 16S rRNA gene, the PCR product showed difference in nine cases from all positive patients at line 446 bp, compared to leader line range from 100 to 2000 bp. This result indicates that 16S rRNA can affect the progression and the severity of the disease by changing the expression level which means that (22.5%) have sever H. Pylori as it showing in (figure 1). A comparative analysis of three different approaches for detecting H.pylori revealed that microbiome diagnostics utilizing next-generation sequencing (NGS) with 16S rRNA sequencing exhibited the highest level of sensitivity in identifying H. pylori. Based on the comparative analysis of NGS-PCR-histopathology, the findings indicate that 16S rRNA sequencing demonstrated a higher detection rate of H. pylori infections in patients, surpassing the efficacy of other examined methodologies by 17.5%. Furthermore, a strong correlation was observed between 16S rRNA sequencing and the evaluation of polymorph activity using the Sydney system scoring. In a particular instance. (22), the presence of H. pylori was identified using NextGeneration Sequencing (NGS) and histological analysis, but Polymerase Chain Reaction (PCR) provided a negative outcome. In a singular instance, the utilization of histology- and PCR-based testing produced affirmative outcomes, however the application of NGS-based 16S rRNA sequencing failed to identify the presence of H. pylori. The utilization of Giemsa staining for microscopic examination of histological samples is seen to possess lower sensitivity compared to other methods. In this study, it was found that Giemsa staining failed to detect the presence of H. pylori in 12 out of 20 patients who were previously classified as H. pylori-positive using 16S rRNA sequencing (23).

Hematological Parameters of H. Pylori

As showen in (table 3), the hematological parameters of the H. pylori test among participants who had positive test compared to participants who had negative test compared. The mean levels of hemoglobin (10.4±3.8vs. 13.1±2.5 g/dL), red blood cell count (RBC 3.9*109±0.9 vs.4.4*109±0.9/µL), white blood cell count (WBC 11.2*109±1.9/L vs.6.7*109±1.8/L) and hematocrit (Hct 34.1±6.2 vs. 39.4±6.0 %) were significantly lower among participant has positive test compared to participant has negative H. pylori test at p-value < 0.0013). However, no significant variations were observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count when comparing cases and controls for p-values > 0.05. this result of this study is agree with (24) that showed there is a significant decrease in hematological parameters of the H. pylori comparative with health people .

Table .3. Hematological parameters of the H. pylori

N	Hematological Parameter	*H. Pylori*		t-Value	p-Value	N-Value
		Positive(n-118)	Negative(n-37)			
1	Hemoglobin(Hb)(g/dL)	10.4±3.8	13.1±2.5	−4.659	0.003	12-18 g/dL
2	RBC ($\times 10^{12}$/L)	3.9±0.9	4.4±0.9	−6.352	0.005	4.5-6.0 cell/ L
3	WBC ($\times 10^{9}$/L)	11.2±1.9	6.7±1.8	−2.061	0.045	5000-10,000 cell/L
4	Hematocrit (Hct) (%)	34.1±6.2	39.4±6.0	−6.259	0.001	38-48 %
5	MCHC (g/dL)	33.5±2.0	34.035±1.35	−2.670	0.009	25.8-33.6 g/dL
6	MCV (fL)	80.5±11.5	80.9±9.0	0.453	0.542	90.4-128 fL
7	MCH (Pg)	29.4±3.4	29.3±2.3	0.283	0.711	26-41.1 Pg
8	RDW (%)	14.0±2.4	12.9±1.9	3.448	0.002	11.4-21.5 %
9	PLT ($\times 10^{9}$/L)	276.7±106.6	261.6±84.5	1.354	0.327	150-400 /L

Risk factor of infection children with H. pylori

The result this study showed many factors that increase the risk of *H. pylori* infection in children (Table .4). A study confirmed that the positivity rates of H. pylori infection were higher among children residing in sizable households (25). In the present study, H. pylori infection did not reveal any significant correlation with age groups, place of residence, source of drinking water, consumption of fast food and chips, regular hand washing before meals, tooth decay, or family income. A study reported that the prevalence of H. pylori can vary among urban and rural populations within the same country (26). In addition, a subsequent investigation revealed a significant decrease in infection prevalence among families with a higher socioeconomic status ($P<0.005$) (27). Another study demonstrated that the seroprevalence of H. pylori was more significant in individuals who relied on unprotected surface water compared to those who utilized piped tap water (28). The findings of a study were indicative of a significant difference in the seroprevalence of H. pylori infection between individuals who did not engage in regular hand washing and those who did ($P=0.021$) (29). As a result, our findings were inconsistent with those reported in previous studies (30).

(Table .4) Risk factor of infection children with H. pylori

Risk factor	H.pylori infection	***Chi-Square test***	P value
Gender			
Male	71(61.8%)	4.11	0.043
female	45(38.1%)		
Residency			
City	41(34.7%)	0.001	0.99
Rural	77(65.3%)		
Source of drinking water			
Tape water	90(76.2%)	1.52	0.23
Mineral water	28(23.7%)		
Fast Food			

Daily	82(69.4%)	1.1	0.79
Weekly	20(16.9%)		
Occasionally	16(13.5%)		
Chips and cakes			
Daily	92(77.9%)	2.04	0.69
Weekly	23(19.4%)		
Occasionally	3 (2.5%)		

Conclusion

The result showed high Prevalence of Helicobacter Pylori among children in in Wasit province and Urea breath test has more sensitivity and specificity than other serological tests including stool antigen and serum antibody test .

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