

Role of miRNA-146a and miRNA-125b in *Helicobacter pylori*

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Background: *Helicobacter pylori* infection is a common gastrointestinal pathogen associated with gastritis and peptic ulcers. The early detection of *H. pylori*-related gastritis is crucial to its effective management, especially in pediatric patients with dyspepsia.

Purpose: This study aimed to assess the expression of miRNA-146a and miRNA-125b as potential indicators of *H. pylori*-associated gastritis in children.

Methods: This cross-sectional study included 70 *H. pylori*-positive children and 50 *H. pylori*-negative controls aged ≤18 years with recurrent abdominal pain who underwent upper gastrointestinal endoscopy. The miRNA-146a and miRNA-125b expression levels in gastric biopsies were determined using quantitative reverse transcriptase-polymerase chain reaction.

Results: *H. pylori*-positive children had significantly higher levels of miRNA-146a than *H. pylori*-negative children across all endoscopic findings (range, 2.2–2.7 vs. 1.07–1.2; $P<0.001$) and histologic grades of gastritis (range, 2.0–2.4 vs. 1.2–1.3; $P<0.001$). Conversely, the *H. pylori*-positive group showed consistently lower miRNA-125b levels across all parameters (range, 0.3–0.8 vs. 0.4–0.9; $P<0.001$). Both miRNAs differentiated *H. pylori* status with an area under the curve of >0.95.

Conclusion: The altered expression of miRNA-146a and miRNA-125b in gastric biopsies of *H. pylori*-positive children suggests their potential role as molecular markers of *H. pylori*-associated gastritis.

Key words: Gastritis, *Helicobacter pylori*, MiRNA-146a, MiRNA-125b

Meaning: The use of miRNA signatures could detect early gastritis, enabling timely *H. pylori* eradication treatment to mitigate growth delays and cancer risk.

Introduction

Helicobacter pylori is a bacterial pathogen characterized by its spiral shape, known to inhabit the gastric environment of over 50% of humans. While prevalence approaches 90%–100% in developing countries, socioeconomic progress has decreased colonization rates to ~30% in industrialized nations.¹⁾

Infection in children is common and varies by country and geographical area. In developed countries, the frequency of *H. pylori* infection among children is 1.2%–12.2%,²⁾ while in developing countries, *H. pylori* is the most commonly isolated infectious agent in children, reaching 82% in some countries.^{3,4)}

According to several studies, person-to-person transmission among family members is common, with mother-to-child being the most common and oral-oral and oral-fecal transmission routes being the most relevant. The main predisposing factors to *H. pylori* infection in children were low socioeconomic status and living in crowded dwellings. Social class was acting as a proxy measure for conditions and practices within the household that increase the transmission of the organism from infected to uninfected subjects.⁵⁾

Despite causing one of the most common chronic bacterial infections worldwide, optimal diagnostics to detect active *H. pylori* gastritis remain elusive. If untreated, protracted inflammation of the gastric epithelium can progress to atrophy, metaplasia, or malignancy.⁶⁾ Therefore, invasive, and accurate diagnostic approaches are urgently needed, particularly to guide treatment in developing

Key message

Question: Why is the early detection of *Helicobacter pylori*-associated gastritis in children important?

Finding: The early detection of *H. pylori*-related gastritis is crucial for its effective management, especially in pediatric patients with dyspepsia.

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regions where access to endoscopy is inadequate.⁷⁾

Micro RNAs (miRNAs) represent a novel class of diagnostic biomarkers capable of distinguishing *H. pylori* gastric pathology before the manifestation of symptomatic disease. These intrinsic small, and noncoding RNAs play crucial roles in regulating inflammation and cellular processes, including growth, differentiation, and apoptosis.⁸⁾ Signature expression patterns of circulating miRNAs responding to *H. pylori* are now being defined through high-throughput profiling. While certain miRNAs appear particularly sensitive to detecting *H. pylori* colonization versus other gastric conditions, further validation is needed for clinical implementation.⁸⁻¹⁰⁾

miRNA-146a regulates innate immunity and the *H. pylori*-induced inflammatory response by modifying the expression of target genes as interleukin (IL)-1 receptor associated kinase 1 (IRAK1) and tumor necrosis factor (TNF) receptor associated factor-6 (TRAF6).¹¹⁾ *H. pylori* results in a reduction in miRNA-125b levels. MiR-125b downregulation targets TNF- α and initiate an inflammatory response.^{12,13)}

We aimed to evaluate the expression of miRNA-146a and miRNA-125b as invasive early biomarkers of *H. pylori*-associated gastritis.

Methods

This study, conducted at the Gastroenterology and Hepatology Unit within the Pediatric Department of Benha University Hospital, Al-Qalyubia, Egypt, (approval No. Ms.27.5.2022), employed a comparative cross-sectional design. Over the duration from April 2022 to March 2023, a total of 120 pediatric patients meeting specific criteria were recruited. Inclusion criteria encompassed children under 18 years old experiencing recurrent abdominal pain warranting upper gastrointestinal endoscopy or gastroscopy.

This encompassed individuals displaying peptic-like dyspepsia (identified by 2 or more symptoms such as periodic pain, pain relief with food or antacid, premeal or hunger-induced pain, nausea/vomiting, and nighttime pain), dysmotility-like dyspepsia (characterized by abdominal distension, anorexia, weight loss, pain worsening with food/milk, and belching), and reflux-like dyspepsia (manifesting as heartburn, chest pain, or acid regurgitation).^{14,15)} Furthermore, children displaying red flag symptoms such as anemia, elevated erythrocyte sedimentation rate (ESR), gastrointestinal bleeding, or failure to thrive were also included.¹⁶⁾

Exclusion criteria encompassed patients with gastrointestinal disorders explaining abdominal pain (e.g., in-

flammatory bowel disease, celiac disease, functional abdominal pain), patients on proton pump inhibitors, as well as those with significant medical comorbidities.

1. Clinical evaluation

A detailed history and physical examination were performed. Anthropometrics encompassed height, weight, and body mass index (BMI) were measured. Percentiles were calculated using Egyptian pediatric references.¹⁷⁾

2. Radiological examination

Abdominal ultrasound scans were performed using curved linear transducers operating at frequencies ranging from 2 to 5 MHz and linear transducers operating at frequencies ranging from 4 to 8 MHz (Xario XG; Toshiba, Japan) to rule out structural and inflammatory conditions.

3. Laboratory analysis

A complete blood count was performed using a 3-mL sample collected in an ethylenediaminetetraacetic acid tube. The analysis was conducted using a CELL-DYN Emerald automated hematology analyzer from Germany to measure hemoglobin (Hb), leukocytes, and platelets. ESR was measured by the Westergren method, using 3 mL anticoagulated blood allowed to separate under gravity for 1 hour. Stool analysis examined color, consistency, and microscopy (1-g sample). Stool culture to detect pathogenic bacteria. Urine analysis examined the chemical properties and sediment (5- to 10-mL midstream sample). Urine culture used blood agar (1-mL urine) to quantify colony forming units.

H. pylori stool antigen testing was done using the qualitative immunochromatographic bioNexia kit (0.15-g stool) based on anti-*H. pylori* antibodies.

4. Diagnostic upper gastrointestinal endoscopy

Upper gastrointestinal endoscopy was conducted under general anesthesia on a cohort of 120 children utilizing an Olympus XQ20 gastroscope. A minimum of 6 gastric biopsy samples were procured from the antrum, body, and duodenum during each procedure for histopathology hematoxylin and eosin (H&E) staining for detection of *H. pylori*, and 1 biopsy for rapid urease and 1 biopsy for miRNA. These biopsies underwent formalin fixation, paraffin embedding, and subsequent examination through H&E and Giemsa staining to identify *H. pylori* presence. Additionally, the rapid urease test was administered on the biopsies using the commercially available Campylobacter-like Organism kit (PYLO-PLUS, Gulf Coast Scientific, USA), with a change in color change from yellow to red within 60 minutes signifying a positive outcome. The nursing staff monitored the color transformation at predetermined

intervals. Endoscopic assessments also encompassed the evaluation of bleeding and tissue damage.

Histopathological analysis was performed to assess *H. pylori* density, chronic inflammation, atrophy, activity, and intestinal metaplasia according to the Updated Sydney System.¹⁸⁾ Parameters assessed included *H. pylori* positivity, histological grade of neutrophil infiltration (activity), mononuclear cell infiltration (chronic inflammation), glandular atrophy, and intestinal metaplasia. These parameters were scored; as null (0), mild (1), moderate (2), and severe (3).

5. miRNA expression analysis

Paraffin-embedded biopsies were sectioned into 10- μ m-thick slices. Two of these slices were deposited into 1.5-mL microcentrifuge tubes and dewaxed via immersion in xylene at 50°C, followed by 100% and 96% ethanol. Total RNA was isolated with TRIzol Reagent (Ambion RNA by Life Technologies; USA), followed by phenol-chloroform extraction. A NanoDrop spectrophotometer (The Epoch Multivolume Spectrophotometer System, USA) measured RNA quality and quantity at 260 and 280 nm, respectively.

The expression levels of miRNA-146a and miRNA-125b were determined through quantitative reverse transcriptase-polymerase chain reaction (PCR). RNA extraction from paraffin-embedded biopsies was conducted using the Qiagen RNeasyPlus Universal Mini Kit (Catalog number: WF10308002), followed by assessment of RNA concentration via ultraviolet-spectrophotometry. MiRNA expression was quantified using either the TaqMan miRNA assay (Applied Biosystems, USA) or the SYBR Green method. Reverse transcription into cDNA was performed using the TaqMan MicroRNA RT Kit (Applied Biosystems, Part No. 4366596), followed by real-time PCR analysis utilizing TaqMan microRNA assays in the StepOne real-time PCR system.

Real-time PCR amplification was carried out in triplicate under the following conditions: a 2-minute incubation at 50°C (for uracil-N-glycosylase activation) was followed by a 10-minute incubation at 95°C (for polymerase activation), followed by 40 cycles of denaturation at 95°C for 15 seconds and alignment and extension at 60°C for 1 minute using a Stratagene Mx3005p qPCR System and MxPro-Mx3005p software (Santa Clara, USA).

The data analysis used the comparative C_t method U6 snRNA as the reference gene. Relative expression (fold change) was calculated using equation 2- $\Delta\Delta C_t$. SYBR dye was used to determine relative quantification during PCR amplification.

6. Sample size calculation

The sample size was calculated according to the follow-

$$n = \left(\frac{(Z_{\alpha/2} + Z_{\beta})^2 \times 2 \times \sigma^2}{\Delta^2} \right)$$

ing equation¹⁹⁾ and based on similar studies²⁰⁾:

Where:

- $Z_{\alpha/2}$ is the Z value for the desired significance level (1.96 for $\alpha=0.05$),
- Z_{β} is the Z value for the desired power (0.84 for 80% power),
- σ is the pooled standard deviation,
- Δ is the effect size.

The final sample size of 120 participants (70 *H. pylori*-positive and 50 *H. pylori*-negative) was determined. The final sample size of 120 participants (70 *H. pylori*-positive and 50 *H. pylori*-negative) was determined using equations based on calculations for both significance and precision objectives.

7. Statistical analysis

Data analysis using SPSS ver. 16 (SPSS Inc, USA). Categorical data were presented as frequencies and percentages, while quantitative data were expressed as mean $\pm$ standard deviation, median, interquartile range, and range. For categorical data analysis, both the chi-square test and Fisher exact test were used. The normality of quantitative data was assessed using Shapiro-Wilk test, with significance set at $P>0.05$. The Student *t* test compared normally distributed variables between 2 independent groups. Correlation analysis between nonparametric variables was done using Spearman correlation coefficient (ρ). Receiver operating characteristic (ROC) curve analysis determined optimal sensitivity and specificity cutoff values. The significance level was set at $P<0.05$.

Results

This study compared the characteristics of 70 *H. pylori*-positive children versus 50 *H. pylori*-negative children. The 2 groups were similar in sex distribution (47.5% males vs 52.5% females), mean age (10.3 $\pm$ 4.3 years), and height percentiles. However, the *H. pylori*-positive group had significantly lower mean weight (32.5 kg vs. 39 kg) and BMI (18.1 kg/m² vs. 19.8 kg/m²) compared to the negative group ($P<0.05$ for all). A greater proportion of *H. pylori*-positive children lived in rural areas (61.4% vs. 40%, $P=0.026$) (Table 1).

In the *H. pylori*-positive group, a significantly higher proportion experienced recurrent abdominal pain (80.0% vs. 28.0%, $P=0.062$), peptic-like dyspepsia symptoms such as periodic pain (80.0% vs. 28.0%, $P<0.001$), pain relieved by antacid (77.1% vs. 36.0%, $P<0.001$), nausea and/or vomiting

Table 1. Demographic and anthropometric characteristics of *Helicobacter pylori*-positive and -negative children

Variable	<i>H. pylori</i> -positive group (N=70)	<i>H. pylori</i> -negative group (N=50)	Test	P value
Sex			$\chi^2=0.95$	0.951
Male	33 (47.1)	24 (48.0)		
Female	37 (52.9)	26 (52.0)		
Age (yr)	10.1±4.3 (3–17)	10.6±4.33 (4–17)	t= 0.63	0.53
Residence			$\chi^2= 4.55$	0.026
Urban	27 (38.6)	30 (60)		
Rural	43 (61.4)	20 (40)		
Weight (kg)	32.5±13.8 (10–62)	39±16.5 (15–65)	t=2.35	0.02
Weight percentile (th)	33.8±19.5 (10th –90th)	48.5±23.7 (25th – 90th)	t=3.7	<0.001
Height (cm)	134±21 (105–158)	140±19 (105–160)	t=1.49	0.139
Height percentile (th)	41±25 (5th–75th)	46±26 (10th –90th)	t=1.16	0.246
BMI	18.1±2.9 (13.1–25.5)	19.8±3.6 (15.4–25.8)	t=2.73	0.008
BMI percentile (th)	30.3±20.4 (3rd –75th)	43.3±19.7 (25th–75th)	t=3.48	<0.001

Values are presented as number (%) or mean±standard deviation (range).

t, Student t test; BMI, body mass index.

Boldface indicates a statistically significant difference with $P<0.05$.

Table 2. Endoscopic and histopathological findings by study group

Endoscopic/histopathological finding	<i>H. pylori</i> -positive group (N=70)	<i>H. pylori</i> -negative group (N=50)	Chi-square test	P value
Endoscopic finding				
Esophagus			6.17	0.187
Normal	66 (94.3)	42 (84.0)		
Esophagitis	2 (2.9)	4 (8.0)		
Esophageal ulcer	0 (0)	2 (4.0)		
Erosions	2 (2.9)	2 (4.0)		
Stomach			30.3	<0.001
Normal	0 (0)	18 (36.0)		
Mild hyperemia	37 (52.9)	14 (28.0)		
Moderate hyperemia	33 (47.1)	18 (36.0)		
Duodenum			8	0.005
Normal	42 (60.0)	42 (84.0)		
Duodenitis	28 (40.0)	8 (16.0)		
Histopathology of biopsies				
Chronic inflammation grade			36.7	<0.001
No	0 (0)	16 (32.0)		
Mild chronic active gastritis	26 (37.1)	18 (36.0)		
Moderate chronic active gastritis	24 (34.3)	16 (32.0)		
Severe active gastritis	20 (28.6)	0 (0)		
Polymorph nuclear cell activity			46.3	<0.001
Present	60 (85.7)	12 (24.0)		
Absent	10 (14.3)	38 (76.0)		
Atrophy			-	-
No	70 (100)	50 (100)		
Yes	0 (0)	0 (0)		
Metaplasia			-	-
No	70 (100)	50 (100)		
Yes	0 (0)	0 (0)		
<i>H. pylori</i> detection			120	<0.001
Negative	0 (0)	50 (0)		
Low (+)	30 (42.9)	0 (0)		
Moderate (++)	28 (40.0)	0 (0)		
Severe (+++)	12 (17.1)	0 (0)		

Values are presented as number (%).

H. pylori, *Helicobacter pylori*.

Boldface indicates a statistically significant difference with $P<0.05$.

Table 3. Levels of miRNA-146a and miRNA-125b by study group

	<i>H. pylori</i> -positive group (N=70)	<i>H. pylori</i> -negative group (N=50)	t test	P value
MiRNA-146a			27.13	<0.001
Median (IQR)	2.5 (2.2–2.6)	1.10 (0.96–1.10)		
Mean±SD (range)	2.58±0.41 (2.18–3.51)	1.1±0.17 (0.95–1.54)		
MiRNA-125b			26.06	<0.001
Median (IQR)	0.37 (0.33–0.39)	0.94 (0.83–0.97)		
Mean±SD (range)	0.38±0.06 (0.32–0.52)	0.95±0.15 (0.82–1.32)		

IQR, interquartile range; SD, standard deviation; *H. pylori*, *Helicobacter pylori*.
Boldface indicates a statistically significant difference with $P<0.05$.

(72.9% vs. 42.0%, $P=0.001$), and night pain (80.0% vs. 44.0%, $P<0.001$). Dysmotility-like dyspepsia also showed a significantly higher occurrence of pain aggravated by food or milk in the *H. pylori*-positive group compared to the negative group (51.4% vs. 32.0%, $P=0.034$). Interestingly, reflux-like dyspepsia demonstrated a significantly lower prevalence of acid regurgitation in the *H. pylori*-positive group compared to the negative group (14.3% vs. 32.0%, $P=0.02$). Moreover, red flag symptoms such as anemia were significantly more common in the *H. pylori*-positive group compared to the negative group (61.4% vs. 32.0%, $P=0.001$).

In the positive group, only 2.9% exhibited esophagitis, while 94.3% showed normal esophageal findings, though this difference was not statistically significant ($P=0.187$). Stomach abnormalities were prevalent, with 52.9% displaying mild hyperemia and 47.1% moderate hyperemia, significantly higher than the negative group's 36.0% normal findings ($P<0.001$). Duodenitis displayed a significantly higher prevalence in *H. pylori*-positive patients compared to significantly higher prevalence in *H. pylori*-positive patients than in the control group ($P=0.005$). Histopathological analysis revealed a significant difference in polymorph nuclear cell activity, with the positive group exhibiting 85.7% compared to the opposing group's 24.0% ($P<0.001$). Furthermore, a significantly greater number of patients with severe active gastritis were observed among *H. pylori*-positive individuals compared to those who were *H. pylori*-negative. Moreover, the positive group showcased diverse levels of *H. pylori* detection, ranging from 42.9% low positivity to 17.1% severe positivity ($P<0.001$) (Table 2).

The miRNA-146a levels were significantly higher in *H. pylori*-positive patients compared to *H. pylori*-negative patients, while miRNA-125b levels were significantly lower in *H. pylori*-positive patients compared to *H. pylori*-negative patients (Table 3, Fig. 1).

miRNA-146a exhibited significant elevation in patients with moderate hyperemia, active gastritis (mild, moderate, and severe), the presence of polymorphonuclear cells (PMN), and various degrees of *H. pylori* detection compared to those with normal endoscopic findings or biopsy reports ($P<0.001$). Conversely, miRNA-125b levels were notably lower in patients with abnormal endoscopic find-

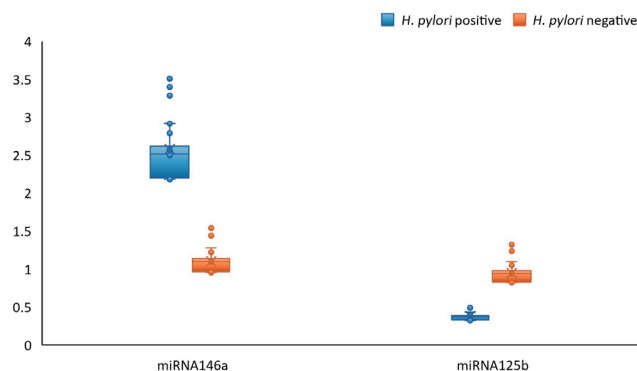


Fig. 1. Levels of miRNA-146a and miRNA-125b by study group. *H. pylori*, *Helicobacter pylori*.

ings or biopsy reports, as well as in those with the presence of PMN and *H. pylori* infection, compared to their respective control groups ($P<0.001$) (Table 4).

The correlation analysis demonstrated associations between miRNA-146a and various clinical parameters, as well as miRNA-125b and other clinical data. For miRNA-146a, significant negative correlations were observed with weight centile ($r=-0.354$, $P=0.006$) and ESR ($r=-0.324$, $P=0.012$). However, there were no significant correlations with age, height centile, BMI centile, Hb levels, platelet count, or white blood cell count. In contrast, miRNA-125b showed significant positive correlations with weight centile ($r=0.284$, $P=0.028$), BMI centile ($r=0.279$, $P=0.031$), and Hb levels ($r=0.306$, $P=0.018$). Additionally, a significant negative correlation was observed with ESR ($r=-0.405$, $P<0.001$). However, there were no significant correlations with age, height centile, platelet count, or white blood cell count.

ROC analysis was conducted to evaluate the performance of miRNA-146a and miRNA-125b in detecting *H. pylori* infection. For miRNA-146a, the area under curve (AUC) was 0.994 (95% confidence interval [CI], 0.950–1.000), with $P<0.001$. At a cutoff point >1.8 , the sensitivity was 96%, and the specificity was 97.1%. Regarding miRNA-125b, the AUC was 0.962 (95% CI, 0.908–1.000), also with a $P<0.001$. At a cutoff point of less than 0.82, the sensitivity was 97%, and the specificity was 94.3% (Fig. 2).

Table 4. Levels of miRNA-146a versus miRNA-125b according to endoscopic and histological findings

Variable	MiRNA-146a				MiRNA-125b			
	Mean±SD	Range	Test	P value	Mean±SD	Range	Test	P value
Endoscopic finding in esophagus			<i>t</i> =0.87	0.385			<i>t</i> =0.27	0.784
Normal	1.7±0.6	1.1-2.2			0.7±0.3	0.3-1.3		
Esophagitis	2±0.8	1-3.5			0.6±0.4	0.3-1		
Endoscopic finding in stomach			<i>F</i> =17.7	<0.001			<i>F</i> =14.9	<0.001
Normal	1.1±0.2	1-1.5			0.9±0.2	0.8-1.2		
Mild hyperemia	2±0.7	1.7-3.1			0.7±0.3	0.3-1.4		
Moderate hyperemia	2.3±0.8	1.9-3.9			0.6±0.3	0.3-1.2		
Endoscopic finding in duodenum			<i>t</i> =1.92	0.058			<i>t</i> =3.54	<0.001
Normal	1.9±0.8	1-3.5			0.7±0.3	0.3-1.3		
Duodenitis	2.2±0.7	1-3.3			0.5±0.2	0.3-1		
Biopsy report of the stomach			<i>F</i> =10.45	<0.001			<i>F</i> =15.34	<0.001
Normal	1.1±0.2	1-1.5			0.9±0.2	0.8-1.3		
Mild chronic active gastritis	2±0.8	1-3.3			0.7±0.3	0.3-1.2		
Moderate chronic active gastritis	2.1±0.9	1-3.5			0.6±0.3	0.3-1.1		
Severe active gastritis	2.4±0.2	2.2-2.8			0.4±0	0.3-0.6		
PMN			<i>t</i> =7.35	<0.001			<i>t</i> =7.75	<0.001
Absent	1.4±0.7	1-3.3			0.8±0.3	0.3-1.2		
Present	2.3±0.7	1-3.5			0.5±0.2	0.3-1.3		
<i>H. pylori</i>			<i>F</i> =189.9	<0.001			<i>F</i> =282	<0.001
No	1.1±0.2	1-1.5			0.9±0.1	0.8-1		
Mild (+)	2.1±0.3	1.9-2.7			0.7±0.1	0.6-1		
Moderate (++)	2.4±0.2	2-2.9			0.6±0.1	0.3-0.8		
Severe (+++)	2.7±0.4	2.2-3.2			0.4±0.1	0.3-0.6		

SD, standard deviation; PMN, polymorphonuclear cells; *H. pylori*, *Helicobacter pylori*.
 Boldface indicates a statistically significant difference with *P*<0.05.

Discussion

Traditional *H. pylori* diagnosis involves invasive endoscopic biopsies and H&E staining. Their sensitivity and accuracy depend on where the tissue sample is collected during the biopsy.^{21,22)}

The detection of *H. pylori* has been demonstrated to be a reliable method using gastric biopsy-based PCR assays, even at low bacterial density, as observed in marginally inflamed gastric mucosa or in proton pump inhibitors recipients, rapid urase not widely available commercial and the miRNA is cheaper.²³⁾

This study evaluated clinical characteristics, endoscopic findings, and miRNA expression profiles among 70 *H. pylori*-positive and 50 *H. pylori*-negative pediatric patients with dyspepsia. We observed significantly lower weight, BMI, and higher rural residence among *H. pylori*-positive children. Moreover, this group exhibited more pronounced recurrent abdominal pain, peptic-like symptoms, warning signs like anemia, and endoscopic evidence of gastritis and duodenitis compared to *H. pylori*-negative cases. Key findings were the differential expression of miRNA-146a and miRNA-125b in the presence of *H. pylori* infection as confirmed via histopathology.

The impaired growth parameters among *H. pylori*-

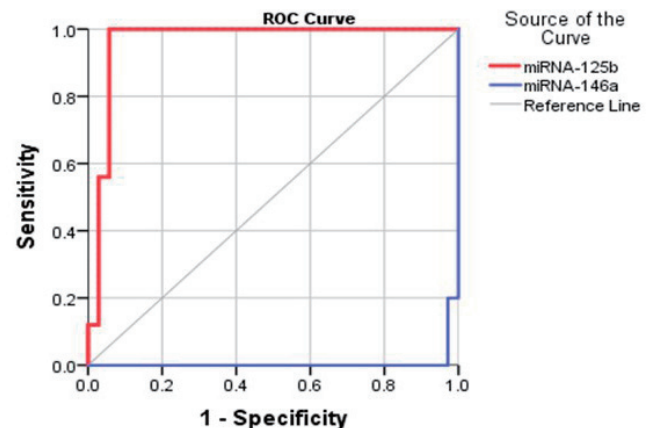


Fig. 2. Receiver operating characteristic (ROC) curve of ability of miRNA-146a versus miRNA-125b to detect *Helicobacter pylori* infection.

positive children align with prior evidence linking chronic gastritis to reduced weight gain and nutritional deficiencies in pediatric populations.²⁴⁾ Proposed mechanisms encompass reduced appetite, chronic inflammation, and micronutrient malabsorption stemming from hypochlorhydria.²⁵⁾ Our rural predominance agrees with higher prevalence seen in resource-limited settings, reflecting socioeconomic, hygiene and overcrowding exposures early in life.²⁶⁾

Of note, the differential miRNA expression we observed appeared to correlate with disease severity markers seen on endoscopic, histological, and special staining evaluations. Specifically, patients exhibiting more advanced visual inflammation on stomach biopsy (moderate hyperemia), worse microscopic injury patterns (active chronic gastritis), heightened immune cell infiltration (PMN presence), and higher tissue loads of visualized bacteria (*H. pylori* grades) tended to harbor more substantially elevated miRNA-146a levels and diminished miRNA-125b compared to their respective negative controls. These findings suggest upregulation and repression of these miRNAs likely occurs on a continuum mirroring the progressive mucosal changes that manifest with persistent *H. pylori* colonization and its resultant immunopathogenesis. The stepwise miRNA alterations may provide not only diagnostic potential but perhaps insight into pathogenesis or even gauge of treatment response not afforded by the traditional dichotomized measures of infection status or presence of gastritis.

The upregulation of miRNA-146a likely represents activation of innate immune pathways attempting to combat the *H. pylori*-induced inflammatory response. Increased miRNA-146a may function to suppress downstream proinflammatory signaling via nuclear factor-kappa B (NF- κ B) and IL-8 that can propagate chronic gastric injury if unrestrained.^{8,27} However, *H. pylori* exhibits an elaborate set of virulence mechanisms that can circumvent host defenses, allowing persistent colonization and inflammation despite miRNA modulatory efforts.¹¹ The subsequent mucosal damage appears partially mediated through repression of miRNA-125b's protective effects, evidenced by our finding of diminished levels impairing cellular proliferation and differentiation programs necessary for restorative healing.⁸ MiR-125b represses TNF- α synthesis, ensuring the suppression of this proinflammatory cytokine, whereas its decrease allows for TNF- α production to occur after toll-like receptors stimulation.²⁸

Liu et al.,²⁹ identified the miR-146a expression in gastric epithelial cells and gastric mucosal tissues from *H. pylori*-induced chronic gastritis in 48 adult patients undergoing gastroscopy. They reported that *H. pylori* upregulated the miR-146a in both gastric epithelial cells and gastric mucosal tissues in NF- κ B-dependent manner. In turn, miR-146a may reduce the expression of target genes, IRAK1 and TRAF6. miR-146a reduced *H. pylori*-triggered IL-8, growth-related oncogene- α , and macrophage inflammatory protein-3 α by inhibiting NF- κ B activity.

Of note, we established strong correlations between miRNA abundance and histological activity, suggesting these small noncoding transcripts may have utility as molecular biomarkers of disease severity. The inverse

association of miRNA-146a with weight/ESR and direct relationship of miRNA-125b with nutritional indices/Hb suggest involvement of these miRNAs in gastric inflammatory pathways known to disrupt growth and absorption.³⁰ Other research has linked altered miRNA participation to interference with ghrelin, leptin, and *H. pylori* neutrophil-activating protein pathways.³¹ Additionally, the accuracy of miRNA profiling in detecting *H. pylori* infection status rival's traditional endoscopy-based techniques, concurring with prior noninvasive formats.^{32,33}

While our study focused on the expression of miRNA-146a and miRNA-125b in gastric tissues of *H. pylori*-infected children, it did not assess their levels in circulation. Tissue-specific miRNAs can be detected in the bloodstream, potentially serving as noninvasive biomarkers for various conditions. Matsuzaki and Suzuki³⁴ concluded that the levels of the 7 circulating miRNAs (miR-128-3p, miR-328-3p, miR-143-3p, miR-144-3p, miR-15a-5p, miR-1-3p, and miR-133b) might represent the tissue miRNA levels and could be promising noninvasive biomarkers to evaluate the carcinogenic process of Barrett's esophagus.

The potential for miRNAs to be released into circulation from inflamed gastric tissues has been explored in other contexts. Noto and Peek³⁵ demonstrated that specific miRNAs, such as miR-146a, are upregulated in response to *H. pylori* infection and modulate the host immune response.

However, this is the first pediatric evaluation of miRNA biomarkers in dyspeptic children to our knowledge. If confirmed through multi-center evaluations, accessible molecular diagnostics via blood sampling could overcome limitations in endoscopic or stool antigen testing among young patients in our setting. Also, our study focused on tissue miRNA expression and did not assess circulating miRNA levels. Future directions include expanding panels and sample sizes to distinguish *H. pylori* gastritis from other causes of dyspepsia among children in primary care settings. While our findings indicate that miRNA-146a and miRNA-125b may serve as potential biomarkers for *H. pylori*-induced gastritis, further validation is required. Future studies should directly compare miRNA expression with conventional diagnostic techniques, such as H&E staining, fecal antigen testing, and the urea breath test, in more extensive and diverse populations. Further studies are needed to investigate the presence and levels of miRNA-146a and miRNA-125b in circulation by correlating tissue and serum miRNA levels in infected individuals to assess their diagnostic potential while exploring their stability and detectability in blood samples to develop reliable, noninvasive tests.

In conclusion, an altered miRNA signature delineates

H. pylori-associated chronic gastritis. Notably, the upregulation of miRNA-146a and the downregulation of miRNA-125b were observed across both endoscopic and histopathological indicators of disease activity. This signature demonstrates promising accuracy in identifying *H. pylori*-associated chronic gastritis among symptomatic children, who may face potential growth-related consequences. The assessment of circulating miRNAs could greatly aid in screening, guiding treatment decisions, and monitoring eradication success, which is particularly crucial during early childhood.

Footnotes

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