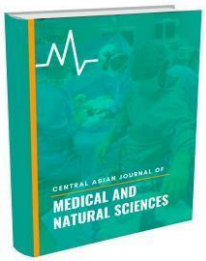

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Article

Clinical Manifestation and Immunological Assays for *H.Pylori* Diagnosis

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Abstract: Date palm (Phoenix dactylifera L.) cultivation is a cornerstone of agriculture in arid and semi-arid regions, with significant cultural and economic importance. Specific Background: In Iraq, the date palm sector faces critical challenges due to traditional propagation methods and limited genetic diversity. Despite the known advantages of tissue culture for mass propagation and disease-free plant production, there is insufficient localized research on its effective implementation in Iraqi varieties. This study aimed to evaluate the role of tissue culture technology in the rapid propagation of elite date palm cultivars and its potential to support the recovery of Iraq's date palm sector. The research demonstrated that tissue culture techniques, specifically somatic embryogenesis and organogenesis, can produce uniform and healthy date palm plantlets, ensuring high propagation rates and genetic fidelity. Additionally, successful acclimatization rates were achieved, indicating the viability of large-scale production. This work provides the first systematic evaluation of tissue culture application for Iraqi date palm cultivars, addressing technical and operational constraints in local laboratories. The findings highlight the potential of biotechnology to revolutionize date palm propagation in Iraq, offering a sustainable solution for germplasm conservation, cultivar improvement, and re-establishment of Iraq's leadership in date production.

Keywords: Date Palm, Tissue Culture, Somatic Embryogenesis, Iraq, Micropropagation

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1. Introduction

Since the groundbreaking discovery of Helicobacter pylori two decades ago, the landscape of diagnosing and treating upper gastro duodenal ailments has undergone a remarkable transformation. Peptic ulcer disease is now regarded as an infectious malady, where the eradication of the offending agent leads to a complete resolution of the issue. The significance of H. pylori infection in the development of gastric cancers is gaining acknowledgment, while its impact on various other disorders of the upper gastrointestinal tract is currently under investigation. Tremendous advancements have been made in unraveling the mechanisms behind this infection's pathogenesis [1].

H pylori, a gram-negative spiral organism, is the main cause of chronic gastritis. Gastritis is diagnosed by histology; the gastric mucosa is infiltrated with mononuclear cells and usually with neutrophils too [2]. *H. pylori* is a fastidious microorganism and requires complex growth media. Often these media are supplemented with blood or serum. These supplements may act as additional sources of nutrients and possibly also protect against the toxic effects of long-chain fatty acids

1.1 *H.pylori*

H. pylori (Helicobacter pylori) is a type of bacteria that infects the stomach and causes inflammation of the stomach lining and surrounding tissues. It is one of the most common bacterial infections worldwide, affecting approximately half of the global population.

1.2 Structure of *helicobacter*

Cell Wall Composition

Peptidoglycan, crafted meticulously by Mur enzymes through a complex multistep journey, stands as a vital pillar of the bacterial cell wall. MurA, the maestro of the initial phase, finds itself in the crosshairs of Fosfomycin's precision. For glutamate racemase MurI, which deftly converts L-Glu into D-Glu, a variety of inhibitors have been unveiled. The widely employed β-lactam antibiotics thwart the intricate process of peptidoglycan cross-linking that unfolds in the concluding chapters of this pathway. Conversely, the succinylase pathway emerges as the singular avenue in Hp for the creation of lysine, an indispensable building block of the bacterial peptidoglycan cell wall. N-succinyl-L,L-diaminopimelic acid desuccinylase (SDAP-deacylase; DapE), a crucial enzyme along this route, has been recognized as vital for the survival of Hp [3].

1.3 Diagnosis

The diverse array of both invasive and noninvasive diagnostic techniques for uncovering the presence of Helicobacter pylori has predominantly found its niche in the heart of developing countries. Cutting-edge molecular methodologies have surfaced, notably a revolutionary assay that detects H. pylori and its clarithromycin resistance straight from fecal samples. The lasting effects of eradication on histological irregularities have been thoroughly scrutinized in a comprehensive meta-analysis, while the predictive value of post-treatment in gastric mucosa-associated lymphoid tissue lymphoma has been meticulously assessed. An innovative operational framework for evaluating gastritis, referred to as the OLGA staging, has also been unveiled. Initiatives to refine the urea breath test procedure have been initiated, alongside the unveiling and comparison of novel stool antigen tests against their predecessors [4].

1.3.1 Laboratory diagnosis

A deep-seated connection unfolds between acute gastritis and H. pylori. As time meanders onward, both gastritis and H. pylori seem to thrive, while the administration of non-steroidal anti-inflammatory medications appears to coincide with unremarkable histological revelations. Curiously, neither the presence of H. pylori nor the intensity of gastritis revealed any ties to gender, the intake of alcohol and tobacco, the motives for endoscopy, or a range of symptoms (including epigastric unease, nausea, vomiting, bloating, belching, heartburn, unpleasant breath, and flatulence) [5].

1.3.2 Clinical diagnosis

1_ Histology: The routine use of hematoxylin and eosin (H&E) staining reveals the hidden world of H. pylori, accompanied by telltale signs of inflammation, notably gastritis. When results lack clarity, one may resort to specialized stains such as Giemsa, Warthin-Starry, acridine orange, toluidine blue, Dieterle, Genta, Romanouski, and McMullen, or delve into the realm of immunochemical techniques. Among these, the Genta stain, a refined concoction of silver, H&E, and Alcian blue, permits the visualization of inflammatory cells alongside H. pylori, though it comes with a significant cost and intricate application process. Conversely, the Giemsa stain dazzles with its elegance, exceptional sensitivity, affordability, and popularity in clinical environments. Alternative methodologies primarily find their niche in the pursuit of research [6].

2_ Endoscopy: A conventional endoscopic examination is customarily performed to unveil ailments associated with H. pylori, such as peptic ulcers, atrophic gastritis, MALT lymphoma, and gastric cancer. This procedure also acts as a vital instrument for obtaining specimens, predominantly gastric mucosa via biopsy, for subsequent investigations

through various invasive tests, including rapid urease test, histological analysis, culturing, and molecular diagnostics. The antrum is typically the preferred site for biopsies aimed at identifying H. pylori infections in most cases, yet for individuals with antral atrophy or intestinal metaplasia, a biopsy from the corpus along the greater curve is advised to reduce the chance of false negatives. The uneven distribution of H. pylori throughout the stomach in various clinical scenarios inevitably leads to sampling errors during biopsy assessments, resulting in several initiatives focused on real-time H. pylori diagnosis during endoscopic procedures [7].

3_ Rapid Urease Test: The rapid urease test (RUT) emerges as a quick, uncomplicated, highly specific, and economically viable instrument for diagnosing H. pylori. However, RUT requires a considerable bacterial concentration; for example, standard commercial kits necessitate a minimum of 10^4 bacterial load in gastric samples. Recent usage of antibiotics, bismuth-containing compounds, and proton pump inhibitors, particularly omeprazole and lansoprazole, can lead to false-negative results, especially in children below five years of age. Collecting biopsies from the corpus instead of the antrum, or employing a combination of both, has proven to enhance the sensitivity of the RUT [8].

2. Materials and Methods

Fifty samples were collected from patients of both genders and various age groups who were diagnosed with *H. pylori*, as well as stomach and intestinal diseases, at Shatra Hospital in January 2023. These patients underwent microscopic examination.

2.1 Urea breath test

The UBT stands as the paramount non-invasive assessment for Helicobacter pylori. Its simplicity in execution, dependability, cost-effectiveness, and safety for patients make it truly remarkable. Primarily, it serves as a follow-up tool after attempts to eradicate H pylori, offering significant advantages over its principal counterpart, serology. Already embraced widely as a research instrument, the UBT merits broader application in everyday clinical settings [9][10].

Helicobacter pylori churns out copious amounts of a formidable urease enzyme, the detection of which is the cornerstone of the UBT. The existence of gastric urease was first unveiled in 1923 by Luck, who revealed the hydrolysis of urea within the stomachs of dogs and various other creatures, and it wasn't long before humans were included in this fascinating discovery. In the 1950s, Kornberg utilized a precursor to the UBT in anesthetized cats, demonstrating that antibiotics obliterated urease activity, leading him to conclude that urease was of bacterial origin. Initially, the identification of H pylori by Warren and Marshall in 1983 bore no connection to gastric urease (the bacterium was initially believed to be urease negative!), but the link soon became apparent. The advantages of urease to H pylori continue to spark debate, yet its diagnostic potential was swiftly acknowledged, with recognition occurring as early as 1985 [3][11].

2.2 Method of work urea breath test

1-At the beginning of the process, patients enjoy a delightful sachet filled with either zesty orange juice or tangy citric acid. This promptly secures the duodenal sphincter, ensuring that the stomach's contents remain perfectly tucked away. Following this, they are encouraged to breathe out through a straw into a glass cylinder sealed with a screw cap. This action produces the initial sample.[12][13]

2-Next, they sip on a potion enriched with 13C-laden urea (around 100 ml), and after a brief pause of 30 minutes, they engage in the blowing ritual once more into a second tube. This generates the post-dose sample. Both samples embark on a journey for a detailed carbon dioxide isotope analysis performed via mass spectrometry, with laboratories typically delivering results within a few days.[14][15]

The concentration of 13C in the initial sample stays within standard boundaries. An increase in levels in the post-dose sample suggests the hidden presence of H. pylori. The enriched urea from the testing kit must have decomposed, leading to heightened 13C levels in the breath, implying the existence of urease-producing H. pylori in the stomach.

3-If the post-dose 13C concentration exceeds the baseline sample by 3.5 parts per 1000, the test is classified as positive for H. pylori. The increase in 13C levels corresponds with the intensity of the infection; values between 30 to 40 parts per 1000 above the baseline are frequently observed in H. pylori infections.

Moreover, two additional backup tubes are usually gathered during both the baseline and post-dose stages.

The diagnostic kits flaunt an impressive sensitivity of 96% and an extraordinary specificity of 100%.

3. Results

The Frequency of male was the height according to table 1 with count 27 and percent 54% comparing to female 23 and percent 46%.

Table 1. Gender distribution for total study groups.

	Gender		P. Value
	Frequency	%	
Male	27	54.0	0.5
Female	23	46.0	
Total	50	100.0	

The Frequency of age group (21-30y) was the height according to table 2 with count 21 and percent 42% followed by age group (31-40y) with count 15 and percent 30% comparing to age groups <10y and >40 y with count 1,6 and percent 2%, 12% respectively.

Table 2. Age groups distribution for total study groups.

		Frequency	%	P. Value
Age Groups	<10 y	1	2.0	0.5
	10-20 y	7	14.0	
	21-30 y	21	42.0	
	31-40 y	15	30.0	
	>40 y	6	12.0	
	Total	50	100.0	

4. Discussion

Helicobacter, the spiral-shaped, Gram-negative gastric microbe, known as Helicobacter pylori, has been in existence for less than twenty years. Initially met with doubt regarding its causal role, this organism is now acknowledged as a key player in a range of prevalent medical issues. Undeniably the instigator of chronic-active gastritis and the root cause of many peptic ulcer cases, this microorganism is also suspected to play a role in the emergence of gastric adenocarcinoma, which ranks as the fourth most widespread cancer globally, as well as mucosa-associated lymphoid tissue lymphoma.

The objective of the investigation is to ascertain the prevalence of *H. pylori* infection among patients diagnosed with colitis, as evidenced by previous research findings indicating a significant incidence rate (50) samples were collected from infected patients. The tally of male individuals afflicted was recorded as (27), while the corresponding count

for female individuals stood at (23). Thus, it can be inferred that males exhibit a greater susceptibility to infection, The age bracket most vulnerable to infection consisted primarily of individuals aged (21-30), followed by those aged (31-40). Conversely, children under the age of <10 exhibited the lowest susceptibility to contracting the infection.

In terms of tests used, blood testing showed the highest rate at 20% of patients. However, it should be noted that this analysis may contain error due to antibodies such as lgG, which can lead to false-negative results for h. Helicobacter pylori infection. On the other hand, while the stool test showed a lower rate of 16%, it may be a little uncomfortable in addition. This analysis occurs after the patient has symptoms;

However, it is useful for young children. Finally, at 14%, the urea test is considered more sensitive and specific in detecting *H. pylori*, in addition to the speed of this analysis. Regarding the medical history of those infected, it is clear that the highest percentage, 28%, was for individuals who had not been infected before.

5. Conclusion

According to the previous results, the present study may conclude the following:

1. *Helicobacter*, a gram-negative bacterium, is considered one of the causative agents of gastric and intestinal diseases.
2. Infection with *Helicobacter pylori* is a highly common condition that tends to increase among young individuals.
3. Infection with *Helicobacter pylori* is the most common cause of gastric inflammation and peptic ulcers worldwide.
4. Recent studies have shown that the prevalence of *Helicobacter pylori* infection is lower among younger individuals.
5. The spread of this infection is more prevalent among individuals with black or Hispanic ethnicity, as well as Asians
6. *Helicobacter pylori* bacteria are capable of producing ammonia, which aids in their protection from gastric acid.

Additionally, *Helicobacter pylori* possesses a unique physiological feature that enables it to generate and transport ammonia outside the cell through the enzyme urease. This capability facilitates the bacteria's ability to penetrate and disrupt the protective mucosal layer

7. Chronic infection with *Helicobacter pylori* bacteria increases the risk of developing gastric cancer

Recommendations

1. Detecting other bacterial virulence factors and conducting polymorphism studies and fragment maps.
2. The bacteria can be transmitted from person to person, especially from individuals infected with *H. pylori* who do not practice proper hand hygiene after using the restroom.
3. It is crucial to select immune and histopathological tests that do not require surgical intervention in order to diagnose and detect Helicobacter infections .
4. Transmission of *H. pylori* can occur through kissing or other forms of close contact
5. Choose the appropriate antibiotic for each infection to reduce antibiotic resistance.
6. Infection with *H. pylori* tends to cluster within families and among individuals living in supervised care facilities and other similar settings.
7. Use the correct method to sterilize surgical instruments and other important parts in hospitals
8. Emphasize the use of modern techniques in hospitals to diagnose bacteria.

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