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Investigation of Helicobacter Pylori in patients suffering from stomach ulcer by BABA Gene: Adherence factor

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Abstract

To identify the presence of the *BabA* adhesion gene in patients who are *Helicobacter pylori* positive and suffering from gastric ulcers and to determine if there is any correlation between its presence and the severity of the ulcers. One hundred patients with diagnosed gastric ulcers were recruited, and each underwent endoscopy to obtain biopsy specimens from their gastric mucosa. The *BabA* gene was detected using a polymerase chain reaction in which specific primers targeting the gene were utilized. Sixty-five percent of the patients were positive for the *BabA* gene, and the results were considerably more significant among those with the most severe forms of ulceration. The authors believe that the *BabA* gene reduces the effectiveness of the mucosal barrier and is thus an essential factor in the development of gastric ulcers. These conclusions underline the significance of genetic testing of the *BabA* gene in subjects suffering from advanced stages of gastric ulcers, particularly for diagnostic procedures. The results highlight the necessity of studies regarding the *BabA* gene in clinical settings to mitigate the risk of significant gastric ulcers and improve treatment options.

Keywords: Baba, Gastric Ulcer, Helicobacter pylori, PCR.

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1. Introduction

Helicobacter pylori is a microaerophilic bacterium found in people's stomachs, one of the Gram-negative bacteria. It causes infections; in addition, it is the primary causative agent of some gastrointestinal disorders such as chronic gastritis, peptic ulcers, and gastric cancer [1]. Almost the entire global population has *H. pylori*, but its occurrence depends on geographic regions, socio-economic status, and age groups [2]. This organism's ability to endure in the harsh, extremely acidic environment of the stomach is related to a unique system that enables it to neutralize stomach acid. It survives by burrowing into the stomach's mucus lining [3]. Because *H. pylori* can adhere to the host's stomach epithelium, it is well-

known that it can survive, which protects it from being expelled through the stomach's peristaltic contractions and from destruction by stomach acid. *H. pylori* uses several adhesion proteins to connect with the stomach epithelium, such as the BabA protein, which is transcribed from the BabA gene. Blood group antigen-binding adhesion (BabA) is a necessary protein found in the outer membrane that facilitates the initial colonization of the gastric mucosa by *H. pylori* by binding the bacteria to the Lewis b antigen [4]. The bacterial colonization of the stomach epithelium depends on this adherence, which enables it to provoke inflammation and ultimately cause ulcers [5, 6].

H. pylori infection does not usually cause symptoms; however, once it causes gastric ulcers, it can lead to significant agony and pain, along with other symptoms like bleeding and stomach perforation. *H. pylori* has been shown to cause 70-90% of all peptic ulcers [7]. BabA had been linked to aggressive *H. pylori*, which were more virulent and which were more likely to result in chronic infections followed by inflammation and ulcer formation [8]. There is evidence that *H. pylori* strains besides the BabA gene can be linked to more serious stomach conditions like gastric ulcers [9, 10].

Concerning the diagnosis, the BabA gene carrier strains of *H. pylori* must be diagnosed by first identifying the presence of the respective gene. In recent years, developed polymerase chain reaction (PCR)-based diagnostic instruments have dramatically expanded, enabling the testing and isolation of the BabA gene in clinical isolates. It enables the detection of *H. pylori* with a BabA adhesion factor. It also allows for diagnosing BabA at much lower levels than other methods, like the urea breath test and histology examinations [11, 12]. This time, we intend to find the BabA gene in patients diagnosed with gastric ulcers and infected with *H. pylori*. Seeking to understand the pathogenesis of ulcers further, we are attempting to improve knowledge regarding the role of this gene in the advancement of gastric ulcers and ascertain whether it may function as a sign for diagnosis.

The primary objective is to look for the BabA adhesion gene in patients suffering from gastric ulcers stemming from *H. pylori* infection, while the secondary objective aims to study the correlation between the presence of the BabA gene and the degrees of gastric ulcers. The goal is to establish the accuracy of clinical diagnosis using PCR methods to detect BabA genes.

2. Literature Review

Human gastric epithelium is home to the most prevalent gastrointestinal pathogen, Helicobacter pylori. A H. pylori infection alone cannot be the causing of the symptoms; rather, the variety of factors, including the virulence factors of the infecting pathogens, the regional weather, virulence genes, immunity of the host, genetic heritage, dietary habits, also the microbiota of the stomach and intestinal tract, lead to the development of serious clinical consequences [13].

The main cause of gastroesophageal reflux disease (GERD) is hyperadherence of H. pylori, and the virulence of these bacteria may also be attributed to this. Gastric pathology includes gastritis, peptic ulcer disease (PUD), and other conditions affecting the digestive system, such as non-erosive reflux disease (NERD) and erosive reflux disease (ERD). The BabA2 gene encodes BabA, one of the primary adhesins that causes H. pylori to attach to the fucosylated blood type antigens Lewis. BabA belongs to a paralogous family whose outer membrane proteins have been characterized. It may also directly contribute to pathogenesis due to its ability to bind to Lewis b, which helps H. pylori colonize the stomach. Bacterial binding to Lewis b is mostly seen in type 1 isolates, suggesting that no single factor can be held entirely accountable for predicting the outcome of an H. pylori infection. However, compared to a strain that lacks these characteristics, an adherent strain may have a greater therapeutic impact [14].

The virulence factors specifically, cagA (cytotoxin-associated gene), vacA (vacuolating cytotoxin A), and outer membrane proteins (OMP), including sialic acid-binding adhesin (SabA), blood group antigen-binding adhesin (BabA), and outer inflammatory protein (OipA), play a crucial role in colonization and the development of a persistent infection that lasts for decades in the host. CagA is a member of the cag pathogenicity island (CagPAI), which harbors numerous virulent genes and is responsible for aberrant cellular signals that result in gastritis linked to H. pylori. Likewise, once vacA enters the host, it is responsible for cellular processes such as cell death, membrane channel creation, and the initiation of the proinflammatory response [15]. OMPs and the Type IV secretory system facilitate H. pylori colonization. Different H. pylori strains vary in the quantity, type, and combination of OMP, which allows H. pylori to adapt and evade host immunity.

The antigens of the ABO/Lewis b (Leb) blood type that are expressed in the gastric epithelium's gastrointestinal lining are bound by BabA. According to a recent study, the protective mechanism of antibodies mimics BabA's binding to the ABO blood group glycans during colonization, resulting in variations in disease severity. This suggests that antibody binding is protective and indicates non-antibiotic treatment regimens for severe gastric diseases [16]. BabA's Leb-carbohydrate binding domain (CBD) demonstrates rapid and varied selection through amino acid substitution, improving binding preferences for various human populations and ABO blood group characteristics. BabA can better adapt for optimal binding or enhance its binding affinity in various sites and microenvironments, such as changes in acidity brought on by disorders like PUD or gastric cancer, thanks to these mutations and selection [17].

Furthermore, in patients with gastritis, BabA, cagA, and vacA cooperate to make clinical results more severe. BabA2 + ve strains were found to be more virulent than BabA −ve infections in Indian patients. A few in-vivo experiments have also shown that several factors, including truncated adhesion, slipped strand mispairing (SSM), changes in the 5′ends of CT repeats, the formation of a non-binding allele of BabA due to amino acid changes in BabA2, which results in the formation of BabA1, or the host's innate immunity, can cause BabA expression and, consequently, its binding to Lewis antigen to be lost [18]. Each infected person has a unique and different sequence, resulting in BabA's worldwide diversity.

In conclusion, mutations that are advantageous to the survival and reproduction of bacteria are persistently transmitted in the population and support the development of phenotypic features when multiple selection forces are at work. A crucial adhesion molecule in H. pylori infection is the BabA protein. Its long-term symbiotic evolution with humans is partially

reflected in its adaptive virulence evolution. It is hoped that a thorough understanding of the BabA protein will help with H. pylori infection prevention and therapy.

3. Materials and Methods

This section details how the patients were sampled, how DNA was obtained, and how the BabA gene was amplified through PCR.

3.1. Study Design

Over the course of six months, recruitment was conducted in a tertiary care facility. Patients' consent was obtained before a sample was collected.

3.2. Patient Selection

- Inclusion Criteria: Illness persons who have endoscopically and biochemically checked for gastric ulcers.
- Exclusion Criteria: The study did not include patients diagnosed with advanced gastric carcinoma or who had taken antibiotics to treat *H. pylori* within the last month.

3.3. Sample Collection

During endoscopy, 140 patients with gastric ulcers had biopsy specimens taken, which were later put into sterile bottles and brought to the laboratory for processing.

3.4. Bacteriological Examination

The biopsy samples were then cultured on blood agar, MacConkey, and Columbia agar plates. After that, the plates were put in an incubator overnight to undergo microaerophilic conditions. Non-lactose fermenters and non-oxidase giving negative result reaction to *Helicobacter pylori* and growing on Columbia agar were cultured on selective media with *Helicobacter pylori* supplement. Then, one presumptive *Helicobacter pylori* colony per selective agar plate was sub-cultured and tested by standard methods. The Gram stain, oxidase and catalase test, hydrogen sulfide production, and nalidixic acid sensitivity using a commercially available species kit, API CAMPY, were done and differentiated at the species level by using microbiological and biochemical procedures [19].

3.5. DNA Extraction

We extracted with the DNA Mini Kit (Qiagen, Germany). The procedure consists of homogenizing tissue samples, followed by DNA purification using the reagents and columns provided with the kit. The spectrophotometer (Nanodrop 2000, Thermo Fisher) was used to measure the extracted DNA's concentration and purity.

3.6. Amplification of the BabA Gene using PCR

Well Defined: Below are the primers for deploying polymerase chain reaction on the BabA gene [20]. Forward Primer: 5'- AGC ATC TCG ACG TGC ATC A -3', Reverse Primer: 5'- CGT GTC CAG GAG GCA GAT G -3'.

PCR reaction conditions: DNA, primers, and PCR Master Mix were combined in a total volume of 50 µL. The melting temperature was 95°C for 5 min. The 30 cycles consisted of 95 degrees for 30 seconds, 55 degrees for 30 seconds (this step is called annealing), and 72 degrees for 1 minute (called elongation). The last step involved extending a PCR product at 72 degrees for 10 min. Electrophoresis on a 1.5% agarose gel was used to separate the reaction products.

3.7. Statistical Statement

Due to the large size of the samples, the Chi-square test was used in statistical analysis, as presented in Table 1.

Table 1.
The Chi-Square test.

	Severe Ulcer	**Mild Ulcer**	**Total**
BabA+	A=30	B=35	65
BabA-	C=10	D=25	35
Total	40	60	100

Calculate the odds Ratio, Equation 1:

$$\text{OR} = \frac{A \times D}{B \times C} \qquad (1)$$

OR is 2.14 > 1, which means the mutation increases the possibility of severe ulcers. To do the Chi-Square test, we have to make an Observed Table by calculating the expected values, Equation 2.

$$E = \frac{(A+B) \times total\ of severe\ ulcer}{100} \qquad (2)$$

After applying the Equation to the rest of the data (Table 2).

Table 2.
The expected value.

BabA+	26	39	65
BabA-	14	21	35
Total	40	60	100

P-value is 0.086, which is considered non-significant due to non-normal distribution. Therefore, we must apply Fisher's exact test in SPSS IBM.30.0.0 @ windows11. The P-value = 0.0103 < 0.05, which is considered significant [21]. Figures 1 and 2 show the relationship between the BAB gene and its severity among patients aged as listed below:

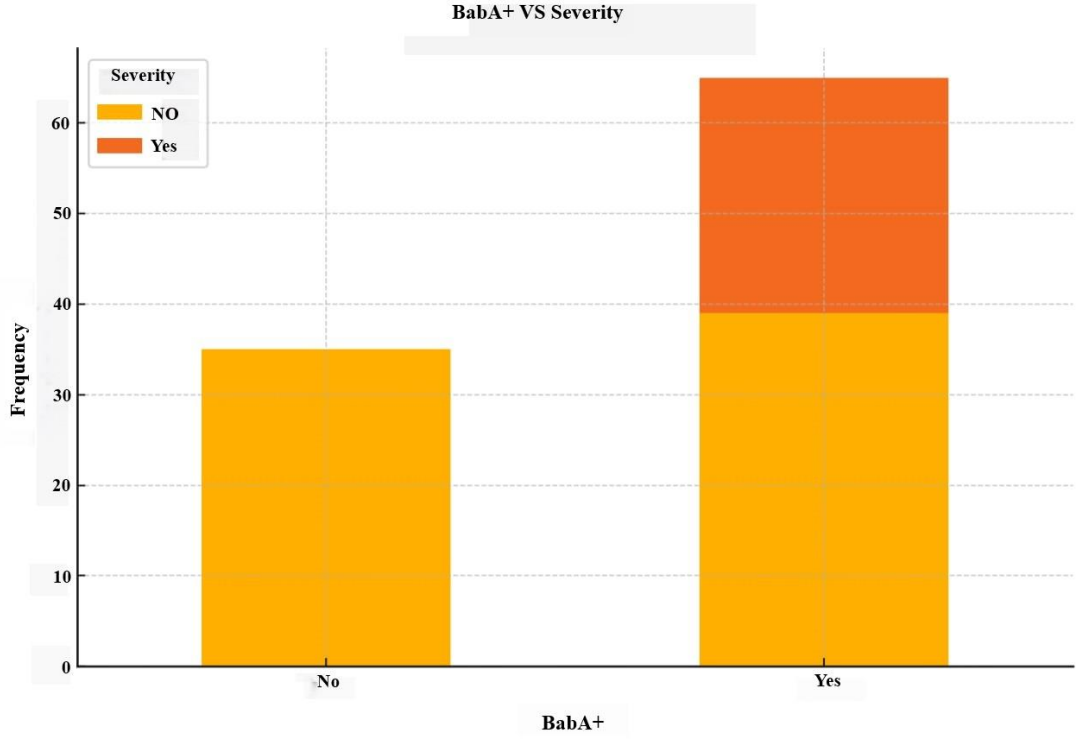

Figure 1.
Relationship between the BAB gene and its severity among patients.

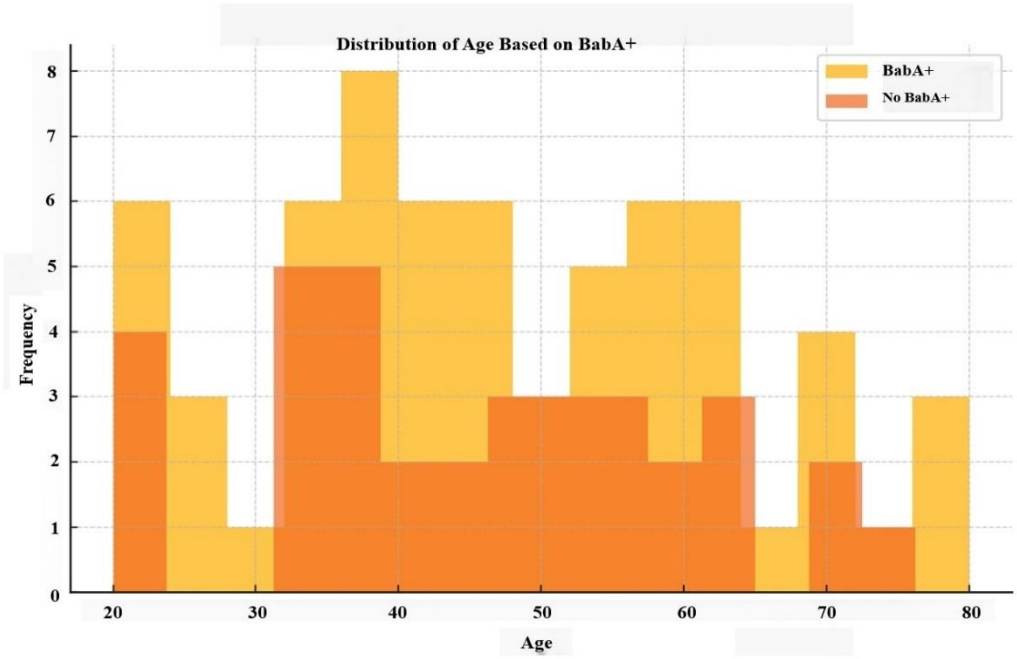

Figure 2.
Distribution of age based on the BAB gene.

4. Results

Hundred bacterial isolates were diagnosed based on the tests used for this purpose, which include the shape of colonies, the color of the colony under a slide stained with Gram stain, the urease enzyme biochemical test, and the oxidase, catalase, and motility tests to observe whether bacteria are motile or non-motile. The isolates were subjected to other tests in addition to the previous tests, which included testing the growth of the bacteria at temperatures of 52 °C and 42 °C, and conducting sensitivity tests for the two antibiotics, cephalothin and nalidixic acid. A hundred isolates were analyzed for the BabA gene. The PCR results and clinical data are listed in Table 3, and Figure 3 shows the Agarose Gel Electrophoresis showing PCR amplification of BabA results.

Table 3.
PCR Results for BabA Gene Detection in *H.* pv/o/Infectious CD Patients with Gastric Ulcers.

Statistic	PCR Result (BabA Gene) Positive	PCR Result (BabA Gene) Negative	Severe Ulcer	Moderate Ulcer	Mild Ulcer	Male	Female
Mean Age	49.20	50.71	49.85	49.25	49.47	50.12	49.23
* (SD)	17.49	17.36	16.84	17.25	18.28	17.49	17.09
** MA	20	20	20	20	20	20	20
** MA	80	80	80	80	80	80	80

Note: *(SD) = Standard Deviation; **(MA) = Maximum Age.

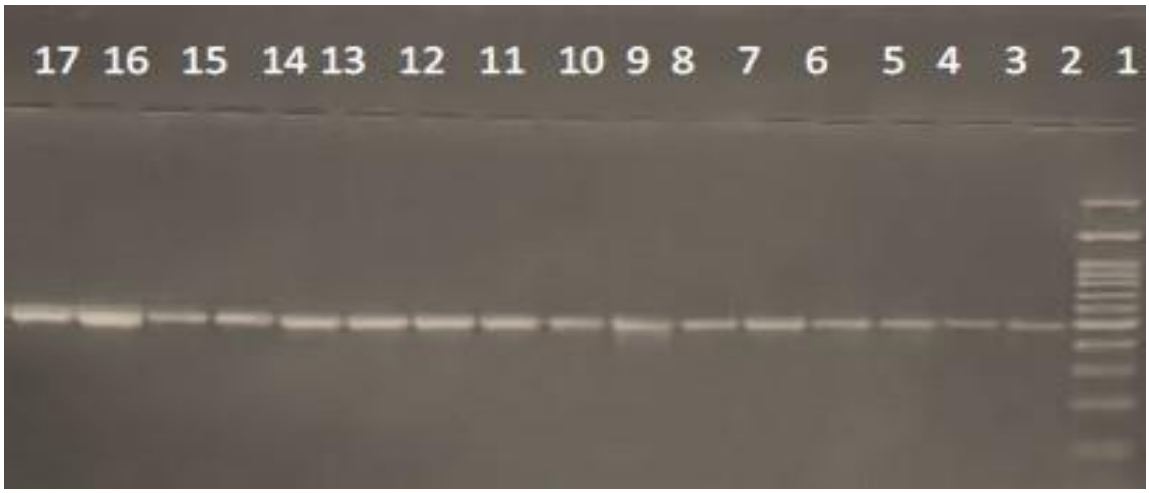

Figure 3. Agarose Gel Electrophoresis Showing PCR Amplification of BabA Gene. Lane 1: DNA ladder (100 bp).
Lane 2-17: Positive samples for BabA gene (band at -500 bp).

5. Discussion

This research verifies the *BabA* gene's essential function in forming gastric ulcers. *BabA* gene PCR detection in *H. pylori* strains of ill persons with gastric ulcers is proportional to the degree of ulceration, which indicates that infection by BabA-positive strains may be more severe.

By binding to the Lewis B antigen found on gastric cell membranes, the BabA gene, which produces the BabA protein, helps bring H. pylori to the stomach epithelium [18]. This adhesion is necessary for establishing a chronic infection that may lead to ulceration. The findings of this research are based on other studies that show that BabA-positive strains are more likely to result in severe gastric pathology [22-24]. In addition to these study findings, the BabA gene PCR detection offers better sensitivity and rapidness for diagnosing than the more conventional methods like the urea breath test or histology [25].

In patients positive for BabA, 40% had severe ulcers, which makes the presence of the BabA gene highly associated with severe ulcerations. These strains are likely more virulent, which makes the hypothesis of H. pylori carrying the BabA gene astonishingly accurate [21]. Using PCR to detect the BabA gene will provide accurate results in diagnosing H. pylori with severe consequences, making it valuable in clinical practice [26, 27].

Detection of BabA contributes massively to clinical practices, especially for patients at higher risk of developing gastric ulcers, so more precise treatment patterns can be administered [28, 29]. Further research should be carried out to cement the link between the presence of the BabA gene and long-term treatment outcomes such as relapse and resistance [30].

6. Conclusion

This study provides compelling reasons to suggest that the BabA gene is fundamental in the pathogenesis of gastric ulcers induced by H. pylori. Identifying the BabA gene through PCR-based assays will undoubtedly aid in the reliable diagnosis and determination of risk for severe gastric pathology. The clinical approach to diagnosing and treating H. pylori-associated infections and gastric ulcers would greatly benefit from implementing BabA gene diagnostic procedures.

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