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EVALUATION OF HEMATOLOGICAL PARAMETERS, AND MOLECULAR ALTERATIONS IN THE EXON-1-3 OF APEX1 GENE AS POTENTIAL CONTRIBUTOR TO GASTRIC CANCER RISK

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Abstract

Background: Gastric cancer remains a significant global health concern, with its progression influenced by a myriad of genetic and environmental factors. Among these factors, the interaction between host genes and infectious agents, such as Helicobacter pylori (H. pylori), has garnered attention for its potential role in gastric carcinogenesis.

Objectives: This study aimed to investigate the association between H. pylori infection and alterations in hematological parameters, as well as the detection of H. pylori APEX-1 genes such as (EXON-1, EXON-2 & EXON-3) using polymerase chain reaction (PCR) targeting the APEX1 gene. Blood samples were obtained from gastric patients and a control group, and hematological parameters including white blood cell counts, red blood cell count, hemoglobin, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were measured.

Results: The results indicated no significant differences in WBC, RBC, HGB, HCT, MCV, MCH, and MCHC between gastric patients and the control group ($p > 0.05$). However, a statistically significant decrease in platelet count was observed in gastric patients compared to the control group (283 ± 67.50 vs. 261 ± 57, $p = 0.034$). Additionally, DNA extracted from H. pylori positive blood samples underwent PCR targeting APEX1 gene, confirming H. pylori positivity. Positive PCR results were observed in samples S1 and S2, displaying a product size of 342 base pairs. Moreover,

samples S35, S36, S37, and S71 exhibited positive PCR results in exon 5 of APEX1, with a product size of 292 base pairs.

Conclusion: This study provides insights into the potential correlation between H. pylori infection, hematological parameters, and genetic alterations in the APEX1 gene. Further research is warranted to explore the clinical implications of these findings in the context of gastric cancer development.

Keywords: H. pylori infection, Hematological parameters, APEX1 gene, Gastric cancer, Polymerase chain reaction (PCR), Genetic alterations

Introduction

Gastric cancer remains a significant global health concern, with its progression influenced by a myriad of genetic and environmental factors (1). Among these factors, the interaction between host genes and infectious agents, such as Helicobacter pylori (H. pylori), has garnered attention for its potential role in gastric carcinogenesis (2). The APEX1 gene, encoding the apurinic/apyrimidinic endonuclease 1, plays a crucial role in DNA repair and maintenance of genomic stability. Emerging evidence suggests that genetic polymorphisms in the APEX1 gene may contribute to individual variations in susceptibility to H. pylori infection and subsequent gastric cancer progression (3).
APEX1, also known as APE1 is a gene pivotal to cellular homeostasis as it encodes a multifunctional protein involved in DNA repair, redox regulation, and transcriptional regulation (4). The multifaceted functions of APEX1 play a critical role in maintaining genomic stability and integrity by actively repairing oxidative DNA damage induced by diverse endogenous and exogenous factors (5).

Among the exogenous factors contributing to oxidative DNA damage in the gastric mucosa, Helicobacter pylori (H. pylori) stands out as a significant pathogen (6). This Gram-negative bacterium colonizes the human stomach and is implicated in chronic gastritis, peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma (7). Notably, H. pylori infection represents the foremost risk factor for gastric cancer, accounting for nearly 90% of non-cardia gastric cancer cases globally (8). Despite this high prevalence, only a fraction of H. pylori-infected individual's progress to gastric cancer, suggesting a complex interplay of host genetic factors in modulating susceptibility and outcomes of the infection (9).

Polymorphisms within the APEX1 gene have emerged as potential determinants influencing the risk of gastric cancer and its precancerous lesions in H. pylori-infected individuals (10). These polymorphisms, capable of altering the expression or activity of the APEX1 protein, have been the focus of numerous studies (11). Additionally, their impact extends beyond susceptibility, as APEX1 gene polymorphisms may shape the biological behavior and treatment response of gastric cancer. Through their influence on DNA repair capacity, apoptosis, angiogenesis, and drug resistance in tumor cells, these genetic variations introduce a layer of complexity to the clinical course of gastric cancer (12, 13).
Understanding the intricate role and mechanisms governed by APEX1 gene polymorphisms in H. pylori-infected individuals is imperative for unraveling the pathogenesis of gastric cancer. The integration of genetic information into clinical decision-making may pave the way for more targeted and effective interventions, thereby advancing the fields of gastric cancer research and precision medicine.

Methodology

Study Population Selection:

- **Inclusion criteria:** Individuals diagnosed with gastric cancer, confirmed H. pylori infection, and available genetic data for APEX1 gene polymorphisms.

- **Exclusion criteria:** Patients with a history of other malignancies, concurrent infectious diseases, or incomplete genetic information.

Genotyping of APEX1 Polymorphisms:

- **DNA Extraction:**
- Genomic DNA was extracted from peripheral blood samples and gastric biopsy specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), respectively, following the manufacturer's protocols (14).
- **APEX1 Genotyping:** Polymerase chain reaction (PCR) amplification was performed for targeted regions of the APEX1 gene associated with known polymorphisms. Genotyping was carried out using validated methods, such as PCR-restriction fragment length polymorphism (RFLP) (15).
- **Gel electrophoresis:** Following the PCR amplification, gel preparation was imperative for the subsequent visualization of DNA bands. A 2% agarose gel was meticulously crafted by dissolving 0.7g of agarose in 35ml of 1X TBE buffer. The solution was subjected to brief boiling in an oven for 1 to 2 minutes. Subsequently, the temperature was reduced to 60oC, and 1.5 to 2µl of ethidium bromide was added to facilitate band visualization under a UV transilluminator. The molten agarose gel was carefully poured into a casting tray, and a comb was strategically inserted to create wells for loading the PCR samples. Solidification at room temperature required approximately 15 to 20 minutes, following which the comb was removed. The gel tank was filled with 1X TBE buffer. Upon gel solidification, 2µl of each PCR product was loaded into individual wells. Gel electrophoresis was conducted at 80V for 50 minutes with a current set at 500 Amps. Post-electrophoresis, bands corresponding to the amplified gene segments were observed under a UV illuminator, adhering to specific running conditions (16).

Exon number	Primer sequence	Primer length	TM (%)	GC (%)	Product size
1	**F** GAGGGAGGCGAGGCTAAG	18	58.87	66.67	342bps
	R CCCTCACCCACGAAACTAGA	20	58.74	55.00	
2	**F** GCTGGTTTCATGATTTCTTTGC	22	57.08	40.91	191bps
	R GCTTCTAGGAAGAAGGGCTGA	21	59.17	52.38	
3	**F** TTGGAAACCACCAGCTTTTT	20	56.24	40.00	299bps
	R GGGGTGACTAAACCCTAAGACC	22	59.76	54.55	

Table 1: Primers designed and optimization used in the study

H. pylori Detection and Confirmation:

- **H. pylori testing:** Presence of H. pylori infection was confirmed through non-invasive methods such as urea breath test, stool antigen test, and serological assays.
- **Confirmation of Gastric Cancer Diagnosis:** Histopathological examination of gastric biopsy specimens confirmed the diagnosis of gastric cancer.

Data Collection:

- **Clinical and Demographic Information:** Comprehensive clinical data, including age, gender, histological subtype of gastric cancer and treatment history were collected.
- **APEX1 Genotype Data:** Genotyping results for APEX1 polymorphisms were recorded and individuals were categorized based on their APEX1 genetic profiles.

Statistical Analysis:

- **Association Analysis:** Statistical tests, such as chi-square tests or logistic regression, were employed to assess the association between APEX1 gene polymorphisms and the risk of gastric cancer in H. pylori-infected individuals.

- **Subgroup Analysis:** Stratified analyses were conducted to evaluate the influence of APEX1 polymorphisms on different aspects including disease progression and response to treatment.

Functional Analysis of APEX1 Variants:

- **In silico Tools:** Computational tools were utilized to predict the potential functional impact of identified APEX1 variants.
- **Functional Assays:** Experimental assays including luciferase reporter assays or functional studies in cell lines were employed to validate the functional significance of identified APEX1 variants.

Biopsy Samples:

Biopsy specimens from the gastric mucosa were collected and utilized for histopathological confirmation of H. pylori infection and gastric cancer diagnosis.

Ethical Considerations:

- **Ethical Approval:** The study protocol was submitted to the Institutional Review Board (IRB) for ethical approval before initiation.
- **Informed Consent:** Informed consent was obtained from all participants, outlining the purpose of the study, potential risks, and benefits.

Results

Evaluation of blood profiling among patients and control

Table 1: Comparison of Complete Blood Count Parameters in Patients and Control Group

Parameter	Reference Range	Patients	Control Group	p-value
WBC	4.0-11.0x10^9/L	8.5 ± 2.23	7.2 ± 2.31	0.067
RBC	4.0-6.0x10^{12}/L	4.2 ± 0.39	4.5 ± 0.62	0.102
HGB	12.0-16.0 g/dL	11.8 ± 1.21	14.1 ± 1.8	0.185
HCT	38%-50%	42 ± 3.20	44 ± 2	0.091
PLT	150-450x10^9/L	283 ± 67.50	261± 57	0.034
MCV	80-100 fL	92 ± 5.41	86 ± 3	0.119
MCH	27-33 pg	30 ± 2.51	31 ± 2	0.271
MCHC	32-36 g/dL	34.5 ± 1.5	35.1 ± 1.5	0.083

**White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin (HGB), Hematocrit (Hct), Platelet (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), MCHC (Mean Corpuscular Hb Concentration)

Analysis of Ethidium Bromide-Stained DNA in H. pylori Positive Samples

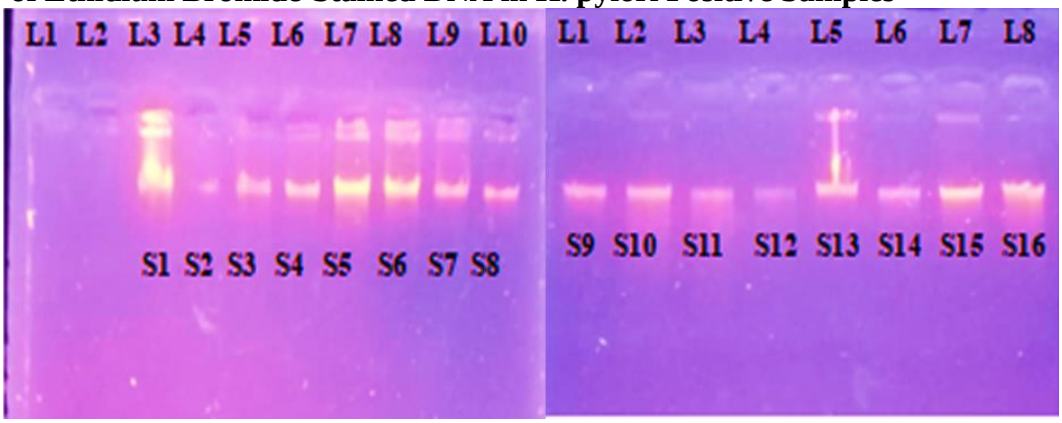

Figure 1: The electropherogram displays ethidium bromide-stained DNA obtained through the DNA extraction process. Sample identities S1, to S8, and S9, to S16 correspond to distinct samples that are confirmed to be positive for H. pylori.

Molecular characterization of H. pylori Positive Blood Samples & PCR Amplification of APEX1 Gene Exon-1

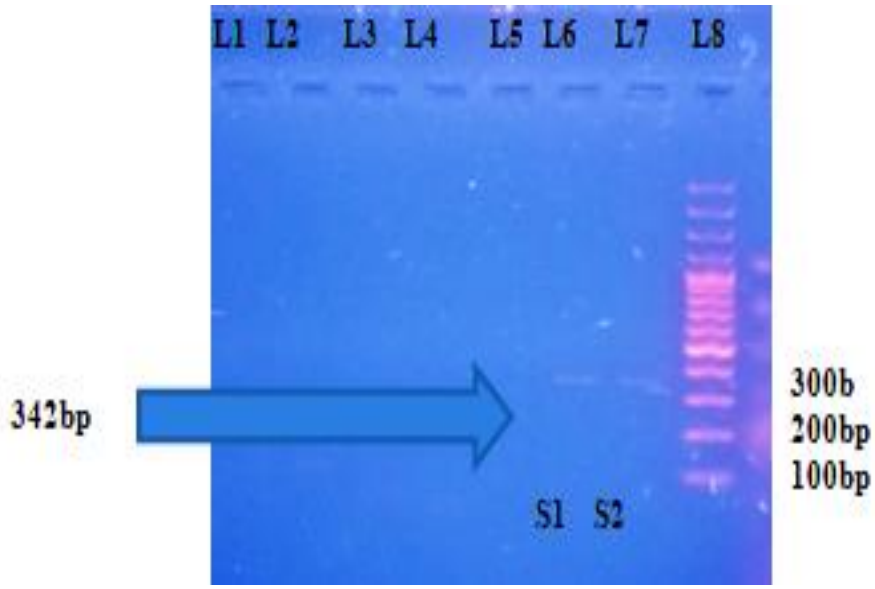

Figure 2: Following the DNA extraction from H. pylori positive blood samples, polymerase chain reaction (PCR) targeting the APEX1 gene was conducted. The analysis confirmed H. pylori positivity, amplifying the specific region corresponding to exon 1 of APEX1. Notably, Lane 6 (S1) and Lane 7 (S2) displayed positive PCR results, exhibiting a product size of 342 base pairs, with Lane 8 serving as a reference for the 100-base pair ladder. The sample identity is denoted as 'S'.

APEX1 Gene Exon-2

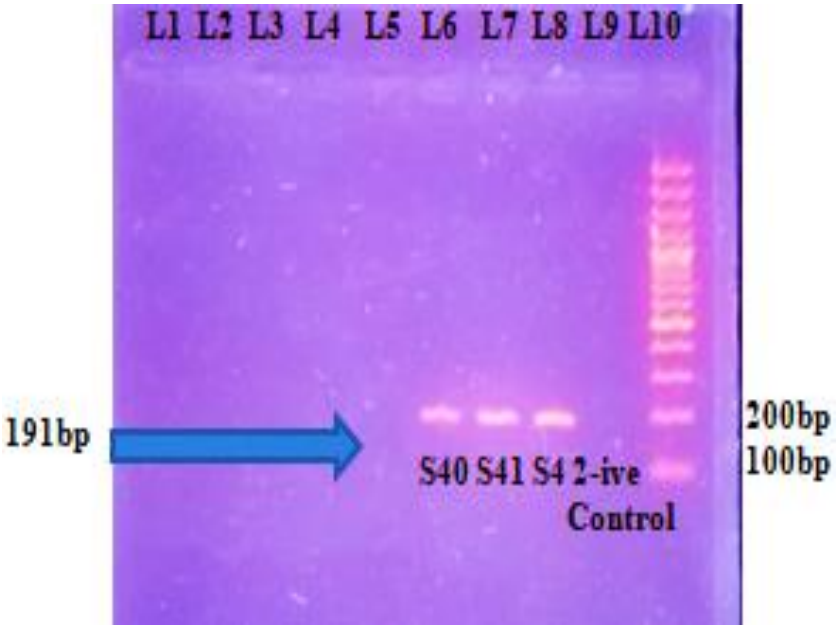

Figure 3: Following the extraction of DNA from both H. pylori positive and negative blood samples, a PCR assay targeting the APEX1 gene was conducted. The results revealed the presence of H. pylori in the samples, with specific amplification occurring in exon 2 of the APEX1 gene. Notably, samples S40 in lane L6, S41 in lane L7, and S42 in lane L8 exhibited positive PCR results, displaying a product size of 191 base pairs. In contrast, sample L9 represented a H. pylori negative sample, and lane L10 served as the reference for a 100-base pair ladder.

APEX1 Gene Exon-3

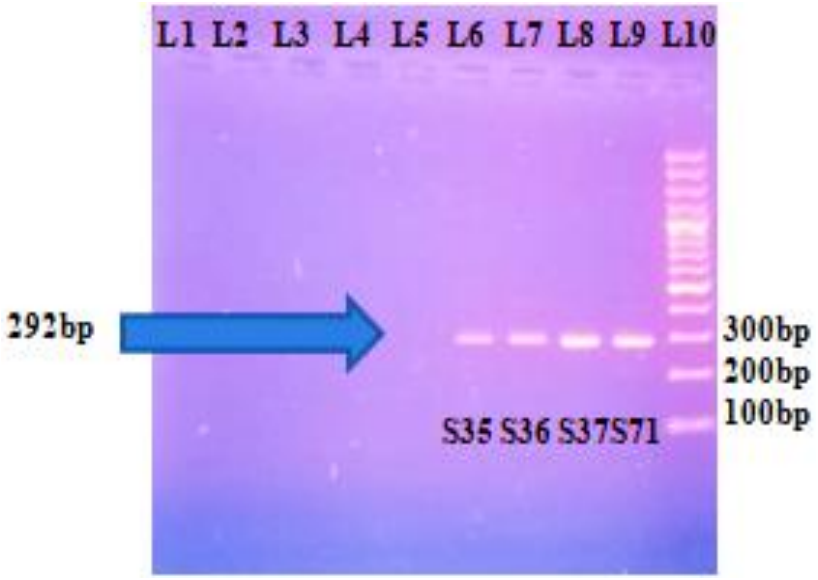

Figure 4: Following the extraction of DNA from an H. pylori positive blood sample, PCR targeting the APEX1 gene was conducted. The samples revealed a positive H. pylori result, with specific amplification occurring in the exon 3 region of APEX1. Noteworthy PCR positive results were observed in samples S35 in lane L6, S36 in lane L7, S37 in lane L8, and S71 in lane L9, all exhibiting a product size of 292 base pairs. Lane L10 served as a reference for a 100-base pair ladder.

Discussion

Chronic inflammation arising from H. pylori infection leads to epigenetic alterations, such as DNA methylation, a crucial mechanism regulating gene expression (6). Despite advancements in understanding these mechanisms, further research is needed to elucidate how H. pylori induces mutations/polymorphisms that contribute to gastric cancer. H. pylori exerts a substantial impact on various molecular mechanisms, resulting in genomic instability.

One mechanism through which H. pylori induces gastric cancer involves DNA damage due to chronic inflammation. DNA repair pathway enzymes, including APEX-1, OGG-1, and PARP-1, play a crucial role in maintaining genetic stability. The interaction of bacterial strains with host genetic polymorphisms may influence the effectiveness of these enzymes in repairing damage, ultimately leading to H. pylori-associated gastric cancer (17).

A study conducted in China aimed to investigate the cause of APEX-1 T1349G polymorphism and its association with gastric cancer. The findings revealed an increased risk of gastric cancer in individuals with APEX-1 polymorphism who consume alcohol. Additionally, evidence suggests a heightened risk of APEX-1 polymorphism in smokers, as smoking induces the production of reactive oxygen species, leading to DNA adduct formation (18).

This study focuses on H. pylori-induced oxidative stress as a significant factor in gastric cancer development. It is hypothesized that H. pylori, in conjunction with smoking, enhances the production of reactive oxygen species, causing polymorphisms in DNA repair pathway genes (such as APEX1), ultimately contributing to gastric cancer.

Genetic variations in DNA repair genes impact DNA repair capacity and increase the risk of various types of cancers. Research is needed to explore the role of DNA polymorphisms in gastric cancer, as their specific contributions remain unclear. The base excision repair pathway, responsible for removing cytotoxic and mutagenic base lesions, is associated with various cancer types (19). APEX1, located on chromosome 14q11.2-q12 and comprising five exons, is a key gene in this pathway, hydrolyzing 3'-blocking fragments from oxidized or alkylated DNA.

Contrary to a meta-analysis by (20), which focused on APEX1 Asp148Glu polymorphism, our study investigates polymorphisms in all coding regions of APEX1. We aim to provide a comprehensive understanding of the relationship between APEX1 gene polymorphisms and gastric cancer risk, considering a broader scope of genetic variations.

Conclusion

Our study investigated the relationship between H. pylori infection, hematological parameters, and genetic alterations in the APEX1 gene. While no significant differences were observed in WBC, RBC, HGB, HCT, MCV, MCH, and MCHC between gastric patients and the control group, a noteworthy decrease in platelet count was identified in gastric patients. The detection of H. pylori DNA using PCR targeting the APEX1 gene confirmed the presence of H. pylori in blood samples from both gastric patients and controls. Positive PCR results in exon 1 and exon 2 of APEX1 were observed suggesting a potential association between H. pylori infection and genetic alterations in these regions. These findings underscore the complex interplay between H. pylori infection, hematological parameters, and genetic factors, particularly in the context of the APEX1 gene. The significant decrease in platelet count among gastric patients may indicate the hematological impact of H. pylori infection. Moreover, the detection of H. pylori DNA in blood samples emphasizes the systemic nature of the infection.

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