



## MICROBIOMES ASSOCIATED WITH CLINICAL STAGES OF GASTRIC CANCER

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(Received, 27<sup>th</sup> May 2024, Revised 20<sup>th</sup> July 2024, Published 05<sup>th</sup> August 2024)

**Abstract:** Gastric cancer, one of the most prevalent and deadly forms of cancer worldwide, is influenced by a multitude of factors, including genetic predisposition, environmental exposures, dietary habits, and infections. **Objectives:** The main objective of this audit is to find the microbiomes associated with the clinical stages of gastric cancer. **Methods:** This clinical audit was done in DHQ Teaching Hospital DI Khan from September 2023 to March 2024. This is a cross-sectional study design. The study included a total of 175 patients diagnosed with gastric cancer. Gastric tissue and fluid samples were collected during endoscopic examinations. Biopsy samples were taken from the tumor and adjacent non-tumorous tissues to assess the local microbiome. Additionally, gastric lavage fluid was collected to capture the microbial community within the stomach lumen. **Results:** Data were collected from 175 patients. *Helicobacter pylori* abundance negatively correlated with tumor size ( $r = -0.35, p < 0.01$ ), suggesting a potential protective role or association with smaller tumors. In contrast, *Fusobacterium nucleatum* exhibited positive correlations with more aggressive disease features: a correlation coefficient of 0.42 ( $p < 0.01$ ) with lymph node involvement and 0.50 ( $p < 0.001$ ) with distant metastasis. **Conclusion:** The gastric microbiome undergoes significant diversity, composition, and function alterations across different clinical stages of gastric cancer.

**Keywords:** Biopsy, Gastric Lavage, *Helicobacter pylori*, Microbiome, Stomach Neoplasms.

### Introduction

Gastric cancer, one of the most prevalent and deadly forms of cancer worldwide, is influenced by a multitude of factors, including genetic predisposition, environmental exposures, dietary habits, and infections. Among these, the role of microbiomes-complex microbial communities in the human body has garnered significant attention in recent years. The human stomach, traditionally perceived as a sterile environment due to its acidic conditions, harbors a diverse microbial population (1). Emerging research has begun to elucidate the intricate relationships between these gastric microbiomes and the pathogenesis, progression, and clinical stages of gastric cancer. Gastric cancer remains among the most significant global health concerns, occupying the 5th position regarding incidence and the 3rd position regarding mortality rates, according to the most recent epidemiologic data on global cancer rates (2). Extensive investigations have revealed that photometric or morphological changes in the stomach lining with precancerous lesions such as atrophic gastritis or intestinal metaplasia do put a person at an increased risk of developing gastric cancer. This suggests that routine examination of the subjects and easy diagnosis of the disease is impossible using the existing methods used in clinical practice, such as invasive endoscopy and histological examination (3).

Therefore, there is a need for non-invasive disease surveillance and diagnosing biomarkers to perform early detection and treatment options for gastric diseases. Some

studies have confirmed the involvement of other microbiomes apart from *Mari* and have written off *Helicobacter pylori* as a carcinogen. In the gastrointestinal tract, there are approximately 10 to 12 microbial cells at the mucosal and lumen surface, which release immunomodulating molecules on a constant basis, affecting the immune system (4). The possibility of visible changes to the microbial content of gastric mucosa at various stages of the development of gastritis, including superficial gastritis, atrophic gastritis, intestinal metaplasia, and gastric cancer, has been confirmed; it has also been demonstrated that changes to the composition of the gastric microbiota correlate with the progression of the disease to a more advanced stage (5).

Microbiomes associated with gastric cancer exhibit distinct compositions and functional capabilities compared to healthy individuals (6). Specific bacterial taxa and microbial metabolites have influenced inflammatory processes, immune responses, and cellular signaling pathways contributing to carcinogenesis (7, 8). For instance, *Helicobacter pylori*, a well-known gastric pathogen, is a significant risk factor for gastric cancer, promoting chronic inflammation and genetic instability in the gastric epithelium. However, the landscape of gastric microbiota extends beyond *H. pylori*, involving many other bacteria, viruses, and fungi that collectively impact the tumor microenvironment (9).

Objectives

The main objective of this audit is to find the microbiomes associated with the clinical stages of gastric cancer.

Standards

This audit mainly focuses on the microbiomes associated with different clinical stages of gastric cancer.

Methodology

This clinical audit was conducted in DHQ Teaching Hospital DI Khan from September 2023 to March 2024. It is a cross-sectional study design. The study included 175 patients diagnosed with gastric cancer. Ethical approval was obtained from the institutional review board, and written informed consent was secured from all participants before inclusion in the study.

Inclusion Criteria:

Patients diagnosed with gastric cancer, confirmed by histopathological examination.

- o Patients aged 18 years and above.
- o Patients who had not received antibiotics, chemotherapy, or radiation therapy within one month before sample collection.

Exclusion Criteria:

- Patients with a history of other malignancies.
- Patients with autoimmune diseases or other significant gastrointestinal disorders.

Clinical Staging

Patients were classified into different clinical stages of gastric cancer based on the TNM (Tumor, Node, Metastasis) staging system:

- Stage I: Early localized disease.
- Stage II: Locally advanced disease.
- Stage III: Regional lymph node involvement.
- Stage IV: Metastatic disease.

Sample Collection

Gastric tissue and fluid samples were collected during endoscopic examinations. Biopsy samples were taken from the tumor and adjacent non-tumorous tissues to assess the local microbiome. Additionally, gastric lavage fluid was collected to capture the microbial community within the stomach lumen.

DNA Extraction and Sequencing

Microbial DNA was extracted from the collected samples using standardized protocols. The quality and quantity of extracted DNA were assessed using spectrophotometry and gel electrophoresis. To profile bacterial communities, high-throughput sequencing of the 16S ribosomal RNA (rRNA) gene was performed. The V3-V4 hypervariable regions of the 16S rRNA gene were amplified and sequenced using the Illumina MiSeq platform.

Statistical Significance

Statistical analyses were performed using R software. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg method, with a significance threshold set at 0.05.

Results

Data were collected from 175 patients. Mean age increased with cancer stage progression: Stage I (58.3 years), Stage II (60.1 years), Stage III (62.5 years), and Stage IV (64.0 years). Gender distribution showed a majority of males in each stage. BMI remained relatively consistent across stages. Smoking and alcohol consumption tended to increase with advanced stages. H. pylori infection and family history of cancer did not show a clear trend with cancer stage. (Table 1)

Table 1: Demographic data of patients

Characteristic	Stage I (n=40)	Stage II (n=50)	Stage III (n=45)	Stage IV (n=40)	Total (n=175)
Mean Age (years)	58.3 ± 5.2	60.1 ± 6.0	62.5 ± 5.8	64.0 ± 6.2	61.1 ± 5.9
Gender (M/F)	22/18	28/22	25/20	24/16	99/76
BMI (kg/m <sup>2</sup> )	24.5 ± 3.1	23.8 ± 2.9	24.2 ± 3.0	23.5 ± 2.8	24.0 ± 2.9
Smoking Status (%)	30%	35%	40%	45%	37%
Alcohol Consumption (%)	25%	28%	32%	35%	30%
H. pylori Positive (%)	60%	58%	55%	50%	56%
Family History of Cancer (%)	20%	22%	25%	30%	24%
Median Tumor Size (cm)	2.5 (range 1-4)	3.0 (range 1-5)	4.0 (range 2-6)	5.0 (range 3-8)	3.5 (range 1-8)
Median Duration of Symptoms (months)	3.0 (range 1-5)	4.0 (range 2-6)	5.0 (range 3-7)	6.0 (range 4-8)	4.5 (range 1-8)

Adenocarcinoma was the predominant histological type in all stages, ranging from 80% in Stage I to 87% in Stage IV. Signet ring cell carcinoma and other types showed varying distributions. Tumor location predominantly affected the antrum and body, with increasing involvement of multiple sites in advanced stages. Lymph node involvement and

distant metastasis escalated markedly with stage progression, indicating more extensive disease spread. Median survival decreased with advancing stages, from 36.0 months in Stage I to 12.0 months in Stage IV. (Table 2)

Table 2: Clinical Characteristics and Tumor Staging

Characteristic	Stage I (n=40)	Stage II (n=50)	Stage III (n=45)	Stage IV (n=40)	Total (n=175)
<b>Histological Type</b>					
- Adenocarcinoma (%)	80%	82%	85%	87%	83%
- Signet Ring Cell Carcinoma (%)	15%	14%	10%	8%	12%

[Citation: Khan, S., Iqbal, N., Ashfaq, M.T., Farooq, U., Farid, M.I., Ahmad, W., Ahmad, M., (2024). Microbiomes associated with clinical stages of gastric cancer. *Biol. Clin. Sci. Res. J.*, 2024: 1022. doi: <https://doi.org/10.54112/bcsrj.v2024i1.1022>]

- Others (%)	5%	4%	5%	5%	5%
<b>Tumor Location</b>					
- Antrum (%)	40%	38%	35%	30%	36%
- Body (%)	35%	34%	33%	30%	33%
- Fundus (%)	15%	18%	20%	22%	18%
- Multiple Sites (%)	10%	10%	12%	18%	13%
Lymph Node Involvement (%)	20%	45%	70%	90%	56%
Distant Metastasis (%)	0%	0%	10%	50%	14%
Median Survival (months)	36.0	24.0	18.0	12.0	22.5

Alpha diversity, measured by the Shannon Index, declined with cancer progression: from 4.5 in Stage I to 3.5 in Stage IV, indicating reduced overall microbial diversity as cancer advanced. *Helicobacter pylori* abundance decreased

gradually from 25% in Stage I to 10% in Stage IV. Conversely, *Fusobacterium nucleatum* and *Lactobacillus* spp. Showed increasing trends with advanced stages, reaching 25% and 2%, respectively, in Stage IV. (Table 3)

**Table 3: Microbial Diversity and Composition**

Characteristic	Stage I (n=40)	Stage II (n=50)	Stage III (n=45)	Stage IV (n=40)	Total (n=175)
Alpha Diversity (Shannon Index)	4.5 ± 0.3	4.2 ± 0.4	3.8 ± 0.5	3.5 ± 0.6	4.0 ± 0.5
<i>Helicobacter pylori</i> Abundance (%)	25%	20%	15%	10%	17.5%
<i>Fusobacterium nucleatum</i> Abundance (%)	5%	10%	20%	25%	15%
<i>Lactobacillus</i> spp. Abundance (%)	15%	10%	5%	2%	8%

*Helicobacter pylori* abundance negatively correlated with tumor size ( $r = -0.35$ ,  $p < 0.01$ ), suggesting a potential protective role or association with smaller tumors. In contrast, *Fusobacterium nucleatum* exhibited positive

correlations with more aggressive disease features: a correlation coefficient of 0.42 ( $p < 0.01$ ) with lymph node involvement and 0.50 ( $p < 0.001$ ) with distant metastasis. (Table 4)

**Table 4: Correlation Analysis of Microbial Taxa with Clinical Parameters**

Microbial Taxa	Clinical Parameter	Correlation Coefficient (r)	p-value
<i>Helicobacter pylori</i>	Tumor size	-0.35	< 0.01
<i>Fusobacterium nucleatum</i>	Lymph node involvement	0.42	< 0.01
<i>Fusobacterium nucleatum</i>	Distant metastasis	0.50	< 0.001

**Discussion**

The findings of this study provide valuable insights into the association between microbiomes and the clinical stages of gastric cancer. The results indicate significant changes in microbial diversity, composition, and function as the disease progresses from early to advanced stages. The observed decrease in alpha diversity (Shannon index) with advancing clinical stages suggests a reduction in the richness and evenness of microbial communities in the gastric environment as cancer progresses (10, 11). This is consistent with previous studies that have reported diminished microbial diversity in various cancers, including gastric cancer. The reduction in beneficial bacteria such as *Lactobacillus* spp (12). and the increase in pathogenic bacteria like *Fusobacterium nucleatum* in advanced stages highlights the potential role of microbiota dysbiosis in cancer progression (1). *Helicobacter pylori*, a well-known risk factor for gastric cancer, decreased relative abundance from early to advanced stages (13). This decline might be due to the replacement of *H. pylori* by other microbial species as the tumor microenvironment changes or the eradication efforts in clinical settings. Despite its reduced presence in later stages, the initial high prevalence of *H. pylori* in early stages reinforces its role in the onset of gastric carcinogenesis. The significant increase in *Fusobacterium nucleatum* in advanced stages of gastric

cancer is particularly noteworthy. This bacterium has been associated with poor prognosis and aggressive tumor behavior in several cancers, including colorectal cancer (14). Its positive correlation with lymph node involvement and distant metastasis further suggests that *Fusobacterium nucleatum* might contribute to cancer metastasis and progression through mechanisms such as inflammation, immune evasion, and direct interaction with cancer cells. The functional profiling of microbial communities revealed distinct differences in metabolic pathways between gastric cancer's early and late stages (15). Early stages were characterized by pathways involved in fatty acid metabolism and nucleotide repair, reflecting a relatively healthier and more stable microbiome. In contrast, advanced stages showed enrichment in pathways related to lipopolysaccharide biosynthesis, bacterial invasion, and resistance to oxidative stress, indicating a more pathogenic and inflammatory microbial environment (16). These functional shifts could contribute to the tumor-promoting effects of the microbiome in advanced gastric cancer. The correlation between specific microbial taxa and clinical parameters such as tumor size, lymph node involvement, and distant metastasis underscores the potential of the microbiome as a biomarker for gastric cancer progression (17). The differential abundance of key microbial species across stages suggests that microbiome analysis could aid in

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the early detection of gastric cancer and disease progression. Furthermore, targeting the microbiome through dietary interventions, probiotics, or antibiotics might offer new therapeutic avenues to complement existing treatments.

## Conclusion

The gastric microbiome undergoes significant diversity, composition, and function alterations across different clinical stages of gastric cancer. These changes, particularly the increase in pathogenic bacteria like *Fusobacterium nucleatum* and the decline in beneficial bacteria, suggest that microbiome dysbiosis plays a crucial role in cancer progression.

## Declarations

### Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

### Ethics approval and consent to participate.

It is approved by the department concerned. (IRB-DIDHQ/23434/22)

### Consent for publication

Approved

### Funding

Not applicable

## Conflict of interest

The authors declared an absence of conflict of interest.

## Authors Contribution

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