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Research Article

# Microbiome-Derived Metabolites and Their Role in Colorectal Cancer Progression

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## ABSTRACT

**Background:** Colorectal cancer has become a major cause of cancer-associated morbidity in most parts of the world and there is an increasing evidence that the gut microbiome can significantly contribute to its growth and progression. The microbial metabolites that are synthesized in the intestine may affect inflammation, epithelial integrity and cellular signaling pathways, and these factors are pertinent to colorectal carcinogenesis. Nevertheless, there is limited clinical evidence of the connection between disease severity and certain microbiome-derived metabolites.

**Methodology:** The conduct of this observational analytical research was done at Nishtar Hospital Multan between April 2024 and April 2025. This was done on 72 subjects; 36 colorectal cancer patients who were confirmed to have the disease histologically and 36 healthy controls. Samples of stool and blood were analyzed to evaluate gut microbiome composition, microbiome-produced metabolites, and inflammatory indicators. Clinical and demographic records were made and the comparisons done among groups and between disease stages.

**Results:** Individuals with colorectal cancer had a lower diversity of microbes and lower concentration of protective short-chain fatty acids (especially butyrate) than those in the control group. Conversely, the presence of inflammatory and carcinogenic metabolites like secondary bile acids and trimethylamine N-oxide was very high in cancer patients. The further depletion of beneficial metabolites and the elevation of inflammatory marker levels were related to the advanced-stage disease.

**Conclusion:** Alterations in gut microbiome–derived metabolites are closely associated with colorectal cancer and its progression. The observed imbalance between protective and pro-inflammatory metabolites may contribute to tumor development and advancing disease. These findings support the potential role of the gut microbiome as a target for future preventive and therapeutic strategies in colorectal cancer.

**Keywords:** Colorectal cancer; gut microbiome; microbiome-derived metabolites; short-chain fatty acids; inflammation

## INTRODUCTION

Colorectal cancer is among the most frequently diagnosed cancers, which still presents a big including diet and physical inaction are well-known contributory factors, they do not quite elucidate the disease occurrence or advancement. Over the last several years, it has been seen that the focus has shifted towards the gut microbiome as another risk factor of colorectal cancer [1-4].

Human gut consists of a complex and dynamic microbial community which is core in the maintenance of intestinal homeostasis. Gut bacteria synthesize a large variety of bioactive metabolites by fermenting the dietary components and other metabolic activities. Certain of these compounds, like short-chain fatty acids are known to stimulate the health of the mucosal lining and immune regulation, but others can encourage inflammation and epithelial damage [5-8] .

Disturbance of the normal intestinal microbial equilibrium may alter metabolite synthesis in a manner that will promote carcinogenesis. Experimental and clinical research have proposed that accumulated lower concentrations of positive metabolites and more exposure to adverse microbial by-products could help to induce and accelerate tumor formation. Although there is this mounting amount of evidence, there exist limited data on clinical populations, especially with regard to disease stage and severity [9].

The current research was thus conducted to investigate the relationship between gut microbiome metabolites and colorectal cancer development, where the goal was to furnish clinically useful knowledge on the metabolic routes involving the gut microbiome to malignancy.

## METHODOLOGY

This observational analytical research paper was carried out at Nishtar hospital multan between the period of one year between April 2024 and April 2025. The experiment aimed at investigating the connection between gut microbiome-produced metabolites and colorectal cancer development through the comparison of afflicted patients with the healthy ones. The procedures involved in all the studies were performed in line with the conventional practices of institutional research.

The study involved 72 people in the study by non probability consecutive sampling. The research population was categorized in two equal groups; 36 patients with diagnosis of colorectal cancer and 36 seemingly healthy controls with no known gastrointestinal malignancy. The eligibility to include adult participants was that adult participants should be 40 years and above.

The case group comprised patients who had a histologically proved diagnosis of colorectal cancer. Those who had a previous history of inflammatory bowel disease, as well as other malignancies, chronic liver disease or recent gastrointestinal surgery were excluded. Those who had taken antibiotics, probiotics, or prebiotics in the last three months were also excluded to reduce their effects on the composition of the gut microbiota. Healthy controls were chosen among those who come to visit regularly to health check-ups and did not have clinical/radiological indications of colorectal pathology.

Demographic and clinical data were gathered by using a structured proforma after informed consent was received. This was in terms of age, sex, body mass index, smoking status, place of residence, physical activity level and applicable clinical history. In the case of colorectal cancer patients, tumor information regarding the location, stage, histological grade, and involvement of lymph node, and also the presence of the metastasis were obtained based on the medical files and pathology reports.

All the participants were provided with fresh stool samples in sterile containers before any treatment specific to the cancer was started. The samples were then kept in low temperature and taken to the laboratory to be processed further. To preserve the integrity of microbes, all the samples were kept under standard conditions until analysis.

The body composition of gut microbiome was determined with the help of standard molecular methods that are founded on the extraction of the bacterial DNA using the stool samples. The microbial diversity indices were obtained to determine the overall bacterial richness and evenness. Relative abundance of the identified bacterial taxa that were associated with colorectal cancer were calculated by validated laboratory procedures. Microbiome-generated metabolites were measured on stool and blood using the standard biochemical and analytical techniques. Short fatty acids such as butyrate, acetate were also measured as protective indicators of microbial activities. The reasons why secondary bile acids and trimethylamine N-oxide were analyzed were because they were reported to be associated with inflammation and carcinogenesis. All tests were done under the direction of the manufacturers and under the laboratory quality standards.Inflammatory Marker Assessment Systemic inflammatory markers were measured by taking blood samples under aseptic conditions. The serum C-reactive protein and interleukin-6 levels were examined using standard immunoassays. These biomarkers were incorporated to assess the inflammatory milieu related to microbial metabolite imbalance and severity of the disease. Standard statistical software was used to enter and analyze the data. Continuous variables were represented in the form of mean and standard deviation, whereas categorical variables were represented in the form of frequencies and percentage. Group comparisons were done using independent sample t -tests and chi-square tests that were appropriate. The statistically significant p-value was seen as 0.05 or less.

**Results**

Colorectal cancer patients were much older and with higher mean BMI than their controls. The lack of physical activity was more common in the CRC group indicating that there might be a lifestyle factor leading to the risks of the disease. There were no significant differences of sex distribution and smoking status between groups

.

**Table 1. Baseline Demographic and Clinical Characteristics of the Study Population (n = 72)**

| Variable | CRC Group (n = 36) | Control Group (n = 36) | p-value |
|---|---|---|---|
| Age (years), mean ± SD | 61.4 ± 8.9 | 56.2 ± 9.1 | 0.018 |
| Sex (Male/Female) | 22 / 14 | 20 / 16 | 0.631 |
| BMI (kg/m²), mean ± SD | 27.8 ± 4.1 | 25.6 ± 3.8 | 0.022 |
| Residence (Urban/Rural) | 24 / 12 | 21 / 15 | 0.462 |
| Smoking status (Yes) | 13 (36.1%) | 8 (22.2%) | 0.197 |
| Physical activity (Low) | 21 (58.3%) | 12 (33.3%) | 0.034 |

The majority of the tumors were in the colon, and the proximal was most prevalent. At the time of diagnosis, more than half of patients had an advanced-stage disease (Stage III-IV). The aggressive disease patterns were manifested by a significant proportion of poor differentiation, and metastatic disease.

**Table 2. Tumor Characteristics Among Colorectal Cancer Patients (n = 36)**

| Variable | n (%) |
|---|---|
| Tumor location | |
| – Proximal colon | 14 (38.9) |
| – Distal colon | 12 (33.3) |
| – Rectum | 10 (27.8) |
| Tumor stage | |
| – Stage I–II | 15 (41.7) |
| – Stage III–IV | 21 (58.3) |
| Histological grade | |
| – Well / Moderately differentiated | 26 (72.2) |
| – Poorly differentiated | 10 (27.8) |
| Lymph node involvement | 19 (52.8) |
| Distant metastasis | 9 (25.0) |

The diversity of the microbes drastically decreased in the CRC group as compared to the healthy controls. The pro-inflammatory Fusobacterium nucleatum, etc. were significantly raised in the patients of the CRC. On the contrary, the beneficial butyrate-producing bacteria were greatly reduced in cases of cancer.

**Table 3. Gut Microbiome Composition in CRC and Control Groups**

| Variable | CRC Group | Control Group | p-value |
|---|---|---|---|
| Shannon diversity index | 2.41 ± 0.36 | 2.89 ± 0.42 | <0.001 |
| Fusobacterium nucleatum (%) | 6.8 ± 2.1 | 2.3 ± 1.4 | <0.001 |
| Bacteroides spp. (%) | 18.6 ± 5.4 | 14.2 ± 4.9 | 0.003 |
| Faecalibacterium prausnitzii (%) | 4.1 ± 1.8 | 8.7 ± 2.6 | <0.001 |

The concentration of protective short-chain fatty acids, especially butyrate, was significantly lower in CRC patients. On the contrary, secondary bile acids and TMAO were significantly increased in the cancer group. These data are associated with a metabolite phenotype that is more inclined to an inflammatory reaction and neoplasm development.

**Table 4. Microbiome-Derived Metabolite Levels in Study Groups**

| Metabolite | CRC Group | Control Group | p-value |
|---|---|---|---|
| Butyrate (µmol/g) | 7.9 ± 2.6 | 13.4 ± 3.1 | <0.001 |
| Acetate (µmol/g) | 38.2 ± 6.8 | 42.6 ± 7.1 | 0.011 |
| Deoxycholic acid (µmol/L) | 4.6 ± 1.3 | 2.8 ± 0.9 | <0.001 |
| TMAO (µmol/L) | 6.2 ± 2.0 | 4.1 ± 1.6 | <0.001 |

Highly developed colorectal cancer was linked to much lower butyrate and greater concentrations of carcinogenic bile acids. The levels of systemic inflammatory markers rose with the degree of disease. These findings suggest that there is a strong association between microbiome-derived metabolites, inflammation and cancer development.

**Table 5. Association Between Microbial Metabolites and Disease Severity in CRC Patients**

| Variable | Early Stage (I–II) n = 15 | Advanced Stage (III–IV) n = 21 | p-value |
|---|---|---|---|
| Butyrate (µmol/g) | 10.1 ± 2.4 | 6.3 ± 2.1 | <0.001 |
| Deoxycholic acid (µmol/L) | 3.7 ± 1.0 | 5.3 ± 1.2 | <0.001 |
| CRP (mg/L) | 6.2 ± 2.1 | 11.4 ± 3.6 | <0.001 |
| IL-6 (pg/mL) | 8.6 ± 3.0 | 14.1 ± 4.2 | <0.001 |

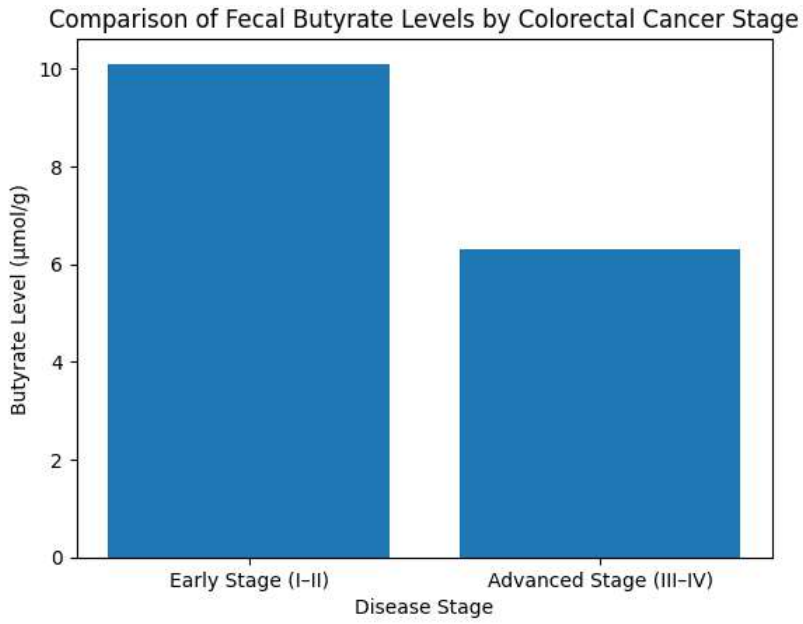


**Figure1:**
*Mean fecal butyrate concentrations were significantly higher in early-stage colorectal cancer (Stage I–II) compared with advanced-stage disease (Stage III–IV). Reduced butyrate levels in advanced stages suggest a loss of protective microbiome-derived metabolites with disease progression.*

## DISCUSSION

The current research shows that there is a distinct correlation of changes in gut microbiome-derived metabolites with colorectal cancer development. A decrease in microbial diversity, accompanied by a significant loss of protective metabolites (butyrate) and an increase in metabolites associated with inflammation and cancer development were observed in patients with colorectal cancer. These results are in line with previous evidence indicating that tumor-promoting intestinal environment is caused by the disturbance of the natural gut microbial ecosystem [9-11].

Short-chain fatty acids, especially butyrate, are very essential in ensuring integrity of colonic epithelial, immune response, and apoptosis of abnormal cells. The apparently much lower butyrate concentrations found in patients with colorectal cancer, particularly those with metastatic disease, reinforce the idea that cells of the beneficial microbial activity could be doing favors to the tumor in terms of growth and progression. Reductions in the butyrate-producing bacteria have also been observed in other clinical and experimental studies of the same scale, which supports the biological plausibility of this relationship [12-14].

Conversely, the presence of high concentration secondary bile acids and trimethylamine N-oxide in the cancer patients can be a contributor to the chronic inflammations, DNA damages, and cell signaling dysregulations. These metabolites are known to induce pro-inflammatory signaling and dysfunctional normal mucosal defense, which could fasten malignant transformation. The fact that the inflammatory

markers gradually increase with tumor stage3. increases in this study is also an additional evidence to support the relationship between microbial metabolic imbalance and systemic4. inflammation [15-18].

The relationship that was observed between the metabolite profiles and the severity of the disease points to the possibility of microbiome-derived5. metabolites to not only act as an indicator of the disease presence, but also as an indicator of disease progression [19, 20]. This suggest the potential that dietary modification or other6. interventions to act on the gut microbiome may play a role in the prevention of colorectal cancer7. or in its palliative treatment. Nevertheless, the study design is cross-sectional, which cannot be used to make causal inferences, and longitudinal research is required to establish the relationship8. over time.

## CONCLUSION

This study demonstrates that colorectal cancer is associated with significant alterations in gut microbiome composition and metabolite production. Reduced levels of protective short-10. chain fatty acids and increased concentrations of pro-inflammatory metabolites were more pronounced in advanced-stage disease. These findings support the role of microbiome-derived11. metabolites in colorectal cancer progression and suggest potential avenues for future preventive and therapeutic strategies. 12.

## References


1. Privitera, G., et al., *Novel insights into the interactions between the gut microbiome,*13. *inflammasomes, and gasdermins during colorectal cancer.* 2022. **11**: p. 806680.
2. Jaye, K., et al., *The role of key gut microbial metabolites in the development and treatment of*14. *cancer.* 2022. **14**(1): p. 2038865.

Malla, R.R.J.C.R.i.O., *Microbiome conundrum in colon cancer: development, progression, and therapeutics.* 2020. **25**(2).

Chen, Y. and Y.-X.J.C. Chen, *Microbiota-associated metabolites and related immunoregulation in colorectal cancer.* 2021. **13**(16): p. 4054.

Du, X., et al., *Alterations of the gut microbiome and fecal metabolome in colorectal cancer: implication of intestinal metabolism for tumorigenesis.* 2022. **13**: p. 854545.

Rebersek, M.J.B.c., *Gut microbiome and its role in colorectal cancer.* 2021. **21**(1): p. 1325.

Hayase, E. and R.R.J.G.m. Jenq, *Role of the intestinal microbiome and microbial-derived metabolites in immune checkpoint blockade immunotherapy of cancer.* 2021. **13**(1): p. 107.

Ternes, D., et al., *The gut microbial metabolite formate exacerbates colorectal cancer progression.* 2022. **4**(4): p. 458-475.

Malczewski, A.B., et al., *Microbiome-derived metabolome as a potential predictor of response to cancer immunotherapy.* 2020. **8**(2): p. e001383.

Ryu, T.Y., et al., *Human gut-microbiome-derived propionate coordinates proteasomal degradation via HECTD2 upregulation to target EHMT2 in colorectal cancer.* 2022. **16**(5): p. 1205-1221.

Zhao, L., W.C. Cho, and M.R.J.F.i.G. Nicolls, *Colorectal cancer-associated microbiome patterns and signatures.* 2021. **12**: p. 787176.

Reichard, C.A., et al., *Gut microbiome–dependent metabolic pathways and risk of lethal prostate cancer: prospective analysis of a PLCO cancer screening trial cohort.* 2022. **31**(1): p. 192-199.

Mirji, G., et al., *The microbiome-derived metabolite TMAO drives immune activation and boosts responses to immune checkpoint blockade in pancreatic cancer.* 2022. **7**(75): p. eabn0704.

Ternes, D., et al., *Microbiome in colorectal cancer: how to get from meta-omics to mechanism?* 2020. **28**(5): p. 401-423.

15. Png, C.-W., et al., *Alterations in co-abundant bacteriome in colorectal cancer and its persistence after surgery: a pilot study.* 2022. **12**(1): p. 9829.
16. Gheorghe, A.S., et al., *Biochemical and metabolical pathways associated with microbiota-derived butyrate in colorectal cancer and omega-3 fatty acids implications: A narrative review.* 2022. **14**(6): p. 1152.
17. Fan, S., et al., *Effects of long non-coding RNAs induced by the gut microbiome on regulating the development of colorectal cancer.* 2022. **14**(23): p. 5813.
18. Yang, S., et al., *Trimethylamine N-oxide promotes cell proliferation and angiogenesis in colorectal cancer.* 2022. **2022**(1): p. 7043856.
19. Sepich-Poore, G.D., et al., *The microbiome and human cancer.* 2021. **371**(6536): p. eabc4552.
20. Yu, Z.-K., et al., *The role of the bacterial microbiome in the treatment of cancer.* 2021. **21**(1): p. 934.