Prevalence of *Campylobacter* Species, *Helicobacter pylori*, Human Papillomavirus, and JC Polyomavirus in Patients with Colorectal Cancer in Iran

Parisa Abedi Elkhichi [1, 2], Ehsan Nazemalhosseini Mojarad (iD) [3], Hossein Dabiri (iD) [1, *], Sama Rezasoltani [4], Abbas Yadegar [4], Mehdi Azizmohamad looha [5], Ali Mojtahedi [1], Mohammad Javad Nasiri (iD) [1]

[1] Department of Medical Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[3] Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding author: Department of Medical Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: h.dabiri@hotmail.com

Abstract

Background: Colorectal cancer (CRC) is a complex disease with diverse gene expression patterns, which can arise from common adenomas or serrated polyps. The role of intestinal microbiota in the development of CRC is still a subject of debate.

Objectives: This study aimed to explore the prevalence of a selection of gastrointestinal microbiota in Iranian patients with CRC.

Methods: A total of 86 biopsy specimens (17 samples from normal tissues and 69 samples from cancer tissues) were analyzed from normal controls and patients with CRC. The presence of *Helicobacter pylori*, *Campylobacter* species (including *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. bovis*, and *C. fetus*), as well as human papillomavirus (HPV) and JC polyomavirus (JCV) in tissue specimens, was examined using PCR.

Results: The prevalence of the targeted bacterial and viral agents in CRC patients exhibited significant variations compared to normal controls. Notably, there was a higher prevalence of the *Helicobacter* genus in patients with CRC compared to normal controls. Patients with CRC were found to be at an increased risk of *Campylobacter* infection, with various *Campylobacter* species identified. Additionally, HPV and JCV genomes were detected in cancer samples at a higher rate than in normal controls.

Conclusions: Our findings demonstrated a higher prevalence of the *Helicobacter* genus, *Campylobacter* species, HPV, and JCV in patients with CRC compared to normal controls. However, further research is required to elucidate the potential role of these bacterial and viral agents in the development of CRC.

Keywords: Colorectal Cancer, *Helicobacter pylori*, *Campylobacter*, Human Papillomavirus, JC Polyoma Virus

1. Background

Colorectal cancer (CRC) is the third most prevalent malignancy and the second leading cause of death globally [1]. The global incidence of colon cancer is high and is rapidly increasing in developing countries [2]. In Iran, CRC has emerged as one of the most common types of cancer, with a noticeable rise in recent years, as reported by the Iranian Cancer Society [3]. Studies have indicated that CRC development or progression is influenced not only by genetic and lifestyle factors such as increased body mass index, obesity, reduced physical activity, and geographic location, but also by microbial and viral infections [4]. Microbial agents disrupt intestinal homeostasis and can induce cellular and genetic alterations [5].

Among the various microbial agents, *Helicobacter pylori* infection is one of the most prevalent infections among the Iranian population (6). This bacterium has been classified as a class I carcinogen by the International Agency for Research on Cancer of the World Health Organization (7). The impact of *H. pylori* extends beyond gastric adenocarcinoma and has been associated with esophageal cancer, liver cancer, and CRC (8).

Campylobacter is recognized as one of the bacterial agents contributing to human digestive disorders (9). Moreover, this bacterium can cause asymptomatic infections in humans and act as a carrier state (10). It adheres to intestinal cells through CadF proteins and produces a genotoxin named cytolethal distending toxin (CDT), consisting of three protein subunits: CdtA, CdtB, and CdtC (11). Notably, the CdtB subunit possesses DNase I properties, which can induce damage to the host's DNA, leading to mutations in intestinal cells and promoting the development of CRC (12).

In addition, viral agents with carcinogenic properties contribute to approximately 10 - 20% of all cancer cases (13). Viral infections, particularly human papillomavirus (HPV) and JC polyomavirus (JCV), can also have a significant impact on CRC development (14, 15). HPV has been associated with various malignancies, including cervical cancer and lung cancer (16, 17). Recent studies have shown the role of HPV in CRC, but the findings have been contradictory (18). On the other hand, JCV, a member of the polyomavirus family, has been identified as a potential factor in the development of lower gastrointestinal neoplasms, including CRC (13).

The impact of bacterial and viral infections on the onset and progression of CRC in the Iranian population remains uncertain and controversial.

2. Objectives

Therefore, in the present study, the prevalence of a selection of gastrointestinal microbiota, including *H. pylori*, different species of *Campylobacter*, HPV, and JCV, in tissue samples of Iranian CRC patients was analyzed using the polymerase chain reaction (PCR) method.

3. Methods

3.1. Human Tissue Sample Collection

In this cross-sectional descriptive study, a total of 86 colon biopsy specimens, including 17 samples from healthy individuals and 69 from CRC patients, were collected at Taleghani Hospital in Tehran, Iran, between 2017 and 2019. A questionnaire was used to record demographic information, medical history, and details regarding physical activity, such as walking and running, prior to colonoscopy. Patients with complex diseases such as diabetes or high blood pressure, a history of cancer, inflammatory bowel disease, or those who had taken antibiotics or non-steroidal anti-inflammatory drugs within the 3 months prior to tissue sampling were excluded.

Suspected CRC patients were identified based on evaluations by expert gastroenterologists, considering the presence of suspicious clinical symptoms related to the disease. Examples of such symptoms include rectal bleeding, blood in the stool, frequent abdominal discomfort such as cramping and bloating, family history, age, and other related factors. Diagnosis of suspected CRC patients was confirmed through colonoscopy and histopathological examination. Normal controls consisted of participants with no abnormal colonoscopy results and a negative family and personal history of gastrointestinal diseases.

For all patients, at least three biopsy specimens were taken. Two samples were sent for histopathological examination, while one sample was used for molecular tests. Biopsy specimens were carefully placed in sterile screw-top test tubes and promptly transported to the laboratory, where they were stored at -80°C for further analysis. This study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, and informed consent was obtained from all patients, indicating their willingness to participate in the study.

3.2. Bacterial Species and Target Genes

The reference strains, including *H. pylori* CCUG 17874, *Campylobacter coli* ATCC 33559, and *Campylobacter jejuni* ATCC 33560, were obtained from the Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. *Campylobacter fetus* ATCC 33561, *Campylobacter lari* ATCC 35221, and *Campylobacter upsaliensis* ATCC 43954, as well as the control DNA samples of HPV and JCV, were provided by Tarbiat Modares University, Tehran, Iran.

The bacterial reference strains were cultured on 10% Columbia blood agar (Merck Co., Hamburg, Germany) and incubated at 37°C under microaerobic conditions (86% N_2, 5% O_2, 5% CO_2, and 4% H2) for 3 - 5 days.

3.3. Total DNA Extraction

Genomic DNA was extracted from tissue samples and harvested colonies of reference strains using a commercially available DNA Extraction Kit (QIAamp DNA Mini, USA) according to the manufacturer's instructions. The quality of the extracted DNA was assessed by measuring the absorbance at 260 and 280 nm using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The samples were also subjected to electrophoresis on 2% agarose gels. All DNA samples were then stored at -20°C until further use.

3.4. PCR Amplification

The presence of bacterial and viral agents was determined using PCR with specific primers. All primer pairs were evaluated using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure their specificity. A no-template reaction mixture was used as a negative control. All PCR reactions were carried out in 0.2 mL tubes using an Eppendorf thermal cycler. Each PCR reaction consisted of a 25 μL solution containing 300 ng of DNA template, 1.0 μM of each primer, 0.25 U of Taq DNA polymerase, 0.5 mM dNTPs, and 1 mM MgCl2. The PCR conditions included an initial denaturation step at 95°C for 5 minutes, followed by 34 cycles of denaturation at 95°C for 45 seconds, annealing at 56°C for 40 seconds, and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was performed. The oligonucleotide sequences used in this work are listed in Table 1.

3.5. Data Analysis

Chi-squared and Fisher's exact tests were employed for the analysis of categorical data, while the *t*-test was used for numerical data. The statistical analyses were performed using SPSS V22 (SPSS, Chicago, IL, USA). A P-value of < 0.05 was considered statistically significant.

4. Results

The demographic and clinical variables of the participants in different study groups were analyzed, and their P-values were assessed between the normal and CRC groups. The mean age of the patients with cancer was 56.85 ± 16 years, while the mean age of the normal subjects was 54.19 ± 14.17 years. The difference in mean age between the two groups was not statistically significant (P = 0.34).

Regarding gender distribution, 46 (66.7%) of the cancer patients and 12 (70%) of the normal subjects were male. There was no significant difference in gender distribution between the two groups (P > 0.05). Additionally, no significant differences were found between the two groups considering factors such as family history of CRC, history of diabetes, smoking, and exercise (P > 0.05) (Table 2).

In terms of the prevalence of the studied bacteria in both groups, 14 (20.3%) of the cancer patients and 2 (12%) of the normal subjects tested positive for the *Campylobacter* genus. Among the positive cases for *Campylobacter*, 6 (8.7%) were identified as *C. coli*, 5 (7.2%) as *C. upsaliensis*, and 3 (4.3%) as *C. lari*. The prevalence of the *Helicobacter* genus and HPV was observed in 27 (39.1%) and 29 (42.0%) of the cancer patients, respectively. Additionally, 33 (47.0%) of the cancer patients and 2 (11.8%) of the control subjects tested positive for JCV (Table 3).

Interestingly, the prevalence of the *Campylobacter* genus, *Helicobacter* genus, HPV, and JCV was significantly higher in patients with CRC compared to the normal controls (P < 0.05). However, there were no statistically significant differences in the rates of other studied bacteria, including *C. coli*, *C. upsaliensis*, and *C. lari*, between the two groups (P > 0.05) (Table 3).

5. Discussion

The human gut hosts a diverse community of microbes that vary in type and abundance across different regions of the intestine (25). Recent research has revealed that an imbalance in the gut microbiota, known as dysbiosis, is associated with the progression of inflammatory bowel disease to CRC (26). Evidence suggests that microbial agents, such as bacteria and viruses, can significantly affect various processes in CRC development, including DNA damage, induction of inflammation, and production of proinflammatory cytokines and interleukins (27, 28). However, it is important to note that the exact mechanisms and causal relationships between host cells and the gut microbiota have not been definitively elucidated (29, 30).

Table 1. Oligonucleotide Sequences Used for Detection of Target Bacteria and Viruses

Target Organism	Gene	Primer	Primer Sequence	Product Size (bp)	Reference
Campylobacter genus	16S rRNA	C412 F	5-GGATGACACTTTTCGGAGC-3	816	(19)
		C1288 R	5-CATTGTAGCACGTGTGTC-3		
Campylobacter coli	askD	CC18F	5-GGTATGATTTCTACAAAGCGAG-3	502	(20)
		CC519R	5-ATAAAAGACTATCGTCGCGTG-3		
Campylobacter jejuni	cj0414	C-1	5-CAAATAAAGTTAGAGGTAGAATGT-3	161	(20)
		C-3	5-CCATAAGCACTAGCTAGCTGAT-3		
Campylobacter fetus	cstA	MG3F	5-GGTAGCCGCAGCTGCTAAGAT-3	359	(20)
		CF359R	5-AGCCAGTAACGCATATTATAGTAG-3		
Campylobacter lari	glyA	CLF	5-TAGAGAGATAGCAAAAGAGA-3	251	(20)
		CLR	5-TACACATAATAATCCCACCC-3		
Campylobacter upsaliensis	lpxA	CU61F	5-CGATGATGTGCAAATTGAAGC-3	86	(20)
		CU146R	5-TTCTAGCCCCTTGCTTGATG-3		
Helicobacter genus	16S rRNA	H276f	5-CTATGACGGGTATCCGGC-3	357	(21)
		H676r	5-ATTCCACCTACCTCTCCCA-3		
Helicobacter pylori	glmM	glmM-F	5-GCTTACTTTCTA ACACTAACGCGC-3	296	(22)
		glmM-R	5-GGATAAGCTTTTAGGGGTGTTAGGGG-3		
JCV	VP	P-F	5-AGGAGGTGCAAATCAAAGATCTG-3	102	(23)
		P-R	5-GGGCCATCTTCATATGCTTCA-3		
HPV	L1	GP5	5-TTTGTTACTGTGGTAGATAC-3	155	(24)
		GP6	5-GAAAAATAAACTGTAAATCA-3		

This study aimed to investigate the relationship between the prevalence of gastrointestinal microbiota, including *H. pylori*, different species of *Campylobacter*, HPV, and JCV in CRC biopsy samples. In our study, *H. pylori* was not found in cancer tissue samples, but the *Helicobacter* genus was detected at a prevalence of 39.1%, which could be related to other *Helicobacter* species. The exact mechanism by which *H. pylori* contributes to the progression of CRC is a subject of debate. Non-pylori *Helicobacter* species such as *H. canis*, *H. marmotae*, and *H. bilis* are a group of opportunistic microorganisms that can stimulate host inflammatory cascades and lead to genetic or epigenetic changes (31, 32).

In a study by Bulajic et al., PCR amplification was used to investigate the prevalence of *H. pylori* in CRC tissue samples (33). In contrast to our study, these researchers detected *H. pylori* DNA in 1.2% of CRC patients and 6% of normal colonic mucosa samples. However, they found no significant association between *H. pylori* and CRC. In another study, Liou et al. identified *H. pylori* infection in 3.6% of CRC and 21.4% of normal mucosa samples in Taiwan (34). Similarly, they found no significant relationship between *H. pylori* infection and an increased risk of CRC.

Campylobacter species are considered endemic in developing countries such as Iran, and infected individuals are often asymptomatic carriers (35). Currently, limited studies have investigated the role of *Campylobacter* species in the development of CRC. However, previous studies have demonstrated an association between CRC and cytolethal distending toxin (Cdt) produced by *Campylobacter* species. Cdt can induce apoptosis and autophagy signaling pathways, leading to increased inflammation and chromosomal instability (36, 37). A study by Pickett and Whitehouse demonstrated that low doses of Cdt (50 pg/mL) can induce early DNA damage and DNA double-strand breaks (DSBs), leading to prolonged arrest in the cell cycle in the G1 and/or G2 phase (38). This event resulted in the production of inflammatory cytokines, promoting tumor progression and metastasis (38). According to our results, the prevalence of the *Campylobacter* genus was 20.3% in CRC patients and 12% in the normal control group. Statistical analysis showed a significant association between the prevalence of *Campylobacter* in CRC tissues compared to normal controls ($P < 0.05$). These findings are consistent with other studies showing a significant association between *Campylobacter* infection and colon malignancies (10, 39, 40). However, further research is required to elucidate

Table 2. Demographic Characteristics and Medical History of Participants in this Study [a]

Characteristics	Normal (N = 17)	Cancer (N = 69)	Total (N = 86)	P-Value
Age	54.19 ± 14.17	56.85 ± 16.00	56.35 ± 15.62	0.348
Sex				1
Male	12 (70)	46 (66.7)	54 (62.8)	
Female	5 (30)	23 (33.3)	28 (32.6)	
Family history of CRC				0.45
Yes	1 (5.9)	12 (17.4)	13 (15.1)	
No	16 (94.1)	57 (82.6)	73 (84.9)	
Diabetes				0.37
Yes	3 (17.6)	6 (8.7)	9 (10.5)	
No	14 (82.4)	63 (91.3)	77 (89.5)	
Alcohol consumption				0.33
Yes	2 (12)	4 (6)	6 (1.2)	
No	15 (88)	65 (94.6)	80 (94.2)	
Smoking				0.4
Yes	3 (20)	7 (8.7)	10 (8.1)	
No	14 (80)	62 (91.3)	76 (91.9)	
Exercise				0.72
Yes	2 (12)	14 (20)	16 (23.3)	
No	15 (88)	55 (80)	70 (76.7)	
Anatomical site of biopsy				0.10
Cecum	2 (11.7)	4 (5.8)	6 (7.0)	
Rectum	6 (35.3)	22 (31.9)	28 (32.5)	
Descending colon	2 (11.7)	11 (15.9)	13 (15.1)	
Rectal	1 (5.9)	2 (2.9)	3 (3.5)	
Rectosigmoid junction	1 (5.9)	12 (17.4)	13 (15.1)	
Colon	1 (5.9)	12 (17.4)	13 (15.1)	
Transverse colon	2 (11.7)	1 (1.4)	3 (3.5)	
Anus mass	1 (5.9)	1 (1.4)	2 (2.3)	
Ascending colon	1 (5.9)	4 (5.7)	5 (5.8)	
Total	17 (100)	69 (100)	86 (100)	

[a] Values are expressed as No. (%) or Mean ± SD.

the potential role of *Campylobacter* infection in the development of CRC.

A considerable number of studies have reported a direct link between high-risk papillomavirus (HPV) infection and CRC ([18], [41]). A recent study in Taiwan showed a high frequency of HPV infection in CRC patients ([42]). The data from Damin's study revealed the presence of HPV in patients diagnosed with CRC, suggesting a potential association between this virus and the development of CRC ([18]). Our research, consistent with other studies, demonstrated a high prevalence of HPV infection among patients with CRC (42.0% in CRC vs. 0% in controls). These findings suggest a potential involvement of HPV in the development of CRC in the Iranian population.

Limited studies have been conducted on the status of JCV in CRC patients. Laghi et al. found that JCV DNA was present in the mucosa of the human colon and CRC, suggesting the virus's involvement in colon cancer pathogenesis and tumor development ([43]). In our study, JCV was detected in almost half (48%) of CRC patients compared to 12% in the normal control, indicating a potential role of JCV in CRC development among Iranian patients (P < 0.05). Similar to our findings, a study by Lin explored the relationship between CRC and JCV, revealing the presence of virus DNA in CRC patients and a high prevalence of JCV infection in colon cancer tissue in Taiwan ([44]). Unlike our observations, Karbalaie Niya et al. reported a low prevalence of JCV infection in the Iranian CRC population ([45]). However, more research is

Table 3. Distribution of Selected Microorganisms Among Cancer and Normal Subjects [a, b]

Target organism	Normal (N = 17)	Cancer (n = 69)	Total (n = 86)	P-Value
Campylobacter genus				0.032
Yes	2 (12)	14 (20.3)	16 (19)	
No	15 (88)	55 (79.7)	70 (81)	
Campylobacter coli				0.594
Yes	0 (0)	6 (8.7)	6 (7.0)	
No	17 (100.0)	63 (91.3)	80 (93.0)	
Campylobacter jejuni				1
Yes	0 (0)	0 (0)	0 (0)	
No	17 (100.0)	69 (100.00)	69 (80.2)	
Campylobacter fetus				1
Yes	0 (0)	0 (0)	0 (0)	
No	17 (100.0)	69 (100.00)	86 (100.0)	
Campylobacter lari				0.610
Yes	0 (0)	3 (4.3)	3 (3.5)	
No	17 (100.0)	66 (95.7)	83 (96.5)	
Campylobacter upsalinsis				0.578
Yes	0 (0)	5 (7.2)	5 (5.8)	
No	17 (100.0)	64 (92.8)	81 (94.2)	
Helicobacter genus				0.001
Yes	0	27 (39.1)	27 (31.4)	
No	17 (100.0)	42 (60.9)	59 (68.6)	
H. pylori				1
Yes	0 (0)	0 (0)	0 (0)	
No	17 (100.0)	69 (100.0)	86 (100.0)	
JCV				0.006
Yes	2 (11.8)	33 (47.8)	35 (40.7)	
No	15 (88.2)	36 (52.2)	51 (59.3)	
HPV				0.001
Yes	0	29 (42.0)	29 (33.7)	
No	17 (100.0)	40 (58.0)	57 (66.3)	

[a] The abundance of different microorganisms in each group. P-value less than 0.05 was considered statistically significant.
[b] Values are expressed as No. (%).

needed to investigate the pathogenic role of these viruses in CRC development and progression.

5.1. Conclusions

In conclusion, our study demonstrated a higher presence of the *Helicobacter* genus, *Campylobacter* species, as well as HPV and JCV, in colorectal specimens from patients with CRC compared to the normal control group. This supports the potential role of these organisms in CRC development among Iranian patients. However, we did not detect *H. pylori* in either patients with CRC or the normal control group, suggesting no correlation between *H. pylori* and CRC development in Iranian patients. It is important to note that our study was conducted with a limited number of samples; therefore, further research with a larger sample size and population is recommended for more precise conclusions.

Acknowledgements

Footnotes

Authors' Contribution: Parisa Abedi Elkhichi: Performed microbiological and molecular tests; Hossein Dabiri: Design of study, conceptualization and methodology; Parisa Abedi Elkhichi, Abbas Yadegar, Hossein Dabiri, Ehsan Nazemolhosseini, Mehdi Azizmohammad Looha, Sama Rezasoltani, Mohamad Javad Nasiri: Statistical analysis; Hossein Dabiri: Data analysis and interpretation; Parisa Abedi Elkhichi, Abbas Yadegar, Hossein Dabiri and Ehsan Nazemolhosseini: Literature review and manuscript writing; Abbas Yadegar: Critical manuscript revision. All authors approved the final version of the manuscript and the authorship list.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The data presented in this study are uploaded during submission as a supplementary file and are openly available for readers upon request.

Ethical Approval: This study is approved under the ethical approval code of IR.SBMU.RIGLD.REC.1395.851.

Funding/Support: This work was supported by a research grant (No. 11949) from School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Informed Consent: Informed consent was obtained from all patients, indicating their willingness to participate in the study.

References

1. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer (Review). *Mol Clin Oncol.* 2021;**15**(6):271. [PubMed ID: 34790355]. [PubMed Central ID: PMC8591689]. https://doi.org/10.3892/mco.2021.2433.

2. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi J, John A, et al. Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. *Cancers (Basel).* 2022;**14**(7). [PubMed ID: 35406504]. [PubMed Central ID: PMC8996939]. https://doi.org/10.3390/cancers14071732.

3. Maajani K, Khodadost M, Fattahi A, Shahrestanaki E, Pirouzi A, Khalili F, et al. Survival Rate of Colorectal Cancer in Iran: A Systematic Review and Meta-Analysis. *Asian Pac J Cancer Prev.* 2019;**20**(1):13-21. [PubMed ID: 30677864]. [PubMed Central ID: PMC6485573]. https://doi.org/10.31557/APJCP.2019.20.1.13.

4. Fang Y, Yan C, Zhao Q, Xu J, Liu Z, Gao J, et al. The roles of microbial products in the development of colorectal cancer: a review. *Bioengineered.* 2021;**12**(1):720-35. [PubMed ID: 33618627]. [PubMed Central ID: PMC8806273]. https://doi.org/10.1080/21655979.2021.1889109.

5. Murphy N, Moreno V, Hughes DJ, Vodicka L, Vodicka P, Aglago EK, et al. Lifestyle and dietary environmental factors in colorectal cancer susceptibility. *Mol Aspects Med.* 2019;**69**:2-9. [PubMed ID: 31233770]. https://doi.org/10.1016/j.mam.2019.06.005.

6. Moosazadeh M, Lankarani KB, Afshari M. Meta-analysis of the Prevalence of Helicobacter pylori Infection among Children and Adults of Iran. *Int J Prev Med.* 2016;**7**:48. [PubMed ID: 27076886]. [PubMed Central ID: PMC4809131]. https://doi.org/10.4103/2008-7802.177893.

7. Ishaq S, Nunn L. Helicobacter pylori and gastric cancer: a state of the art review. *J Gastroenterol Hepatology Bed Bench.* 2015;**8**(Suppl1). S6.

8. Teimoorian F, Ranaei M, Tilaki KH, Shirvani JS, Vosough Z. Association of Helicobacter pylori infection with colon cancer and adenomatous polyps. *J Iranian Pathol.* 2018;**13**(3):325.

9. Riddle MS, Gutierrez RL, Verdu EF, Porter CK. The chronic gastrointestinal consequences associated with campylobacter. *Curr Gastroenterol Rep.* 2012;**14**(5):395-405. [PubMed ID: 22864805]. https://doi.org/10.1007/s11894-012-0278-0.

10. Mahendran V, Riordan SM, Grimm MC, Tran TA, Major J, Kaakoush NO, et al. Prevalence of Campylobacter species in adult Crohn's disease and the preferential colonization sites of Campylobacter species in the human intestine. *PLoS One.* 2011;**6**(9). e25417. [PubMed ID: 21966525]. [PubMed Central ID: PMC3179513]. https://doi.org/10.1371/journal.pone.0025417.

11. Ripabelli G, Tamburro M, Minelli F, Leone A, Sammarco ML. Prevalence of virulence-associated genes and cytolethal distending toxin production in Campylobacter spp. isolated in Italy. *Comp Immunol Microbiol Infect Dis.* 2010;**33**(4):355-64. [PubMed ID: 19195703]. https://doi.org/10.1016/j.cimid.2008.12.001.

12. Lai C, Chen Y, Lin C, Lin H, Kao M, Huang M, et al. Molecular mechanisms and potential clinical applications of Campylobacter jejuni cytolethal distending toxin. *J Frontiers In Cellular Infection Microbiol.* 2016;**6**:9. https://doi.org/10.3389/fcimb.2016.00009.

13. Shavaleh R, Kamandi M, Feiz Disfani H, Mansori K, Naseri SN, Rahmani K, et al. Association between JC virus and colorectal cancer: systematic review and meta-analysis. *Infect Dis (Lond).* 2020;**52**(3):152-60. [PubMed ID: 31766929]. https://doi.org/10.1080/23744235.2019.1692145.

14. Bodaghi S, Yamanegi K, Xiao SY, Da Costa M, Palefsky JM, Zheng ZM. Colorectal papillomavirus infection in patients with colorectal cancer. *Clin Cancer Res.* 2005;**11**(8):2862-7. [PubMed ID: 15837733]. [PubMed Central ID: PMC1479314]. https://doi.org/10.1158/1078-0432.CCR-04-1680.

15. Kimla LJ, Clark TG, Banerjee S, Campino S. JC Polyomavirus T-antigen protein expression and the risk of colorectal cancer: Systematic review and meta-analysis of case-control studies. *PLoS One.* 2023;**18**(3). e0283642. [PubMed ID: 37000859]. [PubMed Central ID: PMC10065230]. https://doi.org/10.1371/journal.pone.0283642.

16. de Oliveira THA, do Amaral CM, de Franca Sao Marcos B, Nascimento KCG, de Miranda Rios AC, Quixabeira DCA, et al. Presence and activity of HPV in primary lung cancer. *J Cancer Res Clin Oncol.* 2018;**144**(12):2367-76. [PubMed ID: 30225539]. https://doi.org/10.1007/s00432-018-2748-8.

17. Petry KU. HPV and cervical cancer. *Scand J Clin Lab Invest Suppl.* 2014;**244**:59-62. discussion 62. [PubMed ID: 25083895]. https://doi.org/10.3109/00365513.2014.936683.

18. Damin DC, Caetano MB, Rosito MA, Schwartsmann G, Damin AS, Frazzon AP, et al. Evidence for an association of human papillomavirus infection and colorectal cancer. *Eur J Surg Oncol.* 2007;**33**(5):569-74. [PubMed ID: 17321098]. https://doi.org/10.1016/j.ejso.2007.01.014.

19. Dep MS, Mendz GL, Trend MA, Coloe PJ, Fry BN, Korolik V. Differentiation between Campylobacter hyoilei and Campylobater coli using genotypic and phenotypic analyses. *Int J Syst Evol Microbiol.* 2001;**51**(Pt 3):819-26. [PubMed ID: 11411703]. https://doi.org/10.1099/00207713-51-3-819.

20. Yamazaki-Matsune W, Taguchi M, Seto K, Kawahara R, Kawatsu K, Kumeda Y, et al. Development of a multiplex PCR assay for identification of Campylobacter coli, Campylobacter fetus, Campylobacter hyointestinalis subsp. hyointestinalis, Campylobacter jejuni, Campylobacter lari and Campylobacter upsaliensis. *J Med Microbiol.* 2007;**56**(Pt 11):1467-73. [PubMed ID: 17965346]. https://doi.org/10.1099/jmm.0.47363-0.

21. Riley LK, Franklin CL, Hook RJ, Besch-Williford C. Identification of murine helicobacters by PCR and restriction enzyme analyses. *J Clin Microbiol.* 1996;**34**(4):942-6. [PubMed ID: 8815113]. [PubMed Central ID: PMC228922]. https://doi.org/10.1128/jcm.34.4.942-946.1996.

22. Bazin T, Nchare Mfondi A, Julie C, Emile JF, Raymond J, Lamarque D. Contribution of genetic amplification by PCR for the diagnosis of Helicobacter pylori infection in patients receiving proton pump inhibitors. *United European Gastroenterol J.* 2018;**6**(8):1267-73. [PubMed ID: 30288289]. [PubMed Central ID: PMC6169049]. https://doi.org/10.1177/2050640618787055.

23. Newcomb PA, Bush AC, Stoner GL, Lampe JW, Potter JD, Bigler J. No evidence of an association of JC virus and colon neoplasia. *J Cancer Epidemiol Biomarkers Prevention.* 2004;**13**(4):662-6. https://doi.org/10.1158/1055-9965.662.13.4.

24. Baay MF, Quint WG, Koudstaal J, Hollema H, Duk JM, Burger MP, et al. Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by PCR in paraffin-embedded cervical carcinomas. *J Clin Microbiol.* 1996;**34**(3):745-7. [PubMed ID: 8904451]. [PubMed Central ID: PMC228883]. https://doi.org/10.1128/jcm.34.3.745-747.1996.

25. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. *Cell Host Microbe.* 2014;**15**(3):317-28. [PubMed ID: 24629338]. [PubMed Central ID: PMC4003880]. https://doi.org/10.1016/j.chom.2014.02.007.

26. Zou S, Fang L, Lee MH. Dysbiosis of gut microbiota in promoting the development of colorectal cancer. *Gastroenterol Rep (Oxf).* 2018;**6**(1):1-12. [PubMed ID: 29479437]. [PubMed Central ID: PMC5806407]. https://doi.org/10.1093/gastro/gox031.

27. Alhinai EA, Walton GE, Commane DM. The Role of the Gut Microbiota in Colorectal Cancer Causation. *Int J Mol Sci.* 2019;**20**(21). [PubMed ID: 31653078]. [PubMed Central ID: PMC6862640]. https://doi.org/10.3390/ijms20215295.

28. Chattopadhyay I, Dhar R, Pethusamy K, Seethy A, Srivastava T, Sah R, et al. Exploring the Role of Gut Microbiome in Colon Cancer. *Appl Biochem Biotechnol.* 2021;**193**(6):1780-99. [PubMed ID: 33492552]. https://doi.org/10.1007/s12010-021-03498-9.

29. Abedi Elkhichi P, Dabiri H, Nazemalhosseini Mojarad E, Rezasoltani S, Asadzadeh Aghdaei HAMID, Pouriran R, et al. Prevalence of Helicobacter pylori in patients with colorectal cancer. *J Inter Molecular Clin Microbiol.* 2018;**8**(2):1001-5.

30. Fireman Z, Trost L, Kopelman Y, Segal A, Sternberg A. Helicobacter pylori: seroprevalence and colorectal cancer. *Israel Med Association : IMAJ.* 2000;**2**(1):6-9.

31. Mueller C, Kwong Chung CKC, Faderl MR, Brasseit J, Zysset D. Helicobacter spp. in Experimental Models of Colitis. *Adv Exp Med Biol.* 2019;**1197**:97-105. [PubMed ID: 31732937]. https://doi.org/10.1007/978-3-030-28524-1_8.

32. Crabtree JE. Gastric mucosal inflammatory responses to Helicobacter pylori. *Aliment Pharmacol Ther.* 1996;**10 Suppl 1**:29-37. [PubMed ID: 8730257]. https://doi.org/10.1046/j.1365-2036.1996.22164003.x.

33. Bulajic M, Stimec B, Jesenofsky R, Kecmanovic D, Ceranic M, Kostic N, et al. Helicobacter pylori in colorectal carcinoma tissue. *Cancer Epidemiol Biomarkers Prev.* 2007;**16**(3):631-3. [PubMed ID: 17372266]. https://doi.org/10.1158/1055-9965.EPI-06-1031.

34. Liou JM, Lin JW, Huang SP, Lin JT, Wu MS. Helicobacter pylori infection is not associated with increased risk of colorectal polyps in Taiwanese. *Int J Cancer.* 2006;**119**(8):1999-2000. [PubMed ID: 16708392]. https://doi.org/10.1002/ijc.22050.

35. Hassanzadeh P, Motamedifar M. Occurrence of Campylobacter jejuni in Shiraz, Southwest Iran. *Med Princ Pract.* 2007;**16**(1):59-62. [PubMed ID: 17159366]. https://doi.org/10.1159/000096142.

36. He Z, Gharaibeh RZ, Newsome RC, Pope JL, Dougherty MW, Tomkovich S, et al. Campylobacter jejuni promotes colorectal tumorigenesis through the action of cytolethal distending toxin. *Gut.* 2019;**68**(2):289-300. [PubMed ID: 30377189]. [PubMed Central ID: PMC6352414]. https://doi.org/10.1136/gutjnl-2018-317200.

37. Saha C, Horst-Kreft D, Kross I, van der Spek PJ, Louwen R, van Baarlen P. Campylobacter jejuni Cas9 Modulates the Transcriptome in Caco-2 Intestinal Epithelial Cells. *Genes (Basel).* 2020;**11**(10). [PubMed ID: 33066557]. [PubMed Central ID: PMC7650535]. https://doi.org/10.3390/genes11101193.

38. Pickett CL, Whitehouse CA. The cytolethal distending toxin family. *Trends Microbiol.* 1999;**7**(7):292-7. [PubMed ID: 10390639]. https://doi.org/10.1016/s0966-842x(99)01537-1.

39. Brauner A, Brandt L, Frisan T, Thelestam M, Ekbom A. Is there a risk of cancer development after Campylobacter infection? *Scand J Gastroenterol.* 2010;**45**(7-8):893-7. [PubMed ID: 20334473]. https://doi.org/10.3109/00365521003734133.

40. Mukhopadhya I, Thomson JM, Hansen R, Berry SH, El-Omar EM, Hold GL. Detection of Campylobacter concisus and other Campylobacter species in colonic biopsies from adults with ulcerative colitis. *PLoS One.* 2011;**6**(6). e21490. [PubMed ID: 21738679]. [PubMed Central ID: PMC3124515]. https://doi.org/10.1371/journal.pone.0021490.

41. Santos NC, Tocantins PDB, Leão-Cordeiro JAB, Ataides FS, Marques LDOR, Silva AMTC. The human papillomavirus in colorectal cancer. *J Med Sci.* 2022;**42**(1):1-7. https://doi.org/10.4103/jmedsci.jmedsci_194_20.

42. Ibragimova MK, Tsyganov MM, Litviakov NV. Human papillomavirus and colorectal cancer. *Med Oncol.* 2018;**35**(11):140. [PubMed ID: 30187207]. https://doi.org/10.1007/s12032-018-1201-9.

43. Laghi L, Randolph AE, Chauhan DP, Marra G, Major EO, Neel JV, et al. JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. *Proc Natl Acad Sci U S A.* 1999;**96**(13):7484-9. [PubMed ID: 10377441]. [PubMed Central ID: PMC22112]. https://doi.org/10.1073/pnas.96.13.7484.

44. Lin PY, Fung CY, Chang FP, Huang WS, Chen WC, Wang JY, et al. Prevalence and genotype identification of human JC virus in colon cancer in Taiwan. *J Med Virol.* 2008;**80**(10):1828-34. [PubMed ID: 18712832]. https://doi.org/10.1002/jmv.21296.

45. Karbalaie Niya MH, Keshavarz M, Tameshkel FS, Taherizadeh M, Esghaei M, Panahi M, et al. Investigation of JC polyomavirus (JCV) genome in colorectal cancer patients from Iran. *J Iran Public Health.* 2020. https://doi.org/10.18502/ijph.v49i3.3153.