How to Cite:
Abbas, H. H., Al-Nasery, M. A. G., Jarullah, B. A., & Abdali, A. K. (2022). Identification of correlation between GIT bacteria and cancer in patients suffer from gastroenteritis in south of Iraq. *International Journal of Health Sciences*, *6*(S1), 6142–6155. https://doi.org/10.53730/ijhs.v6nS1.6260

Identification of correlation between GIT bacteria and cancer in patients suffer from gastroenteritis in south of Iraq

Msc. Hawraa Hamza Abbas
Department of Biology- College of Science- Thi-Qar University

Dr. Mohannad Abdulrazzaq Gati Al-Nasery
Department of Biology- College of Science- Thi-Qar University

Prof. Dr. Basim Abdulhussein Jarullah
Department of microbiology- College of veterinary medicine- Thi-Qar University

Dr. Ali Kamil Abdali
MBChB. FIBMS. FICMS. (Gastroenterologist& Hepatologist)

***Abstract*---**The study was investigated and diagnosis the bacteria that associated with GIT cancer and GIT diseases a total of 200 blood and biopsy samples were collected during the period from September 2020 to June 2021 and samples were included 100 sample for gastrointestinal cancer patients, 50 for healthy people, and 50 for gastrointestinal patients. Gastrointestinal tract diseases patients were diagnosed clinically and the disease was evaluated by specialist physicians, presented with dyspepsia referred to the Esophago Gastroduodeno Scope Unit for endoscopy at AL-Hussein teaching hospital (Consulting digestive tract).In our current study, three types of bacteria were diagnosed that are related to diseases of the digestive system, including: *Streptococcus bovis spp gallolyticus, Fusobacterium nucleatum, Porphyromonas gingivalisbut*by by usig PCR technique and found the highest percentage was patients with cancer as following *F.nucleatum* gene (96%) in patients with cancer, while in patients with GIT diseases (96%) but in the healthy was 36 (72%), whereas the percentage of *S.bovis* gene (58%) in while patients with cancer but (52%)in patients with GIT diseases while the healthy (40%), the presence of *P.gingivalis* gene (31%) in patients with cancer, while in patients with GIT diseases (28%) in the healthy (22%). Depending on the obtained results the hypothesis these bacteria be has a place in etiology of gastrointestinal cancer was supported with our findings.

Keywords---*Fusobacterium nucleatum, Streptococcus bovis, Porphyromonas gingivalis*, GIT cancer, gastric cancer, colon cancer, PCR.

Introduction

Virchow, a German pathologist, discovered a relationship between inflammation and cancer more than 150 years ago. A number of observations clearly suggest that persistent inflammation in the stomach is a prevalent cause of cancer. Cancers of the gastrointestinal tract are more common in diseases that cause a chronically irritated epithelium. The appearance of multifocal tumors in inflamed mucosae contributes to the theory that inflammation is involved in carcinogenesis mechanistically (Virchow, 1863). Cancers of the gastrointestinal tract are a major health problem and represent almost 20% of all cancer related deaths in both men and women (Ferlay *et al.*, 2007). Gastrointestinal Cancer is an important problem in public health worldwide (Rawla and Barsouk, 2019). Colorectal carcinoma is the third most frequent cancer after breast cancer in women and bronchus cancer in men (Thélin and Sikka, 2015). Colorectal carcinoma is the largest cause of death from GIT tumors, in Iraq, colorectal cancer was the seventh top cancers, whereas in Kurdistan, it was the fourth most common cancer for both males and females (Khalil *et al.*, 2018).

Colorectal cancer is a multistep process in which several gene mutations (Nguyen and Duong, 2018). Chronic infection or toxins production, immune evasion, and immunological suppression are all important mechanisms that can lead to carcinogenesis Chronic infection can disrupt the cell cycle, resulting in abnormal cell growth; additionally, toxin production can induce DNA damage from carcinogenic chemicals, which leads to damage to genes, culminating in abnormal cell division and apoptosis (Liardo *et al.*,2021). Gastric cancer is the quart most common malignancy and the second major cause of cancer-associated deaths, accounting for 10% of total cancer deaths worldwide (Sitarz *et al.*, 2018). The spaciously majority of gastric cancers are adenocarcinomas, gastric cancer is also characterized by large geographical variations in its incidence and indeed more than half of the total gastric cancer are in East Asian countries such as Japan, South Korea and China (Rawla and Barsouk, 2019), scientists divide this cancer of stomach into two main classes: -Gastric cardia cancer (cancer of the top inch of the stomach) and non –cardia gastric cancer (cancer in all other areas of stomach).(Ferlay *et al.*, 2010). Colon cancer is a neoplastic illness of the large intestine that can be caused by both inherited and somatic genetic changes that occur throughout a lifetime (Monson *et al.*, 2013). It has been connected to a variety of factors, including socioeconomic level, a drastic shift in eating patterns, refrigeration, chemical preservatives, and environmental changes (Sawicki *et al.,* 2021). Increased harmful bacterial products, decreased helpful bacterial metabolites, and disturbed tissue barriers are the general processes for bacteria-associated (or driven) GI cancer. Cancer progression is further aided by abnormal immunology, persistent inflammation, and hyperpreliferation. Microbial infections and intestinal inflammation can affect the integrity of the intestinal barrier, resulting in increased gut permeability, microbial translocation, and immunological activation (Keku *et al.*, 2015). Some bacteria, such as

Fusobacterium nucleatum, Porphyromonas gingivalis, Helicobacter pylori, and Streptococcus bovis, have been linked to human cancer (Mallika *et al.*, 2020). Oral squamous cell carcinoma (OSCC) can be caused by *P. gingivalis* and *F. nucleatum* (Gholizadeh *et al.*, 2016). Colorectal cancer and pancreatic cancer are more common in F. nucleatum (K Mitsuhashi *et al.*, 2015). S. bovis has been linked to colorectal and colon cancer (Meseeha and Attia, 2018). *S. gallolyticus* (SB biotypes II/2 and I) and *S.infantarius* (biotype II/1). *S. gallolyticus* has a stronger link to colorectal cancers (Kumar *et al.*, 2018) than *S. infantarius*, which has a stronger link to non-colonic cancers (Kaindi *et al.*, 2018). *Porphyromonas gingivalis*, an oral pathogen more closely connected with periodontal disease was linked to digestive tract cancer in addition to *F. nucleatum*. The study was, however, too small to distinguish colorectal cancer from other malignancies (Wang *et al.*, 2021; Abed *et al,* 2020).

Material and method
Study collection:

The study includes 200 samples, 100 samples are patients with cancer, comprising 41 females and 59 males with various histologically proven preoperative GIT carcinomas. The types of cancer in patients with GIT were colon cancer, gastric cancer, and small intestine cancer. The age of patients was between 20-80 years .Two control groups of patients were studied. These included 50 healthy controls and 50 patients suffering from other GIT disease, other than cancer. The non-malignancy conditions were gastric ulcer, and ulcerative colitis. All patients with non-malignant GIT conditions as well as the preoperative GIT cancer patients were initially attending to the Gastroenterology and Hematology Teaching Hospital, during the period between September 2020 to June 2021. Negative control whom are selected after a careful questioning about the general health of each individual especially medical problems related to gastrointestinal diseases.

Gastrointestinal tract diseases in patients were diagnosed clinically and evaluated by specialist physicians, presented at the Esophago Gastroduodeno Scope Unit for endoscopy at AL-Hussein teaching hospital (Consulting digestive tract) in AL-Muthana, Thi-Qar, Basra and Omara provinces. Every participant signed a written agreement after his/her understanding of the project aim and specialist physicians using sterile endoscopy obtained tests that would be performed, tissue biopsies from each person. Biopsy specimens washed and placed in 1 ml of normal saline and /or phosphate buffer saline (PBS) and was preserved at -20°C for molecular analysis.

Table (1)
Primers were used in PCR

Bacteria	Type of Primer	Sequence	PCR product (bp)	Reference
Fusobacterium sp	FUSO1-F	GAGAGAGCTTTGCGT CC	610 bp	Nagano et al., 2007
	FUSO2-R	TGGGCGCTGAGGTTC GAC		
Streptococcus	23S rRNA-F	CCCGGCATGTAATGCATGTC		Kawata, et

bovis	23S rRNA-R	TACAACCCCGATGTGTAAACACA	169 bp	al., 2004
P. gingivalis	16S rRNA-F	AGGCAGCTTGCCATACTGCG	404 bp	Slots et al., 1995
	16S rRNA-R	ACTGTTAGCAACTACCGATGT		

Bacterial DNA was extracted from Biopsy samples by using PrestoTM Mini gDNA Bacteria Kit (Geneaid. USA) and done according to company instruction.

Molecular detection of *Fusobacterium nucleatum* by polymerase chain reaction

The gene of FUSO are used to detection of ***Fusobacterium nucleatum***, amplification and melting conditions were optimized for the PCR using specific primer, this condition produce the most specific and sufficient PCR product, as shown in table (2).

Table (2)
Optimized thermo-cycling condition for FUSO gene of *Fusobacterium nucleatum*

NO.	Stage	Temperature	Time	Number of cycle
1	Initial denaturation	95 ◦C	5 min	1
2	Denaturation	95◦C	45 sec	35
3	Annealing	60 ◦C	45 sec	
4	Elongation	72◦C	45 sec	
5	Final elongation	72◦C	10 min	1

Molecular detection of *S. bovis* by polymerase chain reaction.

The gene of 23SrRNA are used to detection of ***S. bovis***, amplification and melting conditions were optimized for the PCR using specific primer, this condition produce the most specific and sufficient PCR product, as shown in table (3).

Table (3)
Optimized thermo-cycling condition for 23SrRNA gene of *S. bovis*

NO.	Stage	Temperature	Time	Number of cycle
1	Initial denaturation	95 ◦C	5 min	1
2	Denaturation	95◦C	45 sec	35
3	Annealing	57 ◦C	45 sec	
4	Elongation	72◦C	45 sec	
5	Final elongation	72◦C	10 min	1

Molecular detection of *P. gingivalis* by polymerase chain reaction

The gene of 16SrRNA are used to detection of ***P. gingivalis***, amplification and melting conditions were optimized for the PCR using specific primer, this

condition produce the most specific and sufficient PCR product, as shown in table (4).

Table (4)
Optimized thermo-cycling condition for 16SrRNA gene of *P. gingivalis*

NO.	Stage	Temperature	Time	Number of cycle
1	Initial denaturation	95 ◦C	5 min	1
2	Denaturation	95◦C	45 sec	35
3	Annealing	56.5 ◦C	45 sec	
4	Elongation	72◦C	45 sec	
5	Final elongation	72◦C	10 min	1

Result & Discussion
D
etection of *F. nucleatum* gene (*FUSO* gene) by using PCR Technique

The results of detection of *F. nucleatum* among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following *F. nucleatum* was detected in 36 (72%) out 50 of healthy people also *F. nucleatum* was detected in 43 (86%) out 50 in patients with GIT. Finally, the highest percentage recorded in patient with cancer which *F. nucleatum* was detected in 96 (96%) out 100 sample with significant differences between the samples that gave positive and negative to bacteria detection in three groups at p<0.0001 as in table (5) and figure (1):

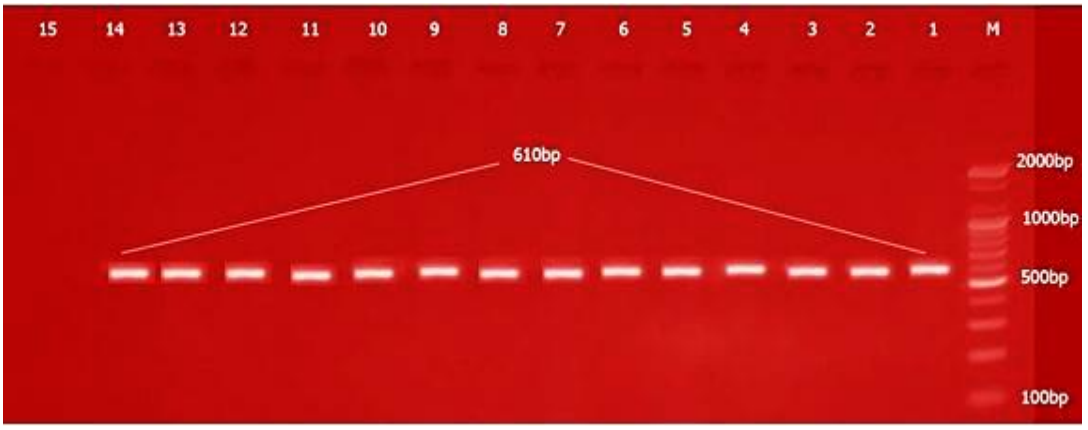

Fig. (1): Agarose gel electrophoresis of PCR product obtained with *Fusobacterium nucleatum* strains -specific primers that generated 610bp amplicon. Lanes (1-14); positive, Lanes (15); negative, Lane M represent 100bp DNA ladder.

Table (5)
Identification of *Fusobacterium nucleatum* among three studied groups (Healthy, patients with GIT and patients with Cancer)

Results	Healthy N=50	*GIT N=50*	Cancer N=100	P value[a]
Positive	36 (72)	43 (86)	96 (96)	<0.0001*

Negative	14 (28)	7 (14)	4 (4)	
P value[b]	<0.0001*	<0.0001*	<0.0001*	

* represent a significant difference at p<0.05.

A, among all three studied groups. B, between positive and negative results. These findings corroborated the findings of (Cuellar-Gómez et al., 2021). The researchers collected tissue from 30 patients (18 men and 12 women) who had colorectal cancer (CRC) and 30 normal people to delicate the relationship between F. nucleatum and CRC. The results showed that the accumulation of F. nucleatum determined in CRC was significantly greater than in the normal control, which was also found in the current study. Furthermore, the findings agreed with those of (Suehiro *et al.*, 2017), who conducted a study to detect *F. nucleatum* in stool and investigate its association with colorectal tumors, feces sample were obtained from 60 healthy individuals , 11 patients with colorectal non advanced adenomas, 19 patients with colorectal advanced adenoma/carcinoma, and 158 patients with colorectal cancer.

PCR results recorded more than 260 copies of F. nucleatum were discovered in only 10% of the healthy persons in the control group, but *F. nucleatum* was found in 55% of the non-advanced adenoma group, 32% of the advanced adenoma/CIS group, and 54% of the colorectal cancer group. The current investigation, on the other hand, discovered F. nucleatum in GIT patients. Lee *et al.,* (2016) found that *F. nucleatum* was less prevalent in GIT. In a study of 54 patients with inflammatory bowel disease (IBD), including 26 with Crohn's disease (CD), 25 with ulcerative colitis (UC), and 3 with Behcet's disease (BD), 10 Fusobacterium spp were identified: six with F. mortiferum, two with F. varium, and one with *F. nucleatum* and *F. mort* .Su *et al.* (2020), on the other hand, discovered that when they investigated the mechanism by which F. nucleatum promotes intestinal epithelial cell (IEC) mortality, they analyzed *F. nucleatum* abundance in 44 (ulcerative colitis) UC tissues from patients and 9 normal tissues. *F. nucleatum* was found in a larger proportion of UC tissues than in normal tissues. Huh *et al.*, (2020) found *F. nucleatum* played a role in Inflammatory bowel diseases in 1526 fecal samples, the results reported the *F. nucleatum* was commonly discovered in inflammatory bowel disease (IBD) participants with low microbial diversity; these variations were linked to the patient's health state, sample type, and geographical location.

Furthermore, *F. nucleatum* was found in patients with cancer in numerous studies, including (Kashani *et al.*, 2020), which found that 35 (43 percent) of 80 patients with colorectal cancer (33 male and 47 female) had positive *F.nucleatum* biopsies. According to (Boehm *et al.,* 2020), *Fusobacterium spp.* and *F. nucleatum* were more commonly detected in tumorous tissue of colorectal cancer (CRC) and gastric cancer (GC) compared to non-tumorous tissues. In CRC patients, the frequency and bacterial load were greater than in GC patients. Abed *et al.*, (2020) the circulatory system appears to be the most efficient pathway for *F. nucleatum* to reach colon cancers, as demonstrated in a pre-clinical model.

This most likely affects humans during transitory physiologic bacteremias coming from the mouth. *F.nucleatum*, a gram-negative bacteria, is a widespread part of the oral microbiota (Zhou *et al.*, 2014) *F. nucleatum* bacteria interact with one

another by expressing several virulence factors, and they may attach to a wide range of mammalian cell types, including epithelial and endothelial cells, polymorph nuclear neutrophil, monocyte, erythrocytes, fibroblast, and natural killer (NK) cells (Y. Liu *et al.*, 2019). The cell surface protein FadA is a crucial virulence factor in *F. nucleatum*, regulating the bacterium's adherence and invasion. FadA gene expression was substantially greater in human CRC specimens than in neighboring normal tissues (Rubinstein *et al.*, 2013).

This protein allows *F. nucleatum* to bind to E-cadherin in CRC and epithelial cells, activate the -catenin pathway, and trigger the production of transcription factors lymphoid enhancer factor (LEF)/T cell factor (TCF), all of which promote tumor cell proliferation (Chen *et al.*, 2017). The presence of *F. nucleatum* in the gut has been linked to the development of cancer, namely colorectal cancer (CRC), *F.nucleatum* promotes tumor formation in the CRC microenvironment by causing inflammation and a host immunological response. The cell surface proteins FadA, Fap2, and RadD produced by *F. nucleatum* can drive the host to create inflammatory factors and attract inflammatory cells, providing an environment favorable to tumor formation. Furthermore, *F. nucleatum* can cause immunological suppression of the gut mucosa by reducing the activity of immune cells such as macrophages, T cells, and natural killer cells, which contributes to cancer progression.

Detection of *Streptococcus bovis* gene (*23S rRNA* gene) by using PCR Technique

The results of detection of *S. bovis* among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following *S. bovis* was detected in 20 (40%) out 50 of healthy people also *S. bovis* was detected in 26 (52%) out 50 in patients with GIT. While, the highest percentage recorded in patient with cancer which *S. bovis* was detected in 58 (58%) out 100 sample with significant differences between the samples that gave positive and negative to bacteria detection in three groups at $p<0.05$ and significant differences between those groups as in table (6) and figure (2).

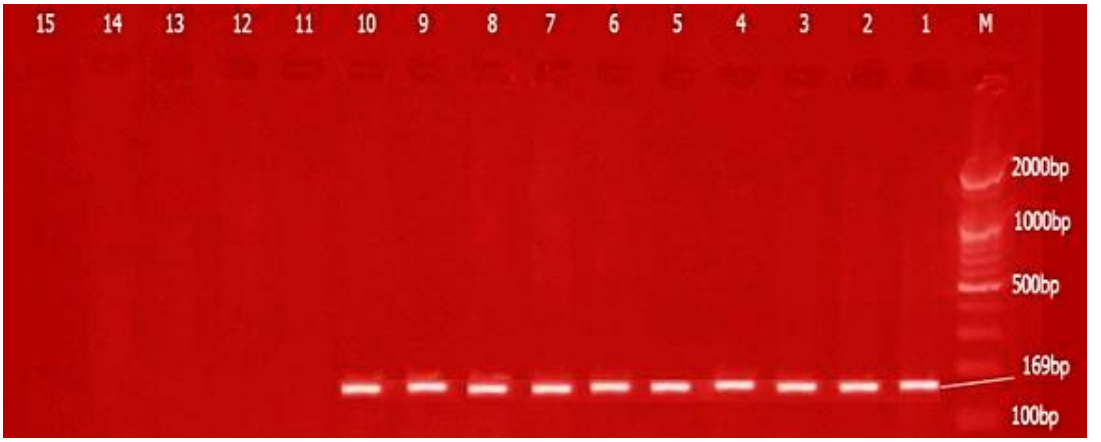

Figure (2): Agarose gel electrophoresis of PCR product obtained with *Streptococcus*

bovis strains -specific primers that generated 169bp amplicon. Lanes (1-10); positive, Lanes (11-15); negative, Lane M represent 100bp DNA ladder.

Table (6)
Identification of *Streptococcus bovis* among three studied groups (Healthy, patients with GIT and patients with Cancer)

Results	Healthy N=50	*GIT N=50*	Cancer N=100	P value[a]
Positive	20 (40)	26 (52)	58 (58)	0.035*
Negative	30 (60)	24 (48)	42 (42)	
P value[b]	0.046*	0.689	0.110	

* represent a significant difference at p<0.05.

A, among all three studied groups. B, between positive and negative results.
The current study's findings were consistent with those of (Al-Jashamy *et al.*, 2010), who analyzed a total of 166 stool specimens taken from ill and healthy patients. Out of 166 cases investigated, the overall prevalence of *S. bovis* was determined to be 41 (24.7 percent). 41 (48.6 percent) of these *S. bovis* isolates were discovered in individuals with colonic polyps, adenocarcinomas, inflammatory bowel disease (IBD), and chronic gastrointestinal tract illness (GIT). In the same study, it was discovered that the incidence of colorectal cancer was 24.7 percent, with adenocarcinomas accounting for 51 percent and having the highest incidence in the sigmoid region of the colon. In the majority of IBD and chronic GIT patients, ulcerative colitis was present (41.4 percent).

Our findings were consistent with those of (Heidarian *et al.*, 2017), who collected 29 fecal samples from IBD patients (22 with UC and 7 with CD) and 29 healthy volunteers, and reported on the relative abundance of *streptococcus spp* and its association with disease activity in the inflammatory bowel. As the relative abundance of *Streptococcus spp*. is greater in IBD patients compared to controls, Streptococcus spp. overgrowth was found in 27% of UC (6/22) patients and 42% of CD (3/7).

Streptococcus spp overgrowth was seen in 5% (5/29) of the control groups. On the other hand, the proportion of cancer patients infected with *S. bovis* was 58 (58 percent) out of 100 samples, which is lower than the percentage of cancer patients infected with *F. nucleatum*, which was 96 (96 percent) out of 100 samples. This proportion was agreed upon (Eshaghi *et al.,* 2020) during the identification of *S.bovis* in 55 individuals with colon illnesses the findings found In terms of DNA of *S. bovis*, 3 biopsy samples (5.5 percent) with a 95 percent confidence interval were declared positive and 52 (94.5 percent) were reported negative in 55 biopsy samples from patients with colon illnesses.

Little and colleagues (2019) found in a quality assurance study that 70% of patients were referred for colonoscopy following an episode of *S. bovis* bacteremia, suggesting the need for greater identification of the link of S. bovis bacteremia and malignancy as CRC. *S. bovis* is a Gram-positive group D streptococcus detected in 11% of asymptomatic people' gastrointestinal tract (Krishnan, and Eslick, 2014). McCoy and Mason discovered a link between colorectal cancer

(CRC) and group D streptococci in individuals with endocarditis in 1951. Streptococci are the most common bacteria in the oral cavity and one of the most common bacteria in the human GIT; yet, little is known about their prevalence and functions in the GIT of IBD patients.

Streptococcal interactions with a variety of host cells frequently result in pro-inflammatory responses. *S. suis* subtilisin-like protease (SspA) can generate a pro-inflammatory response in macrophages via Toll-like receptor 2 (TLR2) (Frolova *et al.*, 2008). TLR2 expression was shown to be greater in the intestines of IBD patients compared to healthy persons, according to Sybille Landwehr-Kenzel et al. (2014). This observation might explain our patients' higher activity index score indirectly by interacting TLRs with high levels of Streptococci in their gut. Streptococcus group A M protein, rhamnose glucose polymers, glucosyltransferase, and *S. mutans* AgI/II polypeptides are further Streptococci components that can trigger inflammatory responses in human tissue (Landwehr-Kenzel *et al.*, 2014). This bacterium's lipoproteins and other components can cause a pro-inflammatory reaction in the colon. Streptococcus metabolites and structural components interact with several cellular equivalents implicated in the inflammatory response in IBD patients, either directly or indirectly (Heidarian *et al.*, 2017).

Detection of *Porphyromonas gingivalis* gene (*16SrRNA* gene) by using PCR Technique

The results of detection of *P. gingivalis* among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following *P. gingivalis* was detected in 11 (22%)out 50 of healthy people also *P. gingivalis* was detected in 14 (28%)out 50 in patients with GIT. While, in patient with cancer which *P. gingivalis* was detected in 31 (31%)out 100 sample which recorded the highest value between groups, however there was significant difference between the samples that gave positive and negative to bacteria detection in three groups at $p<0.05$ and non significant differences between those groups as in table (7) and figure (3).

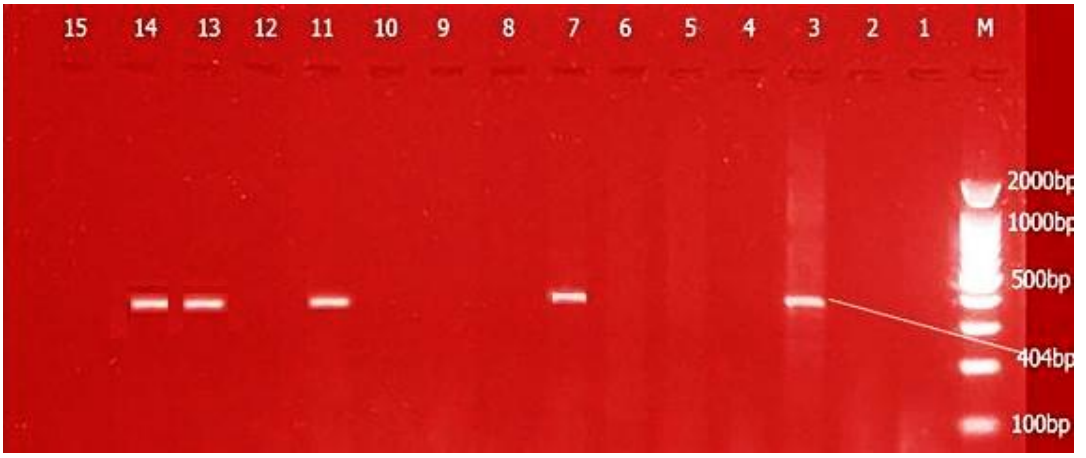

Figure (3): Agarose gel electrophoresis of PCR product obtained with *Porphyromonas gingivalis* strains -specific primers that generated 404bp

amplicon. Lanes (3, 7, 11, 13, 14); positive, other Lanes are negative, Lane M represent 100bp DNA ladder.

Table (7)
Identification of *Porphyromonas gingivalis* among three studied groups (Healthy, patients with GIT and patients with Cancer)

Results	Healthy N=50	*GIT N=50*	Cancer N=100	P value[a]
Positive	11 (22)	14 (28)	31 (31)	0.345
Negative	39 (78)	36 (72)	69 (69)	
P value[b]	<0.0001*	<0.0001*	<0.0001*	

* represent a significant difference at p<0.05.

A, among all three studied groups. B, between positive and negative results.
The associated the *P. gingivalis* with GIT and with cancer agreed with (Wang *et al.*, 2021) in their results to detect *P. gingivalis* in feces 77 subjects divided into 22 control individuals and 23 subjects with colorectal cancer once they compared the levels of *P. gingivalis* in the feces from the individuals in each group , observed an increased abundance of *P. gingivalis* in the colorectal cancer group compared with the adenoma and healthy donor groups which indicate that the increased abundance of *P. gingivalis* in the gut microbiota may be a general feature of colorectal cancer.

Kong *et al.*, (2021) used different methods to detect P. gingivalis in different digestive system cancers. The specimens (tissues)from patients diagnosed with histologically confirmed primary tumors were included 50 cases of oral squamous cell carcinoma cancer (OSCC), 50 cases of esophageal squamous cell carcinoma cancer (ESCC), 30 cases of gastric cardia adenocarcinoma (GCA), 30 cases of gastric cancer, the positive samples were identified by PCR as oral squamous 28 (56.00 percent), esophageal squamous cell carcinoma (ESCC) 21 (42.00 percent), gastric cardia adenocarcinoma (GCA) 5 (16.675 percent), gastric cancer (GC) 1 (3.33 percent), and colorectal cancer (CRC) 1 (2.86 percent).

Moreover in our study , *P. gingivalis* was detected in 14 (28%)out 50 in patients with GTI such as inflammatory bow disease , there many studies in model mice provide the relationship between ulcerative colitis and *P. gingivalis* ,Tsuzuno and colleagues, (2021), discovered that oral treatment of P. gingivalis to mice dramatically exacerbated the severity of colitis, and that ingested P. gingivalis damaged the colonic epithelial barrier by lowering the expression of tight junction proteins in vivo. *P. gingivalis*-specific epithelial barrier breakdown was suggested by in vitro permeability experiments utilizing the intestinal epithelial cell line, the disruption of the epithelial barrier by *P. gingivalis* was proposed. *P.gingivalis* aggravates gastrointestinal inflammation in vulnerable hosts by interacting directly with the intestinal epithelial barrier. The same findings were achieved by (Zhao *et al.,* 2021), who discovered that *P. gingivalis* enhanced the severity of UC in part via peptidylarginine deiminase (PPAD) in a mouse model. This aggravation was due to PPAD, which caused an aberrant immunological response and increased the Th17/Treg ratio. *P. gingivalis* has the ability to adhere to and invade

host cells, and oral mucosal epithelial cells are thought to be the most significant intracellular environment for P. gingivalis (Lee *et al.*, 2018).

Bacterial invasion occurs in four stages: (a) entrance, (b) survival, (c) replication, and (d) departure from the host cell (Casadevall, 2008). *P. gingivalis* is linked to a bad prognosis in human colorectal cancer because it colonizes and gets enriched in tumor tissue, activating the NLRP3 inflammasome in the immune microenvironment and eventually promoting colorectal carcinoma growth (Wang *et al.,* 2021). *P. gingivalis* increases distant metastasis and chemoresistance to anti-cancer therapies, as well as the proliferation of oral tumor cells, via altering defensin gene expression, peptidyl-arginine deiminase, and noncanonical -catenin activation. Furthermore, *P. gingivalis* has been linked to precancerous stomach and colon lesions, esophageal squamous cell carcinoma, head and neck carcinoma (larynx, throat, lip, mouth, and salivary glands), and pancreatic cancer (Olsen *et al.*, 2019).

Conclusion

The study found that *S. bovis spp gallolyticus, F. nucleatum, P. gingivalis* participated in causing cancer in the digestive system were found in the samples of people who do not have cancer and people who have problems in the digestive system by using the PCR technique, but the presence of the genes of these bacteria in people who have cancer was higher than it is in patients with Gastrointestinal system problems, and these results supported the theory and previous studies about the role of these bacteria in causing ulcers in the digestive tract.

References

1. Abed, J., Maalouf, N., Manson, A. L., Earl, A. M., Parhi, L., Emgård, J. E., ... & Bachrach, G. (2020). Colon cancer-associated Fusobacterium nucleatum may originate from the oral cavity and reach colon tumors via the circulatory system. Frontiers in cellular and infection microbiology, 10, 400.
2. Al-Jashamy, K., Murad, A., Zeehaida, M., Rohaini, M., & Hasnan, J. (2010). Prevalence of colorectal cancer associated with Streptococcus bovis among inflammatory bowel and chronic gastrointestinal tract disease patients. Asian Pac J Cancer Prev, 11(6), 1765-8.
3. Boehm, E. T., Thon, C., Kupcinskas, J., Steponaitiene, R., Skieceviciene, J., Canbay, A., ... & Link, A. (2020). Fusobacterium nucleatum is associated with worse prognosis in Lauren's diffuse type gastric cancer patients. Scientific reports, 10(1), 1-12.
4. Casadevall A. (2008). Evolution of intracellular pathogens. Annu. Rev. Microbiol. 62, 19–33. doi: 10.1146/annurev.micro.61.080706.093305
5. Chen, Y., Peng, Y., Yu, J., Chen, T., Wu, Y., Shi, L., ... & Fu, X. (2017). Invasive Fusobacterium nucleatum activates beta-catenin signaling in colorectal cancer via a TLR4/P-PAK1 cascade. Oncotarget, 8(19), 31802.
6. Cuellar-Gómez, H., Ocharán-Hernández, M. E., Calzada-Mendoza, C. C., & Comoto-Santacruz, D. A. (2021). Association of Fusobacterium nucleatum infection and colorectal cancer: A Mexican study. Revista de Gastroenterología de México (English Edition).

7. Eshaghi, F., Bashi Zadeh Fakhar, H., Ghane, M., & Shokry, J. (2020). Diagnostic Evaluation of Streptococcus gallolyticus Infection in Patients with Colon Diseases by Polymerase Chain Reaction (PCR) and Culturing Methods. International Journal of Cancer Management, 13(6).
8. Frolova, L., Drastich, P., Rossmann, P., Klimesova, K., & Tlaskalova-Hogenova, H. (2008). Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. Journal of Histochemistry & Cytochemistry, 56(3), 267-274.
9. Heidarian, F., Noormohammadi, Z., Asadzadeh Aghdaei, H., & Alebouyeh, M. (2017). Relative abundance of streptococcus spp. and its association with disease activity in inflammatory bowel disease patients compared with controls. Archives of Clinical Infectious Diseases, 12(2).
10. Huh, J. W., & Roh, T. Y. (2020). Opportunistic detection of Fusobacterium nucleatum as a marker for the early gut microbial dysbiosis. BMC microbiology, 20(1), 1-17.
11. Kaindi, D. W. M., Kogi-Makau, W., Lule, G. N., Kreikemeyer, B., Renault, P., Bonfoh, B., ... & Jans, C. (2018). Colorectal cancer-associated Streptococcus infantarius subsp. infantarius differ from a major dairy lineage providing evidence for pathogenic, pathobiont and food-grade lineages. Scientific reports, 8(1), 1-11.
12. Kashani, N., Abadi, A. B., Rahimi, F., & Forootan, M. (2020). FadA-positive Fusobacterium nucleatum is prevalent in biopsy specimens of Iranian patients with colorectal cancer. New microbes and new infections, 34, 100651.
13. Khalil, K. H., Al-Hassawi, B. A., & Abdo, J. M. (2018). Histopathologicalevaluation Of Colorectal Carcinoma. Duhok Medical Journal, 12(2), 45-67.
14. Kong, J., Yuan, X., Wang, J., Liu, Y., Sun, W., Gu, B., ... & Gao, S. (2021). Frequencies of Porphyromonas gingivalis Detection in Oral-Digestive Tract Tumors. Pathology and Oncology Research, 27, 64.
15. Krishnan, S., & Eslick, G. D. (2014). Streptococcus bovis infection and colorectal neoplasia: a meta-analysis. Colorectal Disease, 16(9), 672-680.
16. Kumar, R., Herold, J. L., Taylor, J., Xu, J., & Xu, Y. (2018). Variations among Streptococcus gallolyticus subsp. gallolyticus strains in connection with colorectal cancer. Scientific reports, 8(1), 1-10.
17. Landwehr-Kenzel, S., & Henneke, P. (2014). Interaction of Streptococcus agalactiae and cellular innate immunity in colonization and disease. Frontiers in immunology, 5, 519.
18. Lee K., Roberts J. S., Choi C. H., Atanasova K. R., Yilmaz O. (2018). Porphyromonas gingivalis traffics into endoplasmic reticulum-rich-autophagosomes for successful survival in human gingival epithelial cells. Virulence 9 (1), 845–859. doi: 10.1080/21505594.2018.1454171.
19. Lee, Y., Eun, C. S., Lee, A. R., Park, C. H., & Han, D. S. (2016). Fusobacterium isolates recovered from colonic biopsies of inflammatory bowel disease patients in Korea. Annals of laboratory medicine, 36(4), 387-389.
20. Liardo, R. L. E., Borzì, A. M., Spatola, C., Martino, B., Privitera, G., Basile, F., ... & Vacante, M. (2021). Effects of infections on the pathogenesis of cancer. The Indian journal of medical research, 153(4), 431.

21. Little, D. H., Onizuka, K. M., & Khan, K. J. (2019). Referral for Colonoscopy in Patients with Streptococcus bovis Bacteremia and the Association with Colorectal Cancer and Adenomatous Polyps: A Quality Assurance Study. Gastrointestinal Disorders, 1(4), 385-390.
22. Liu, Y., Baba, Y., Ishimoto, T., Iwatsuki, M., Hiyoshi, Y., Miyamoto, Y., ... & Baba, H. (2019). Progress in characterizing the linkage between Fusobacterium nucleatum and gastrointestinal cancer. Journal of gastroenterology, 54(1), 33-41.
23. Mallika, L., Augustine, D., Rao, R. S., Patil, S., Alamir, A. W. H., Awan, K. H., ... & Prasad, K. (2020). Does microbiome shift play a role in carcinogenesis? A systematic.
24. McCoy, W. C., & Mason 3rd, J. M. (1951). Enterococcal endocarditis associated with carcinoma of the sigmoid; report of a case. Journal of the Medical Association of the State of Alabama, 21(6), 162-166.
25. Meseeha, M., & Attia, M. (2018). Streptococcus bovis Bactremia and Colon Cancer: A Well Established but Frequently Forgotten Association: 1456. Official journal of the American College of Gastroenterology| ACG, 113, S835-S836.
26. Mitsuhashi, K., Nosho, K., Sukawa, Y., Matsunaga, Y., Ito, M., Kurihara, H., ... & Shinomura, Y. (2015). Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. Oncotarget, 6(9), 7209.
27. Nguyen, H. T., & Duong, H. Q. (2018). The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncology letters, 16(1), 9-18.
28. Olsen, I., & Yilmaz, Ö. (2019). Possible role of Porphyromonas gingivalis in orodigestive cancers. Journal of oral microbiology, 11(1), 1563410.
29. Rawla, P., & Barsouk, A. (2019). Epidemiology of gastric cancer: global trends, risk factors and prevention. Przeglad gastroenterologiczny, 14(1), 26.
30. Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., & Han, Y. W. (2013). Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell host & microbe, 14(2), 195-206.
31. Sawicki, T., Ruszkowska, M., Danielewicz, A., Niedźwiedzka, E., Arłukowicz, T., & Przybyłowicz, K. E. (2021). A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers, 13(9), 2025.
32. Sitarz, R., Skierucha, M., Mielko, J., Offerhaus, G., Maciejewski, R., & Polkowski, W. P. (2018). Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer management and research, 10, 239–248.
33. Su, W., Chen, Y., Cao, P., Chen, Y., Guo, Y., Wang, S., & Dong, W. (2020). Fusobacterium nucleatum Promotes the Development of Ulcerative Colitis by Inducing the Autophagic Cell Death of Intestinal Epithelial. Frontiers in cellular and infection microbiology, 10, 739.
34. Suehiro, Y., Sakai, K., Nishioka, M., Hashimoto, S., Takami, T., Higaki, S., ... & Yamasaki, T. (2017). Highly sensitive stool DNA testing of Fusobacterium nucleatum as a marker for detection of colorectal tumours in a Japanese population. Annals of clinical biochemistry, 54(1), 86-91.

35. Thélin, C., & Sikka, S. (2015). Epidemiology of colorectal cancer—Incidence, lifetime risk factors statistics and temporal trends. Screening for colorectal Cancer with colonoscopy. London: IntechOpen Limited, 61-77.
36. Tsuzuno, T., Takahashi, N., Yamada-Hara, M., Yokoji-Takeuchi, M., Sulijaya, B., Aoki-Nonaka, Y., ... & Yamazaki, K. (2021). Ingestion of Porphyromonas gingivalis exacerbates colitis via intestinal epithelial barrier disruption in mice. Journal of Periodontal Research, 56(2), 275-288.
37. Wang, X., Jia, Y., Wen, L., Mu, W., Wu, X., Liu, T., ... & Wang, Z. (2021). Porphyromonas gingivalis promotes colorectal carcinoma by activating the hematopoietic NLRP3 inflammasome. Cancer Research, 81(10), 2745-2759.
38. Wu, J., Li, Q., & Fu, X. (2019). Fusobacterium nucleatum contributes to the carcinogenesis of colorectal cancer by inducing inflammation and suppressing host immunity. Translational oncology, 12(6), 846-851.
39. Zhao, X., Liu, J., Zhang, C., Yu, N., Lu, Z., Zhang, S., ... & Pan, Y. (2021). Porphyromonas gingivalis exacerbates ulcerative colitis via Porphyromonas gingivalis peptidylarginine deiminase. International journal of oral science, 13(1), 1-12.
40. Zhou, X., Liu, X., Li, J., Aprecio, R. M., Zhang, W., & Li, Y. (2015). Real-time PCR quantification of six periodontal pathogens in saliva samples from healthy young adults. Clinical oral investigations, 19(4), 937-946.
41. Monson, J.R.; Weiser, M.R.; Buie, W.D.; Chang, G.J.; Rafferty, J.F.; Buie,W.D. and Rafferty, J. (2013). Standards practice task force of the American society of colon and rectal surgeons. Practice parameters for the management of rectal cancer. Dis Colon Rectum, 56: 535-550.
42. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. 2007. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 18:581–592.
43. Ferlay, J.; Shin, HR. and Bray, F. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer 2010; 127(12):2893–2917.
44. Virchow R. Die krankhaften Geschwu¨lste, vol 1. Berlin: August Hirschwald,1863.
45. T.O. Keku, S. Dulal, A. Deveaux, B. Jovov, X. HanThe gastrointestinal microbiota and colorectal cancerAm J Physiol Gastrointest Liver Physiol, 308 (2015), pp. G351-G363