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# Prevalence Of Some Viruses Related To GIT Cancers Among Patients In South Area Of Iraq

**Msc. Hawraa Hamza Abbas* , Dr. Mohannad Abdulrazzaq Gati Al nasery* , Prof. Dr. Basim abdulhussein jarullah****

* Department of Biology- College of Science- Thi-Qar University.

** Department of microbiology- College of veterinary medicine- Thi-Qar University.

**Abstract**

The study was investigated and diagnosis the viruses 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), the results revealed, three types of viruses were diagnosed that are related to diseases of the digestive system, with regard to viruses, including: Human papilloma viruses (HPVs), The Epstein-Barr virus (EBV), Polyoma virus (MCPV) and Human herpes virus (HHV) by PCR technique, however the percentage of existence of these viruses was higher in patients with cancer than in patients with GIT disease as following : the present of *Hpv18* gene was (13%) in patients with cancer, while in patients with GIT diseases (13%) but in the healthy (0 %) , while the results of *EBV* genes showed *EBNA1* in patients with cancer (24%) but (14%) in GIT diseases patients , in the healthy was (10%), whereas the percentage of *Polyoma virus* genes (*VP1* and *VP2* genes) (28 %) in people with cancer, but in patients with GIT diseases (26%), healthy people (20%) with significant differences between those groups .Our findings supported the hypothesis these viruses can be has a place in etiology of gastrointestinal cancer.

**Key words:** HPV / EBV / Polyoma virus / GIT cancer / gastric cancer/ colon cancer/ PCR

## Introduction

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). Human viruses, such as Epstein-Barr virus (EBV) and human papillomavirus (HPV) are also thought to play a role in different cancers (Metwally et al., 2021; Ursu et al., 2021). Human papillomavirus (HPV) is a type of non-enveloped DNA virus with a virion size of _55 nm that causes benign cancers in people who are sexually active. HPV infection, on the other hand, can sometimes lead to the development of malignant lesions (Graham, 2017). There are more than 80 HPV subtypes, which are divided into low-risk and high-risk categories. Low-risk HPVs (types 6, 11, and 33, for example) are linked to the development of warts and benign lesions. High-risk HPVs (including 16, 18, and 31) have been linked to a variety of malignancies, including cervical and anal carcinoma (Rantshabeng et al., 2017). The Epstein–Barr virus (EBV) was the first virus to cause cancer in humans, and it has been linked to a variety of human malignancies arising from epithelial cells, lymphocytes, and mesenchymal cells (Ko, 2015). The virus moves via the oropharyngeal epithelium to B cells, where it creates a lifetime latent infection, and infection spreads from host to host by salivary contact. EBV has three primary latency patterns, each of which helps the virus dodge the host's immune response while increasing B-cell survival and proliferation (Mesri et al., 2014).

**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.

**Blood sample collection**

By using disposable syringes, a four milliliter of venous blood were drowning from redial vein of each person. The blood was placed in EDTA tubes and do not allowed to clot at room temperature.

**Table (1): Primers were used in PCR**

| Virus | Type of Primer | Sequence | PCR product (bp) | Reference |
|---|---|---|---|---|
| Human Pailoma Virus | HPV18-F | GACACCTTAATGAAAAACGACGA | 103 bp | Osman, et al. 2019 |
| | HPV18-R | CGTCGTTGGAGTCGTTCCTG | | |
| Epstein–Barr virus | EBNA1-F | AAGGAGGGTGGTTTGGAAAG | 297 bp | Aboulkassim, et al, 2015 |
| | EBNA1-R | AGACAATGGACTCCCTTAGC | | |
| Polyomavirus | VP1 gene-F | GGAGGAGTAGAAGTTCTAGAA | 434 bp | Whiley, Mackay and Sloots, 2001 |
| | VP1 gene-R | TCTGGGTACTTTGTYCTGTA | | |
| | VP2 gene-F | CACTTTTGGGGGACCTAGT | 131 bp | |
| | VP2 gene-R | CTCTACAGTAGCAAGGGATGC | | |

**Viral Nucleic Acid Extraction**

Viral DNA was extracted from blood samples by using Viral Nucleic Acid Extraction Kit II (Geneaid. USA) and done according to company instruction.

**Molecular detection of HPV by polymerase chain reaction:**

The gene of HPV18 are used to detection of **HPV**, 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 HPV18 gene of HPV**

| NO. | Stage | Temperature | Time | Number of cycle |
|---|---|---|---|---|
| 1 | Initial denaturation | 95 ◦C | 5 min | 1 |
| 2 | Denaturation | 95◦C | 45 sec | 35 |
| 3 | Annealing | 58◦C | 45 sec | |
| 4 | Elongation | 72◦C | 45 sec | |
| 5 | Final elongation | 72◦C | 10 min | 1 |

**Molecular detection of Epstein–Barr virus by polymerase chain reaction:**

The genes of EBNA1 gene were used to detection of **Epstein–Barr virus**, 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 EBNA1 gene of **Epstein–Barr virus**

| NO. | Stage | Temperature | Time | Number of cycle |
|---|---|---|---|---|
| 1 | Initial denaturation | 95 ◦C | 5 min | 1 |
| 2 | Denaturation | 95◦C | 45 sec | 35 |
| 3 | Annealing | 55 ◦C | 45 sec | |
| 4 | Elongation | 72◦C | 45 sec | |

| | | | | |
|---|---|---|---|---|
| 5 | Final elongation | 72◦C | 10 min | 1 |

**Molecular detection of Polyomavirus by polymerase chain reaction.**

The genes of VP1 and VP2 gene were used to detection of Polyomavirus, 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 VP1 and VP2 gene of Polyomavirus.**

| NO. | Stage | Temperature | Time | Number of cycle |
|---|---|---|---|---|
| 1 | Initial denaturation | 95 ◦C | 5 min | 1 |
| 2 | Denaturation | 95◦C | 45 sec | 35 |
| 3 | Annealing | VP1(52 ◦C)<br>VP2(56◦C) | 45 sec | |
| 4 | Elongation | 72◦C | 45 sec | |
| 5 | Final elongation | 72◦C | 10 min | 1 |

**Result & Discussion**

**Detection of Human Papiloma Virus gene (HPV18 gene) by using PCR Technique**

The results of detection of HPV among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following HPV was not detected (0% ) out 50 of healthy people which were negative for HPV DNA but HPV was detected 6 (12%)out 50 in patients with GIT. While, in patient with cancer which HPV was detected 13 (13%)out 100 sample of patients with Cancer , however there was significant difference between the samples that gave positive and negative to HPV DNA detection in three groups at $p<0.05$ and significant differences between those groups as in table (5) and figure (1) :

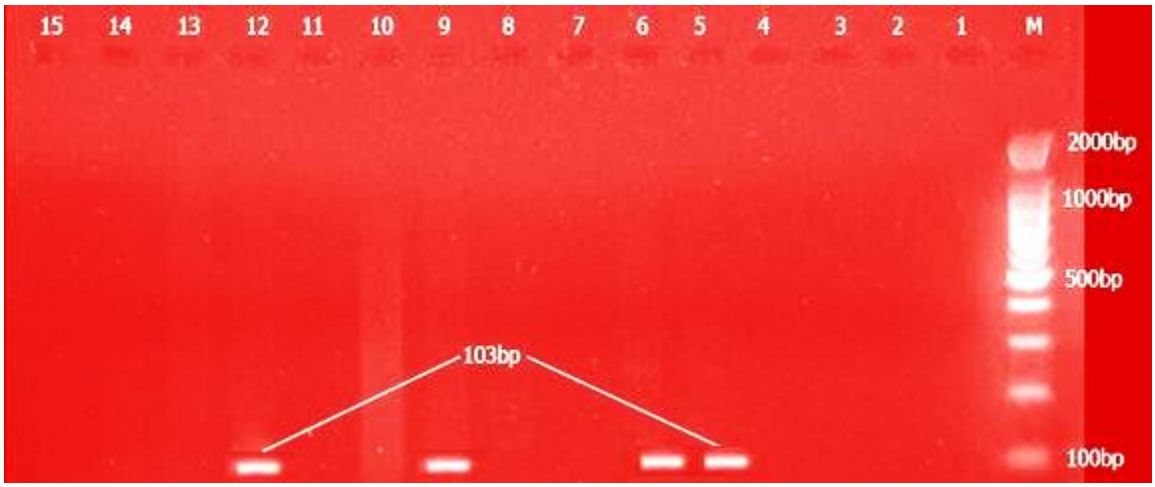


Figure (1): Agarose gel electrophoresis of PCR product obtained with HPV -specific primers that generated 103bp amplicon. Lanes (5, 6, 9, 12); positive, other Lanes are negative, Lane M represent 100bp DNA ladder.

**Table (5): Identification of HPV among three studied groups (Healthy, patients with GIT and patients with Cancer).**

| Results | Healthy<br>N=50 | GIT<br>N=50 | Cancer<br>N=100 | P value[a] |
|---|---|---|---|---|
| Positive | 0 (0) | 6 (12) | 13 (13) | 0.030* |
| Negative | 50 (100) | 44 (88) | 87 (87) | |
| 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 current study's findings in the GIT group were consistent with those obtained in the CD and UC groups when compared to a control group of patients with diverticulitis (Suk et al., 2018). Individuals with IBD were more likely to develop HPV-related malignancies than those with diverticulitis, and the connection between HPV-related cancer and IBD is substantially stronger. Bernabe-Dones et al. (2016) discovered that when fresh frozen tissue was collected from 45 non-familial, sporadic CRC patients (cases) and 36 cancer-free individuals (controls), PCR revealed that HPV DNA was detected in 19 of the 45 (42.2 percent) CRC samples and 1 of the 36 (2.8 percent) control samples studied. This decrease in the control group was observed in our data, which corresponded with (Bodaghi et al., 2005) who discovered that colon tissues (51%) patients with colorectal cancer were positive for HPV DNA but all control persons' colorectal tissues tested negative for HPV DNA. Patients with inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are likely to be at high risk of human papillomavirus (HPV) infection

due to therapeutic immunosuppression and changes in mucosal immunity, according to Suk et al. (2018); however, the association of IBD and HPV-related cancers is unknown. However, the absence of HPV DNA in healthy persons was related to Human papillomavirus (HPV) is the most prevalent sexually transmitted infection (STI) in the world, with a significant detrimental influence on individual social life. Sexually active women and men will get infected at least once in their lives (Chesson et al., 2014), however the persons in our research had no sex encounter with infected. The integration of the HPV genome into a host chromosome is also a mechanism of HPV-induced carcinogenesis. HPV genome integration is frequently found at common human genome vulnerable spots (Thorland et al., 2003). When it comes to the HPV genome, integration follows a more distinct pattern. HPV genome integration has been associated with particular changes in host cellular gene expression (Alazawi et al, 2001). Cells expressing E6/E7 from integrated HPV sequences outperform cells with episomal HPV genomes in terms of selective growth. Experiments reveal that repressing E2 in cervical cancer cell lines produces growth suppression, lending credence to the idea that loss of E2 repressor activity may be crucial for malignant development (Yeo-Teh et al., 2018).

**Detection of Epstein-Barr virus gene (EBNA1 gene) by using PCR Technique**

The results of detection of EBV among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following EBV was detected in 5 (10 %) out 50 of healthy people also EBV was detected in 7 (14%) out 50 in patients with GIT. Finally, the percentage EBV detection of in patient with cancer which was 24 (24 %)out 100 sample with significant differences between the samples that gave positive and negative to DNA detection in three groups at $p<0.05$ also there was significant difference between three groups as in table (6) and figure (2).

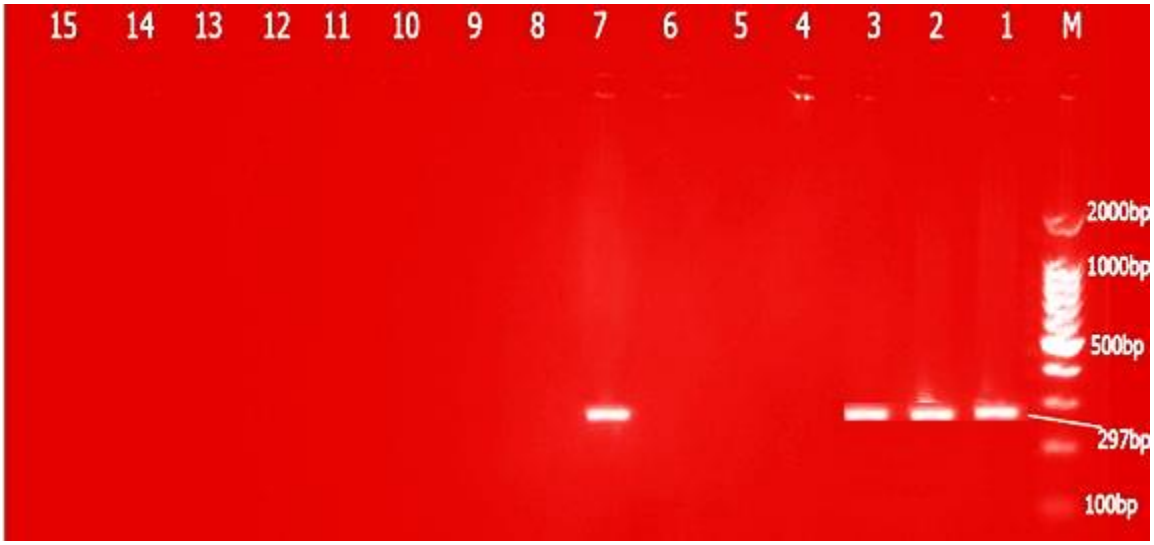


Figure (2): Agarose gel electrophoresis of PCR product obtained with Epstein-Barr virus (EBV) -specific primers that generated 297bp amplicon. Lanes (1, 2, 3, 7); positive, other Lanes are negative, Lane M represent 100bp DNA ladder.

**Table (6):** Identification of Epstein-Barr virus (EBV) 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 | 5 (10) | 7 (14) | 24 (24) | 0.021* |
| Negative | 45 (90) | 43 (86) | 76 (76) | |
| 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.

This study collected peripheral blood and intestinal samples from 92 consecutive UC patients and normal colonic mucosal tissues from 20 as controls, which agreed with several previous investigations (Xu et al., 2020). EBV testing and evaluation were carried out using EBV-DNA polymerase chain reaction (PCR) and immunohistochemistry. Among the 92 UC patients in the research, 36 (39.1%) were found to have superimposed EBV colitis (EBER > 2/HPF) (compared to 0% for controls), which was also agreed with (Li et al., 2019) EBV was found in 33 of 99 IBD patients (33.3 percent).

The frequency of EBV among controls was 7.5 percent (3/40). Finally, Derakhshan et al., (2018) agreed that in their investigation, 30 samples of intestinal biopsy from patients with UC in the active phase of illness and 30 biopsy samples from healthy participants were analyzed using the PCR technique. EBV was identified in 10 of 30 biopsies from UC patients and 3 of 30 biopsies from non-UC individuals (33.3 % vs. 10 %). On the other hand, the presence among cancer patients was 24 (24%) out of 100.

This matched with several research, such as Liu et al., (2021), who disclosed in their study that blood EBV DNA was evaluated in 399 patients with colonic ulcers, with 30 instances being positive. The EBV-encoded RNA (EBER) was found in the intestinal tissues of 13 EBV-positive individuals (EBER-positive group). In 17 cases, the test came out negative (EBER-negative group). The risk of acute EBV infection in patients with colonic ulcer was 7.52 percent. According to (Lima et al., 2012), 73 out of 151 (48.3 percent) stomach cancer tissue cases submitted to the PCR method were EBV-positive. Nagi and his colleagues (2021) discovered EBV and HPV in the same tissue when they collected 107 (13 normal and 94 cancer) colorectal samples and discovered that 60 of the 94 CRC samples (63.8 %) were positive for highrisk HPVs. the existence of EBV, it was discovered that 27/94 (28.7 %) of the CRC samples tested positive for this oncovirus, whereas all normal colorectal samples tested negative. Gupta et al., (2020) reported similar results, with samples from 64 (60%) male and 42 (40%) female patients included in the research.

The large bowel data revealed the presence of high-risk HPVs (80%) and EBVs (14%–25%) in CRC samples. Morever , EBV causes infectious mononucleosis as well as a variety of epithelial and

lymphoproliferative cancers such as nasopharyngeal and gastric carcinoma, as well as multiple B-cell lymphomas and T-cell/NK lymphomas (Bedri et al., 2019).

**Detection of Human Polyoma Virus genes (VP1 gene and VP2 gene) by using PCR Technique**

The results of detection of Human Polyoma Virus among three studied groups (Healthy, patients with GIT and patients with Cancer) by using PCR , revealed as the following Human Polyoma Virus was detected in 10 (20%)out 50 of healthy people also Human Polyoma Virus was detected in 13 (26%)out 50 in patients with GIT.

Finally, the highest percentage recorded in patient with cancer which Human Polyoma Virus was detected in 28 (28%) out 100 sample with significant differences between the samples that gave positive and negative to DNA detection in three groups at $p<0.05$ also there was significant difference between three groups as in table (7) and figures (3) and (4)..

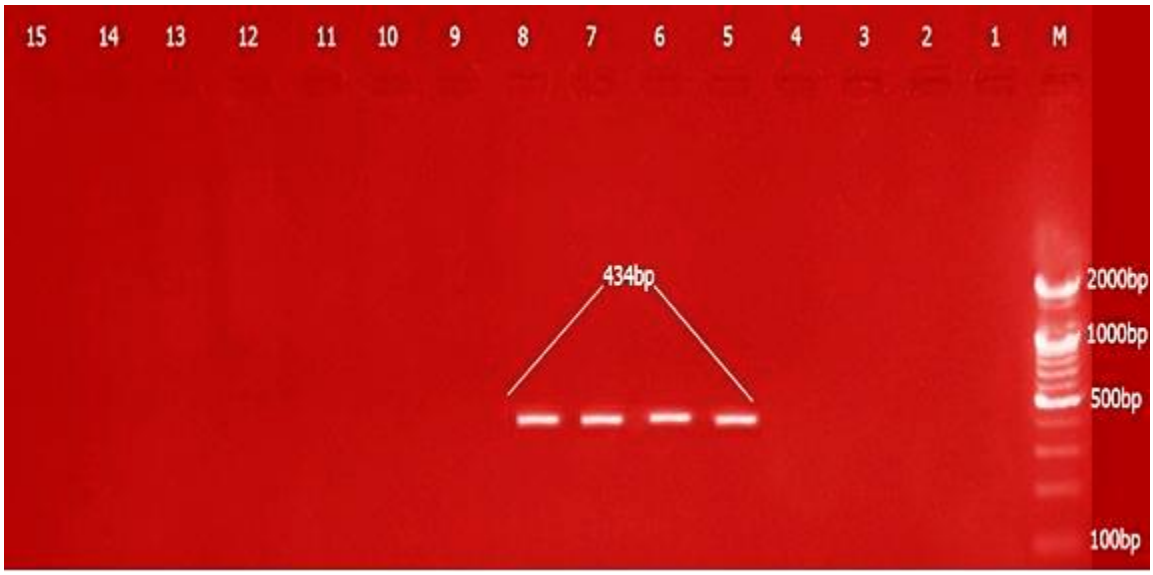


Figure (3): Agarose gel electrophoresis of PCR product obtained with Polyoma Virus-specific primers that generated 434bp amplicon of Capsid Protein VP1. Lanes (5-8); positive, other Lanes are negative, Lane M represent 100bp DNA ladder.

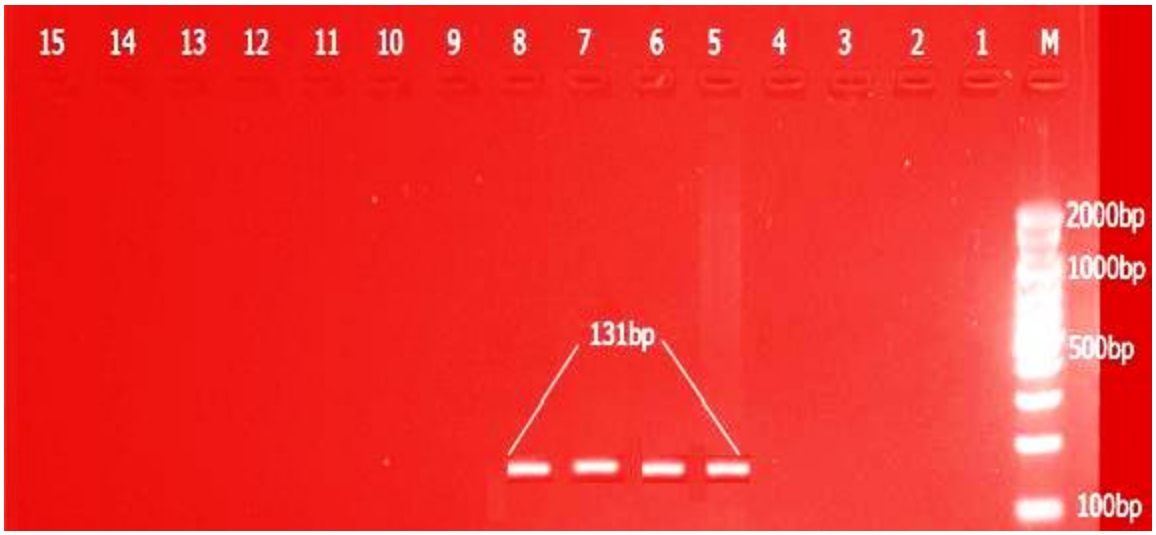


Figure (4): Agarose gel electrophoresis of PCR product obtained with Polyoma Virus-specific primers that generated 131bp amplicon of Capsid Protein VP2. Lanes (5-8); positive, other Lanes are negative, Lane M represent 100bp DNA ladder.

**Table (4-18):** Identification of Polyoma Virus among three studied groups (Healthy, patients with GIT and patients with Cancer).

| Results | Healthy<br>N=50 | GIT<br>N=50 | Cancer<br>N=100 | P value[a] |
|---|---|---|---|---|
| Positive | 10 (20) | 13 (26) | 28 (28) | 0.393 |
| Negative | 40 (80) | 37 (74) | 72 (72) | |
| 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 current study found agreement in the group with GI with (Flores et al., 2014) when they studied the prevalence of BKV infection in patients with inflammatory bowel disease (IBD). The study included 53 patients with IBD whose underlying diseases included Crohn's disease (60.4 %) and ulcerative colitis (39.6 %), and discovered that KV viruria was considerably more prevalent in IBD patients than in controls (54.7 % versus 11.3 % ). Moreover our findings agreed with different studies that focused on the association between BKV with gastric cancer as (Dolci et al., 2021) , reported when investigated the association between colon cancer and six polyomavirus which collected from125 colon cancer patients' different specimens, 110 tumor tissues, 55 negative surgical margins, and 39 peripheral blood samples were analyzed for the presence of six HPyVs: JCPyV, BKPyV, MCPyV, HPyV 6, 7, and 9, the findings revealed HPyVs genome was detected in

33/204 samples ,the significant higher positivity was found in tumor tissues, also agreed with (Enam et al., 2002) who investigated the link between Human Polyomavirus JCV and colon cancer. A total of 27 colon cancers were paraffin-embedded, and gene amplification confirmed the presence of the viral early genome in 22 of the 27 samples. In their review research on the incidence of JC and BK viruses in patients with colorectal cancer, Shoraka and his colleagues (2021) discovered that out of 1461 relevant papers, 24 publications were suitable and included. Infection with JCV was considerably greater in CRC patients compared to healthy controls, normal surrounding mucosa, and colorectal adenoma, but not in non-CRC patients. HPyVs have been related to particular severe diseases, primarily in immunocompromised people.

The expanding number of people infected with HIV or using immunosuppressive medicines enhances the possibility of novel illnesses related with known or yet-to-be-discovered HPyVs. In fact, there is a chance that additional HPyVs are linked to other cancers in humans. Several research undertaken over the last decade have looked at the presence of practically all known HPyVs in human malignancies (Prado et al., 2018). A recent research found that the TSPyV mT antigen interacts with PP2A via a Zn2+-binding domain motif, and that this connection is essential for the activation of the pro-proliferative MEK/ERK/MNK1 signaling axis (Wu JH et al., 2017). As previously stated, the PP2A-MAPK-regulated pathway is essential for controlling apoptosis and cell growth. As a result, disruptions in this system may result in uncontrolled cell proliferation (Silverstein et al., 2002).

**Conclusions:**

The study found HPV / EBV / Polyoma virus / 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 viruses 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.

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