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Molecular and Phylogenic study of some Gastrointestinal Bacteria and Viruses associated with Cancer in the South area of Iraq

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Abstract

This study investigated the diagnosis the bacteria that associated with GIT cancer and GIT diseases. A total number of 200 blood and biopsy samples were collected during the period from September 2020 to June 2021. Samples 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 the current study, three types of bacteria were diagnosed which relate to diseases of the digestive system, including: *Streptococcus bovis spp gallolyticus, Fusobacterium nucleatum, Porphyromonas gingivalisbut*by by usig PCR technique. It was found that the highest percentage was for patients with cancer. 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 the existence of these viruses was higher in patients with cancer than in patients with GIT disease. The DNA sequencing analysis showed a clear genetic variation between bacteria associated with GIT cancer based on 16SrRNA gene according to phylogenetic tree analysis that analyzed compared Standard NCBI-BLAST but in viruses associated with GIT cancer based on specific gene according to phylogenetic tree analysis compared Standard NCBI-BLAST. Except the *HPV18* and *Jc Polyomavirus* the similarity was 100% homology which genetic closed related to NCBI-Blast *HPV18* and *Jc Polyomavirus.*

Keywords* :*Fusobacterium nucleatum / Streptococcus bovis / Porphyromonas gingivalis/ HPV / EBV / Polyoma virus/GIT cancer / gastric cancer/ colon cancer/ PCR/ DNA sequence

1. 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). 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 (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).

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).

2. Materials and methods

2.1 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. 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.

2.2 Blood sample collection

By using disposable syringes, four milliliters of venous blood were drowning from redial vein of each participate. Each blood sample was placed in EDTA tubes and do not allowed to clot at room temperature.

2.3 Biopsy sample Collection

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 were 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 of Bactria:

Bacteria	Type of Primer	Sequence	PCR product (bp)
Fusobacterium sp	FUSO1-F	GAGAGAG CTTTGCGT CC	610 bp
	FUSO2-R	TGGGCGC TGAGGTT C GAC	
Streptococcus bovis	23S rRNA-F	CCCGGCA TGTAATG CATGTC	169 bp
	23S rRNA-R	TACAACC CCGATGT GTAAACA CA	
P. gingivalis	16S rRNA-F	AGGCAGC TTGCCAT ACTGCG	404 bp
	16S rRNA-R	ACTGTTA GCAACTA CCGATGT	

Table (2): Primers were used in PCR of Virus:

Virus	Type of Primer	Sequence	PCR product (bp)	Refe

Human Pailoma Virus	HPV18-F	GACACCTTAATGAAAAACGACGA	103 bp	Thongsen, et al. 2019
	HPV18-R	CGTCGTTGGAGTCGTTCCTG		
Epstein–Barr virus	EBNA1-F	AAGGAGGGTGGTTTGGAAAG	297 bp	Abdulkasim, 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		

2.4 DNA Bacterial Extraction

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

Molecular detection of *Fusobacterium nucleatum* by polymerase chain reaction.

The gene of FUSO was used for the detection of ***Fusobacterium nucleatum***, amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 3.

Table (3): Optimized thermo-cycling condition for FUSO gene of *Fusobacterium nucleatum:*

NO.	Stage	Tem.	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

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

The gene of 23SrRNA was used for the detection of ***S. bovis***, amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 4.

Table (4): Optimized thermo-cycling condition for 23SrRNA gene of *S. bovis*

NO	*Stage*	*Tem.*	*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*

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

The gene of 16SrRNA was used for the detection of ***P. gingivalis***, amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 5.

Table (5): Optimized thermo-cycling condition for 16SrRNA gene of *P. gingivalis*

NO.	*Stage*	*Tem.*	*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*

Viral Nucleic Acid Extraction

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

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

The gene of HPV18 was used to detect of ***HPV***, amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 6.

Table (6): Optimized thermo-cycling condition for HPV18 gene of *HPV*

NO.	Stage	Tem.	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

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

Genes of EBNA1 were used to detect ***Epstein–Barr virus***, amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 7.

Table (7): Optimized thermo-cycling condition for EBNA1 gene of ***Epstein–Barr virus***

NO.	Stage	Tem.	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

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

Genes of VP1 and VP2 were used for the detection of *Polyomavirus,* amplification and melting conditions were optimized for the PCR using specific primer, these conditions produce the most specific and sufficient PCR product, as shown in Table 8.

Table (8): Optimized thermo-cycling condition for VP1 and VP2 gene of *Polyomavirus.*

NO.	Stage	Tem.	Time	Number of cycle
1	Initial denaturation	95 °C	5 min	1
2	Denaturation	95°C	45 sec	35
3	Annealing	VP1(52 °C) VP2(56°C)	45 sec	
4	Elongation	72°C	45 sec	
5	Final elongation	72°C	10 min	1

3. Results

3.1 DNA Sequancing and Phylogentic tree analysis of bacteria

The DNA sequencing analysis showed a clear genetic variation between bacteria associated with GIT cancer based on 16SrRNA gen according to phylogenetic tree analysis that analyzed *F.nucleatum*, *S.bovis* and *P. gingivalis* with Standard NCBI-BLAST CABKNP010000002.1 *F.nucleatum*, *S.bovis* (AB168118.1) and *P. gingivalis* W50/1125722.3.

3.2 DNA Sequancing and Phylogentic tree analysis of *F.nucleatum*

The findings of phylogentic tree analysis of 5 *F.nucleatum* isolated based on 16SrRNA gen found *F.nucleatum* isolate No.4 and 5 were showed genetic closed related to NCBI-Blast *F.nucleatum* (CABKNP010000002.1 *F.nucleatum*). On the other hand, the other isolates were showed differences at total genetic variation (0.002 -0.0020%) as shown in Figure 1.

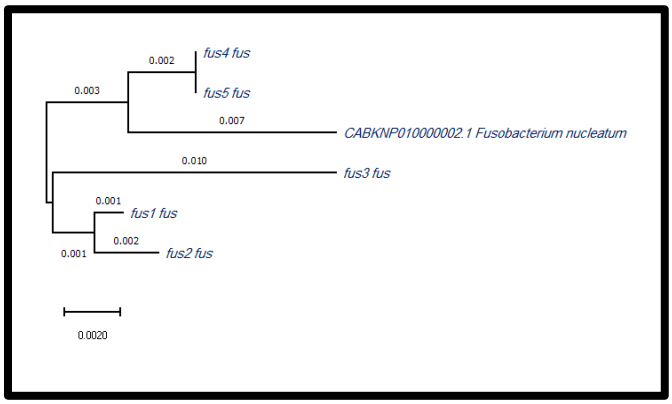

Figure 1: Phylogeny of 5 *Fusobacterium nucleatum* strains based on 16SrRNA gen. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with a reference.

3.3 DNA Sequancing and Phylogentic tree analysis of *S.bovis*

The findings of phylogentic tree analysis of 5 ***S.bovis*** isolated based on based on 16SrRNA gen found ***S.bovis*** isolate No.4 showed genetic closed related to NCBI-Blast ***S.bovis*** (AB168118.1). However, the other isolates showed differences at total genetic variation (0.006 -0.0031%) as shown Figure 2.

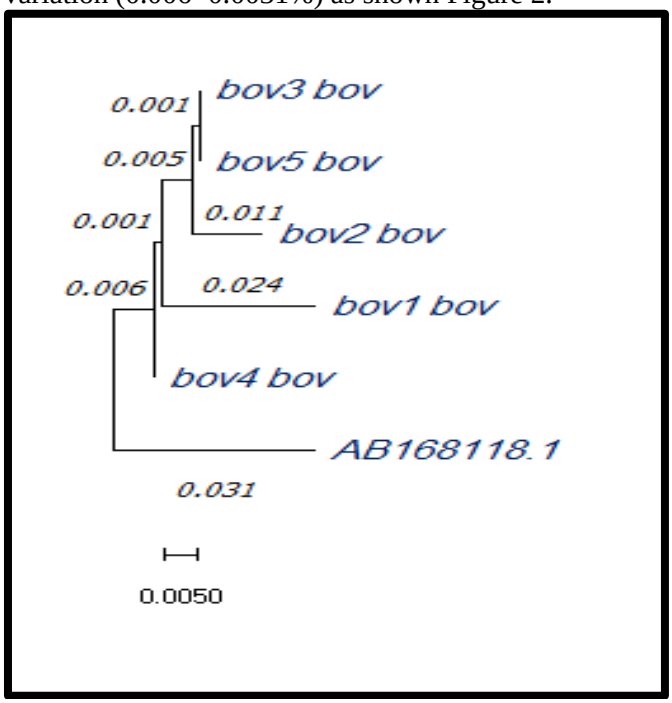

Figure 2: Phylogeny of 5 *Streptococcus bovis* strains based on 16SrRNA gen. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with a reference.

3.4 DNA Sequancing and Phylogentic tree analysis of *P. gingivalis*

The findings of phylogentic tree analysis of 5 *P. gingivalis* isolates based on based on 16SrRNA gen found *P. gingivalis* isolate No.4 showed genetic closed related to NCBI-Blast *P. gingivalis* (*P. gingivalis* W50/1125722.3). On the other hand, the other isolates were showed differences at total genetic variation (0.003 -0.0010%) as shown in Figure 3.

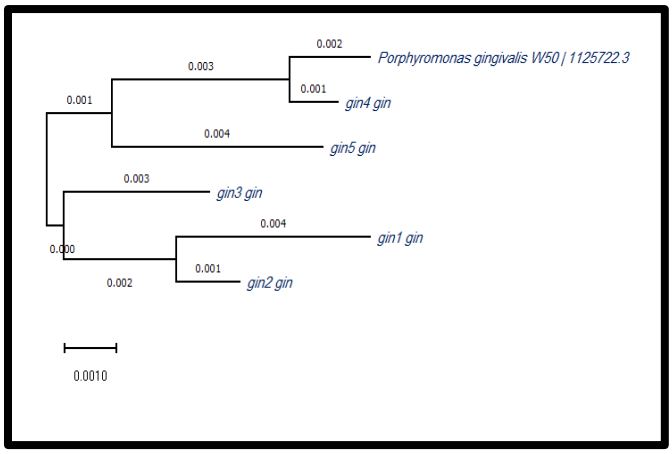

Figure 3: Phylogeny of 5 *Porphyromonas gingivalis* strains based on 16SrRNA gen. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with a reference.

3.5 DNA Sequancing and Phylogentic tree analysis of viruses

The DNA sequencing analysis showed clear genetic variation and similarity between viruses associated with GIT cancer based on specific gene according to phylogenetic tree analysis that analyzed HPV-18, *EBV* ,BK *Polyomavirus* and JC *Polyomavirus* with Standard NCBI-BLAST *NC001357.1 Human papillomavirus 18*, *NC007605.1 human gammaherpersviruses-4* , *NC001538.1* BK *Polyomavirus* Complete genome and *NC001699.1* JC *Polyomavirus complete genome*.

3.6 DNA Sequancing and Phylogentic tree analysis of *Human papillomavirus 18*

The findings of phylogentic tree analysis of 5 HPV-18 based on based on specific gene found all sequences of HPV18 are similar, there is 100% homology which genetic closed related to NCBI-Blast *HPV18* (*NC001357.1 Human papillomavirus 18)* as shown in Figure 4.

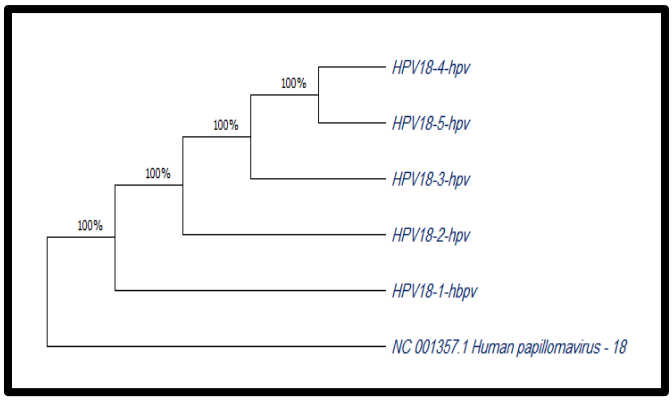

Figure 4: Phylogeny of 5 HPV18 strains based on specific gene. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with reference. Note: all sequences of HPV18 are similar, there is 100% homology.

3.7 DNA Sequancing and Phylogentic tree analysis of *Epstein-Barr virus (EBV)*

The findings of phylogentic tree analysis of 5 *EBV* based on based on specific gene found *EBV* strain No.3 showed genetic closed related to NCBI-Blast *EBV* (*NC007605.1 human gammaherpersviruses-4*) while the other strains were showed differences at total genetic variation (0.003 - 0.0020%) as shown in Figure 5.

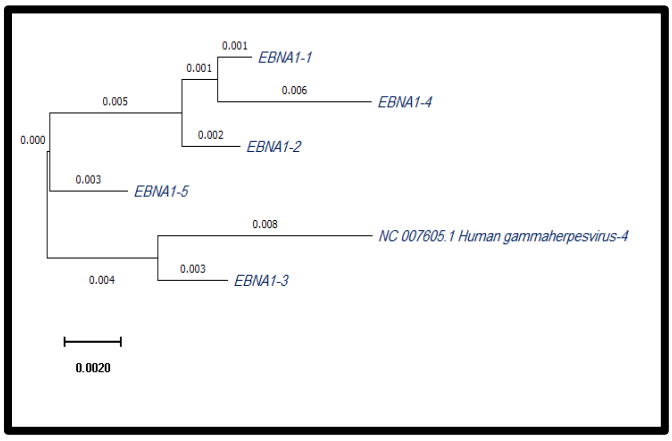

Figure 5: Phylogeny of 5 *Epstein-Barr virus (EBV)* strains based on specific gene. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with a reference.

3.8 DNA Sequancing and Phylogentic tree analysis of BK *Polyomavirus*

The findings of phylogentic tree analysis of 5 **BK *Polyomavirus*** based on based on VP1 gene gen found **BK *Polyomavirus*** strain No.1 and 3 showed genetic closed related to NCBI-Blast BK *Polyomavirus* (*NC001538.1* BK *Polyomavirus* Complete genome) while the other strains were showed differences at total genetic variation (0.002 - 0.0010%) as shown in Figure 6.

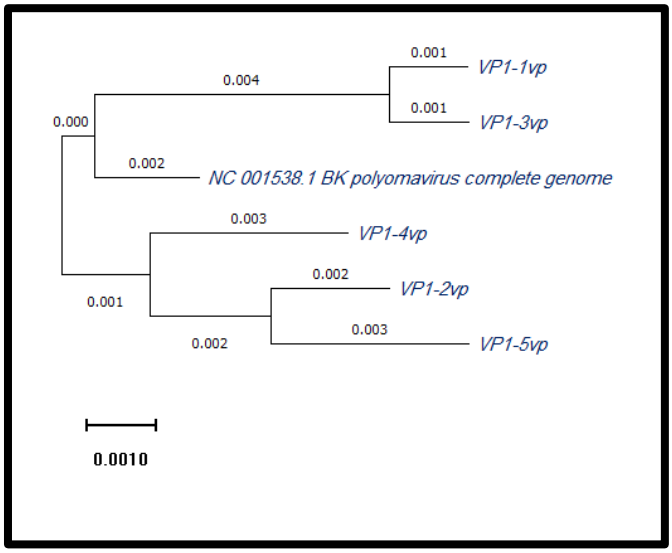

Figure 6: Phylogeny of 5 BK *Polyomavirus* strains based on VP1 gene. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with a reference.

3.9 DNA Sequancing and Phylogentic tree analysis of JC *Polyomavirus*

The findings of phylogentic tree analysis of 5 JC *Polyomavirus* based on based on VP2 gene found all sequences of JC *Polyomavirus* are similar, there is 100% homology which genetic closed related to NCBI-Blast *HPV18* (*NC001699.1* JC *Polyomavirus complete genome*)as shown in Figure 7.

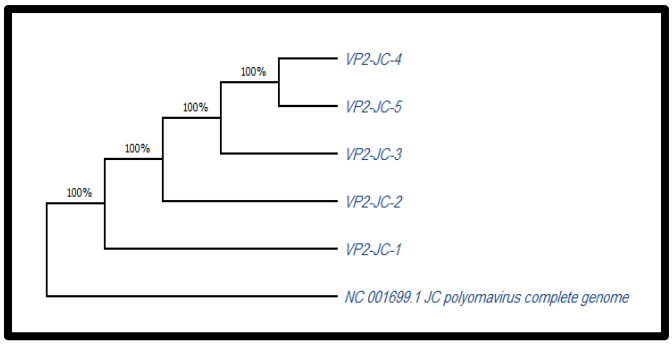

Figure 7: Phylogeny of 5 JC *Polyomavirus* strains based on VP2 gene. This tree was built by Neighbor joining method. Branch lengths were added for each sample to illustrate the distance among studied isolates in comparison with reference. Note: all sequences of JC *Polyomavirus* are similar, there is 100% homology.

4. Discussion

4.1 DNA Sequancing and Phylogentic tree analysis of *F.nucleatum*

The findings of phylogentic tree analysis of 5 *F.nucleatum* isolated based on 16SrRNA gen found *F.nucleatum* isolate No.4 and5 showed genetic closed related to NCBI-Blast *F.nucleatum* (CABKNP010000002.1 *F.nucleatum)* while the other isolates were showed differences at total genetic variation of (0.002 -0.0020%) as shown in Figure 1.

The results agreed with the findings of Richardson et al., 2020 reported during the study of diverse group of Fusobacterium, and it is postulated that F. nucleatum in the GI tract originate from the mouth according to 16 RNA gen the oral communities demonstrate the highest level of variation and have the richest pool of unique sequences, with certain nodes/strains enriched in the GI tract and others diminished during translocation but the bacteria in the gastric and colon/pouch communities showed reduced diversity and are more closely related, this attributed to selective pressure in the GI tract.

4.2 DNA Sequancing and Phylogentic tree analysis of *S.bovis*

The findings of phylogentic tree analysis of 5 *S.bovis* isolated based on based on 16SrRNA gen found *S.bovis* isolate No.4 showed genetic closed related to NCBI-Blast *S.bovis* (AB168118.1) while the other isolates were showed differences at total genetic variation (0.006 -0.0031%) as shown in Figure 2.

This genetic variation was noticed by Lin et al, (2011) when studied the sequencing and comparative genome analysis of two pathogenic Streptococcus gallolyticus subspecies: genome plasticity, adaptation and virulence found S.gallolyticus atcc 43143, S. gallolyticus ucn34 and S. pasteurianus atcc 43144 were of the bovis group with atcc 43143 phylogenetically more related to ucn34 (both biotype i) than to atcc 43144 (biotype ii) of the different subspecies. This variation attributed environmental and host adaptation, moving from a herbivore to man (Lin et al., 2011).

4.3 DNA Sequancing and Phylogentic tree analysis of *P. gingivalis*

The findings of phylogentic tree analysis of 5 *P. gingivalis* isolates based on based on 16SrRNA gen found *P. gingivalis* isolate No.4 showed genetic closed related to NCBI-Blast *P. gingivalis* (*P. gingivalis* W50/1125722.3) while the other isolates were showed differences at total genetic variation (0.003 -0.0010%) as shown Figure 3.

A study revealed that there is a genetic variation and similarity when studies 19 Porphyromonas gingivalis strains deepening on four copies of 16S rRNA gene sequences were identified in each of the eight complete genomes and one in the other 11 unfinished genomes and revealed Phylogenomic comparison based on shared proteins and whole genome nucleotide sequences consistently showed two groups with closely related members (Chen et al., 2017).

4.4 DNA Sequancing and Phylogentic tree analysis of Human papillomavirus 18

The findings of phylogentic tree analysis of 5 HPV-18 based on based on specific gene found all sequences of HPV18 are similar, there is 100% homology which genetic closed related to NCBI-Blast HPV18 (NC001357.1 Human papillomavirus 18) as shown Figure 4.

During Investigation the identification intratypic variants of HPV16 and HPV18 among women with cervical lesions in Tunisia based on Based on E6 and L1 genes noticed HPV16 E6 sequences clustered with the European German lineage (A2) whereas one isolate diverged differently in the L1 region and clustered with the African sub-lineage (B1). HPV 18 E6 sequences clustered with the European sub-lineage (A1) but L1 sequences clustered as a new clade which diverged from A1-A5 (Jendoubi-Ferchichi et al., 2018).

4.5 DNA Sequancing and Phylogentic tree analysis of Epstein-Barr virus (EBV)

The findings of phylogentic tree analysis of 5 EBV based on based on specific gene found EBV strain No.3 showed genetic closed related to NCBI-Blast EBV (NC007605.1 human gammaherpersviruses-4) while the other strains were showed differences at total genetic variation (0.003 -0.0020%) as shown in Figure (5). Lengths were added for each sample to illustrate the distance among studied isolates in comparison with reference.

Shen et al.,(2015) noticed the variations of Epstein-Barr virus (EBV)-encoded small RNAs (EBERs) in NPC biopsies and TW samples of healthy donors The sequence variations of EBER in all cases and healthy donors,also this genetic variation agreed with in (Kwok et al.,2012) in Epstein-Barr Virus isolated associated with nasopharyngeal carcinoma .

However, the EBV genomes and proteins appeared to be highly diverse regardless of types of EBV-associated disease (Choi et al., 2018).

4.6 DNA Sequancing and Phylogentic tree analysis of BK Polyomavirus

The findings of phylogentic tree analysis of 5 BK Polyomavirus based on based on VP1 gene gen found BK Polyomavirus strain No.1 and 3 showed genetic closed related to NCBI-Blast BK Polyomavirus (NC001538.1 BK Polyomavirus Complete genome) while the other strains were showed differences at total genetic variation (0.002 - 0.0010%) as shown in Figure 6.

Our findings agreed (Gorish et al., 2019), identification the BKV subtype that circulates among sudanese patients with PCa found All the BKV LTAg gene sequences derived from Sudanese patients were classified with Subtype-1 BKV strains from Iran and Japan the results recorded that some isolates had identical amino acids with Iranian strains and Japanese strains, whereas other strains had a silent mutation.

4.7 DNA Sequancing and Phylogentic tree analysis of JC Polyomavirus

The findings of phylogentic tree analysis of 5 JC Polyomavirus based on based on VP2 gene found all sequences of JC Polyomavirus are similar, there is 100% homology which genetic closed related to NCBI-Blast HPV18 (NC001699.1 JC Polyomavirus complete genome)as shown Figure 7.

The similarity of Among the 10 isolated JCPyV 7B sequences was large which seven of them has identical sequences to CY AB038249 VP1. Hu and his colleagues (2018) investigate the epidemiology and subtype distribution of JCPyV in HIV-1-infected patients found Among the 10 isolated JCPyV 7B sequences; seven samples had identical sequences to CY AB038249 VP1.

5. Conclusions:

The results of the present study found that *S. bovis spp gallolyticus, F. nucleatum, P. gingivalis* and *HPV / EBV / Polyoma viruses* participated in causing cancer disease in the digestive system. The presence of the genes of these bacteria and virus in people who have cancer was higher than it is in patients with gastrointestinal system problems. The results support the theory and previous studies about the role of these bacteria in causing ulcers in the digestive tract.

Reference:

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