Whole Genome Sequencing of KI Polyomavirus Strain from Patient with Breast Cancer in Basrah City

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Abstract

Background: Polyomaviruses are small, circular, non-enveloped viruses with a diameter of about 40–50 nm, and have a double-stranded DNA molecule descended from the Polyomaviridae. 32 PyV strains are described, more than 10 of which are reported to infect humans. The current study aims to identify the sequence of the complete genome of the KI polyomavirus strain to study phylogenetic analysis and determine variation in the KI Polyomavirus sequence. **Materials and Methods:** One purified viral DNA sample of positive cases of KI Polyomavirus was sent for next-generation sequencing to identify whole genome sequencing. **Results**: The KI Polyomavirus sequence has been registered in the NCBI GenBank under the accession number LC776086.1 KI Polyomavirus. The current study showed that the KI Polyomavirus sequence has a length of 5040 bp. By comparing the sequences, it was found that there were many mutations, some of which were silent, and the other mutations caused a change in the amino acid. Ten reference strains from NCBI were used to create a phylogenetic tree for the studied clinical sample. **Conclusion**: The present results revealed that the studied strain LC776086.1 KI Polyomavirus showed great similarity with one sequence, EF127907 KI polyomavirus Stockholm 350, complete genome.

Keywords: *Human polyomavirus; KI polyomavirus; MSA; NGS; WGS.*

Introduction

In 1953, Polyomaviruses were first discovered in mice and then in various types of rabbits, birds, bats, cattle, sea lions, hamsters, monkeys, and humans (1). The organization of the PyV genome is highly conserved, which comprises three genes, the viral non-coding control regions of approximately 500 bp, and the early and late regions. PyVs are widespread in the population of humans. PyV infection occurs in the early stage of life, but most infections remain asymptomatic (2). Polyomaviruses are small DNA viruses that are capable of persistent infection and have the potential to cause cancer. Worldwide, two polyomaviruses are known to naturally infect humans, BK virus (BKPyV) and JC virus (JCPyV), where both viruses were discovered in 1971 (3). KI polyomavirus (KIPyV) was first discovered and identified at the Karolinska Institute in 2007, hence the name, and was detected as part of a systemic "molecular screening" search for unknown viruses in clinical respiratory samples, the researchers screened the cell-free floats of 20 random nasopharyngeal samples for diagnostics of respiratory tract infections in the Karolinska University laboratory. KIPyV was detected in the blood samples of about 2/62 (3.2%) HIV-positive individuals and 4/130 (3.1%) healthy

blood donors (4). In recent years, many studies have been conducted in Basrah Governorate on the most important pathogenic medical viruses, as in the studies conducted by Shihab *et al.,* 2020 (5), Salman *et al.,* 2021 (6) and, ATBEE *et al.,* 2020 (7), The current study aims to identify the sequence of the complete KIPyV genome strain to analyze the phylogeny to identify variation in KIPyV sequences.

Materials and Methods

Sample collection

In the current study, 195 samples were collected from October 6, 2022, to the end of April 2023. They were collected from Basrah Teaching Hospital, Al-Fayhaa Teaching Hospital, Al-Sadr Teaching Hospital, and the Chest Diseases Center in Basrah City. 89 paraffin-embedded tissue samples from neoplastic and non-neoplastic lesions obtained from the histological examination laboratory and taken from the gastrointestinal tract (GIT) of the stomach, intestine, colon, jejunum, ileum, and rectum were retrospectively collected. As well as the kidneys, breasts, thyroid, tonsils, and lungs. 106 samples of nasal swabs and pleural fluids for respiratory infections were taken after obtaining official approvals for ethical considerations.

DNA extraction

DNA of KIPyV was extracted according to the FavorPrepTM mini tissue genomic DNA extraction kit and following the manufacturer's instructions for Formalin-fixed paraffin-embedded (FFPE) samples, while for respiratory samples, DNA was extracted according to the Bio-DNA purification kit (spin column) following the manufacturer's instructions. The purity concentration of the extracted samples was read using a Nanodrop device.

Molecular identification of KIPyV

Real-time-PCR detected the KIPyV strains with SolGentTM2X Real-Time PCR Smart mix, a qPCR amplification kit with SYBR ® Green I in the mixture. The reaction was performed by mixing 10 µl of SYBR Green, with DNA (4 µl), forward and reverse primer (1 µl of each one), and 4 µl of nuclease-free water. The KI-B-4603- forward primer (5’- GAATGCATTGGCATTCGTGA -3'), the KI-B-4668- reverse primer (5’-GCTGCAATAAGTTTAGATTAGTTGGTGC -3') were used to amplify small T antigen (ST-Ag) gene. The real-time PCR conditions including the first step of the reaction submitted to denaturation for 10 minutes at 95˚C, after 45 cycles, the denaturation step is 20 seconds at 95 ˚C, then 58 ˚C for 30 seconds for the annealing step, then 72 ˚C for 40 seconds for extension step, finally, the extension step was done for 10 minutes at 72˚C. The reaction analysis was subjected to a melting curve to ensure the primer specificity.

The KIPyV strains were also detected by conventional PCR with a SolGentTM 2X Taq PLUS PCR Smart mix 1 kit from SolgGent Biotechnology [Cat. No. STD01B-M50h]. The reaction component in the 12.5 µl includes SolGentTM Taq PLUS DNA Polymerase, Buffer, dNTP, and tracking dye according to the instructions recommended by the manufacturer. The reaction was performed by mixing 12.5 µl of2X Taq PLUS PCR Smart mix1, 2.5 µl of Tracking dye, 3 µl of DNA, 1 µl of Primers (F & R:100 pmol/ µl), and 5 µl of Distilled water. The KI-B-4603- forward primer (5’-GAATGCATTGGCATTCGTGA-3'), the KI-B-4668- reverse primer (5’-GCTGCAATAAGTTTAGATTAGTTGGTGC -3') were used to amplify target sequence 114 bp. The PCR condition was set as an initial denaturation step at 95 °C for 10 minutes, then 45 cycles, the denaturation step is 20 seconds at 95 ˚C, then 58 ˚C for 40 seconds for the annealing step, then 72 ˚C for 45 seconds for the extension step, finally, the extension step was done for 10 minutes at 72˚C. The products of PCR were visually under a UV source on a 2% agarose gel.

Whole genome sequences (WGSs)

The KIPyV DNA sample was sent to Apical Scientific Laboratories/ Malaysia to determine the sequence using next-generation sequencing (NGS) for determining WGSs using a strategy of long PCR amplicon.

Results

Molecular detection of KIPyV

Of the 195 samples, only 18 and 6 were positive by real-time PCR and conventional PCR, respectively (figure 1&2). Several PCR products of approximately 114 bp were amplified. Six samples were sent for sequencing to Macrogen/Korea to confirm the presence of KIPyV DNA, and two samples were successfully sequenced. The first sample was from a patient (34 years old) suffering from breast cancer at Al-Fayhaa Teaching Hospital, and the second sample was from a patient (78 years old) suffering from a respiratory infection at the Chest Diseases Center in Basrah City.

Whole genome sequences

One purified KIPyV DNA sample (the first sample) was sent to determine the sequence of the complete genome, and sequencing of the sample was successful. The KIPyV DNA has been registered in the NCBI GenBank under the accession number LC776086.1 KI Polyomavirus BASRAH88 complete genome. Using the UPGMA method, the evolutionary history of the target sample was inferred (10). An ideal tree is shown with the sum of branch length = 0.00712. The tree was drawn using a scale and calculation of evolutionary distances by the composite maximum likelihood method, with a length of branches in the same units to infer the phylogenetic tree and are in units of base substitutions per site (11). The sequences of 11 references are used in this analysis. All ambiguous positions for each sequence pair were removed (pairwise deletion option). A total of 5040 base pairs were found in the final dataset. The analyses of phylogenetics are performed by using MEGA X (12).

Phylogenetic analyses

Genome structure

According to the clinical strain assembly using the template, EF127907 KI polyomavirus Stockholm 350, complete genome, the target sample in the study was found to be 5040 base pairs in length. The genome structure in the study sample is typical of KIPyV as the genome can be divided into 6 genes. The Open reading frame (ORF) map of the studied sample is shown in Figure (3).

Analyzes of genomic variation

The multiple sequence alignments (MSA) of LC776086.1 KI Polyomavirus BASRAH88 have been done (Figures, 4 and 5) with 10 reference strains including; OQ254786, NC 009238, MK049348, KU746835, KM085447, KC571691, EU358767, EF520289, EF127908, EF127907 to identify any mutations and find any changes in the amino acid expression (Table 1).

The sequences showed the presence of many different mutations, some of them were silent, and most caused a change in the amino acid. There were 38 mutations in the LC776086.1 KI Polyomavirus BASRAH88 sequences, 16 of them were missense which caused changes in the amino acid, and 13 were silent. Importantly, most substitutions lead to a change in amino acids, the nucleotide positions 11 and 12 were mutated from C-to-G and C-to-T substitutions, respectively, leading to a change in amino acids Serine to Cysteine. On the other hand, the LC776086.1 KI Polyomavirus sequences have substitutions often completely different from other reference strains. Using the maximum composite likelihood method, a phylogenetic tree consisting of the complete nucleotide sequences of the target clinical sample with 10 reference strains was constructed, and the evolutionary distances were highlighted. As shown in Figure 6, the studied sample was compared with the reference strains at the level of evolutionary relationships, and the genetic distance between the 11 KIPyV strains was calculated. All samples showed similarity in the sequences. Analysis of the phylogenetic tree showed 7 main clades, the present results revealed that the studied strain LC776086.1 KI Polyomavirus showed great similarity of about 99.25% with one reference strain, EF127907 KI polyomavirus Stockholm 350, complete genome with genetic distance or sequence divergence 0.00712 reflecting a close relationship of these strains.

Table (1): mutations among studied sample sequence compared with reference strains

Accession number											Site	Mutations	
LC776086	EF127907	EF127908	EF520289	EU358767	KC571691	KM085447	KU746835	MK049348	NC 009238	OQ254786		Type	Amino acids
G	C	C	C	C	C	C	C	C	C	C	11	Missense	Serine to Cysteine
T	C	C	C	C	C	C	C	C	C	C	12		
C	T	T	T	T	T	T	T	T	T	T	13	Missense	Cysteine to proline
C	G	G	G	G	G	G	G	G	G	G	14		
C	C	A	T	T	C	T	A	C	T	C	76	Silent	
A	T	T	T	T	T	T	T	T	T	T	83	Missense	Valine to Aspartic acid
C	A	A	A	A	A	A	A	A	A	T	84		
T	C	C	C	C	C	C	C	C	C	C	354	Silent	
A	G	G	G	G	G	G	G	G	G	G	414	Silent	
A	C	C	C	C	C	C	C	C	C	C	417	Silent	
G	A	A	A	A	A	A	A	A	A	A	418	Missense	Threonine to Alanine
G	G	C	G	C	C	C	C	G	C	G	1177	Missense	proline to Alanine
C	T	T	T	T	T	T	T	T	T	T	1251	Silent	
C	T	T	T	T	T	T	T	T	T	T	1252	Missense	Cysteine to Histidine
A	G	G	G	G	G	G	G	G	G	G	1253		
G	C	C	C	C	C	C	C	C	C	C	1400	Missense	Proline to Arginine
G	C	C	C	C	C	C	C	C	C	C	1401		
A	C	C	C	C	C	C	C	C	C	C	1402	Missense	Histidine to Serine
G	A	A	A	A	A	A	A	A	A	A	1403		
T	C	C	C	C	C	C	C	C	C	C	1404		
T	A	A	A	A	A	A	A	A	A	A	1405	Missense	Isoleucine to Phenylalanine
T	A	A	A	A	A	A	A	A	A	A	2217	Silent	
C	T	T	T	T	T	T	T	T	T	T	2229	Silent	
A	A	G	G	G	G	G	G	G	G	G	2599	Missense	Alanine to Threonine
C	T	T	T	T	T	T	T	T	T	T	3099	Silent	
C	T	T	T	T	T	T	T	T	T	T	3105	Silent	
G	A	A	A	A	A	A	A	A	A	A	3327	Silent	
C	T	T	T	T	T	T	T	T	T	T	3546	Silent	
A	T	T	T	T	T	T	T	T	T	T	3547	Missense	Tryptophan to Arginine
C	T	T	T	T	T	T	T	T	T	T	3804	Silent	
T	G	G	G	G	G	G	G	G	G	G	3839	Missense	Cysteine to Phenylalanine
A	C	C	C	C	C	C	C	C	C	C	4302	Silent	
A	C	C	C	C	C	C	C	C	C	C	4303	Missense	Glutamine to Arginine
G	A	A	A	A	A	A	A	A	A	A	4304		
G	C	C	C	C	C	C	C	C	C	C	4485	Missense	Asparagine to Lysine
T	C	C	C	C	C	C	C	C	C	C	4672	Missense	Proline to Leucine
T	C	C	C	C	C	C	C	C	C	C	4673		
T	G	G	G	G	G	G	G	G	G	G	5029	Missense	Glycine to Tryptophan

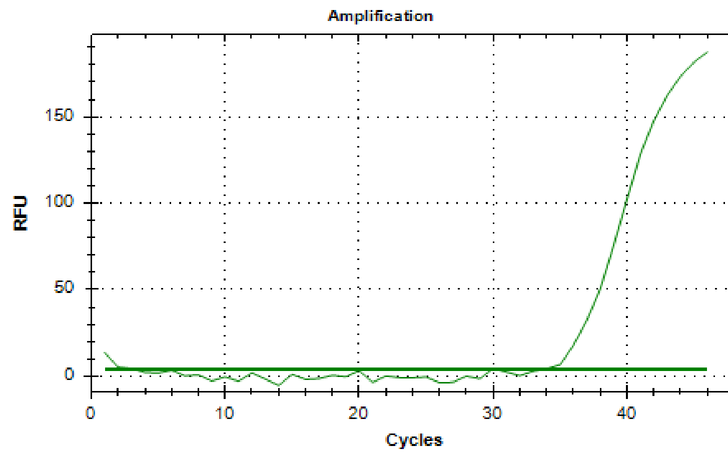

Figure (1): amplification curve of KI Polyomavirus. A representative amplification curve of the threshold was set automatically by the machine at the typical CTs range.

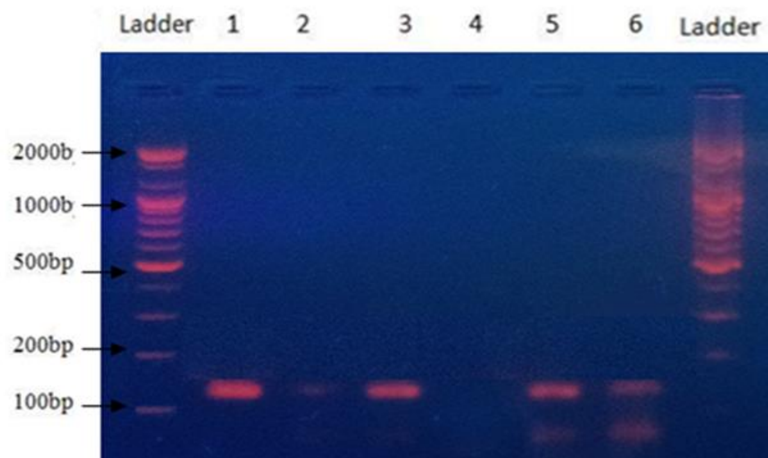

Figure (2): Agarose gel electrophoresis at (70 V) for (50 min) of PCR amplified products of KIPyV. First and last lane = DNA ladder (100-2000 bp), Lanes 1,3,5,6 = positive samples, Lanes 2,4 = negative samples

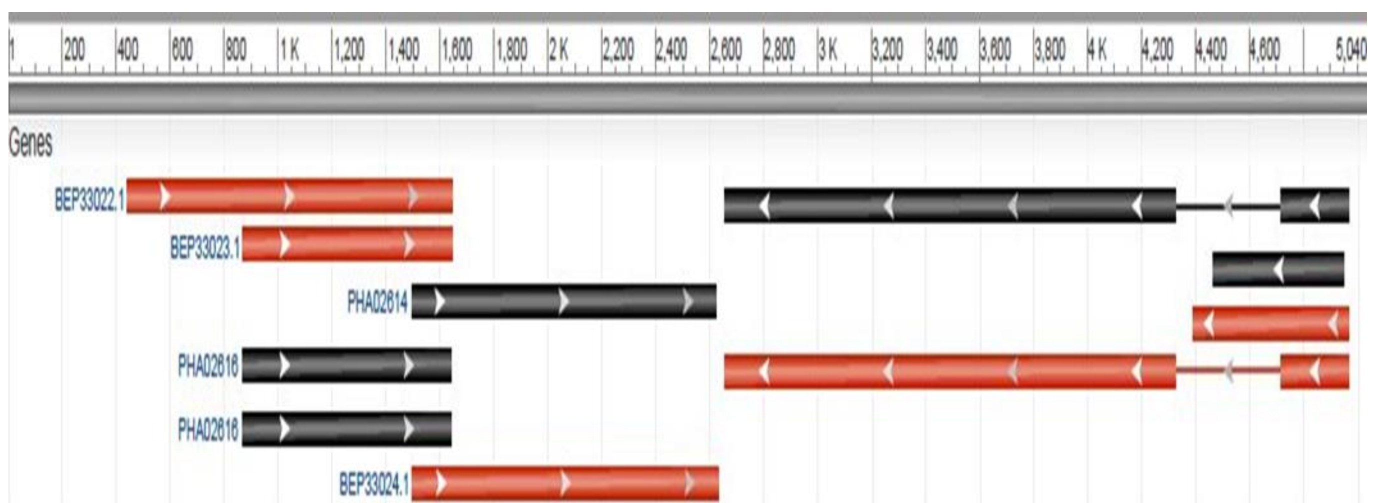

Figure (3): map of the studied sample.

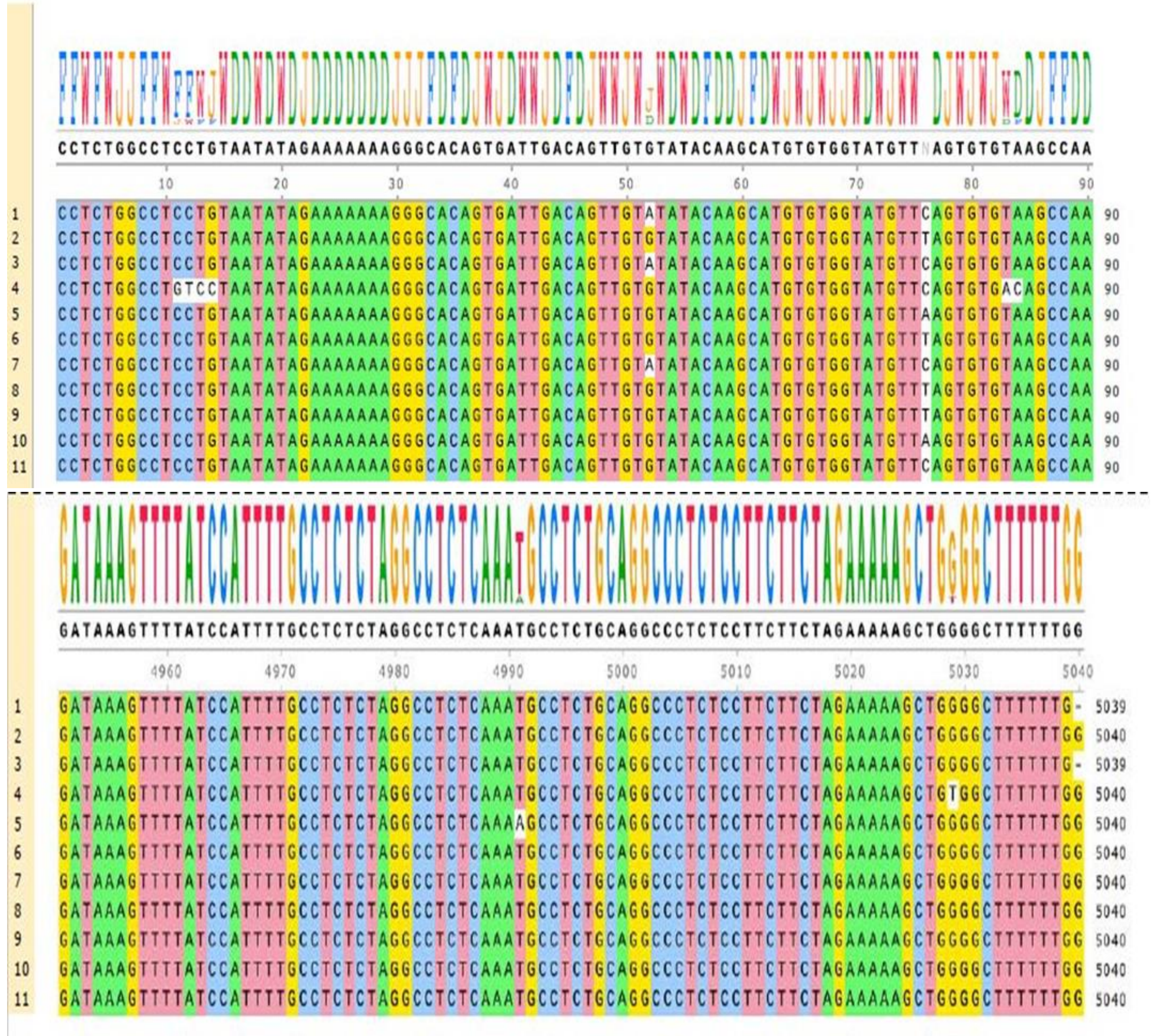

Figure (4): MSA of the DNA sequences of the studied sample with the reference strains. This figure represents part of the complete genome.

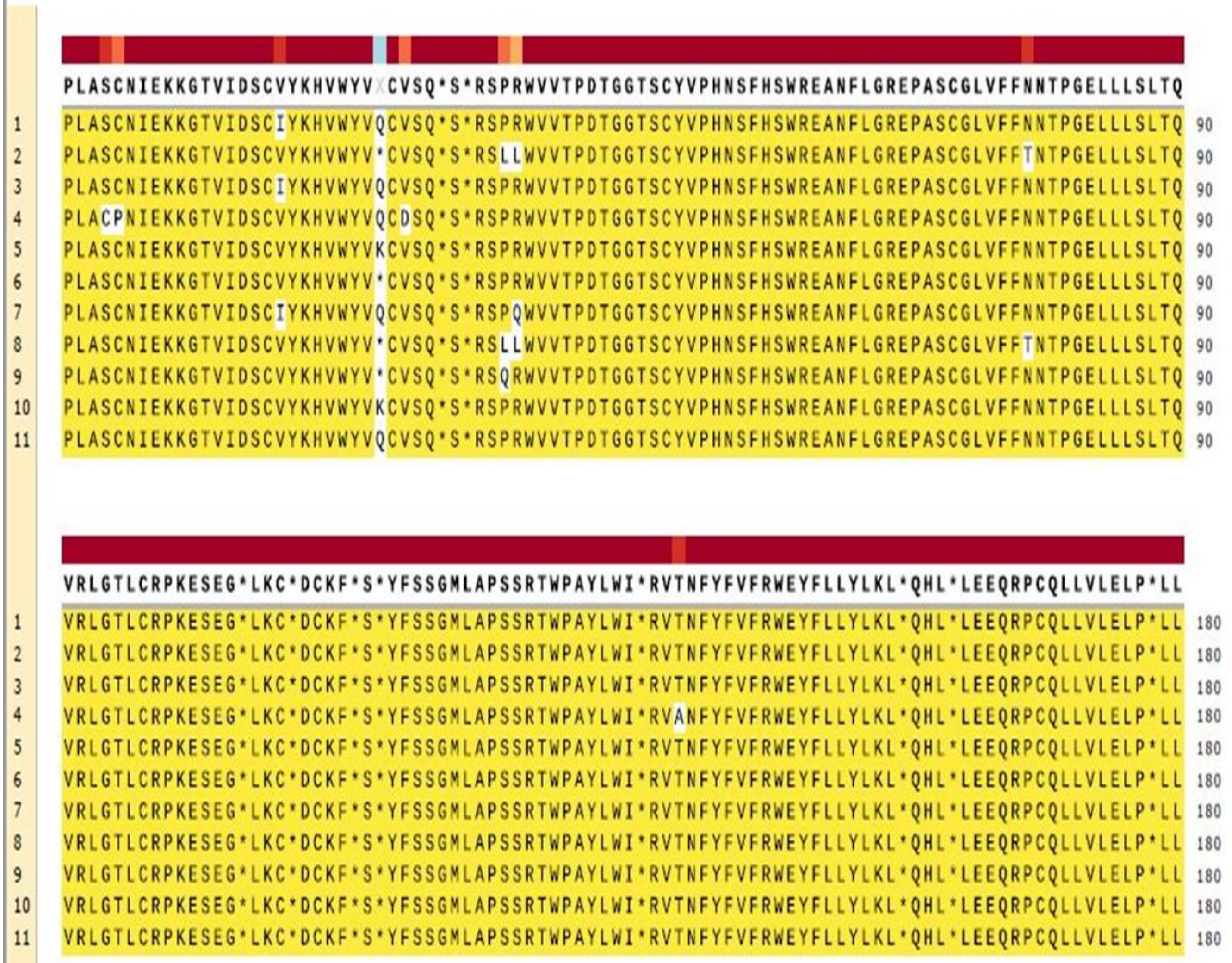

Figure (5): MSA of amino acids in the sample studied with the reference KI Polyomavirus strains.

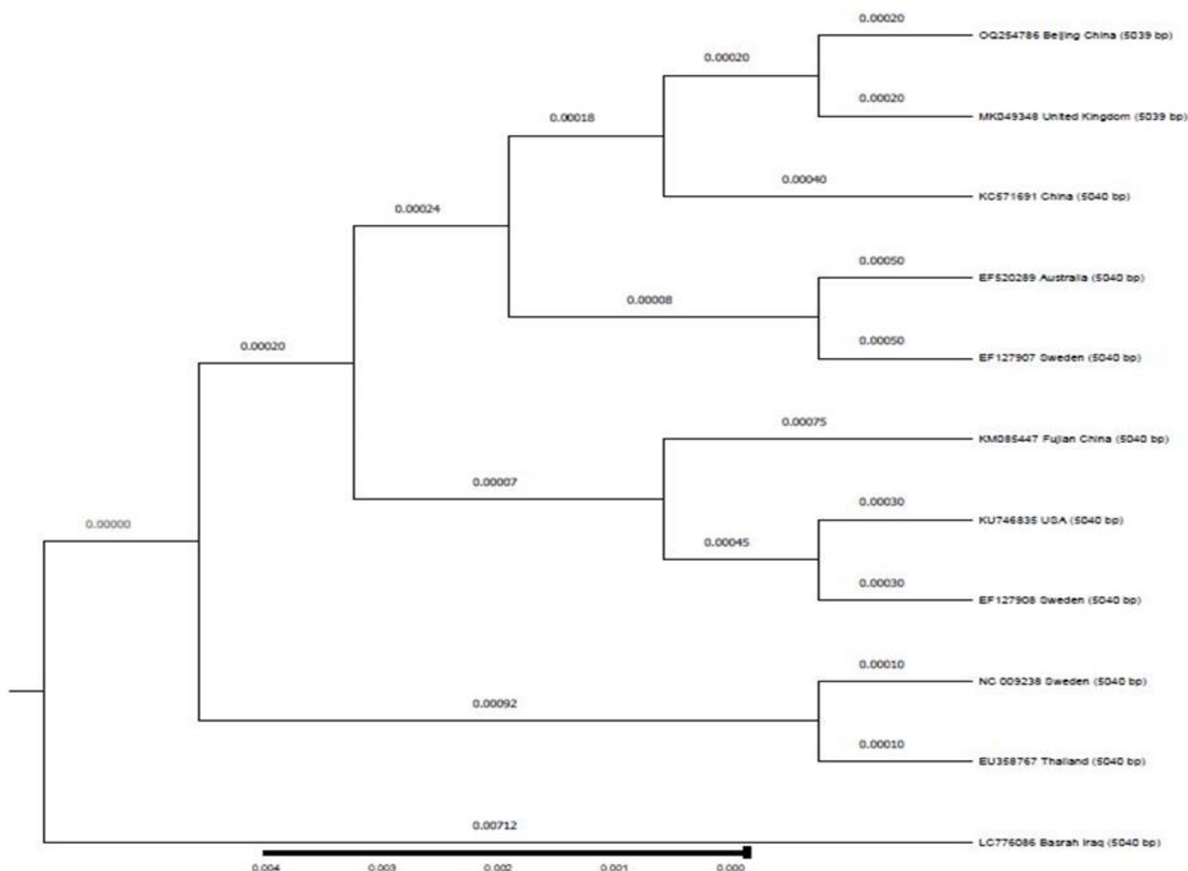

Figure (6): Phylogenetic analysis of KIPyV strains.

Discussion

Polyomaviruses are a growing family that infects birds, fish, rodents, humans, and non-human primates. The discovery of new species has been accelerated due to the development of modern molecular diagnostic tools during the past decade (2). Two new viruses have emerged within the family Polyomaviridae, KI (Karolinska Institutet) and WU (University of Washington). These viruses were first discovered in respiratory specimens in children in 2007, HPyV infection usually occurs in early life or adolescence, and HPyV infection is often asymptomatic, leading to initial viral replication and persistence near the point of infection (14). The organization of the genomic DNA of KIPyV is typical of the PyV family, which consists of 3 regions, the early and late regions separated by the non-coding control region (NCCR). The early region encodes small T antigen (ST-Ag) and large T antigen (LT-Ag) (regulatory proteins), and the late region encodes structural proteins. The size of the KIPyV genome is within the range of Polyomaviridae. The similarity of amino acid sequence of nonstructural proteins is high to those of other major polyomaviruses (3). This study focused on individual clinical samples from 195 patients. During the current study, KI polyomavirus was detected in 6 out of 195 patient tissue samples at a rate of about 3.0% taken from infected patients, which is in agreement with what most research indicates such as that published by researchers in Tobias Allander and his colleagues.

KIPyV infection has been occurring through the respiratory transmission route. Primary replication of KIPyV occurs when it enters the respiratory system. The infection usually occurs without clinical symptoms, but immunocompromised individuals may exhibit symptoms (13). Kean and colleagues

found that the seroprevalence rates of KIPyV in a cohort of healthy adult blood donors and pediatric blood samples are about 55%, it was found that the seroprevalence rate in children aged 1-5 years was 44.6%, and this percentage rise to 60.9% in the age group between 10-15 years, which indicates the childhood stage in which primary exposure to the virus occurs (15). The amino acid sequences of the VP1 protein in HPyVs are highly identical. For example, BKPyV identity shares 78.2% with JCPyV of VP1 amino acid sequence, while the identity of KIPyV with WUPyV shares about 65% of the amino acid sequence, and lower similarity to BKPyV (28%), JCPyV (27%), and SV40 (28%) (16).

The KIPyV was isolated from adult patients with breast, thyroid, and pleural fluid infections in the current study. This may be evidence of the involvement of KIPyV as a carcinogenic agent in infected patients. When molecular testing was performed, it was found that the infection rate decreased to about 3.0%. This may be due to geographical spread or the season of sample collection, such that the genetic DNA of the virus is low compared to seroprevalence, which indicates the role of the immune system of infected people against viral infection. Some studies have reported virus detection and clinical symptoms in immunocompromised or co-infected patients. By PCR amplification, viral loads were low in every sample in which amplification was delayed, indicating latency and persistence of infection rather than active virus replication (17).

By performing the whole genome sequencing and phylogenetic analysis, it was found that the virus has the same typical genomic organization, but with a difference in some nitrogenous bases (mutations) from all KIPyV used in the multiple sequence alignment (MSA) of approximately 0.75. %. Comparing the amino acid expression of KIPyV proteins to the expression of reference strains using MSA, it was found that there are some differences in these proteins (Table, 1) with a high percentage of identity of approximately 99.05%. This indicates the occurrence of many different mutations. In the generated sequence, there are 38 mutations, 16 of which are missense, and 13 of which are silent. The incidence of KIPyV infection in Iraq approximately (3.0%) was compared to that established in Sweden (1%) (3), Australia (2.6%) (20) the United Kingdom (1.4%) (21), and Thailand (1.99%) (22).

KIPyV was separated from other known polyomaviruses by whole-genome evolutionary analysis and obtained contradictory results when early and late genes were analyzed separately. KIPyV is predicted to express ST-Ag, LT-Ag, VP1, and VP3 according to the analysis of ORF, while MT-Ag and Agno-protein are missing. The absence of MT antigen is not surprising, given that most PyVs lack expression of this specific protein, including the major PyVs. All of BKPyV, JCPyV, SA12, and SV40 express the Agno-protein. The lack of an ORF for the Agno-protein in other polyomavirus types is interesting. The Functional effects of the Agno-protein are unclear (3).

Allander and colleagues demonstrated in 2007 that KIPyV is phylogenetically related to other major PyVs in the early region and with little homology of the late region of the genome. They identified the presence of KIPyV in 6 (1%) by PCR of a total of 637 nasopharyngeal aspirates (NPAs) and in 1 (0.5%) of 192 stool samples (18). Xiaobo Song and his colleagues (2016) found that 10.6% of nasopharyngeal samples (n = 94) from patients with respiratory symptoms or infections contained DNA of KIPyV, while 12.5% (n = 96) positive for VP1 DNA of KIPyV of blood samples from healthy individuals, and about 50% of the NCCR sequences from samples were identical to Stockholm NCCR 60, suggesting that this strain may represent the optimal strain, also found that the greater number of isolates carrying mutations from nasopharyngeal samples compared to isolates from blood may indicate a higher mutation rate in the former, suggesting that these mutations may be specific to KIPyV circulating

in the respiratory tract (19). The non-coding regulatory regions include the insertion of 10 nucleotides with many differences in the sequence of those nucleotides, and in the coding regions, there are many non-synonymous mutations, which can be considered causative factors in the occurrence of new viruses (22).

Conclusions

All KI polyomaviruses used in the multiple sequence alignment with our strain have the same length 5040 bp, with 99.25% similarity in DNA sequences and 99.05% identity of amino acid sequences. The KIPyV has been registered in the NCBI GenBank under the accession number LC776086 WU Polyomavirus BASRAH88 complete genome.

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Availability of data

The data are available for any further communication.

Ethics approval and consent to participate

Since our paper was based on using biopsy samples from human subjects, we followed the ethical protocols to obtain agreements from the general manager of the health office and the research unit in the same office to start the work.

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Competing interests

We confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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