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Epstein-Barr Virus Genotypes and Phylogeny among Cancer Patients in Sana'a City, Yemen

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Abstract

Epstein-Barr virus (EBV) is a ubiquitous virus that infects more than 90% of the world's population. It is a tumorigenic herpes virus that causes infectious mononucleosis (IM) and has been linked to the development of several malignant tumours such as Burkitt`s lymphoma, Hodgkin`s lymphoma, Nasopharyngeal carcinoma and Gastric carcinoma. This study was designed to detect EBV DNA and to identify the EBV genotypes and phylogeny among cancer patients' group (Case) and healthy individuals' group (Control) in Sana'a city, Yemen. One hundred subjects were enrolled in the study. Fifty individuals were clinically diagnosed to have cancer. The remaining 50 individuals were healthy controls. Serum IgM antibody against EBV viral capsid antigen (VCA) were tested by an enzyme-linked immunosorbent assay (ELISA).EBV-DNA detection was done using conventional polymerase chain reaction while genotyping and sub-genotyping were performed by Nested polymerase chain reaction of EBNA-2 gene and LMP-1 gene, respectively. Results showed that the prevalence rate of EBV-VCA IgM antibodies among cancer patients was 12% while in healthy individuals was 8%. EBV-DNA positivity were 66.7% (4/6) and 50% (2/4) for cancer patients (cases) and controls, respectively. Also, all EBV-DNA positive cases in both cancer patients and controls were genotype 1 and sub-genotype Med- with a rate of 100% both of them. The results presented genotypes and sub-genotype of EBV circulating in Sana'a city. It is worth mentioning that genotype 1 and Med- strain was first time recorded in Yemen. This study concluded that genotype 1 and Med- strain being predominant in Sana'a city, Yemen. Clinical significance of these finding have not been investigated and shall be evaluated in future studies.

Keywords: anti-EBV-VCA IgM antibodies, Cancer, DNA, Epstein-Barr Virus, PCR, Sana'a city, Yemeni.

1. Introduction

Cancer is a major public health problem in developing countries and worldwide with increasing frequency, especial with increased modernization and predisposition to a large number of carcinogenic agents (Alwan, 1997; AL-Nabhi *et al*., 2017), whereas cancer is a major public health problem in Yemen. AL-Nabhi *et al*. (2017) mentioned that cancer registry in Yemen is still a big challenge in absence of national cancer surveillance. In 2021, the World Health Organization (WHO) suggested that out of Yemen's population (33,28 million inhabitants), approximately 35,000 Yemeni people currently have cancer, and more than 11,000 are newly diagnosed with the disease every year (O'Neill, 2021; WHO, 2021). The link between virus and cancer was one of the pivotal discoveries in cancer research. Therefore, it is generally agreed that viruses are involved in 10-20-% of all cancers (zur Hausen, 2001; Parkin, 2006).

The Epstein-Barr virus (EBV) is a ubiquitous oncogenic virus (Ayee *et al.*, 2020) initially discovered by electron microscopy within a cultured African Burkitt's lymphoma (BL) cell line in 1964 (Epstein *et al.*, 1964). EBV, a double-stranded, 170-kbp DNA virus packaged within an icosahedral capsid surrounded by an envelope, belongs to the subfamily gammaherpesvirinae in Herpesviridae family (Peh *et al*., 2003; Habibian *at al*., 2018). The viral genome exists in linear form in mature virions and in circular episomal form in latently infected cells, and encodes for more than 85 genes. This virus is ubiquitous, and infects more than 90% of the human population worldwide with a life-long, asymptomatic, latent infection (Peh *et al*., 2003). EBV is the causative agent of infectious mononucleosis, playing a significant role as a cofactor in the process of tumorigenesis and has consistently been associated with a variety of malignant tumours, including endemic Burkitt's lymphoma, nasopharyngeal carcinoma, certain B and T-cell lymphomas, Hodgkin's disease, gastric carcinomas, and other lymphoproliferative diseases (Mendes *et al*., 2008). Primary EBV infection usually occurs subclinically during childhood, and thereafter the virus establishes a latent infection of B lymphocytes that persists for life (Habibian *at al*., 2018). In latency, only small subset of viral genes is expressed, which include the six EBV nuclear proteins:

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EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, and three latent membrane proteins: LMP-1, LMP-2A, LMP-2B (Santpere *et al*., 2014).

There are two different EBV genotypes: Type 1 and Type 2, also known as Type A and B, distinguished by

the differences in the EBNA-2 gene, where the rest of the EBV genes differ only by less than 5% in their sequence. The divergence in EBNA-2 reveals only 54% of homology between the two types, facilitating the distinction between each EBV type (Smatti *et al*., 2018). Two genotypes of the virus, namely genotypes 1 and 2, exist and exhibit variation in geographical distribution. Although EBV genotype 1 is globally distributed, it is predominantly found in American, Chinese, European and South-East Asian (SEA) populations, whereas genotype 2 is predominantly found in Africa (Ayee *et al.*, 2020). The two genotypes also vary in biological properties; EBV genotype 1 is more efficient in immortalizing B cells while genotype 2 has a higher lytic ability (Walling *et al*., 2003; Saechan *et al*., 2006). EBV genotypes 1 and 2 can further be subdivided into different virus strains based on the genetic diversity of LMP-1gene, which shows greater degree of polymorphism than most EBV genes (Walling *et al*., 2003). LMP-1 is a 356-amino acid protein, which consists of a short cytoplasmic N-terminus, six membrane spanning domains, and a long cytoplasmic C- terminal domain (Li and Chang, 2003). LMP1 plays an important role in signal transduction and cell survival (Bouvard *et al*., 2009). Variants in LMP-1 were classified into 7 main groups: B95-8, Alaskan, China 1, China 2, Mediterranean (Med+) and (Med−), and North Carolina (NC) (Bouvard *et al*., 2009; Tzellosand Farrell, 2012; Yakovleva *et al*., 2015). However, new strains were subsequently reported from different origins, including two new strains from Thailand, Southeastern Asia 1 (SEA1), and Southeastern Asia 2 (SEA2), which have unique amino acid substitutions (Saechan *et al*., 2006; Saechan *et al*., 2010). Multiple EBV variants could be detected within one individual, which could affect disease induction and prognosis (Walling *et al*., 2003). For example, a variant LMP-1 gene with 30 bp deletion gene was detected in virus isolated from NPC tumor and was associated with a higher transforming activity compared to the typical prototype LMP-1 (B95-8) (Hu *et al*., 1991; Blake *et al*., 2001).

Attempts to develop preventative vaccines against EBV have been largely unsuccessful. Thus, it increases the risk of EBV spread. The vast majority of published studies on EBV prevalence are focused on serological analysis rather than viremia detection (Adjei, *et al*., 2008; Van-Lant and Knipe, 2009; Suntornlohanakul, *et al*., 2015; de Paor *et al*., 2016; Cohen, 2018). Clearly, detection of circulating EBV DNA is a better indication of infection status, which can contribute to improving the level of medical care prevention measures (Kondo, *et al*., 2004; Lin, *et al*., 2004). Previous studies from Middle Eastern countries such as Kuwait (Makar *et al.,* 2003), Saudi Arabia (Al-Diab *et al.,* 2003), Jordan (Vasef *et al.,* 2004), the UAE (Al-Salam *et al.,* 2008), Egypt (Audouin *et al.,* 2010), and Syria (Al Moustafa *et al.,* 2016) have investigated EBV and its association with certain diseases such as Hodgkin's lymphoma (ranging from 28% to 87%), but not among other cancer patients and healthy individuals. To the best of our knowledge, no studies have been conducted in Sana'a, Yemen or Middle East countries concerning EBV detection and genotypes and sub-genotypes identification in neither cancer patients or healthy individuals except in Qatar, where the circulating genotypes and sub-genotypes of EBV in healthy blood donors were determined by Smatti *et al.* (2017). Nasher (2012) determined the prevalence of the high-risk HPV type 16 and 18 and EVB in some Yemeni patients with oral squamous cell carcinoma. From this point, this study aimed at detection of EBV using ELISA and PCR method and identifying the EBV genotypes and sub-genotypes (strains) circulating within various study groups in Sana'a city, Yemen. Furthermore, this information will enable the health officials in Sana'a city to consider the development of new policies that aim at reducing the burden of communicable diseases related to malignancies.

2. Materials and Methods

2.1. Study Population and Design

This study is a case-control study. Sample size was calculated by Epi info version 7 (CDC, Atlanta, USA). A total number of 100 participants were included in this study. The study was conducted in two groups. The first group consisted of 50 cancer patients, who were clinically diagnosed with Burkitt's lymphoma, Hodgkin's lymphoma, Nasopharyngeal and Gastric carcinoma by a physician and also considered clinically suspected cases of EBV infection, in addition to those who attended the national oncology center in Sana'a city. The second group consisted of 50 healthy individuals from the general population who were considered controls.

2.2. Sample Collection

Venous blood (5 mL) was collected from each participate using venous puncture techniques and divided into equal shares. One part (2.5 ml of blood specimen) was added into vacutainer serum tube free from anticoagulant agent and left to dot at room temperature, then the blood was centrifuged for five minutes at 3000 rpm. The gel in the tubes formed a physical barrier between the serum and the red blood cells during centrifugation. Then each serum sample was separated into Eppendorf tube, until performing serological assay. The other 2.5 ml of the blood specimen was added into an EDTA tube and stored as a whole blood sample until performing molecular assay. The sera and whole blood samples were stored at -20^{o}C until performing tests.

2.3. Serological Assay

Sera from all specimens were analyzed for Epstein-Barr virus IgM viral capsid antigen (VCA) using opened system (manual) Enzyme-Linked Immunoassay (ELISA) diagnostic kits provided by (DIA. PRO, Italy). Quality control was performed according to manufactured instructions (DIA. Pro, Italy). According to the information included in the kits insert, the immunoassay used has sensitivity >98% and specificity >98%.

2.4. Molecular assay

Molecular identification of Epstein-Barr virus was performed using Conventional Polymerase Chain Reaction (PCR). The EBV IgM positive specimens were used in the molecular assay.

2.4.1. DNA Extraction

Total viral DNA were extracted from 200 µl of human whole blood specimens using the *AccuPrep®* Genomic DNA Extraction kit (K-3032) (Bioneer, Inc., Korea) according to the manufacturer's instruction. Extracted DNA samples were then stored at -20 °C for further testing.

2.4.2. Primers used in molecular assay

Specific genomic sequences (primers) synthesized by Bioneer, Inc., Korea were used in this investigation to detect EBV DNA, and to identify EBV genotypes and sub-genotypes table (1).

Table (1): The sequences of EBV virus primers used for EBV detection, genotyping and sub-genotypes during this study (Smatti *et al.*, 2017).

Primer type	Primer sequences	Amplicon Size
E2p1	5'-AGGGATGCCTGGACACAAGA-3'	596pb
E2p2	5'-TGGTGCTGCTGGTGGTGGCAA T-3'	
Ap1	5'- TCTTGATAGGGATCCGCTAGGATA-3'	497pb
Ap2	5'-ACCGTGGTTCTGGACTATCTGGATC-3'	
Bp1	5'-CATGGTAGCCTTAGGACATA-3'	150pb
Bp2	5'-AGACTTAGTTGATGCCCTAG-3'	
A1	5'-AGTCATAGTAGCTTAGCTGAA-3'	602pb
A2	5'-CCATGGACAACGACACAGT -3'	
B1	5'-AGTCATAGTAGCTTAGCTGAA-3'	587pb
B2	5'- CAGTGATGAACACCACCACG-3'	

2.4.3. EBV DNA detection by PCR

Detection of EBV DNA in all extracted samples were performed by a Conventional PCR using the *AccuPower®HotStart* PCR PreMix kit (K-5050) (Bioneer, Inc., Korea), and primers (E2p1 and E2p2) that have previously been reported (Table, 1). PCR amplification was preformed according to the manufacturer's instructions (Bioneer, Inc., Korea). Briefly, primer solutions were thawed, and genomic DNA was prepared. 4 µl(5Mm/µl) of diluted primers mix was distributed into the each *AccuPower®HotStart* PCR PreMix tube. 4 µl (100ng/µl) of genomic DNA was added to the individual PCR tube. Distilled water was added to *AccuPower®HotStart* PCR PreMix tube until the total volume of mixture became 20 µl. The lyophilized blue pellet was dissolved by vortexing and spin-down. The PCR tube was placed in TProfessional *TRIO* Thermocycler (Biometra Ltd, Germany) and the cycling program was started, whereas PCR reaction cycling conditions involved initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification with denaturation at 95°C for 0.45 s, annealing at 56°C for 0.45 s, extension at 72°C for 0.45 s, and a find extension at 72°C for 5 min. A negative control (without genomic DNA) was used. Afterward, PCR amplified products were separated on 1.5% ethidium bromide-stained agarose gel and visualized using UV light trans illuminator, then a photo was taken by a Sony digital camera.

2.4.4. EBV genotyping by nested PCR of the EBNA-2 gene

Extracted samples that showed EBV DNA positive results were used in the genotypes assay. EBV genotyping was performed by nested PCR targeting the EBNA-2 gene as described by Smatti *et al.* (2017) and Ayee *et al.* (2020), using specific primers as previously reported (Table, 1), with slight modification to cycling conditions. The first round of the PCR was done by amplifying a common region of EBNA-2 using Apl and Ap2 as sense and antisense primers, respectively. Each PCR reaction mixture of 20 µl contains the following components with the final concentrations: *AccuPower®HotStart* PCR PreMix (produced by Bioneer, Inc. Korea), 2 µl (5Mm/µl) each of the forward and reverse primers, and 3 µl (100ng/µl) of genomic DNA. The volume was made up with nuclease-free water. The cycling conditions for the PCR reaction were as follow: initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification with denaturation at 95°C for 0.45 s, annealing at 56°C for 0.45 s, extension at 72°C for 0.45 s, and a find extension at 72°C for 5 min. A second round PCR (nested) was performed using 0.5 µl of the amplicons from the first round as template; all other reaction components were the same as the first-round reaction mixture, except for the primers. A forward primer (Bp1) and reverse primers (Bp2) were used for the second-round amplification. The reaction was carried out at initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification with denaturation at 95°C for 0.45 s, annealing at 56°C for 0.45 s, extension at 72°C for 0.45 s, and a find extension at 72°C for 5 min. Afterward, PCR amplified products were separated on 1.5% ethidium bromide-stained agarose gel and visualized using UV light trans illuminator then a photo was taken by a Sony digital camera. In all experiments, a negative control (without genomic DNA) was used.

2.4.5. EBV sub-genotyping by sequencing of LMP-1 gene

EBV sub-genotyping was done using nested PCR targeting the LMP-1 gene as described by Smatti *et al.* (2017), using specific primers as previously reported (Table, 1), with slight modification to cycling conditions. The first round of the PCR was done by amplifying a common region of LMP-1 using Al and A2 as sense and antisense primers, respectively. Each PCR reaction mixture of 20 µl contains the following components with the final concentrations: *AccuPower®HotStart* PCR PreMix (produced by Bioneer, Inc. Korea), 2 µl (5Mm/µl) each of the forward and reverse primers, and 4 µl(100ng/ µl)of genomic DNA. The volume was made up with nuclease-free water. The cycling conditions for the PCR reaction were as follow: initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification with denaturation at 95°C for 0.45 s, annealing at 56°C for 0.45 s, extension at 72°C for 0.45 s, and a find extension at 72°C for 5 min. A second round PCR (nested) was performed using 0.5 µl of the amplicons from the first round as template; all other reaction components were the same as the first-round reaction mixture, except for the primers. A forward primer (B1) and reverse primers (B2) were used for the second-round amplification. The reaction was carried out at initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification with denaturation at 95°C for 0.45 s, annealing at 56°C for 0.45 s, extension at 72°C for 0.45 s, and a find extension at 72°C for 5 min. Afterward, PCR amplified products were separated on 1.5% ethidium bromide-stained agarose gel as previously described and visualized using UV light trans illuminator,

then a photo was taken by a Sony digital camera. In all experiments, a negative control (without genomic DNA) was used.

2.5. Phylogenetic analysis

LMP-1 PCR products (587 pb) were sent out to Germany at Eurofins Genomics for purification and determination of DNA sequence. Then, CLC Main Workbench 5 was used to run sequence alignments and construct the phylogenetic tree. For sequence homology comparison, sequences were compared to reference sequences representing the seven main EBV strains available in the GenBank database: B95.8 prototype strain (V01555), Med + with 30-bp deletion (AY337721), Med - without 30-bp deletion (AY493810), China 1 (AY337723), ina 2 (AY337724), Alaskan (AY337725), and NC strain (AY337726). As described by Lorenzetti *et al.* (2012) and Smatti *et al*. (2017). The phylogenetic tree was generated using the neighbor joining method. Bootstrapping and reconstruction were carried out with 100 replicates to obtain the confidence level of the phylogenetic tree.

3. Results and Discussion

3.1. Seroprevalence of EBV among study groups

Epstein-Barr virus VCA-IgM antibody were detected by Enzyme Linked Immunosorbant Assay (ELISA) test in 6 (12%) of the 50 cancer cases and in 4(8%) of the 50 controls (Table 2). In contrast, Salehi *et al.* (2016) reported that only 12/673 (8%) of blood samples were anti-VCA IgM antibodies positive, while 35% of the case group and 6% of the control group were positive for this antibody.

Table (2): Seroprevalence of EBV IgM antibody among cancer patients and control groups.

Groups	Seropositive of EBV IgM	
	Number	%
Cancerpatients (n=50)	6	12
Control groups (n=50)	4	8
Total	10	10

3.2. Molecular detection of EBV among study groups

All seropositive specimens of acute EBV infection (EBV IgM antibody) (n=10) were screened for the present of EBV-DNA using Conventional Polymerase Chain Reaction (PCR) assay. The number of EBV-DNA positive were 66.7% (4/6) and 50% (2/4) for cancer patients (cases) and controls, respectively (Figure1). In our study, not all samples classified serologically with reactive EBV infection (EBV-IgM positive) were positive by PCR. This could be explained by the cross-reaction with other antigenically related viruses, especially CMV (de Ory *et al.*, 2011and Guerrero-Ramos *et al.*, 2014). Also, serological testing only cannot confirm reactivation status or the exact reactivation time (Maurmann *et al.*, 2003). Therefore, EBV-DNA detection and viral load quantification is used to assist in the diagnosis of EBV reactivation, although discrepancies can be found between PCR and serology.

In the same respect, many studies have detected EBV-DNA in different types of cancer and healthy individuals. In Brazil, 43% (13/30) of the patients with HL had EBV-DNA and 8% (1/13) of healthy individuals were positive for EBV-DNA (Musacchio *et al.*, 2006). In India,EBV-DNA was detected in 49% (16/33) of HL patients and not in controls (Sinha *et al.*, 2016).In Iran, 35/56 (62.5%) of GC patients and 3/56 (5.4%) of controls, were EBV-DNA positive (Amoueian *et al.*, 2018). In Ghana, the number of EBV-DNA positives were 67% (37/55) and 92% (48/53) for NPC patients and controls, respectively (Ayee *et al.*, 2020). The differences in results might be due to:1. the small sample size of the reactivation group (n=10); 2.EBV detection by PCR is highly affected by the specimen used (whole blood versus PBMC versus serum) and the variation in sample types must always be considered when comparing different studies (Smatti *et al*., 2017); 3. several factors including; the methodology employed (e.g. relative sensitivities and the specificity of the tests used), undefined socioeconomic conditions, the geographic distribution and the immunity disturbance occasionally seen in cancer patients (Musacchio *et al*., 2006; Nasher, 2012; Sinhal *et al.*, 2016).

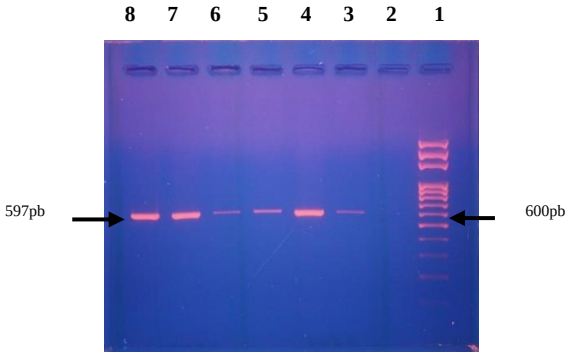


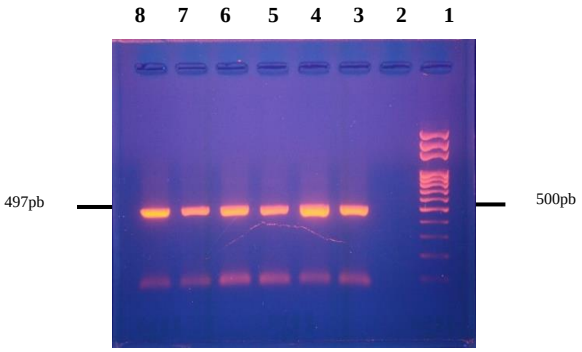
Figure (1): Electrophoretic pattern of EBV-DNA detection. Lane 1: molecular weight marker- 100 pb (Bioneer, Inc., Korea); lane 2: negative control (without genomic DNA); lane 3,4,5,6,7 and 8: positive samples

3.3. EBV Genotypes in cancer patients and controls of the study participants

All positive samples of EBV-DNA detected were tested for EBV genotyping using nested PCR targeting the EBNA2 gene showed 497pb and 150pb fragment which are characteristic of genotypes 1 and 2, respectively.

Results in figure (2), showed that the frequencies of EBV genotype 1 in blood samples of both cancer patients and controls were predominant 100%(6/6), while frequencies of EBV genotype 2 in blood samples were 0% (0/6) in both cancer patient and control samples, whereas genotype 1 is usually more prevalent in Europe, America, China, and South Asia (Hu *et al.*, 1991; Tzellos and Farrell, 2012), compared to genotype 2 that is more prevalent in African and Papua New Guinean populations (Bouvard *et al.*, 2009; Kwok *et al.*, 2015).Our findings are in agreement with the results of two studies in Iran and Malaysia. Only genotype 1 was detected in Iranian HL and NHL samples (Habibian *et al.*, 2018), and in Malaysian NPC, HL and BL patients (Peh *et al.*, 2003). In contrast, other studies reported the prevalence of genotypes 1&2 in several countries. In India, the prevalence of type A, B and both A and B was reported to be 32(45.7%), 2(2.9%) and 1(1.4%), respectively (Janani *et al.*, 2015). However, 37(72.5%) of type 1, 2(3.5%) of type 2 and 4% of both types were reported in Qatar (Smatti *et al.*, 2017). In Brazil,54(71.1%) EBV1, 13(17.1%) EBV 2 and 9(11.8%) EBV 1&2 were reported by Monteiro *et al.*

(2020). In China, type 1was detected in 59 (72%) of leukemia cases and in 31 (88.6%) of myelodysplastic syndrome (MDS), while type 2 was detected in 7 (8.5%) of



leukemia and in 3 (8.6%) of MDS, and both types 1&2 were detected in 16 (19.5%) of leukemia and 1 (2.8%) of MDS (Wanga *et al.*, 2021).

Figure (2): Electrophoretic pattern of EBV genotype 1. Lane 1: molecular weight marker- 100 pb; lane 2: negative control (without genomic DNA); lane 3,4,5,6,7 and 8: EBV positivity samples of genotypes 1.

3.4. EBV sub-genotypes in cancer patients and controls of the study participants

The 6 genotyped samples were further sub-genotyped by sequence analysis of the LMP1 gene C-terminus region. Nested PCR was used to amplify a 602pb and 587pb products as shown in figure (3&4). The second cycle of PCR products (587pb products) were sent out to Germany at Eurofins Genomics for purification and determination sequence DNA. After that, five DNA sequences were obtained from the sent samples, while the determination of the DNA sequence of one sample failed. Generated sequences were aligned in comparison to previously reported EBV strains (prototype B95-8(V01555), Med+(AY337721), Med-(AY493810), China 1(AY337723), China 2(AY337724), Alaskan(AY337725), NC (AY337726) using CLC Main Workbench 5. The phylogenetic tree was generated using the neighbor joining method.

Data in figure (5) showed that all EBV genotype positive cases in both cancer patients and controls were Med- (Mediterranean -) strain with a rate of 100%. In contrast to our results, there are several previous studies that have reported different strains of EBV in many countries including Hong Kong where LMP1-defined strains, China 1, China 2 and Mediterranean+ were reported to be the most common strains observed among infectious mononucleosis (IM) patients and asymptomatic individuals (AS) with primary EBV infection (Kwok *et al*., 2015). In Qatar, Smatti *et al*. (2017) revealed the presence of four variants among healthy blood donors including Mediterranean, B95.8, China 1 and North Carolina strains. In China, Wang *et al.* (2021) reported that four distinct sequence patterns were found in the specimens of patients with leukemia and myelodysplastic syndrome (MDS): B95-8, China 1, China 2, and Med (Mediterranean). These differences in results could be explained according to the suggestion of Gurtsevitch and Smirnova (2021) that direct sequencing of the C-terminal domain of LMP1(showing a high degree of heterogeneity compared to other EBV genes) in biological materials (blood, saliva, and tumor tissue) of cancer patients and healthy individuals from different geographic regions mismatched LMP1variants.

To the best of our knowledge, this study is the first which determined the genotype and the sub-genotype of EBV in Sana'a city, Yemen and provides a baseline information on the prevalence and co-infection patterns of the genotypes and sub-genotype among four types of cancer patients and healthy individuals in Sana'a city, Yemen and Middle East countries.

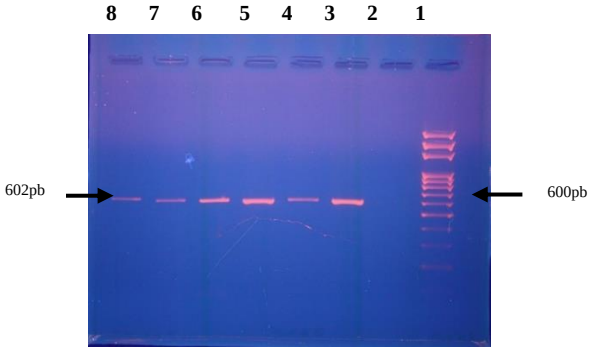


Figure (3): Electrophoretic pattern of EBV sub-genotype. Lane 1: molecular weight marker- 100 pb; lane 2: negative control (without genomic DNA); lane 3,4,5,6,7 and 8: EBV genotyped samples tested of sub-genotypes with amplicon of 602pb.

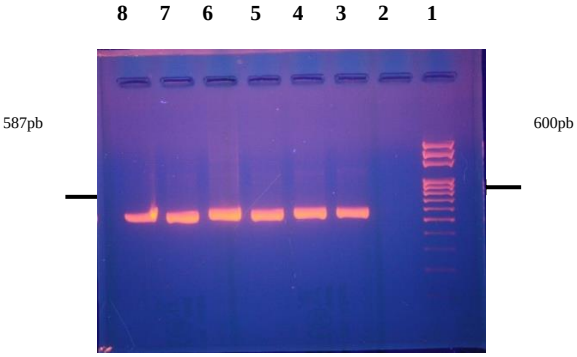


Figure (4): Electrophoretic pattern of EBV sub-genotype. Lane 1: molecular weight marker- 100 pb; lane 2: negative control (without genomic DNA); lane 3,4,5,6,7 and 8: EBV genotyped samples tested of sub-genotypes with amplicon of 587pb.

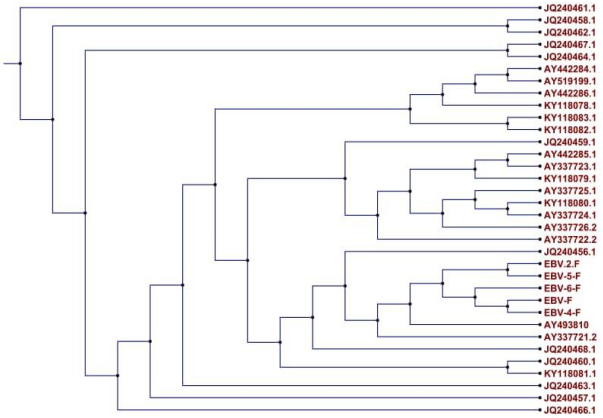


Figure (5): Phylogenetic tree of the C-terminus of LMP-1.

4. Conclusion

This is the first report for the predominance of EBV genotype 1, and sub-genotype Med- strain in the studied

Yemeni population. Identification of the virulent EBV genotype 1 in Yemen indicates a possible risk factor in the development of cancer in Yemeni patients.

5. Ethical approval

Ethics statement of the study a known ledged distinctive consent forms the Committee of Biological Sciences Department, Faculty of Science, Sana'a University and from the National Oncology Center of sample collection in Sana'a city, Yemen.

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Conflict of interest

The authors verify having no interest in competition and no conflict of interests.

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