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SMSA designed; EAA performed experiments and analyzed data. QYMA and AAA reviewed and checked the paper for errors. SMSA gave final approval for publication.

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The Association of Epstein-Barr Virus Antibodies with Cancer Patients among Yemeni Patients

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Abstract:

The Epstein–Barr virus (EBV) is the causative agent of infectious mononucleosis and has been linked to several malignancies. Seroepidemiological studies have shown that approximately 90%–95% of individuals worldwide are infected with EBV. This study aimed to determine the seroprevalence of EBV among cancer patients compared with healthy controls in Sana'a city, Yemen, and determine the clinical characteristics and some risk factors associated with EBV infection. One hundred subjects were enrolled in the study. Fifty individuals were clinically diagnosed to have cancer. The remaining 50 individuals were healthy controls. Serum IgG and IgM antibodies against EBV viral capsid antigen (VCA) were tested by an enzyme-linked immunosorbent assay (ELISA). The results showed that the prevalence rate of EBV-VCA IgG and IgM antibodies among cancer patients was 94% and 12% while in healthy individuals was 82% and 8%, respectively. Most common symptoms were pharyngitis, fever, and fatigue. Moreover, educational level, income status, residency, consuming Shammah, and blood transfusion were found as significant risk factors for EBV infection. Therefore, this study concluded that Sana'a city is one of the endemic areas in Yemen, and the incidence of cancer types in the present study is associated with EBV infection.

INTRODUCTION

Cancer is a major public health problem in developing countries and worldwide with increasing frequency, especially with increased modernization and predisposition to a large number of carcinogenic agents (Alwan, 1997; AL-Nabhi *et al*., 2017; Amjad *et al.,* 2020a,b; Iqbal and Ashraf, 2020). Genetic mutations contribute to the development of various cancer types (Ali *et al.,* 2015; Ashraf *et al.,* 2018; Iqbal, 2020). Cancer is a major public health problem in Yemen. The cancer registry in Yemen is still a major challenge in absence of national cancer surveillance (AL-Nabhi *et al*., 2017). In 2021, the World Health Organization (WHO) suggests that out of Yemen's population (33,28 million inhabitants), approximately 35,000 Yemeni 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 belonging to the family Herpesviridae (Ayee *et al.*, 2020), that was initially discovered by electron microscopy within a cultured African Burkitt's lymphoma (BL) cell line in 1964 (Epstein *et al.,* 1964). It is estimated that this ubiquitous virus has infected more than 90% of the world's population (Gequelin *et al.,* 2011). EBV is a tumorigenic herpes virus that causes infectious mononucleosis (IM) and is associated with a variety of human tumors of lymphoid (Burkitt lymphoma, Hodgkin disease, and B lymphoproliferative disease) and epithelial cell (nasopharyngeal carcinoma and gastric carcinoma) origin. Primary EBV infection usually occurs subclinically during childhood, and thereafter the virus establishes a latent infection of B lymphocytes that persists for life (Higgins *et al*., 2007; Smatti *et al*., 2017). EBV is now associated with 1% of global cancers, which are mostly lymphomas and carcinomas; approximately 140,000 people die of EBV-associated cancers each year (Bakkalci *et al.*, 2020).

A few studies have attempted to identify the responsible risk factors (Higgins *et al.,* 2007). Whereas, previous studies showed that the factors influencing the seroprevalence of EBV infection were age, gender, familial socioeconomic status, ethnicity, country or region of residence, household educational level, kissing, smoking habit, and the place of child-rearing. The analysis of risk factors for the acquisition of primary EBV infection would help identify those susceptible populations (Mekmullica *et al.,* 2003; Chen *et al*., 2015). Previous large-scale seroepidemiological surveys in Europe and United States showed that over 50% of adolescents would have been seropositive to EBV. High EBV seropositive rate ranging from 60% to 93% was reported in some regional studies (Chen *et al*., 2015). Furthermore, the vast majority of published studies on EBV prevalence are focused on serological analysis rather than viremia detection (Adjei, *et al*., 2008; Suntornlohanakul, *et al*., 2015).

Our study aimed to investigate the association between EBV and cancer patients via measuring EBV-VCA IgG and IgM in cancer patients compared with healthy controls, determine the clinical characteristics, and identify some risk factors associated with EBV infection.

MATERIALS AND METHODS

Study Population and Design

This study is a case-control study. The sample size was calculated by Epi info version 7 (CDC, Atlanta, USA). A total number of 100 individuals 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 the 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 from the general population (healthy individuals) as controls.

Data Collection

Data collection was done from each studied case and control by using a specially designed questionnaire that included socio-demographic data and some associated risk factors.

Sample Collection

Five milliliters of venous blood was collected from each participant using venous puncture techniques. Blood specimens were added into vacutainer serum tubes that are free from the 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 an Eppendorf tube, and stored at -20°C until tested (Othman *et al*., 2017).

Serological Assay

Sera from all specimens were analyzed for Epstein-Barr virus IgG and IgM by using opened system (manual) Enzyme-Linked Immunoassay (ELISA) diagnostic kits (DIA. PRO, Italy). The serum sample of each study participant was examined in vitro qualitative determination of IgM class and IgG class antibodies to EBV viral capsid antigen (EBV-VCA) by ELISA technique. Quality control was performed according to manufactured instructions (DIA. Pro, Italy). According to the information included in the kit insert, the immunoassay used has sensitivity >98% and specificity >98%.

Statistical Analysis

The data were entered and analyzed using SPSS version 21. Chi-square was performed at a 95% confidence interval and a significance level of >0.05 was used to determine the relationships between data collection and seropositive samples; *P*-values of 0.05 or less were considered to be significant. An odd ratio was performed to assess associated risk factors (Yusuf *et a*l., 2019).

RESULTS

General characteristics of study samples

Table (1) showed general characteristics of cancer cases and controls groups in this study. It is apparent from the table that out of 50 cancer cases, 36(72%) were male and 14(28%) were female. Regarding age, the age range of cases was from 4-72 years. However, 45(90%) of study participants were having a secondary or less level of education, 34(68%) were poor (low) and limited income status with 29(58%) married and 35(70%) lived in rural areas. While in the control group, out of 50 controls, 33(66%) were male and 17(34%) were female. The age range of control was from 8–68 years. However, 32(64%) of study participants were having a university level of education, 40(80%) moderate-income status with 31(62%) married, and 45(90%) lived in urban areas.

Table 1. General characteristics of study samples (n = 100).

Personal data	Cancer cases (n=50)		Controls (n=50)	
	Number	%	Number	%
Gender				
Male	36	72	33	66
Female	14	28	17	34
Age group (years)				
<20	19	38	9	18
20 – 29	6	12	10	20
30 – 39	5	10	9	18
40 – 49	6	12	12	24
50 – 59	9	18	7	14
>60	5	10	3	6
Educational level				
Secondary or less	45	90	18	36
University	5	10	32	64
Income status				
Moderate	16	32	40	80
Poor (low)	34	68	10	20
Marital status				
Married	29	58	31	62
Single	21	42	19	38
Residency				
Rural	35	70	5	10
Urban	15	30	45	90

Seroprevalence of EBV among study groups

Data in table (2) showed that the EBV IgG antibody was detected by the Enzyme-Linked Immunosorbent Assay (ELISA) test in 47(94%) of the 50 cancer cases and 41(82%) of the 50 controls. Also, data showed that the EBV IgM antibody was detected in 6(12%) of the 50 cancer cases and 4 (8%) of the 50 controls.

Seroprevalence of EBV in different types of cancers

From data in table (3), it was obvious that out of 22 Hodgkin's lymphoma patients 20(91%) were positive for EBV IgG, out of 23 Nasopharyngeal carcinoma patients 23(100%) were positive for EBV IgG, and out of 5 Gastric carcinoma patients 4(80%) were positive for EBV IgG. While Burkitt's lymphoma patients, no cases were found during the study period. Our results revealed that out of 22 Hodgkin's lymphoma patients, 1(4.5%) were positive for EBV IgM, out of 23 Nasopharyngeal carcinoma patients 4(17.4%) were positive for EBV IgM, and out of 5 Gastric carcinoma patients 1(20%) was positive for EBV IgM. While Burkitt's lymphoma patients, no cases were found during the study period.

Table 2. Seroprevalence of EBV IgG and IgM antibodies among cancer patients and control groups.

Groups	Seropositive of EBV IgG		Seropositive of EBV IgM	
	Number	%	Number	%
Cancer patients (n=50)	47	94	6	12
Control groups (n=50)	41	82	4	8

Table 3. Seroprevalence and relative risk of EBV IgG and IgM antibodies in different types of cancer.

Type of cancer	Positive EBV IgG		Positive EBV IgM	
	No.	%	No.	%
Burkitt's lymphoma (n=0)	0	0	0	0
Hodgkin's lymphoma (n=22)	20	91	1	4.5
Nasopharyngeal carcinoma (n=23)	23	100	4	17.4
Gastric carcinoma (n=5)	4	80	1	20
Total	47	53.4	6	60

General characteristics Associated with EBV Infection

EBV infection according to the general characteristics of cancer patients and control groups is shown in Table (4). Males were more likely to have EBV infection than females in (70.2%) and (73.2%) of cancer patients and controls, respectively. Whereas, this difference was not statistically significant (*P* = 0.759). However, cancers patients and controls aged from <20 and 40-49 years old were more susceptible to have the previous infection of EBV in (38.3%) and (24.4%) of cases, respectively. While, recent EBV infection was high in both cancer patients and control ranging from 50-59 years old in (33.2%) and (50%), respectively with no significant statistically difference (*P*=0.094). The secondary or less level of education of cancer cases showed the higher level of EBV previous and recent infection in (89.4%) and (83.3%), respectively. While, the university level of education of controls showed the higher level of EBV previous and recent infection in (68.3%) and (75%), respectively with a significant association (*P*=0.000). Cancer patients with low economic level were more susceptible to have EBV previous and recent infection in (66%) and (66.7%), respectively. Contrary, controls with moderated economic level were more susceptible to have EBV previous and recent infection in (75.6%) and (75%), respectively with a significant association (*P*=0.000). Cancer patients and controls who married were more likely to have both EBV previous and recent infection in (57.4%) and (65.9%) of EBV previous infection, and (83.3%) and (75%) of EBV recent infection, respectively

with no a significant statistically (*P*=0.419). Moreover, Cancer patients who lived in rural areas showed higher level of EBV previous and recent infection in (68.1%) and (83.3%), respectively. Conversely, population controls of urban areas showed higher level of EBV previous and recent infection (87.8%) and (100%), respectively with a significant association (*P*=0.000) as shown in the same table.

Symptoms of EBV Infection

Symptoms of EBV infection were Rash (10.5%), Fever (9.8%), and Fatigue (9.1%) most commonly in cancer patients, while Pharyngitis (28.6%) and Fatigue (22.2%) most commonly in healthy controls, from all EBV IgM positive cases shown in Table 5.

Table 4. The ratio of EBV infection with general characteristics associated with infection in study samples.

Patients characteristics	Number of IgG positive (%)				P-value	Number of IgM positive (%)			
	Cancer cases		Controls			Cancer cases		Controls	
	No.	%	No.	%		No.	%	No.	%
Gender									
Male	33	70.2	30	73.2	0.759	5	83.3	3	75
Female	14	29.8	11	26.8		1	16.7	1	25
Age group (years)									
<20	18	38.3	5	12.3	0.094	1	16.7	1	25
20 – 29	6	12.8	9	22		0	0	1	25
30 – 39	5	10.6	7	17		1	16.7	0	0
40 – 49	6	12.8	10	24.4		1	16.7	0	0
50 – 59	7	14.9	7	17		2	33.2	2	50
>60	5	10.6	3	7.3		1	16.7	0	0
Educational level									
Secondary or less	42	89.4	13	31.7	0.000	5	83.3	1	25
University	5	10.6	28	68.3		1	16.7	3	75
Income status									
Moderate	16	34	31	75.6	0.000	2	33.3	3	75
Poor (low)	31	66	10	24.4		4	66.7	1	25
Marital status									
Married	27	57.4	27	65.9	0.419	5	83.3	3	75
Single	20	42.6	14	34.1		1	16.7	1	25
Residency									
Rural	32	68.1	5	12.2	0.000	5	83.3	0	0
Urban	15	31.9	36	87.8		1	16.7	4	100

Table 5. Symptoms of EBV infection (n=100).

Symptoms	EBV IgM Positive				Total			
	Cancer cases		Controls		Cancer cases		Controls	
	No.	%	No.	%	No.	%	No.	%
Lymphadenopathy	3	8.3	0	0	36	72	1	2
Pharyngitis	3	8.8	2	28.6	34	68	7	14
Fever	4	9.8	1	14.3	41	82	7	14
Fatigue	4	9.1	2	22.2	44	88	9	18
Rash	2	10.5	0	0	19	38	4	8

Risk Factors Associated with EBV Infection

Some associated risk factors were studied evaluating the emergence of EBV infection, risk factors that were strongly and statistically significant with EBV infection were consuming Shammah and blood transfusion (*P*-value= 0.001 and 0.002, and OR of 16.970 (2.119 - 135.895) and 6.989(1.859 – 26.277), respectively) in a confidence interval of 95%. While the rest of the risk factors have not shown a significant association with EBV infection clarified in Table 6.

Table 6. Some risk factors associated with EBV infection of study samples (n=100).

Risk factors	Number of IgG positive (%)				P-value	Number of IgM positive (%)			
	Cancer cases		Controls			Cancer cases		Controls	
	No.	%	No.	%		No.	%	No.	%
Smoking									
Yes	16	94	11	84.6	0.464	4	23.5	1	7.7
No	31	93.9	30	81.1		2	6.1	3	8.1
Chewing qat									
Yes	27	93	30	93.8	0.123	4	13.8	2	6.3
No	20	95	11	61		2	9.5	2	11.1
Consuming Shammah									
Yes	14	100	1	100	0.001	4	28.6	0	0
No	33	92	40	81.6		2	5.6	4	8.2
Blood transfusion									
Yes	17	94.5	3	100	0.002	2	11	0	0
No	30	93.8	38	80.9		4	12.5	4	8.5

DISCUSSION

Epstein-Barr virus (EBV) is a human herpesvirus that is usually carried lifelong as an asymptomatic infection. EBV is the causative agent of infectious mononucleosis and has been linked to the development of several malignant tumors, including B-cell neoplasms such as Burkitt's lymphoma and Hodgkin's disease, certain forms of T-cell lymphoma, and some epithelial tumors, such as undifferentiated nasopharyngeal carcinoma and a proportion of gastric cancers (Hafez *et al.*, 2006).

Our study showed that 47(94%) and 6(12%) of 50 cancer patients were positive for IgG and IgM antibodies to EBV-VCA (infected with EBV), respectively. While, in control groups, 41(82%) and 4(8%) were positive for IgG and IgM antibodies, respectively. Nearly similar results were recorded in Taiz, Yemen by Ali (2019) who found that the prevalence of EBV IgG antibodies among various types of cancer was (80.1%). In contrast to our results, another study conducted in Sana'a city, Yemen by Al-Akwa'a (2011) reported that the seropositivity of EBV IgG antibodies were (67.4%) of cancer patients and (40.9%) of control groups.

Regarding cancer types, the present study found that out of 22 Hodgkin's lymphoma (HL), 20(91%) and 1(4.5%) patients, out of 23 Nasopharyngeal carcinomas (NPC), 23(100%) and 4(17.4%) patients and out of 5 Gastric carcinomas (GC), 4(80%) and 1(20%) patients were positive for EBV IgG and IgM VCA antibodies, respectively. While Burkitt's lymphoma (BL) no found any case during period collection samples. Our results of the EBV prevalence in HL agree with a study in UK by Swanson (2007), but the contrast with findings of Nath *et al*. (2016) in India and de Mattea *et al*. (2019) in Argentina. Similarity for NPC was agreed to a study from UK (Swanson, 2007) and Sudan (Adan *et al*., 2014), but the contrast with (Umar and Ahmed, 2014) in Pakistan and (Edreis *et al.*, 2016) in Sudan. In contrast to our results of GC, there were two studies with a high frequency from UK (Swanson, 2007) and Portugal (Nogueira *et al.*, 2017). Also, there were other studies with a low frequency from

Portugal (Ribeiro *et al.*, 2017) and Iran (Amoueian *et al.*, 2018). So, these differences between any type of cancer and infection of EBV might be probably due to the small size of our samples.

No significant association was observed between EBV infection and gender, although males were more susceptible to have EBV infection, our result agreed with a study in Jordan (Sughayer *et al.,* 2014) and Portugal (Nogueira *et al.,* 2017). This could be due to the most studies indicated that lifestyle or occupational factors may exist among males (Omer *et al.*, 2016).

Moreover, age group in the present study indicated that the highest frequency of EBV previous infection rates was seen among age group < 20 years (childhood group) of cases group, this agree with Palma *et al.* (2012) in Mexican. Thus, may be related to increasing viral load which caused in child age as a result to replication and exposure as various type of malignancies (Hutajulu *et al.*, 2017). Other age groups of cases and control group of EBV previous and recent infection showed a high frequency of infection rates after <20 years were seen among the age group 40-49 years and 50-59 years (adults), this age category agrees with Omer *et al.* (2016) in Sudan. This may be due to those seniors which may be related to older subjects had more exposure to EBV triggers along with the weakening of immune response which may facilitate EBV reactivation (Hutajulu *et al.,* 2017).

Regarding educational level, income status, and place of residency results showed a strongly significant association with *P*-value = 0.000 for EBV previous infection both cases and control group. This is due to economic status is one of the critical factors which determent whenever it is suitable to exposure EBV (Chabay and Preciado, 2016) and associated with factors such as education as well as race/ethnicity and other environments such as nutritional status (Crowcroft *et al.*, 1998; Dowd *et al.*, 2013; Fang *et al.*, 2015). While cancer patients and controls who married were more likely to have both EBV previous and recent infection, this may be due to the virus has also been reported in both male and female genital secretions, suggesting that sexual transmission may occur (Higgins *et al.*, 2007).

Regarding the signs and symptoms risk estimate category as present in our study, pharyngitis, fever, and fatigue have been the highest risk estimate in EBV recent infections, this supports and confirms other studies that recorded symptoms of EBV infection (Valachis and Kofteridis, 2012).

Our findings indicate a strongly significant association between consuming Shammah and blood transfusion and EBV infection. This may be due to the consuming Shammah is very common practice in Yemen, and have been proven its relation with the development of oral and esophagus cancers (Scheifele *et al.*, 2007). While in blood transfusion results might to explained be the EBV can spread through the blood, utilizing blood transfusion and organ transplantations (Smatti *et al.*, 2018).

Finally, our findings showed no association of the smoking, chewing qat, and history of malaria included in this study with the EBV infection. The same observation was reported by Al-Akwa'a (2011) and Nasher (2012).

CONCLUSION

Our study corroborated previous studies that showed a high seroprevalence of EBV, affecting more than 90% of the population in Sana'a city, Yemen, and the incidence of cancer types in the present study is associated with EBV infection. More importantly, we have shown a strongly statistically significant relation between EBV infection, and educational level, income status, residency, consuming Shammah, and blood transfusion as significant risk factors.

ETHICAL APPROVAL

Ethics statement of the study a known ledged distinctive consent forms the Committee of

Biology 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 have no conflicts of interest.

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