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## RESEARCH ARTICLE

### IMMUNOHISTOCHEMICAL DETECTION OF EPSTEIN - BARR VIRUS IN NASOPHARYNGEAL CARCINOMA LESIONS AMONG SUDANESE PATIENTS USING LMP-1 MONOCLONAL ANTIBODY

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#### ABSTRACT

**Objective:** this was a retrospective descriptive study aimed to assess the role of latent membrane protein-1 (LMP-1) Monoclonal Antibody in the detection of *Epstein- Barr* virus (EBV) in nasopharyngeal carcinoma (NPC) lesions in Sudanese patients.

**Methods:** Forty-five paraffin-embedded formalin-fixed tissue sections of nasopharyngeal carcinoma lesions were stained by Anti- EBV LMP-1 and results were correlated with the available clinical data.

**Results:** EBV was positive in 23 cases and negative in 22 cases. Most positive results were in elderly males with undifferentiated carcinomas.

**Conclusion:** Anti- EBV LMP-1 is effective in detecting EBV in nasopharyngeal carcinoma lesions.

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#### INTRODUCTION

About 15 % of all cancers worldwide result from chronic viral infections with higher incidence in developing countries (Thompson and Kurzrock, 2004). Epstein - Barr virus (EBV) is a human herpes virus primarily causes infectious mononucleosis (Liebowitz et al., 1993). Persistent EBV infection is associated with various lymphoid and epithelial malignancies, including Burkitt lymphoma (Zur Hausen, et al., 1970), Hodgkin lymphoma (Chen et al., 2007), peripheral T-cell lymphoma (Niedobitek et al., 1997), nasopharyngeal carcinoma (SbihiLammali et al., 1996), and mature natural killer (NK) cell malignancies (Jaffe et al., 1996). The EBV genome encodes a series of products interacting with or exhibiting homology to a wide variety of antiapoptotic molecules, cytokines, and signal transducers, hence promoting EBV infection, immortalization, and transformation (Elgui de Oliveira, 2007). Different methods have been used to find out the association between EBV and nasopharyngeal carcinoma. These include serology (Lanier et al., 1981), polymerase chain

reaction (Akao et al., 1991), immunohistochemistry (Niedobitek et al., 1992), in situ hybridization (Ameera AM Adam, et al., 2014), and immunofluorescence techniques (MOA Malik, et al., 1979). Nasopharyngeal carcinoma is common in Sudan; incidence is higher in males and all age groups are affected including children (Abuidris et al., 2008 and Abdullah et al., 2011). This study aimed to assess the role of immunohistochemistry in detecting Epstein -Barr virus in nasopharyngeal carcinoma lesions in Sudanese patients.

#### MATERIALS AND METHODS

This was a retrospective descriptive study conducted during the period between January and April 2014. Forty five paraffin-embedded formalin-fixed tissue blocks of nasopharyngeal carcinoma were included in this study. These tissue blocks were obtained from the archives of Radiation Isotope Center Khartoum (RICK) hospital after taking the numbers of blocks from the hospital records of the years 2011, 2012, and 2013. Two 3-4  $\mu$  m-thick tissue sections were cut from each block using a rotary microtome. One section was put on a frosted-end glass slide and stained by Hematoxylin and Eosin stain for confirmation of diagnosis found in the records and for grading of the tumor. The other section was placed on positively charged slide and dried overnight at

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58° C. Recommended Ventana®\* Staining Procedure (iView™) using Ventana stainer machine was applied (Index). All quality control measures were adopted during specimen collection and processing. Statistical Package for Social sciences (SPSS) software version 16 was used for data entry and analysis. Results were tabulated and presented in percentage form. Parameters analyzed were age, sex, tumor grade, and IHC results.

## RESULTS

The total number of the obtained tissue blocks was 45 blocks with all their records studied and analyzed. Age of patients ranged between 15 and 80 years with median age of 47.5 years (Table No 1).

**Table 1. Age distribution of Patients in the study**

Age Group	No of Patients	Frequency
10-20	5	11%
21-30	4	9%
31-40	5	11%
41-50	17	39%
51-60	6	13%
61-70	6	13%
71-80	2	4%

Patients were 36 males and 9 females; the male to female ratio was 4: 1. Twenty-two cases were poorly differentiated squamous cell carcinoma (grade III) and the remaining 23 were of grade IV type (undifferentiated carcinoma). Immunohistochemical staining was positive for EBV in 23 cases (51%) and negative in 22 (Table No 2).

**Table 2. Results of Immunohistochemical Staining**

IHC	PD	UnDif
Positive	9 (7M and 2 F)	16 (11 M and 5 F)
Negative	13 (11 M and 2 F)	7 (All M)

Note: PD= Poorly Differentiated, UnDif= Undifferentiated, M= Male, and F= Female.

## DISCUSSION

In this study, median age and sex distribution of NPC were not different from what was reported in the literature; most of patients were men over 40 years (Vokes *et al.*, 1993). To our knowledge, this is the first study in Sudan that investigated the presence of EBV in nasopharyngeal carcinoma lesions using LMP-1 Monoclonal Antibody. Swapnil Karnik *et al* (2003) investigated the association of Epstein-Barr virus (EBV) latent membrane protein-1 (LMP-1) in 100 cases of Hodgkin's lymphoma in South India and reported positive results in 82 % of cases. Wu Mingyao *et al* (1992) studied 80 cases of esophageal carcinoma lesions using EBV LMP-1 monoclonal antibody immunohistochemistry with 6.3% of cases were positive. Of the 5 cancer tissue positive cases, 2 were undifferentiated carcinoma and 3 were poorly differentiated squamous cell carcinoma. Saul Grinstein *et al* (2002) found immunohistochemically EBV in some carcinomas of malignant tumors of the breast, lung, colon, and prostate. PCR analysis of selected cases confirmed the presence of EBV. Eliane Pedra Dias *et al* (2009) studied twenty-four paraffin-

embedded tonsil specimens (recurrent tonsillitis) using viral protein LMP-1 and reported 37.5% of specimens positive for EBV. Hila *et al.* (2008) studied 60 cases of undifferentiated nasopharyngeal carcinoma from north of Tunisia using latent membrane protein (LMP1) and found EBV positive in 66.7% of cases.

## Conclusions

In conclusion, EBV is important in the pathogenesis and screening of nasopharyngeal carcinoma which has high frequency among Sudanese patients. In addition, EBV LMP-1 monoclonal antibody can be used as diagnostic and prognostic tumor marker for nasopharyngeal carcinoma. Further studies with larger number of specimens are recommended.

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