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ORIGINAL ARTICLE

Diagnostic Significance of Latent Membrane Protein 1 (LMP-1), EMA, CD45, CD20 and CD3 in Epstein-Barr Virus-Associated Nasopharyngeal Carcinoma

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ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma which differ from other head and neck cancers and linked to the Epstein Barr virus (EBV) and its encoded oncoproteins, such as EBNA1 and LMP1.These viral oncogenes have been found to be an important factor in its pathogenesis. We aimed to diagnose NPC and differentiate it from lymphoma, also to assess the expression pattern of LMP1 in the different histological types of NPC in a sample of the Egyptian population and to introduce serologic methods for screening and early detection of NPC. **Results:** Eighty-eight trans-nasal biopsies were examined. Carcinoma of the nasopharynx was detected in 79 of them. Of NPC patients 74/79 (93.6 %) showed positive test against IgA/ VCA. Late Membrane Protein was detected in 37/43(74 %) of cases of NPC tumor. Using histopathology as the gold standard for NPC, the sensitivity and specificity was 84% and 86% respectively with highly significant (p = 0.018). Epithelial membrane antigen was detected in 32/43 (64 %) NPC biopsies, the sensitivity and specificity were 72 % and 86 % respectively (p= 0.018). CD45 was positive in all 50 biopsies in the background leukocytes and negative in the tumor cells. **Conclusion:** Type III undifferentiated carcinoma is the commonest type of NPC. The antibodies to EBV-VCA IgA are of diagnostic value in early detection and are recommended for NPC screening at the population level.

Keywords: Diagnosis, Epstein-Barr virus, Nasopharyngeal carcinoma.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a rare cancer that is characterized by squamous cell carcinomas which differ from other head and neck cancers. The exact pathogenesis of NPC is still unknown, but it is linked to the Epstein Barr virus (EBV) and its encoded oncoproteins, such as EBNA1 and LMP1.These viral oncogenes have been found to be an important factor in the development of NPC [1]. LMP-1 regulates a number of signaling pathways in the pathogenesis of NPC, mainly through the three functional domains of its C terminal activating regions 1, 2, and 3. Through the NF-κB signaling pathway, LMP-1 regulates cell proliferation, apoptosis, transformation, and distant metastasis, causing immortalization through the p65 subunit [2].

NPC is characterized with poor prognosis after metastasis, with a 91% fatality rate within 1 year after metastasis with a high recurrence rate. According to the International Agency for Research on Cancer, NPC has a significant regional bias, with 129,000 new NPC cases worldwide in 2018, more than 70% of which occurred in Southeast and East Asia [3,4]. Although sensitivity of NPC to radiotherapy and chemotherapy, patients with metastatic spread exhibit poor prognoses, with a median survival of 13 months [5]. The etiology of nasopharyngeal carcinoma is complex and is not yet completely

understood. Carcinogenesis is however known to be associated with high titers of EBV. Genetically predisposed patients may develop NPC when exposed to a viral and/or environmental factor. NPC is considered rare; however, its incidence is higher in some endemic regions include Southwest Asia (Canton and Hong Kong), the Mediterranean region, North Africa, and Greenland and Alaska [6].

NPC is classified into 3 subtypes; Keratinizing squamous cell carcinoma (WHO type 1) which is associated with EBV infection in around 70% to 80% of cases. Nonkeratinizing squamous cell carcinoma (WHO type 2) and Undifferentiated or poorly differentiated carcinoma, including lymphoepithelioma and anaplastic variants (WHO type 3). Both type 2 and type 3 are highly responsive to treatment and are predominantly associated with EBV infection [7]. We aimed to diagnose NPC and differentiate it from lymphoma, also to assess the expression pattern of LMP1 in the different histological types of NPC in a sample of the Egyptian population and to introduce serologic methods for screening and early detection of NPC.

METHODS

This is a case-control study. The study was conducted at the E.N.T and pathology departments, Zagazig university faculty of medicine during the period between April 2019-May 2023.

Inclusion criteria: Patients with histologically proven NPC, who signed a written and informed consent.

Exclusion criteria: Patients with lymphoma and other tumors.

Sample size: Eighty-eight patients were included in this study with clinical features of NPC, subjected to histopathological investigation for confirmation of the disease by biopsy and enrolled in the study. Seven tissue specimens were selected from patients with (adenoid, hyperplastic polyp) as controls.

Data Collection and Procedures:

Information collected including demographic and clinical data. After signing an informed consent, the patients were investigated for the presence of NPC by histopathology of a biopsy from the nasopharynx. Biopsies from the nasopharynx were taken from all cases under local anesthesia; this was done in 97.4 % of the cases. The remaining 2.6 % were re-biopsied under general anesthesia.

Solid Phase Enzyme-Linked Immuno-Sorbent Assay (ELISA):

Five ml of venous blood were collected at the time of enrollment; Blood samples were immediately centrifuged and sera were stored at -20Co.The stored sera were tested serologically for EBV IgA early antigen (EA), IgA viral capsid antigen (VCA) using Solid Phase Enzyme-Linked Immuno-Sorbent Assay (ELISA) based on the sandwich principle (HAMBURG kits). The antibody was Epstein –Barr Virus EA IgA ELISA RE 57301. The antibody is used for the immunoassay for the qualitative and quantitative determination of IgA antibodies against the early antigen (EA) of Epstein –Barr Virus in human serum and plasma.

EBV capsid antigen (VCA gp 125 affinity purified from P3HR1- cells IBL company, Hamburg, Germany) is bound on the surface of the microtiter strips. Diluted patient serum ready to use, calibrator and controls were pipetted into the wells of the microtiter plate. A binding between the IgA antibodies of the samples and immobilized antigen takes place during the first incubation.

In the second incubation, ready to use anti human IgA Peroxidase conjugate was added. This binds to IgA antibodies captured on the microtiter wells. Subsequently, substrate (TMB) was pipetted, inducing the development of the blue dye in the wells. The colour develops was terminated by the addition of the stop solution, which courses the colour change from blue to yellow. The resulting dye was measured spectrophotometrically at the wave length of 450 nm. The concentration of the Ig antibodies is directly proportional to the intensity of the colour.

- Results of samples were determined directly using cut- off –value (COV) recommended by the manufacturer
- The same methods were applied to test EBV early antigen IgA.
- An EBV-negative serum and NPC serum were used as negative and positive control, respectively in each micro titer assay.
- Nasopharyngeal examination:

Diagnostic procedures include clinical examination and imaging techniques. Computed tomography with contrast and magnetic resonance imaging with contrast, are helpful for detection of the presence of a tumor, as well as for delineation of the extent of disease. Computed tomographic scanning (CT) is sensitive for detecting bone changes & tumor staging. Magnetic resonance imaging, owing to its multiplanar imaging capability, is a more sensitive modality than CT scanning for detecting small lesions and extent of invasion [8].

Endoscopic examination with directed biopsy for

a definitive histological diagnosis. This is done by: -

1. Posterior rhinoscopy: Mirror examination of the nasopharynx (this is done under local or general anesthesia).
2. Rigid flexible endoscopy: It allows close-up view of the nasopharynx and its recesses.
3. Flexible fiberoptic nasopharyngoscopy: this is the most useful endoscopy for nasopharyngeal and upper aerodigestive system examination [8]
4. Cases were diagnosed and treated at the Zagazig University Hospital and were followed up in Clinical and Medical oncology departments. Patients underwent treatment according to staging either definitive radiation therapy alone or concurrent chemoradiotherapy. Cisplatin, Carboplatin, 5-flurouracil and Gemcitabine are commonly used to treat nasopharyngeal cancer.

-Immunohistochemistry staining:

Formalin-fixed paraffin-embedded tissue blocks were serially sectioned into 3–5 μm sections followed by deparaffinization in xylene and rehydration in descending grades of alcohols. For antigen retrieval, 10mM citrate buffer (pH 6.0) at the microwave for 20 min was used. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 min. After repeat washing in PBS, the slides were incubated with Primary antibodies used in this study were anti-LMP1 (1:100 dilution) (clone CS1–4, Dako). CD20 (clone L26, Dako, Carpinteria, CA), EMA (E29, Biocare), CD3 (CP 215 A, C, dilution 1: 100; Biocare)

Binding site of primary antibodies was visualized by using the polymer detection system; the Dako EnVision™ kit (Dako, Copenhagen, Denmark). Finally, the tissue sections were counterstained with Meyer's hematoxylin, dehydrated and mounted. Negative controls were done by replacement of the primary antibodies with a non-immune serum. Positive and negative controls were stained in the same setting with the studied cases. The immuno-staining included: LMP, EMA, CD45, CD3, CD20. Staining was performed in 43 NPC cases and 7 tumor free biopsies. For positive EBV LMP control, Reed Sternberg cells were used. For the control of CD45, CD3, CD20, tonsillar tissue was used & breast carcinoma for EMA.

Ethical and administrative considerations: The ethical standards were approved by the Institutional Review Board Faculty of Medicine, Zagazig University, Egypt (ZU- IRB#116/25-Feb 2024).

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using Microsoft Office Excel 2010 for windows (Microsoft Cor., Redmond, WA, USA) and SPSS 22.0 for windows (IBM Inc., Chicago, IL, USA). Categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Categorical data were compared using Chi-square test or Fisher's exact test when appropriate. Validity of viral markers and immunohistochemistry was calculated using diagnostic performance depend on sample 2x2 contingency tables generation. The sensitivities (SN), specificities (SP), positive predictive values (PPV), negative predictive values (NPV), and accuracies with their respective 95% confidence intervals were calculated. All tests were two sided. p-value < 0.05 was considered statistically significant.

RESULTS

A total of 79 patients who presented in this study; their median age was 39.0 years with a range of 5-80 years. The age distribution of the study population, appeared to be of two peaks, one in late adolescence (15-19 years, n=15) whereas the other peak was among adult patients (50-54 years, n=16). Males were more affected than females with a ratio of 2:1. The commonest symptoms and signs at presentation in order of frequency were audiological, cervical lymphadenopathy and nasal symptoms. However, cranial nerves involvement was present in a considerable number of patients.

Eighty-eight trans-nasal biopsies were examined. Carcinoma of the nasopharynx was detected in 79 cases. When applying WHO classification to describe the histological pattern. Type I was not found, type II accounted for 13 (16.5 %) patients and type III accounted for 59 (74.7%) patients. Seven (8.9 %) cases showed a mixed histological pattern of type II & III. Statistically, type III is significantly more common than type II and the mixed type (p value = 0.001 and p value =0.001 respectively), but no significant difference was observed between type II and the mixed type (p value = 0.28). In 9 patients who were clinically suspected as cases of NPC, biopsies showed no tumor; showed only dysplastic and hyperplastic changes.

Serology results:

Viral Capsid Antigen (VCA/ IgA) results:

Seventy-four of NPC patients 74/79 (93.6 %) showed positive test against IgA/ VCA (fig.1).

According to the WHO classification of NPC, 13/13 (100%) patients of type II, 55/59 (93.2%) of type III, and 6/7 (85.7 %) of the mixed type II and III cases were positive for VCA/ IgA antibody. There was no significant correlation between VCA test performance and the histological subtypes (*p value > 0.05* for all types). Sera of 5/9 (55.6 %) patients in whom no tumor was detected histologically showed positive serology. Seventy eight out of 79 sera from the control group were negative. The sensitivity and specificity were 94 % and 99 % respectively (table ٣) with highly significant reading ($p < 0.001$) (fig. 2).

Serum of one healthy individual showed a positive test for VCA/ IgA antibodies (Examination of his nasopharynx showed no abnormality. He was followed up)

Early antigen (EA/ IgA) results:

The antibodies to IgA Early antigen were detected in sera of 40/79 (50.6 %) of the cases with nasopharyngeal carcinoma (fig. 3).

All healthy controls (n=79) sera gave negative test for EA IgA antibodies. The sensitivity and specificity were 50 % and 100 % respectively (table 3).

When applying WHO classification; Seven of 13 type II patients (53.8%) showed a positive reaction, of 30/59 type III patients (50.8%) had a positive reaction. In biopsies of mixed type II and III 3/7 (42.8 %) patients were found to be positive for EA/ IgA antibodies. There was no significant correlation between EA test performance and the histological subtypes (*p value > 0.05* for all types). In nine tumor free patients had non-reactive sera against IgA Early antigen (fig. 4).

Immunohistochemistry results:

Interpretation of immunohistochemical staining of Late Membrane Protein (LMP-EBV):

Late Membrane Protein was detected in 37/43(74 %) of cases of NPC tumor. Using histopathology as the gold standard for NPC, the sensitivity and specificity was 84% and 86% respectively with highly significant ($p = 0.018$). Applying WHO classification of NPC positive tests were detected in 6/9 (66.7%) cases of type II. Of type III 27/31 (87%) of the cases were positive. In the mixed type of type II and type III all of the cases (3/3) were positive for LMP. For the histological subtypes there was no significant difference between the association of the LMP and the histological subtype ($p > 0.05$). (table 1, 3)

Interpretation of immunohistochemical staining of Epithelial Membrane Antigen (EMA), CD45, CD3, CD20:

Epithelial membrane antigen was detected in 32/43 (64 %) NPC biopsies, the sensitivity and specificity were 72 % and 86 % respectively ($p= 0.018$). This was irrespective of the histological subtype and in mixed type II and type III EMA was found in 2/3 (67.7%) cases. The cases that were negative for EMA proved to be positive for cytokeratin. CD45 was positive in all 50 biopsies in the background leukocytes and negative in the tumor cells, The sensitivity and specificity were 88% and 14 % respectively (Table 2). We used this test to exclude the possibility of a lymphoma in which large cells were CD 45 positive.

Table (2) showed the presence of CD3 positive cell were detected 39/50(78 %) of the cases, the sensitivity and specificity were 81% and 43% respectively. In type III NPC, T cells infiltrated the tumor in 24/31(77.4 %) of the biopsies, type II was 8/9 (88.9 %) positive biopsies for CD3. All of the three cases of the mixed type showed immuno-staining for T cells.

There was no significant difference in the presence of CD 3 cells and the histological subtypes (p value > 0.05). (fig7, Table 3).

Table (2) showed CD20 B lymphocytes marker performed in 43 nasopharyngeal biopsies. Of all 50 biopsies 44 (88.0 %) of the cases were positive for the B cell marker CD20 irrespective of the presence or absence of tumor. B cells were detected in biopsies that showed hyperplasia of the lymphoid tissue, dysplasia of epithelium or granuloma formation in tumor negative biopsies. In type III NPC B cells were found in 29/31 (93.5 %) of the biopsies. In type II B cells were found in 6/9 (66.8 %) biopsies. All of the three cases of the mixed type were positive for B cells. There was no significant difference in the presence of CD 20 cells and the histological subtypes (p value > 0.05). In biopsies that were tumor free, B cells were detected in 6/7 (85.7 %) of the samples. The sensitivity and specificity were 88% and 14% respectively (Table 3).

In hematoxylin and eosin-stained section one nasopharyngeal biopsy was negative for tumor but the surface epithelium showed hyperplasia. Using Immunoperoxidase staining for LMP the virus was detected in the hyperplastic epithelium.

Table (1): Relationship between histopathogical subtypes and viral markers among the studied subjects.

	Type II (N=13)		Type III (N=59)		Mixed (N=7)		Control (N=79)		p-value
	No.	%	No.	%	No.	%	No.	%	
IgA VCA									
Negative	0	0%	4	6.8%	1	14.3%	78	98.7%	<0.001
Positive	13	100%	55	93.2%	6	85.7%	1	1.3%	
IgA EA									
Negative	6	46.2%	29	49.8%	4	57.2%	79	100%	<0.001
Positive	7	53.8%	30	50.2%	3	42.8%	0	0%	

Categorical variables were expressed as number (percentage); a: Chi-square test; p-value<0.05 is significant.

Table (2): Relationship between histopathogical subtypes and immunohistochemistry (IHC) among the studied subjects.

	Type II (N=9)		Type III (N=31)		Mixed (N=3)		Control (N=7)		p-value
	No.	%	No.	%	No.	%	No.	%	
LMP									
Negative	3	33.3%	4	12.9%	0	0%	6	85.7%	<0.001
Positive	6	66.7%	27	87.1%	3	100%	1	14.3%	
EMA									
Negative	1	11.1%	10	32.3%	1	33.3%	6	85.7%	0.018
Positive	8	88.9%	21	67.7%	2	66.7%	1	14.3%	
CD45									
Negative	0	0%	0	0%	0	0%	0	0%	1.000
Positive	9	100%	31	100%	3	100%	7	100%	
CD20									
Negative	3	33.3%	2	32.3%	0	0%	1	14.3%	0.156
Positive	6	66.7%	29	67.7%	3	66.7%	6	85.7%	
CD3									
Negative	1	11.1%	7	22.6%	0	0%	3	42.9%	0.355
Positive	8	88.9%	24	77.4%	3	100%	4	57.1%	

Categorical variables were expressed as number (percentage); a: Chi-square test; p-value<0.05 is significant.

Table (3): Diagnostic performance of viral markers and IHC among the studied subjects (N=50).

	TP	FP	TN	FN	SN (95%CI)	SP (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
IgA VCA	74	1	78	5	93.7% (85.8-97.9)	98.7% (93.1-99.9)	98.7% (91.3-99.8)	93.9% (86.9-97.3)	96.2% (91.9-98.6)
Ig EA	40	0	79	39	50.6% (39.1-62.1)	100%	100%	66.9% (61.8-71.7)	75.3% (67.8-81.8)
LMP	36	1	6	7	83.7% (69.2-93.2)	85.7% (42.1-99.6)	97.3% (85.4-99.6)	46.2% (28.9-64.3)	84% (70.9-92.8)
EMA	31	1	6	12	72.1% (56.3-84.7)	85.7% (42.1-99.6)	96.9% (83.3-99.5)	33.3% (22.1-46.9)	74% (59.7-85.4)

TP: True positive; FP: False positive; TN: True negative; FN: False negative; SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; 95%CI: 95%Confidnce Interval; p-value<0.05 is significant.

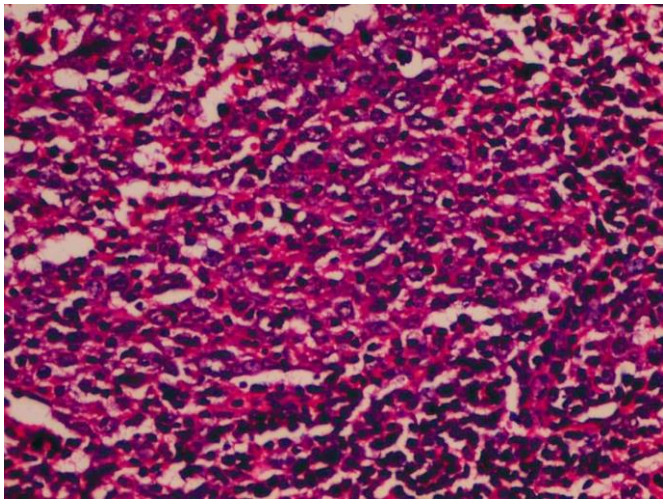

Figure (1)

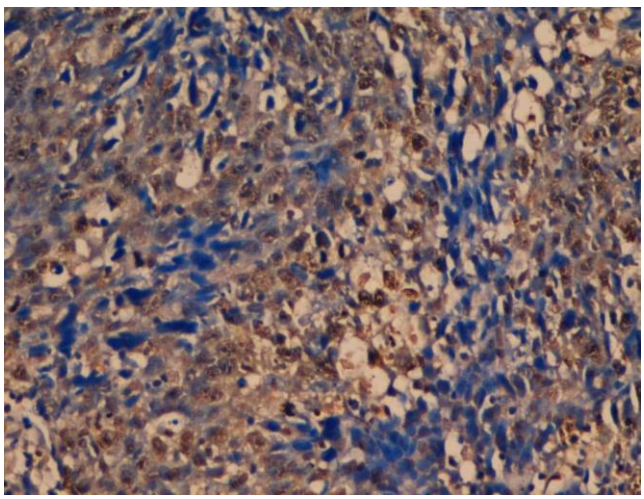

Figure (2)

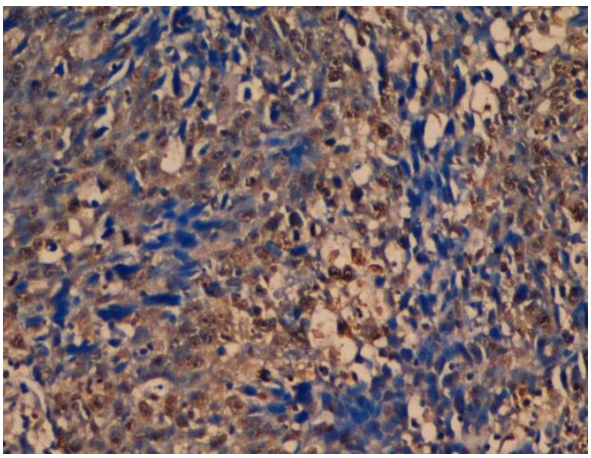

Figure (3)

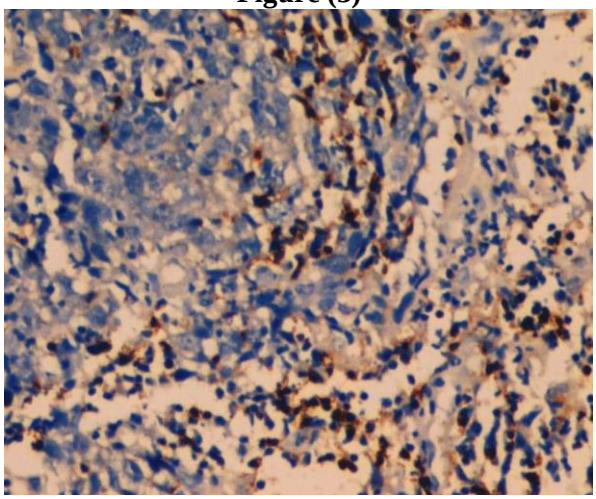

Figure (4)

DISCUSSION

Nasopharyngeal carcinoma (NPC) is one of the most prevalent squamous cell carcinomas of the head and neck, and Epstein-Barr virus (EBV) infection is one of main pathogenic factors involved in its development and progression. Approximately 129,000 new NPC cases we re reported in 2018, and more than 70% of patients with NPC are diagnosed at late stage. Radiation therapy (RT) is the preferred treatment for NPC. However, in about 20% of patients, therapy fails to give adequate pathologic response due to radiotherapy resistance, recurrence, and distant spread. Therefore, new molecular mechanisms controlling NPC development and behavior is needed to reach new strategies & lines for NPC management [9, 10].

The age distribution in this study is bi-modal with two peaks at the age groups of 15-19 years and 50-54 years respectively. This is similar to the age distribution of patients in North Africa. Although nasopharyngeal carcinoma is reported to be a rare tumor in young patients [11]. Our finding showed that two cases less than 14 years old. However, the exact incidence of pediatric NPC in Egypt is still unknown. In our study the ratio of males to females was 2:1, which is in agreement with Wang et al., 2021[12]. Undifferentiated nasopharyngeal carcinoma (WHO III classification), in the present study is the most common type of nasopharyngeal carcinomas accounting for 74.7 % of all cases. This finding is similar to that reported from Tse *et al*., 2006 [13]. Our findings showed that WHO type II is the second predominant histological type of NPC, accounting for (16.5 %) of all NPC patients. Seven biopsies (8.9 %) in this study showed both types II and III in the same biopsy. Li *et al*. stated that p53 accumulates abnormally in EBV-positive NPC to promote EBV latency. In addition to the common mechanisms by which LMP1 initiates p53 stabilization and accumulation through the inhibition of MDM2, LMP1 could also stabilize p53 through K63-linked ubiquitination catalyzed by TRAF2 [14]. Notably, Zhu et al. found that TRAF2 could also stimulate cell proliferation and radio-resistance in EBV-negative NPC [15]. So, since TRAF2 is regulated by multiple EBV proteins in NPC, targeting TRAF2 for treatment is expected to counteract the role of EBV in NPC.

Although infection with the EBV and genetic susceptibility appear to play major roles in the

etiology of NPC in high-incidence populations, migrant studies suggest that environmental factors may also be important [16]. Epstein-Barr virus capsid antigen (EBV-VCA/IgA), one of EBV lytic cycle antigens, is one of the most commonly used markers for diagnosis of nasopharyngeal carcinoma [17]. The monoclonal antibody used to detect VCA by ELISA in this study was combined IgG/IgA antibody. Out of 88 patients examined, sera of 76 (93.8 %) were positive. Five sera from 9 biopsies that were negative histopathologically for NPC were serologically positive. In Indonesia 50% of biopsy negative patients and most controls were VCA positive [18]. Under such condition serology will be of no value in diagnosing NPC. In the present study only one out of 79 healthy controls showed positive serology. The measurement of IgA and/ or IgG antibodies against VCA by ELISA was shown to be more sensitive than other techniques in the detection of antibodies against VCA in NPC patients [19].

Serum EBV-VCA/IgA titer may be used as an independent prognostic marker of NPC (Ling *et al*. 2009) [17] suitable for routine diagnosis and early detection of NPC recurrence (Wiley *et al*. 2005) [20]. Improvement in the ELISA method for EBV detection has led to an increased use of the method for diagnosis of new cases and follow up of treated patients (Karray *et al*. 2005) [21]. A study from North America suggests that IgA anti VCA antibodies alone or in combination with early antigen are of potential value in diagnosis of undifferentiated carcinoma (Pearson, *et al*. 1983) [22]. This was also reported in Italian patients (Cevenini *et al*. 1986) [23]. This work showed that the use of early antigen was not as sensitive as VCA in the diagnosis of NPC.

LMP expression in epithelial tumor cells was detected in (74 %) of NPC cases in the present study. Similar finding were reported by Hila *et al*. and Bai *et al* [24, 25]. Bai and Hila found expression of LMP in the tissues in 66.7% and 66.4% of their NPC cases respectively. In other studies, LMP was detected in all biopsies of NPC (Preciado *et al*. 2002) [26]. This may reflect variation in the techniques used or could possibly be a specific attribute of the virus that may vary from one area to another.

In the present study, the biopsies of NPC patients in the age group of 15 to 20 years, 10 of 12 cases expressed LMP antigen. Similar results were reported by Preciado *et al.* (2002) who found LMP expression in epithelial tumor cells in 9/10 of biopsies in patients aged 15 to 20 years [28]. It appears that the juvenile form of NPC has specific features regarding not only cellular composition but also viral gene expression [27]. Regarding the association of EBV with types II and III NPC agree with the findings reported by others, Ling *et al*. 2009, Preciado *et al*. 2002 and Yi *et al*. 2009 [17,26,28].

EMA is useful to differentiate type III NPC (poorly differentiated) from malignant Lymphoma, the three types of nasopharyngeal carcinoma arise from nasopharyngeal epithelial cells and should be positive for at least one epithelial cell marker. EMA in this study was positive 88.9 % of types II and of 67.7 % type III tumours. Similar finding was reported by Gusterson who found 57.1% of NPC cases showed positive staining. Staining for cytokeratin was the most reliable epithelial marker for identifying NPC and excluding lymphoma (Gusterson *et al*. 1983; Meng *et al*., 2023) [29,30]. In four type III NPC biopsies, the cells were negative for EMA but were positive for cytokeratin (data not shown)

Immuno-staining for leucocytes common antigen, T and B lymphocyte markers showed leucocytic infiltration in all NPC cases and in tumor free biopsies. The latter would be expected since the nasopharynx is a lymphoid organ. The infiltration of the type III carcinoma is well known ever since the tumor was called lymphoepithelioma. The infiltrating cells in the tumor may be reactive against the tumor antigens but they may also be residual normal lymphoid cells. This can only be settled by the isolation of lymphocytes from the tumor and demonstration of their proliferation response in vitro to EBV.

Declaration of interest:

The authors report no conflicts of interest. The authors along are responsible for the content and writing of the paper.

Funding information:

None declared.

CONCLUSION

In conclusion, our results suggest that Type III, undifferentiated carcinoma (WHO classification) is the commonest type of nasopharyngeal carcinomas. The antibodies to EBV-VCA IgA are of diagnostic value in early detection of NPC cases and are recommended for NPC screening at the population level.

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