Pathology & Oncology Research (2020) 26:2185–2190
https://doi.org/10.1007/s12253-020-00808-0

ORIGINAL ARTICLE

Comparison of Epstein-Barr Virus Serological Tools for the Screening and Risk Assessment of Nasopharyngeal Carcinoma: a Large Population-based Study

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Received: 18 October 2019 / Accepted: 17 March 2020 / Published online: 28 March 2020

Abstract

Epstein-Barr virus (EBV)-based serologic antibody testing has been found to be a feasible alternative for nasopharyngeal carcinoma (NPC) screening in endemic areas. The purpose of this study was to evaluate the performance of ELISA based on VCA IgA antibody, EA-IgA and Rta-IgG antibody specific to EBV in the diagnosis of NPC. A total of 2155 untreated NPC patients and 6957 healthy volunteers without nasopharyngeal disorder were recruited, and all subjects received EBV VCA-IgA, EA-IgA and Rta-IgG antibody tests simultaneously. The diagnostic efficiency of three testing alone or in combination for the diagnosis of NPC was evaluated. The prevalence of IgA antibody against EBV-VCA, IgA antibody against EBV-EA and IgG antibody against EBV-Rta was 89.9%, 46.6% and 63.2%. The sensitivity, specificity, positive predictive value, negative predictive value and Youden index were 89.88%, 89.65%, 73.18%, 96.63% and 0.79 for the EBV VCA-IgA antibody test, 46.59%, 96.89%, 82.5%, 85.42% and 0.43 for the EA-IgA antibody test, and 63.25%, 94.87%, 79.48%, 89.29% and 0.58 for the Rta-IgG antibody test in the diagnosis of NPC, and ROC curve analysis revealed the greatest diagnostic efficiency for EBV VCA-IgA antibody test and the lowest efficiency for EBV EA-IgA antibody test in the diagnosis of NPC. In addition, the simultaneous triple positivity of VCA-IgA, EA-IgA and Rta-IgG antibodies specific to EBV indicated the highest risk of NPC, and the simultaneous triple negativity of the three types of anti-EBV antibodies suggested the lowest risk of NPC. Our data demonstrate that EBV VCA-IgA antibody test shows a higher diagnostic efficiency than EA-IgA and Rta-IgG antibody tests for the screening of NPC, and triple positivity of is a better biomarker for the diagnosis of NPC.

Keywords Nasopharyngeal carcinoma · Epstein-Barr virus · VCA-IgA · EA-IgA · Rta-IgG · Serological screening · Diagnostic efficiency · ROC curve analysis

Introduction

Nasopharyngeal carcinoma (NPC), the most common cancer originating from the nasopharynx [1], is a rare malignancy worldwide but common in China, notably in southern China [2]. Worldwide, NPC accounted for 0.7% of total new cancer cases and 0.8% of all cancer deaths in 2018 [3]. In China, the new NPC incident cases and deaths were estimated to be 44.6 and 24.2 thousand, and the crude incidence and mortality was 3.26/100000 and 1.77/100000, while the cumulative incidence and mortality was 0.25% and 0.14% in people at ages of 0 to 74 years in 2014, respectively [4].

Although the exact pathogenesis of NPC has not been fully understood, smoking, occupational exposure to dust, consumption of salted fish, and genetic factors have been proved to contribute to the development of NPC [5, 6]. In addition, Epstein-Barr virus (EBV) infection has been strongly linked to NPC pathogenesis [7, 8].

Currently, radiotherapy is the first choice for the treatment of NPC [9], and early detection is of great significance to improve the survival and prognosis in NPC patients [10]. The definitive diagnosis of NPC still depends on biopsy [1]; however, EBV-based serologic antibody testing has been found to be a feasible alternative for

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NPC screening in endemic areas [11–13]. The purpose of this study was to evaluate the performance of enzyme-linked immunosorbent assay (ELISA) based on viral capsid antigen (VCA) IgA antibody, early antigen IgA antibody (EA-IgA) and Rta protein IgG antibody (Rta-IgG) specific to EBV in the diagnosis of NPC.

Subjects and Methods

Ethical Statement

This study was approved by the Ethical Review Committee of Fujian Provincial Cancer Hospital. Written informed consent was obtained from all participants following a detailed description of the purpose of the study, and all experimental procedures described in this study were conducted in accordance with the Declaration of Helsinki.

Subjects

A total of 2155 untreated NPC patients were recruited from the Department of Head and Neck Radiotherapy, Fujian Provincial Cancer Hospital (Fuzhou, China) during the period from June 2011 through August 2015, and all NPC diagnoses were confirmed by pathological examinations, while 6957 healthy volunteers without nasopharyngeal disorder that were sampled from the Physical Examination Center of Fujian Provincial Cancer Hospital during the study period served as controls. All study subjects received EBV VCA-IgA, EA-IgA and Rta-IgG antibody tests simultaneously for the first time during the study period. The TNM classification was performed according to the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) (7th edition) [14]. Demographic and clinical features were captured from patients' medical records.

EBV VCA-IgA, EA-IgA and Rta-IgG antibody Testing

Fasting venous blood was sampled and centrifuged, and serum samples were collected for the subsequent experiments. Serum VCA-IgA, EA-IgA and Rta-IgG antibodies specific to EBV were tested using the EBV VCA IgA ELISA Kit (EUROIMMUN AG; Luebeck, Germany), EBV EA IgA ELISA Kit (EUROIMMUN AG; Luebeck, Germany) and EBV Rta IgG ELISA Kit (Tarcine BioMed, Inc.; Beijing, China) following the manufacturers' instructions.

Statistical Analysis

All data were entered into Microsoft Excel 2010 (Microsoft Microsoft Corporation; Redmond, WA, USA), and all statistical analyses were done using the statistical software SPSS version 11.5 (SPSS, Inc.; Chicago, IL, USA). Differences of proportions were tested for statistical significance with chi-square test. The diagnostic efficiency was assessed using receiver operating characteristic (ROC) curve analysis, and the risk of NPC was evaluated with odds ratio (*OR*). A P value < 0.05 was considered statistically significant.

Results

Subject Characteristics

The NPC patients included 1577 men and 578 women, and had a median age of 48 years (range, 11 to 84 years). The controls included 3788 men and 3179 women, and had a median age of 44 years (range, 15 to 81 years). Overall, the prevalence of IgA antibody against EBV-VCA, IgA antibody against EBV-EA and IgG antibody against EBV-Rta was 89.9%, 46.6% and 63.2%, respectively. In addition, there was age- ($\chi^2 = 19.393$, $P = 0$), pathological type- ($\chi^2 = 9.575$, $P = .008$), N stage- ($\chi^2 = 40.056$, $P = 0$) and clinical stage-specific prevalence of IgA antibody against EBV-VCA ($\chi^2 = 32.639$, $P = 0$), T stage- ($\chi^2 = 20.545$, $P = 0$), N stage- ($\chi^2 = 27.06$, $P = 0$) and clinical stage-specific prevalence of IgA antibody against EBV-EA ($\chi^2 = 43.327$, $P = 0$), and age- ($\chi^2 = 20.304$, $P = 0$), N stage- ($\chi^2 = 36.816$, $P = 0$) and clinical stage-specific prevalence of IgG antibody against EBV-Rta ($\chi^2 = 22.081$, $P = 0$) in the NPC patients (Table 1).

Diagnostic performance of EBV VCA-IgA, EA-IgA and Rta-IgG Antibody Testing for the Diagnosis of NPC

The sensitivity, specificity, positive predictive value, negative predictive value and Youden index were 89.88%, 89.65%, 73.18%, 96.63% and 0.79 for the EBV VCA-IgA antibody test, 46.59%, 96.89%, 82.5%, 85.42% and 0.43 for the EA-IgA antibody test, and 63.25%, 94.87%, 79.48%, 89.29% and 0.58 for the Rta-IgG antibody test in the diagnosis of NPC (Table 2). The three antibody tests varied significantly in the diagnostic sensitivity and specificity for NPC ($P < .01$). In addition, ROC curve analysis revealed the greatest diagnostic efficiency for EBV VCA-IgA antibody test and the lowest efficiency for EBV EA-IgA antibody test in the diagnosis of NPC (Fig. 1).

Value of Combined Anti-EBV Antibody Tests in the Assessment of Risk of NPC

The simultaneous triple positivity of VCA-IgA, EA-IgA and Rta-IgG antibodies specific to EBV indicated the highest risk of NPC, and the simultaneous triple negativity of the three types of anti-EBV antibodies suggested the lowest risk of NPC (Table 3).

Table 1 Prevalence of IgA antibody against EBV-VCA, IgA antibody against EBV-EA and IgG antibody against EBV-Rta stratified by demographic and clinical features

Characteristics		No. cases	VCA-IgA		EA-IgA		Rta-IgG	
			No. positives	Positive rate (%)	No. positives	Positive rate (%)	No. positives	Positive rate (%)
Total number		2155	1937	89.9	1004	46.6	1363	63.2
Gender	Men	1577	1425	90.4	744	47.2	1006	63.8
	Women	578	512	88.6	260	45.0	357	61.8
Age (years)	≤ 20	33	23	69.7	13	39.4	10	30.3
	21–40	499	441	88.4	240	48.1	298	59.7
	41–60	1297	1184	91.3	611	47.1	841	64.8
	≥ 61	326	289	88.7	140	42.9	214	65.6
Pathological type	Non-keratinizing and differentiation	218	191	87.6	99	45.4	133	61.0
	Non-keratinizing and non-differentiation	1905	1722	90.4	889	46.7	1214	63.7
	Others	32	24	75.0	15	46.9	15	46.9
T stage	T1	382	335	87.7	148	38.7	232	60.7
	T2	504	456	90.5	223	44.2	328	65.1
	T3	633	578	91.3	297	46.9	397	62.7
	T4	636	568	89.3	336	52.8	406	63.8
N stage	N0	149	117	78.5	53	35.6	74	49.7
	N1	409	348	85.1	155	37.9	222	54.3
	N2	1199	1105	92.2	589	49.1	789	65.8
	N3–N4	398	366	92.0	206	51.8	278	69.8
M stage	M0	2011	1804	89.7	932	46.3	1266	63.0
	M1	144	133	92.4	72	50.0	97	67.4
Clinical stage	I	33	24	72.7	7	21.2	15	45.5
	II	142	112	78.9	37	26.1	68	47.9
	III	1015	928	91.4	461	45.4	643	63.3
	IV	965	873	90.5	499	51.7	637	66.0

Discussion

Although rare worldwide, NPC is highly prevalent in southern China [4]. NPC has no specific clinical syndromes or signs at early stage, which increases the difficulty of diagnosis and has a high possibility of misdiagnosis, and lymph node metastasis frequently occurs upon definitive diagnosis [1]. Since late-stage NPC has an unsatisfactory prognosis and a low survival

Table 2 Diagnostic value of EBV VCA-IgA, EA-IgA and Rta-IgG antibody testing for NPC

Variable		EBV VCA-IgA antibody test	EBV EA-IgA antibody test	EBV Rta-IgG antibody test
NPC patients	No. positive	1937	1004	1363
	No. negative	218	1151	792
Health controls	No. positive	710	213	352
	No. negative	6247	6744	6605
Specificity (%)		89.65	96.89	94.87
Sensitivity (%)		89.88	46.59	63.25
Positive predictive value (%)		73.18	82.50	79.48
Negative predictive value (%)		96.63	85.42	89.29
Youden index		0.79	0.43	0.58

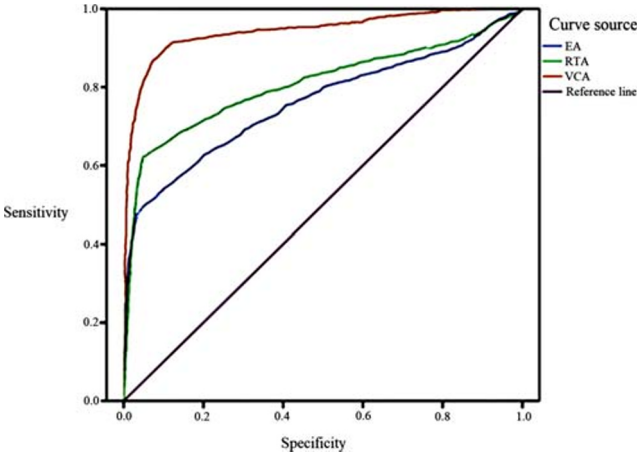

Fig. 1 ROC curve of EBV VCA-IgA, EA-IgA and Rta-IgG antibody testing for the diagnosis of NPC

rate, early identification and diagnosis is of great importance to extend the survival and improve the clinical outcomes in NPC patients [5, 6].

To date, the definitive diagnosis of NPC still depends on nasopharyngoscopy and pathological examinations; however, these approaches are invasive, high in cost and complicated to perform, which are unsuitable for NPC screening [15]. Infection with EBV, a member of the herpesvirus family and one of the most common human viruses, has been found to strongly correlate with NPC [7, 8]. In addition, previous studies have detected EBV in almost all NPC cell lines, and high-titer antibodies specific to EBV in NPC patients, and serum anti-EBV antibodies have therefore been employed as markers for early diagnosis of NPC [16–18]. Currently, EBV latent membrane protein (LMP) and nuclear antigen have been widely used for serological screening of NPC, including LAMP1, VCA, EA, Epstein-Barr nuclear antigen (EBNA), Rta, Zta, membrane antigen and lymphocyte-detected membrane antigen (LYDMA) [18], and detection of either IgA or IgG antibody against EBV antigens has been applied for NPC screening [19–21].

Previous studies have shown an anti-EBV antibody-specific diagnostic efficacy for NPC. In 211 untreated NPC patients, 203 non-NPC ear-nose-throat patients, and 210 healthy controls, the sensitivity, specificity, Youden index and area under ROC curve were 98.1%, 82.8%, 0.81 and 0.98 (95% *CI*: 0.96–0.99) for EBV VCA IgA antibody test, 89.1%, 98.5%, 0.88 and 0.94 (95% *CI*: 0.92–0.96) for EA IgA antibody test, and 90.5%, 85.2%, 0.76 and 0.92 (95% *CI*: 0.89–0.95) for Rta IgG antibody test in detection of NPC [21]. Among 64 NPC patients, 60 benign rhinitis and 60 healthy individuals, the sensitivity, specificity and area under ROC curve were 75%, 90.83%, and 0.897 (95% *CI*: 0.846–0.949) for EBV Rta IgG antibody test, 71.88%, 96.67%, and 0.882 (95% *CI*: 0.813–0.95) for EA IgA antibody test, and 79.69%, 95%, and 0.951 (95% *CI*: 0.912–0.989) for VCA IgA antibody test in diagnosis of NPC [22]. In another study recruiting 64 NPC patients, 60 benign rhinitis and 60 healthy individuals, the sensitivity, specificity and area under ROC curve were 76.79%, 92.22%, and 0.914 (95% *CI*: 0.862–0.967) for EBV Rta IgG antibody test, 73.21%, 98.89%, and 0.873 (95% *CI*: 0.802–0.943) for EA IgA antibody test, and 78.57%, 90%, and 0.928 (95% *CI*: 0.877–0.979) for VCA IgA antibody test in diagnosis of NPC [20].

In this study recruiting 2155 untreated NPC patients and 6957 healthy controls, there was no gender-specific prevalence of the antibodies against the three EBV antigens in NPC patients, which was in agreement with previous reports [20–22], and no M stage-specific prevalence was seen, indicating no correlation between EBV infections and distal metastases in NPC [21]. However, we detected N stage- and clinical stage-specific prevalence of IgA antibody against EBV-VCA, T stage-, N stage- and clinical stage-specific prevalence of IgA antibody against EBV-EA, and age-, N stage- and clinical stage-specific prevalence of IgG antibody against EBV-Rta in NPC patients, which was inconsistent with previous studies [21]. Our data further demonstrate that the seroprevalence of EBV VCA IgA, EA IgA and Rta Ig antibodies correlates strongly with the TNM stage and clinical stage of NPC [23, 24].

Table 3 Combinations of EBV VCA-IgA, EA-IgA and Rta-IgG antibody testing to assess the risk of NPC

Combination of anti-EBV antibody testing			Positive rate (%)		*OR*	95% *CI*
VCA-IgA	EA-IgA	Rta-IgG	NPC patients	Healthy controls		
Negative	Negative	Negative	6.54	85.37	0.08	0.065–0.09
Positive	Negative	Negative	17.54	6.77	2.59	2.283–2.94
Negative	Positive	Negative	0.23	0.49	0.47	0.186–1.212
Negative	Negative	Positive	3.16	3.88	0.81	0.626–1.056
Positive	Positive	Negative	12.44	2.31	5.37	4.447–6.494
Negative	Positive	Positive	0.19	0.06	3.23	0.808–12.897
Positive	Negative	Positive	26.17	0.92	28.45	22.069–36.675
Positive	Positive	Positive	33.74	0.2	167.64	99.008–283.853

Like the results from previous small-scale studies [20–22, 25], our data also showed the greatest diagnostic efficiency of EBV VCA-IgA antibody test and the lowest diagnostic efficiency of EA-IgA antibody test in the detection of NPC, as revealed by ROC curve analysis. This is consistent with the results from meta-analyses revealing that the presence of VCA-IgA in peripheral blood is a valuable predictor for NPC [19, 26]. Then, we assessed the value of combined detection of antibodies against EBV antigens for the diagnosis of NPC. Simultaneous triple positivity of VCA-IgA, EA-IgA and Rta-IgG antibodies specific to EBV suggested the highest risk of NPC, followed by triple positivity of EBV VCA-IgA and Rta-IgG antibodies, and simultaneous triple negativity of the three types of anti-EBV antibodies indicated the lowest risk of NPC. Our data were similar to previous studies showing that combined detection of these serum indexes may improve the diagnostic efficacy of NPC [20–22].

Previous studies have shown that EBV DNA has a higher sensitivity and specificity than VCA-IgA antibody against EBV in the diagnosis of NPC [27, 28], and combined detection EBV DNA and VCA-IgA antibody improves the accuracy of NPC screening [29, 30]. In addition, positivity for both EBV-DNA and VCA-IgA was reported to be a better biomarker for the prognosis of NPC [31]. In this study, however, EBV DNA-based assay was not included. Further studies recruiting a large-scale population to assess the diagnostic efficacy and prognostic value of combined EBV antibody and DNA-based assays seem justified.

In summary, the results of the present study demonstrate that EBV VCA-IgA antibody test shows a higher diagnostic efficiency than EA-IgA and Rta-IgG antibody tests for the screening of NPC, and triple positivity of is a better biomarker for the diagnosis of NPC.

Funding Information This study was funded by the grants from the Natural Science Foundation of Fujian Province (grant nos. 2016J01511, 2017J05120 and 2018J01273), the Science and Technology Program of Fujian Province (grant no. 2018Y2003) and the Joint Funds for the Innovation of Science and Technology, Fujian Province (grant no. 2017Y9072).

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interests.

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