RESEARCH COMMUNICATION

Systematic Review on Epstein-Barr Virus (EBV) DNA in Diagnosis of Nasopharyngeal Carcinoma in Asian Populations

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Abstract

Objective: To conduct a meta-analysis to investigate the value of EBV DNA in diagnosis of nasopharyngeal cancer (NPC) in Asian populations, and provide important evidence for screening. Methods: Prospective or respective case-control or cohort studies regarding the detection role of EBV DNA for NPC were included in our study. We conducted a comprehensive literature search in PubMed, EMBASE, and the Chinese Biomedical Database (CBM database between January 1980 and March 2012. Results: A total of 18 studies with 1492 NPC cases and 2641 health controls were included. Almost of the included studies were conducted in China, and only one other conducted in Thailand. The overall results demonstrated that the pooled sensitivity, specificity, positive likelihood (+LR) and negative likelihood (-LR) were 0.73 (0.71-0.75), 0.89 (0.88-0.90), 8.84 (5.65-13.84) and 0.19(0.11-0.32), respectively. The overall EBV DNA detection showed the largest area of 0.932 under the summary receiver operator curve (SROC). The accuracy of detection by plasma for NPC (0.86) was higher than in serum (0.81), with largest areas under the SROC of 0.97 and 0.91, respectively. Conclusion: Our results demonstrated the EBV DNA detection in plasma or serum has high sensitivity and specificity in diagnosis of NPC, especially in Chinese populations with a high risk of cancer.

Keywords: Epstein-Barr virus (EBV) DNA - diagnosis - nasopharyngeal carcinoma - Asian population

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Introduction

Nasopharyngeal carcinoma (NPC) has been recognized for over 100 years, with the first published report of the disease in 1901. In 2002, there were 80,000 new cases worldwide, accounting for 0.7% of all cancers and making it the 23rd most common new cancer in world (Parkin et al., 2005; Wei et al., 2010; Sharma et al., 2011). In contrast, it was the seventh most common new malignancy in Hong Kong. Based on geographic distribution, the age-standardised incidence rate of nasopharyngeal carcinoma for both males and females is < 1 per 100,000 person-years in most regions. However, dramatically elevated rates are observed in the Cantonese population of southern China (including Hong Kong) (Parkin et al., 2005). In terms of sex distribution, nasopharyngeal carcinoma is diagnosed more frequently in males than in females with an approximate ratio of 2 to 3:1 (Parkin et al., 2005).

Infection with EBV is most strongly associated with NPC, as shown by the raised levels of antibodies against EBV in most patients with NPC, presence of EBV DNA or RNA in all tumour cells, and the presence of EBV in a clonal episomal form and precursor lesion of nasopharyngeal carcinoma. The prognosis of advanced nasopharyngeal carcinoma is not very satisfaction with the two-years survival rate of 50% for patients with stage III or IV NPC. However, the two years survival rate could reach to 90% or even higher in patients with stage I and II NPC. Therefore, there is a clear need for early diagnosis and treatment method to reduce the mortality of NPC.

Although several prior studies (Zeng et al., 1982; Zeng et al., 1983; Zeng et al., 1985; Tsang et al., 2004) have demonstrated on the use of EBV serology for screening and early detection of NPC, the role of EBV DNA detection as a screening test for NPC remains inconclusive. Previous research has reported that cell-free EBV DNA can be quantitatively measured in the blood of NPC patients using polymerase chain reaction (PCR) technique (Lo et al., 1999; Shotelersuk et al., 2000; Lin et al., 2001; Chan and Lo, 2002; Chan et al., 2003; Fan et al., 2004). Recent studies have demonstrated that the plasma EBV-DNA level might be a sensitive and reliable biomarker for the diagnosis of NPC at a molecular level/ clinical practice (Fan et al., 2004; Shao et al., 2004; Zhang et al., 2004; Yang et al., 2006). Samples which showed positivity for serum DNA were also positive for tissue DNA, which suggests that the serum EBV DNA originated from NPC and could be use as a marker for tumor DNA. However, although previous studies on early screening strategies and treatment have shown a high survival rate,

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Table 1. Characteristics of Included Studies

Study ID	Country	Sample size (Case/Control)	Method	Study design	Selection bias	Measurement bias
Feng 2009	China	65/29	PCR, plasma	Case-control	Yes	No
Kong 2010	China	56/60	PCR, Plasma	Case-control	Yes	No
Liao 2010	China	34/30	FR-PCR, Plasma	Case-control	Yes	No
Luo 2009	China	160/76	PCR, Serum	Case-control	Yes	No
Sun 2010	China	62/62	PCR, Plasma	Case-control	Yes	No
Sun 2008	China	68/90	PCR, Plasma	Case-control	Yes	No
Tan 2010	China	12340	FQ-PCR, Serum	Case-control	Yes	No
Wai 2010	China, Hong Kong	18/1181	PCR, Serum	Case-control	No	No
Zhang 2012	China	40/50	PCR, Serum	Case-control	Yes	Unclear
Zhu 2012	China	168/60	PCR, Plasma	Case-control	Yes	No
Fan 2004	China	65/68	RQ-PCR, Serum	Case-control	Yes	No
Shao 2004	China	147/78	RQ-PCR, Plasma	Case-control	Yes	No
Chang 2008	China	156/264	RQ-PCR, Plasma	Cohort	No	No
Chan 2003	China	55/163	RQ-PCR, Serum	Case-control	Yes	No
Mai 2002	China	66/58	PCR, serum	Case-control	Yes	No
Leung 2004	China, Hong kong	139/178	RQ-PCR, plasma	Case-control	Yes	No
Lo 1999	China, Hong kong	57/43	Q-PCR, plasma	Case-control	Yes	No
Mutirangura 1998	Thailand	13/111	PCR, serum	Case-control	Yes	No
Total						

Table 2. The Diagnostic Characteristics of Included Studies

Study ID	TP	FP	FN	TN	Sensitivity	Specificity	+LR(95% CI)	-LR(95% CI)
Feng 2009	45	1	20	28	0.69	0.97	6.28(3.07-12.82)	0.32(0.22-0.46)
Kong 2010	41	7	15	53	0.73	0.88	5.88(1.94-17.85)	0.30(0.19-0.47)
Liao 2010	20	3	14	27	0.59	0.9	5.81(3.12-10.82)	0.46(0.30-0.69)
Luo 2009	110	9	50	67	0.69	0.88	14.75(5.71-38.12)	0.35(0.28-0.45)
Sun 2010	59	4	3	58	0.95	0.94	14.34(6.60-31.11)	0.05(0.02-0.16)
Sun 2008	65	6	3	84	0.96	0.93	22.15(1.39-353.54)	0.05(0.02-0.14)
Tan 2010	33	0	90	40	0.27	1	6.43(4.98-8.29)	0.74(0.66-0.83)
Wai 2010	15	153	3	1028	0.83	0.87	3.375(1.86-6.12)	0.19(0.07-0.54)
Zhang 2012	27	10	13	40	0.68	0.8	10.36(2.61-41.10)	0.41(0.26-0.65)
Zhu 2012	58	2	110	58	0.35	0.97	2.31(1.75-3.05)	0.68(0.60-0.76)
Fan 2004	64	29	1	39	0.98	0.57	6.10(3.62-10.29)	0.03(0.01-0.19)
Shao 2004	138	12	9	66	0.94	0.85	23.88(12.51-45.58)	0.07(0.04-0.14)
Chang 2008	127	9	29	255	0.81	0.97	30.62(9.75-96.23)	0.19(0.14-0.27)
Chan 2003	31	3	24	160	0.56	0.98	8.20(3.82-17.62)	0.44(0.33-0.60)
Mai 2002	56	6	10	52	0.85	0.9	42.26(16.03-111.44)	0.17(0.10-0.30)
Leung 2004	132	4	7	174	0.95	0.98	13.83(4.64-41.24)	0.05(0.03-0.11)
Lo 1999	55	3	2	40	0.96	0.93	3.66(2.64-5.07)	0.04(0.01-0.15)
Mutirangura 1998	13	29	0	82	1	0.74	6.28(3.07-12.82)	0.05(0.01-0.74)
Pooled results	1089	290	403	2351	0.73 (0.71-0.75)	0.89 (0.88-0.90)	8.84(5.65-13.84)	0.19(0.11-0.32)

this has been demonstrated to be inadequate to assess the effectiveness of screening or treatment due to lead-time and length-time biases, and the evidence of role of EBV DNA in diagnosis of NPC is inconclusive, especially for Asian population who are with high incidence of NPC. Therefore, we aimed to conduct a meta-analysis to investigate the value of EBV DNA in diagnosis of NPC in Asian population, and provide important evidence for screening method of NPC.

Materials and Methods

Selection criteria

Prospective or respective case-control or cohort studies regarding on the detection role of EBV DNA for NPC were included in our study. When the results of a study were published more than once, only the study that contained the most complete data was included in the analysis. Moreover, participants with NPC were eligible for inclusion, and the controls were non-cancer or disease-free subjects.

Identification of studies

To identify studies regarding on the role of EBV DNA in diagnosis of NPC, we conducted a comprehensive literature search in PubMed, EMBASE, and the Chinese Biomedical Database (CBM database between January 1980 and March 2012. A comprehensive and exhaustive search strategy was formulated in an attempt to identify all relevant studies regardless of language or publication status using the following terms: 'Epstein-Barr Virus', 'EBV', 'DNA', 'serological test', 'nasopharyngeal carcinoma' and 'NPC'.

Two reviewers independently examined abstracts of all candidate articles to decide whether to include or exclude them in the subsequent detailed review. We also

Table 3. The Diagnostic Characteristics of EBV DNA in Plasma and Serum

Subgroup	TP	FP	FN	TN	Pooled Sensitivity	Pooled Specificity	Pooled +LR (95% CI)	Pooled-LR (95% CI)
Plasma	740	51	212	843	0.78(0.75-0.81)	0.94(0.92-0.96)	12.51(7.88-19.86)	0.14(0.05-0.37)
Serum	349	239	191	1508	0.65(0.60-0.69)	0.86(0.85-0.88)	5.42(3.40-8.64)	0.27(0.13-0.54)

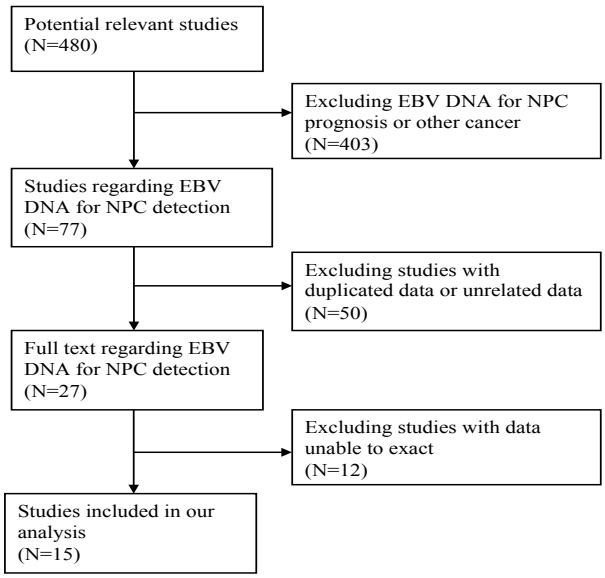

Figure 1. Flow Chart of Literature Selection

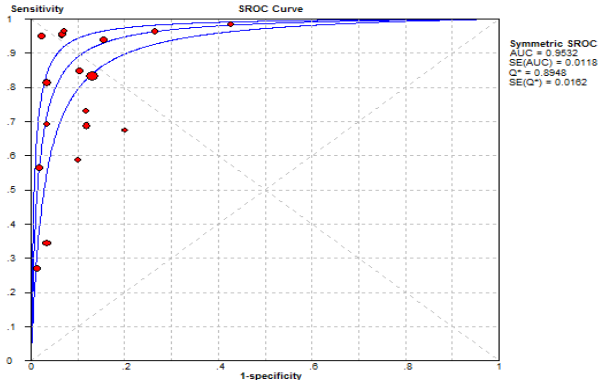

Figure 2. SROC for the Pooled Accuracy of EBV-DNA for NPC Detection

attempted to identify additional studies by searching the reference lists of relevant trials, and scrutinized author names, location, setting, number of participants, study data and selection bias as well as measurement bias to ensure most complete data in our study (Table 1).

Statistics

We used standard methods recommended for meta-analysis of diagnostic studies. For each study we computed measures of test accuracy using standard methods: sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–), and diagnostic odds ratio (DOR). These measures were pooled using the random effects model. Each study in the meta-analysis contributed using a summary receiver operating characteristic (SROC) curve. Unlike a traditional ROC plot that explores the effect of varying thresholds (cut points for determining test positives) on sensitivity and specificity in a single study, each data point in the SROC plot represents a separate study. The SROC curve presents a global summary of test

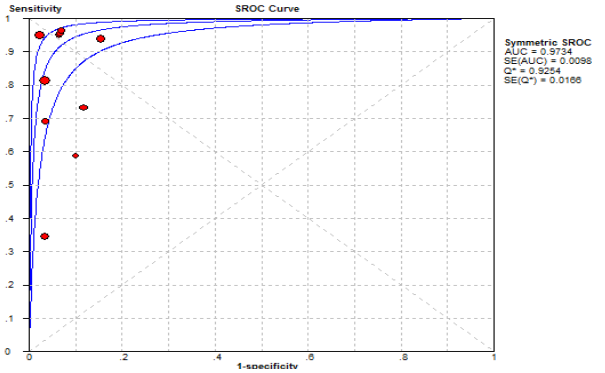

Figure 3. SROC for the Pooled Accuracy of EBV-DNA in Plasma for NPC Detection

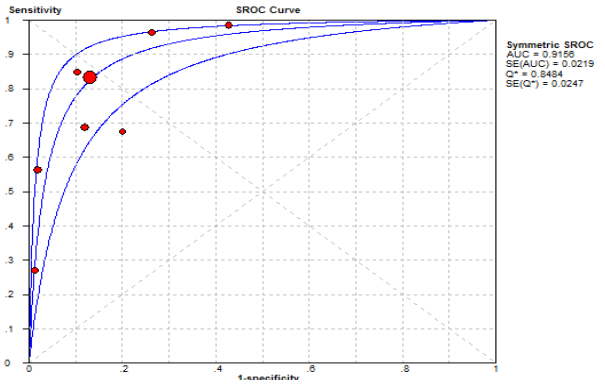

Figure 4. SROC for the Pooled Accuracy of EBV-DNA in Serum for NPC Detection

performance, and shows the trade off between sensitivity and specificity. The area under the SROC curve is a global measure of overall performance. An area under the curve of 1 indicates perfect discriminatory ability. Heterogeneity was tested using the I^2 with significance set at $P < 0.05$, and its possible sources were assessed by subgroup analyses. Statistical analysis was conducted by using Meta-DiSc statistical software version 1.4 (Unit of Clinical Biostatistics, Ramony Cajal Hospital, Madrid, Spain).

Results

A total of 480 titles or abstracts were selected, but 465 studies were excluded due to unrelated, duplicated or inappropriate data. Finally, a total of 18 studies with 1492 NPC cases and 2641 health controls were included. Almost of the included studies were conducted in China, and only one study conducted in Thailand. For the methods of included studies, 10 studies used EBV DNA detection in Plasma, and other 8 studies used serum (Table 1). The overall results demonstrated that the pooled sensitivity, specificity, positive likelihood (+LR) and likelihood negative (-LR) were 0.73(0.71-0.75), 0.89(0.88-0.90), 8.84(5.65-13.84) and 0.19(0.11-0.32), respectively. The overall EBV DNA detection showed the largest area of

0.932 under the summary receiver operator curve (SROC). In the pooled analysis, the heterogeneity across studies showed significant difference ($p<0.05$, $I^2>50\%$), therefore, the random effect model was used in our analysis.

The subgroup analysis was conducted regarding the detection method, by plasma or serum, to investigate the heterogeneity within the included studies. For the studies detected by plasma, the pooled sensitivity, specificity, positive likelihood (+LR) and likelihood negative (-LR) were 0.78(0.75-0.81), 0.94(0.92-0.96), 12.51(7.88-19.86) and 0.14(0.05-0.37), respectively. Moreover, for detection by serum, the pooled sensitivity, specificity, positive likelihood (+LR) and likelihood negative (-LR) were 0.65(0.60-0.69), 0.86(0.85-0.88), 5.42(3.40-8.64) and 0.27(0.13-0.54), respectively. The accuracy of detection by plasma for NPC (0.86) is higher than by serum (0.81) (Table 3). The largest area under the SROC was 0.97 for plasma and 0.91 for serum (Figure 3 and Figure 4). The subgroup analysis for plasma and serum showed no heterogeneity within studies, with the p value of 0.07 for plasma and 0.13 for serum.

Discussion

NPC is often difficult to diagnose because of the non-specific nature of its clinical symptoms and the difficulty in visualizing the nasopharynx (Zong et al., 1992). Submucosal primary lesions often escape endoscopic examination (Wei et al., 1991). However, the diagnostic accuracy of endoscopic examination is not high, as a result, the five years survival of NPC patients is about 10% to 20%. In our meta-analysis, the results showed EBV DNA detection by plasma or serum was an effective method for NPC. The sensitivity and specificity of the EBV DNA detection could be as high as 0.78 (0.75-0.81) and 0.94 (0.92-0.96) for NPC diagnosis, which indicated people who suspected with NPC would be diagnosed by EBV DNA detection in plasma or serum, especially for high risk area of NPC such as south China, Taiwan and elsewhere in Southest Asia (Lo et al., 1999; Lo et al., 2000; Chan et al., 2002). Moreover, the pooled results showed the EBV DNA detection had higher accuracy in plasma than in serum, which suggested the EBV DNA in plasma could be a better detection tool in high risk areas.

Although the etiological factors leading to the development of NPC are incompletely understood, it has been well established that there is a strong association between EBV infection and NPC. Since EBV DNA has been shown to be present in the tissues of NPC biopsies, the possibility of detecting EBV DNA in the circulation of NPC patients has been raised (Lung et al., 1992). In 1998, Mutirangura et al. showed that, in a group of 42 NPC patients, circulating extracellular EBV DNA could be detected, by PCR amplification, in one third of them but in none of the 82 control subjects (Mutirangura et al., 1998). Moreover, previous studies showed association between the EBV DNA in the serum of NPC patients and their apoptosis (Mutirangura et al., 1998).

Our study showed the accuracy of EBV DNA in plasma was higher than in serum. The possible reason might be plasma with the higher concentration of cell free DNA than in serum. Previous studies indicated the plasma had higher level of EBV than serum (Fan et al., 2004). However, there is another study showed the serum samples had more cell-free DNA levels than plasma samples, and the level was more higher even in samples stored below 4 °C. Therefore, further studies on the difference between plasma and serum EBV DNA are needed.

In conclusion, our results demonstrated the EBV DNA detection in plasma or serum had higher sensitivity and specificity in diagnosis of NPC, especially in Chinese population with high risk of cancer. Furthermore, the EBV DNA in plasma had higher accuracy than in serum, which provide important evidence for further screening method for NPC.

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