Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

EBNA-1 - BIOMARKER FOR NASOPHARYNGEAL CARCINOMA: A META-ANALYSIS

Thuan Duc Lao[1], Thuy Ai Huyen Le[1], Dung Huu Nguyen[2*]

1. *Department of Pharmaceutical and Medical Biotechnology, Faculty of Biotechnology, Ho Chi Minh City Open University, Ho Chi Minh City, Vietnam.*
2. *University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam.*

ARTICLE INFO

Received:
08 Oct 2019
Received in revised form:
02 Feb 2020
Accepted:
10 Feb 2020
Available online:
28 Feb 2020

Keywords: EBNA-1, nasopharyngeal carcinoma, meta-analysis

ABSTRACT

Background: Epstein-Bar virus (EBV) is reported to be intimately associated with the development of nasopharyngeal carcinoma (NPC), the most common and high incidence cancer of the head and neck in Asian countries. Among the EBV DNA genome, *the EBNA-1* gene plays an important role in NPC. **Objective:** a meta-analysis was performed according to the PRISMA guideline to systematically evaluate the association between the EBNA-1 and NPC. **Materials and methods:** Relevant articles were identified by searching the MEDLINE database. The frequency and Odds ratio (OR) were applied to estimate the effect of EBNA-1 based on random-/fix-effects models. **Results:** eight studies included 1,372 samples: 588 samples from NPC cancer patients, and 784 samples from non-cancerous samples, were enrolled in the meta-analysis. The overall frequency of *EBNA-1* in NPC patients and controls was 88.72% and 4.32%, respectively. The current meta-analysis suggested that individuals with the presence of *EBNA-1* are associated with NPC risk under the fix-effects model (pooled OR = 331.09, 95%CI = 139.95-783.30). Our findings underscore the correlation among *EBNA-1* and all subgroups, including region, method, and source of samples under the fix-effects model. **Conclusion:** Based on those findings, the presence of EBNA was suggested as a promising biomarker for the diagnosis and screening of NPC.

To Cite This Article: Thuan Duc Lao, Thuy Ai Huyen Le, Dung Huu Nguyen, (2020), "EBNA-1 - Biomarker for Nasopharyngeal Carcinoma: a Meta-Analysis", ***Pharmacophore, 10(1),*** *92-99.*

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor of the nasopharynx, which has remarkably pronounced differences in distribution according to geography and ancestry, gravitating toward Southern Asia, especially in China and Vietnam. [1-7] A wide range of non-cancerous diseases, benign and malignant originated from the squamous and mesenchymal epithelium occur in the laryngopharynx and among these types , polyps, vocal cord nodules, papilloma, and squamous cell carcinoma are more common. [8] Cancer cells have higher levels of reactive oxygen species (ROS) than normal cells, resulting in increased oxidative stress. [9] Updated to 2018, a total of 129,079 new nasopharyngeal cases were recorded in the world, of which 72,987 nasopharyngeal death cases have occurred. In the Southeast Asian region, a total of 34,681 new cases, of which 22,231 nasopharyngeal death cases were recorded. [6] Notably, the symptoms of NPC were unclear, thus, NPC is commonly diagnosed at a late stage (stage 3 or 4). [10-12] Therefore, the major obstacle to early diagnosis and screening of NPC is the different access due to the deeply seated location of the nasopharynx, as well as the unclear presenting symptoms.

A well-established etiology factor of NPC is strongly associated with Epstein-Barr virus (EBV), also known as human gammaherpesvirus 4 (HHV4), has been postulated. [13-16] The infection of EBV has been reported to be the early step in nasopharyngeal tumorigenesis and act as an important role in the carcinogenesis of the disease. [16-18] Since then, based on the detection of genomic EBV has been received as much attention and represented a prospective biomarker for diagnosis and early screening of NPC. EBV has a double-strand DNA genome consisted of approximately 170-kilobases, encoded more than 85 genes. [19, 20] Among them, *EBNA-1* (*Epstein-Barr nuclear antigen-1*) gene plays an important role in EBV infection. As a function, *EBNA-1* is essential for the EBV immortalization of cells and responsible for EBV DNA episome replication, segregation, and persistence of the viral genome, which achieved through sequence-specific DNA binding. [19,

Corresponding Author: Dung Huu Nguyen; University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam. Email: drnguyenhuudung @ gmail.com

21] However, due to the different sensitivities and intra/interassay coefficients of variation of methods, the reported frequency of EBNA-1, and its prognostic value is highly variable, and also remain controversial. Therefore, we performed the present study to carry out a systematic review and a meta-analysis, notably, the first systematic review and a meta-analysis, to summarize the previously published studies and to evaluate *EBNA-1* as prognostic markers for NPC risk.

Materials and Methods

Search strategy and inclusion/exclusion criteria

The current meta-analysis was performed according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). [22] The related published articles were searched and collected in MEDLINE database (updated on December 2019), by using separation or combination of following keywords: "Nasopharyngeal carcinoma", "Epstein-Barr Virus", "EBV", "Epstein-Barr Antigen 1", "EBNA-1", "prognosis", "diagnosis". Additional studies were also identified via the references listed in the articles.

Studies were considered eligible only when they met all of the following inclusion criteria: i) The articles were limited to studies written in English; ii) case-control study designed; iii) provided that data about the frequency of *EBNA-1* as well as the sample size in both case and control group. Exclusion criteria were as follows: i) The articles were written in other languages; ii) abstracts, case reports, letter to the editor or unpublished articles were eliminated; iii) studies were related to other tumors and not specific for NPC; iv) studies lacked vital information for analysis.

Data Extraction

The eligibility of each study, the relevant data from the eligible studies were independently retrieved by two authors. Disagreements were resolved through discussion within the third author or our research team. The relevant data were extracted from each study according to the data form, including the first Author's last name, year of publication, the country where the study was performed, sample type, experimental methods to assess EBNA-1 detection, and some cases and controls subjects.

Statistical Analysis

All data were statistically analyzed using the MedCalc® software by MedCalc Software Ltd. (https://www.medcalc.org/). The frequency of EBNA-1 was calculated in both case and control groups. The strength of the association between *EBNA-1* and NPC was evaluated by Odds ratio (OR) with 95% confidence intervals (95%CI). In the present study, the heterogeneity among the included studies was estimated by the Cochran Q test and I^2 statistics. [23] The cut-off point: p = 0.05 for the Q test and I^2 were used to test the heterogeneity between studies. [24, 25]. The scale of I^2 value is classified as following: $I^2 <$ 25%: no heterogeneity, 25% $\leq I^2 \leq$ 50%: moderate heterogeneity, and $I^2 >$ 50%: strong heterogeneity. [24, 25] The random-effects model was applied if the heterogeneity among studies existed ($p < 0.05$ for Q test, $I^2 >$ 50%). In the case of no between-study heterogeneity, a fixed-effects model was applied to compute the pooled ORs. To determine the presence of publication bias, the symmetry of the funnel plots in which ORs were plotted against their corresponding standard errors were assessed by the Begg's funnel plot and Egger's test ($p < 0.05$ indicates statistically significant). [26, 27]

Results

The characteristics of eligible studies

A total of 409 articles were retrieved from the database. After exclusion of studies that not met the inclusion criteria, finally, eight studies included 1,372 samples: 588 samples from NPC cancer patients, and 784 samples from non-cancerous samples, were enrolled in the meta-analysis. The characteristics of the included studies of *EBNA-1* and NPC risk were summarized in Table 1.

The number of patients in the included studies ranged from 13 to 105 (mean: 98). These patients came from Asia (five countries: Taiwan, India, Malaysia, China, Vietnam), Europe (one country: Netherlands) and Africa (one country: Morocco). All the studies were published between 2004 and 2017. The source of NPC samples consisted of biopsy tissue, Biopsy of fine-needle aspiration of neck masses, nasopharyngeal swab, and blood samples. Of this, the biopsy tumor sample was preferentially used in the evaluation of *EBNA-1* frequency in NPC. Among the included eight studies, six studies used PCR method, two studies used Realtime-PCR to explore the frequency of *EBNA-1* in NPC and corresponding controls.

The frequency of *EBNA-1*, and the association between *EBNA-1* and NPC

Considering the significant heterogeneity between studies (Case: Q = 159.28, $p < 0,0001$, I^2 = 94.35%; Control: Q = 63,35, $p < 0,0001$, I^2 = 85.79%, 95% CI for I^2 = 91.48-96.25), the random-effects model was used to explore the frequency of EBNA-1 in NPC and corresponding controls (Fig. 1, Fig. 2).

As shown in Fig. 1, Fig. 2, the frequency of *EBNA-1* in NPC patients and controls was 88.72%, and 4.32%, respectively. The meta-analysis results suggested that the frequency of *EBNA-1* in NPC patients was significantly higher than the corresponding controls. We also evaluated the association between the presence of *EBNA-1* and NPC by analysis OR. The

presence of *EBNA-1* was associated with an increased NPC risk with a pooled OR of 277.07 (95% CI = 66.0-1,163.16), based on the random-effects model (Q = 39.70, p < 0.0001, I^2 = 77.33%, 95% CI for I^2 = 58.37-87.65) (Fig. 3). The funnel plot of pooled analysis, which was quite asymmetric, indicated that there was significant bias among the included studies, therefore there was a factor of influence on the current meta-analysis (Fig. 4). Aiming to remove the influent factor of the current meta-analysis, the sensitivity analysis was applied. The sensitivity analysis found that the study by Krishna et al. (2006) was a relatively poor-quality study. When the study by Krishna et al. (2006) was removed, the between heterogeneity decreased to I^2 = 8.39% (p = 0.37). Notably, the association between EBNA-1 and NPC increased, which was indicated by the increased OR of 331.09 in the fix-effects model (Fig. 5).

Subgroup analysis was performed according to the region, source of samples and methods for EBNA-1 detection. The association between *EBNA-1* and NPC was confirmed in each subgroup (Table 2). Considering the source of samples, the significant association between presence of *EBNA-1* and NPC was observed among the NPC biopsy tissue group and non-biopsy group in the fix-effect model (NPC biopsy tissue group: OR = 377.61, 95% CI = 144.36-987.74; non-biopsy group: OR = 295.35, 95% CI = 76.69-1,137.57). With the method for *EBNA-1* detection, significant correlation between *EBNA-1* and NPC was found among method of PCR group and Real-time PCR in fix-effects model (PCR: OR = 254.62, 95% CI = 99.62-50.78; Real-time PCR: OR = 789.83, 95% CI = 104.02-5,997.52). Regarding to the subgroup of region, there was the significant association between *EBNA-1* and NPC among the group of Asia in random-effects model and non-Asia group in fix-effects model (Asia: OR = 361.19, 95% CI = 131.02-995.72; non-Asia: OR = 243.04, 95% CI = 47.28-1,249.42).

Sensitivity analysis and publication bias

Aiming to evaluate the stability and reliability of the conclusions, the sensitivity analysis was performed according to the leave-one-out method by excluding one study. As results, the pooled OR was ranged from 288.63 (95% CI = 107.26-776.71) to 502.46 (95%CI = 208.45-1,211.178) under the fix-effects model within the I2 ranged from 0.00 (95%CI = 0.00-59.55, p = 0.59) to 4.55 (95% CI = 0.00-69.38, p = 0.40) (Table 3). Additionally, the quite symmetric in the funnel plot, indicating that publication bias could not be completely excluded, was observed (Fig. 6). Therefore, the results, and conclusion of the present meta-analysis, which was to evaluate the association between EBNA-1 and NPC risk, were stable and reliable.

Discussion

In the current study, a large pool of clinical studies, based on the previously published studies, was used to evaluate the association between *EBNA-1* and NPC risk. Eight studies, including 588 samples from NPC cancer patients, were combined to 784 samples from non-cancerous samples, to evaluate the potential of *EBNA-1* as a biomarker for screening and diagnosis of NPC. The overall frequency of *EBNA-1* in NPC patients and controls were 88.72% (95% CI = 75.87-97.09) and 4.32% (95% CI = 1.07-9.61), respectively. Additionally, the current meta-analysis suggested that individuals with the presence of *EBNA-1* are associated with NPC risk (pooled OR = 331.09, 95%CI = 139.95-783.30) (Fig. 5, Table 2). Previous studies reported that *EBNA-1* plays an important role in the nasopharyngeal tumorigenesis by the infection of EBV. In detail, *EBNA-1* is essential for EBV immortalization of cell and responsible for EBV DNA episome replication, segregation and persistence of viral genome as well as the maintenance of EBV genome in circular episome with multi copies in the infected cell [15, 19, 21]. This indicated that the EBNA-1 gene might play a crucial role in the pathogenesis of NPC, and may be included in diagnosis and screening models of NPC.

After omitting the relatively poor-quality study by Krishna et al. (2006), the heterogeneity of current meta-analysis was decreased to 8.39% (< 25%), indicating no heterogeneity among the included studies. This indicated that the *EBNA-1* might play a crucial role in the pathogenesis of NPC. Additionally, a significant association between *EBNA-1* and NPC risk was found among in all subgroups, including region, method, and source of samples (Table 2): PCR method was applied to determine the presence of *EBNA-1* of a favorable clinical sample of nasopharyngeal carcinoma biopsy samples, which are comprised of tumor cells. Additionally, a significant association between *EBNA-1* and NPC was found among the Asian region and the Non-Asia region, which once again confirmed that nasopharyngeal cancer is native to the Asian region.

However, the current meta-analysis exhibited some limitations due to the data of non-English language studies that may contribute to some bias, as well as the evaluation of the correlation between *EBNA-1* and clinicopathological features.

Conclusion

Our meta-analysis indicated the presence of *EBNA-1* was significantly associated with NPC, which suggested that the EBNA-1 plays a crucial role in the nasopharyngeal tumorigenesis. Additionally, our findings underscore the correlation among EBNA-1 and all subgroups, including region, method, and source of samples. Based on those findings, the presence of EBNA was suggested as a promising biomarker for the diagnosis and screening of NPC.

Table 1. The characteristics of studies included in the meta-analysis of *EBNA-1* and NPC risk

Author, Reference	Year	Region	Case		Control		Method	Source of	
			P	N	P	N		Case	Control
Hao et al. [11]	2004	Taiwan	64	70	6	354	PCR	B	Sb
Krishna et al. [28]	2006	India	20	29	6	26	PCR	B	Se
Steven et al. [29]	2006	Netherlands	67	78	0	67	RT-PCR	Sb	Sb
Yap et al. [30]	2007	Malaysia	34	35	0	35	PCR	B	B
Yap et al. [30]	2007	Malaysia	30	34	1	34	PCR	Bf	Bf
Zhang et al. [31]	2012	China	49	49	0	20	PCR	B	B
Zhang et al. [31]	2012	China	48	49	0	20	PCR	Sb	Sb
Lourembam et al. [32]	2015	India	105	105	24	115	RT- PCR	Bl	Bl
Nawaz et al. [33]	2015	Morocco	36	44	1	18	PCR	B	B
Lao et al. [16]	2017	Vietnam	44	95	1	95	PCR	Sb	Sb

Note: B: Nasopharyngeal carcinoma biopsy tissue; Bf: Biopsy of fine-needle aspiration of neck masses; Sb: Nasopharyngeal swab; Bl: Blood sample.

Table 2. Summary of subgroup analysis in the meta-analysis of *EBNA-1* and NPC risk

Group	**Case**		**Control**		**Model, OR, 95% CI (Fix-effects model)**	**Heterogeneity**	
	P	**N**	**P**	**N**		I^2 **(%)**	**p**
Total	477	559	33	758	331.09, 139.95-783.30	8.39	0.37
Region							
Asia	374	437	32	673	361.19, 131.02-995.72	3.82	0.40
Non-Asia	103	122	1	85	243.04, 47.28-1,249.42	43.27	0.18
Method							
PCR	305	376	9	576	254.62, 99.62-50.78	32.01	0.19
Real-time PCR	172	183	24	182	789.83, 104.02-5,997.52	0.00	1.00
Source of sample							
Biopsy sample	213	232	8	461	377.61, 144.36-987.74	21.66	0.28
Non-biopsy	264	327	25	297	295.35, 76.69-1,137.57	10.12	0.34

Table 3. Sensitivity analysis of EBNA-1 and NPC risk by the fix-effects model

	OR, 95% CI	**Heterogeneity**	
		I^2**, 95% CI**	***p***
Omitting Hao et al., 2006	288.63, 107.26-776.71	8.43, 0.00-70.62	*0.37*
Omitting Steven et al., 2006	302.02, 122.97-741.82	8.64, 0.00-61.67	*0.82*
Omitting Yap et al., 2007	310.21, 128.62-748.17	11.99, 0.00-71.76	*0.34*
Omitting Yap et al., 2007	399.27, 135.33-850.54	18.47, 0.00-61.24	*0.28*
Omitting Zhang et al., 2012	311.09, 129.86-745.25	4.55, 0.00-69.38	*0.40*
Omitting Zhang et al., 2012	314.91, 130.77-758.32	14.13, 0.00-56.96	*0.32*
Omitting Lourembam et al., 2015	289.40, 118.01-709.67	20.42, 0.00-62.80	*0.27*
Omitting Nawaz et al., 2015	392.96, 150.13-1,028.57	0.00, 0.00-63.81	*0.52*
Omitting Lao et al., 2017	502.46, 208.45-1,211.178	0.00, 0.00-59.55	*0.59*

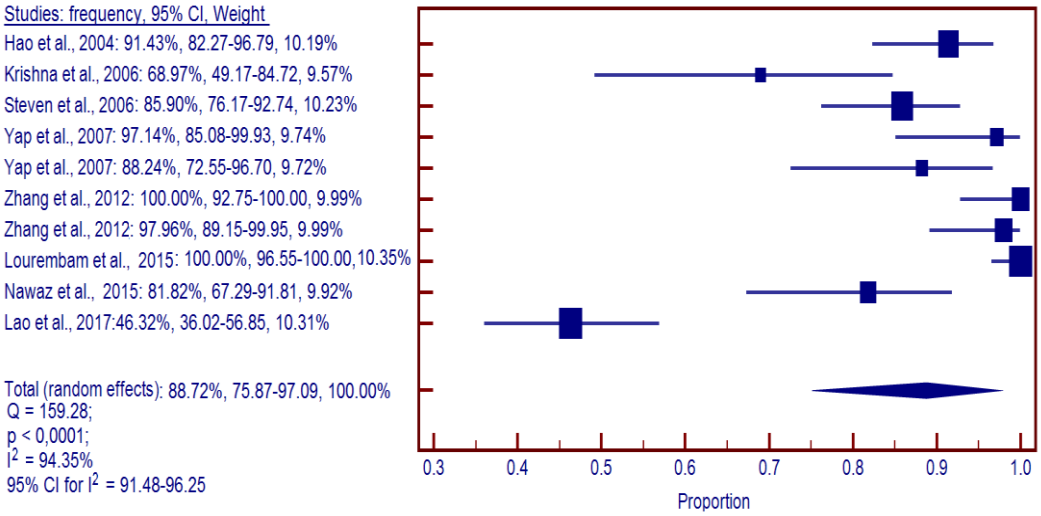

Figure 1. Forest plot of *the EBNA-1* gene detected in NPC samples

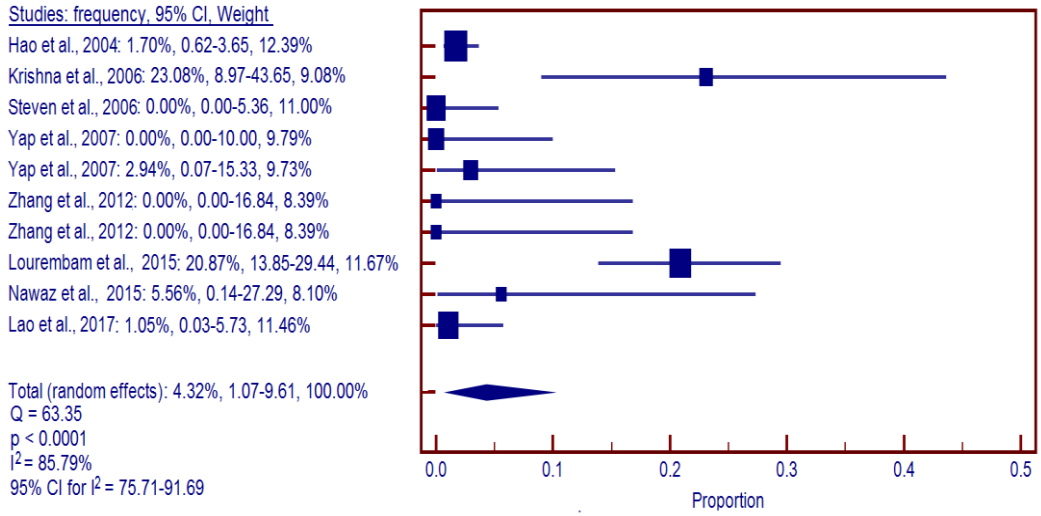

Figure 2. Forest plot of the *EBNA-1* gene detected in nasopharyngeal control

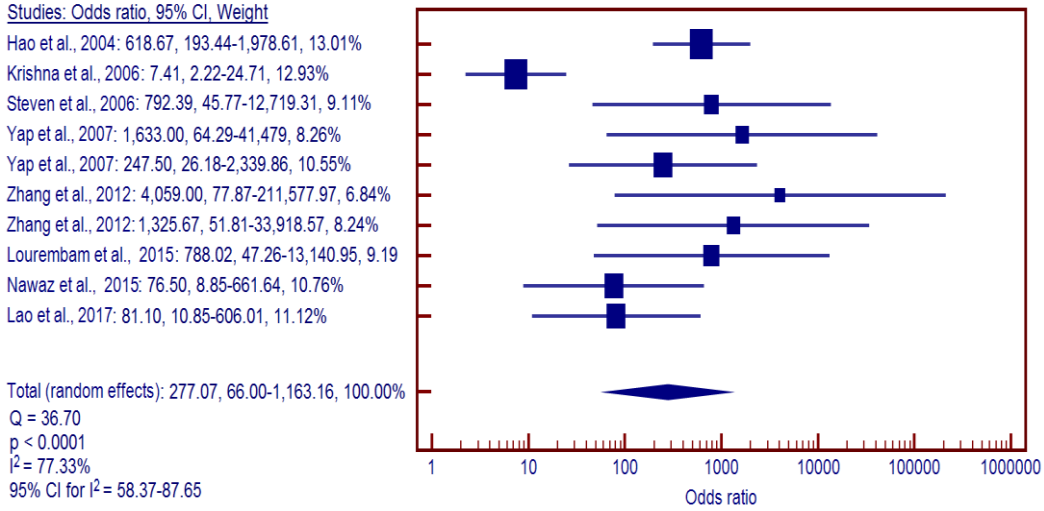

Figure 3. Forest plot of the association between the presence of EBNA-1 gene and NPC through OR based on the random-effects model.

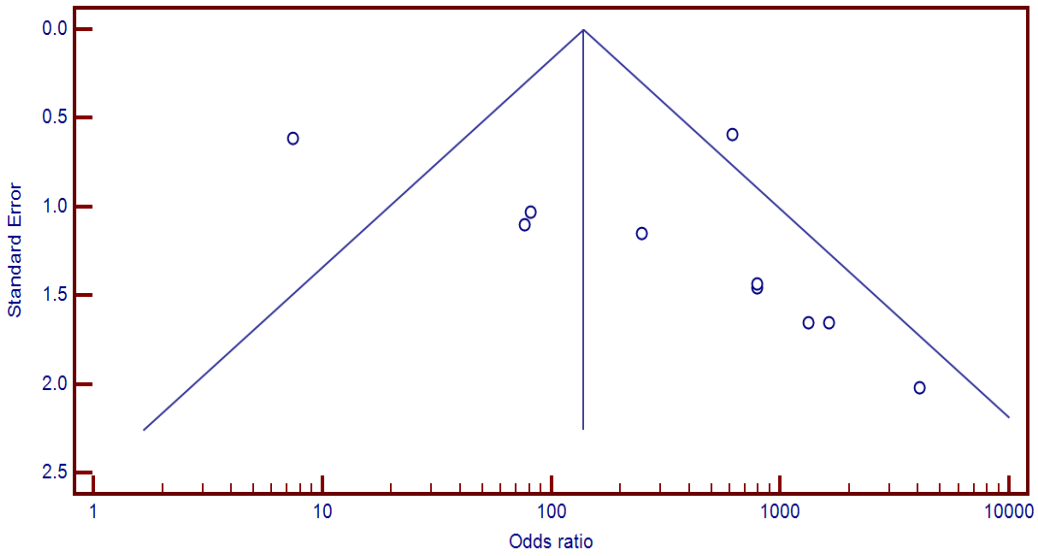

Figure 4. Forest plot of the association between the presence of *EBNA-1* gene and NPC through OR based on the random-effects model.

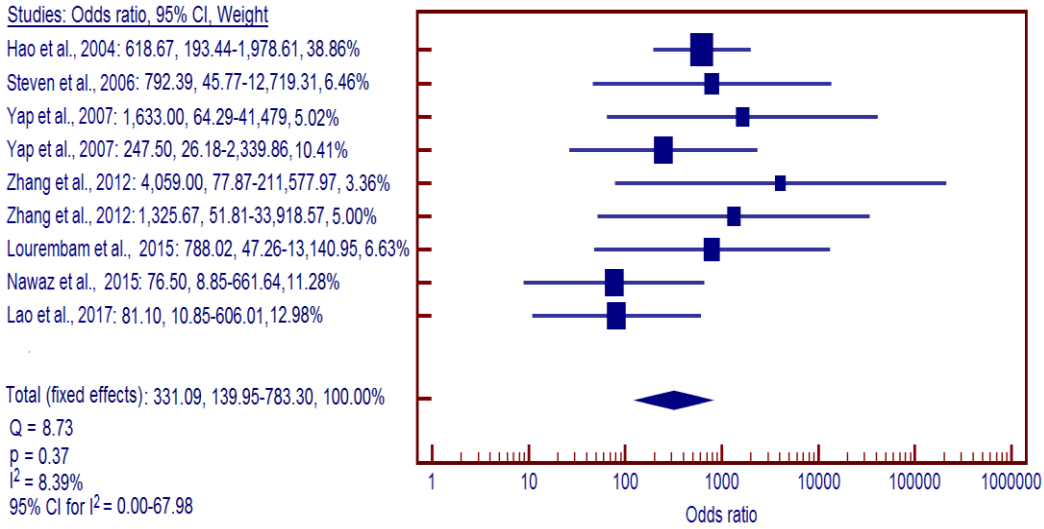

Figure 5. Forest plot of the association between the presence of EBNA-1 gene and NPC through OR based on the fix-effects model

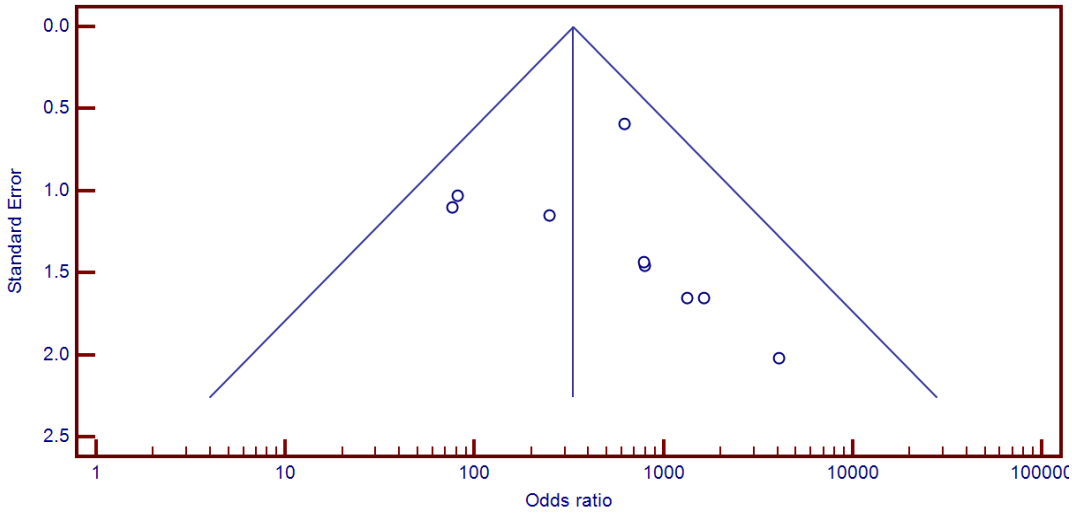

Figure 6. Forest plot of the association between the presence of *EBNA-1* gene and NPC through OR based on the fix-effects model

References

1. Sham JS, Wei WI, Zong YS, Choy D, Guo YQ, Luo Y, et al. Detection of subclinical nasopharyngeal carcinoma by fibreoptic endoscopy and multiple biopsy. Lancet. 1990; 335(8686): 371-374. DOI: DOI: 10.1016/0140-6736(90)90206-k.
2. Chang CM, Yu KJ, Mbulaiteye SM, Hildesheim A, Bhatia K. The extent of genetic diversity of Epstein-Barr virus and its geographic and disease patterns: a need for reappraisal. Virus Res. 2009; 143(2): 209-221. DOI: 10.1016/j.virusres.2009.07.005.
3. Mahdavifar N, Ghoncheh M, Mohammadian-Hafshejani A, Khosravi B, Salehiniya H. Epidemiology and Inequality in the Incidence and Mortality of Nasopharynx Cancer in Asia. Osong Public Health Res Perspect. 2016; 7(6): 360-372. Doi: 10.1016/j.phrp.2016.11.002.
4. Lao TD, Nguyen TV, Nguyen DH, Nguyen MT, Nguyen CH, Le THA. miR-141 is up-regulated in biopsies from Vietnamese patients with nasopharyngeal carcinoma. Braz Oral Res. 2018a; 32:e126. Doi: 10.1590/1807-3107bor-2018.vol32.0126.
5. Lao TD, Nguyen DH, Le THA. Study of mir-141 and its Potential Targeted mRNA PTEN Expression in Nasopharyngeal Carcinoma: From in Silico to Initial Experiment Analysis. AJPRHC, 2018b; 10(3):, 66-74. Doi: 10.18311/ajprhc/2018/22341.
6. Lao TD, Le THA. Epidemiology, incidence, and mortality of Nasopharynx Cancer in Southeast Asia: an updated report. Adv life sci. 2020; 7(2): 86-90.
7. 33. Shrihari T.G. Chronic Inflammatory Mediators in Tumor Microenvironment Induced Tumor Progression. Int. J. Pharm. Phytopharm. Res. 2019;9(2):28-31.
8. Saki N, Menhas K, Nikakhlagh S, Lotfinia M, Abshirini H. Compare the accuracy of diagnosis based on the stroboscopy and pathological report. J. Adv. Pharm. Edu. Res. 2019;9(S2):131-134.
9. Noureldeen AF, Gashlan HM, Qusti SY, Ramadan RM. Antioxidant Activity and Histopathological Examination of Chromium and Cobalt Complexes of Bromobenzaldehydeiminacetophenone Against Ehrlich Ascites Carcinoma Cells Induced in Mice. Int. J. Pharm. Phytopharm. Res. 2017;7(4):7-12.
10. Epstein JB, Jones CK. Presenting signs and symptoms of nasopharyngeal carcinoma. Oral Surg Oral Med Oral Pathol. 1993; 75(1): 32-36. DOI: 10.1016/0030-4220(93)90402-P.
11. Hao SP, Tsang NM, Chang KP, Ueng SH. Molecular diagnosis of nasopharyngeal carcinoma: detecting LMP-1 and EBNA by nasopharyngeal swab. Otolaryngol Head Neck Surg. 2004; 131(5): 651-654. Doi: 10.1016/j.otohns.2004.04.013.
12. Wu L, Li C, Pan L. Nasopharyngeal carcinoma: A review of current updates. Exp Ther Med. 2018; 15(4): 3687-3692. Doi: 10.3892/etm.2018.5878.
13. Rowe M, Lear AL, Croom-Carter D, Davies AH, Rickinson AB. Three pathways of Epstein-Barr virus gene activation from EBNA1-positive latency in B lymphocytes. J Virol. 1992; 66(1): 122-131.
14. Vera-Sempere FJ, Burgos JS, Botella MS, Cordoba J, Gobernado M. Immunohistochemical expression of Epstein-Barr virus-encoded latent membrane protein (LMP-1) in paraffin sections of EBV-associated nasopharyngeal carcinoma in Spanish patients. Eur J Cancer B Oral Oncol. 1996; 32B(3): 163-168. DOI: 10.1016/0964-1955(95)00093-3.
15. Young LS, Dawson CW, Eliopoulos AG. The expression and function of Epstein-Barr virus-encoded latent genes. Mol Pathol. 2000; 53(5): 238-247. Doi: 10.1136/mp.53.5.238.
16. Lao TD, Nguyen DH, Nguyen TM, Le TAH. Molecular Screening for Epstein-Barr virus (EBV): Detection of Genomic EBNA-1, EBNA-2, LMP-1, LMP-2 Among Vietnamese Patients with Nasopharyngeal Brush Samples. Asian Pac J Cancer Prev. 2017; 18(6): 1675-1679. Doi: 10.22034/APJCP.2017.18.6.1675.
17. Niedobitek G, Meru N, Delecluse HJ. Epstein-Barr virus infection and human malignancies. Int J Exp Pathol. 2001; 82(3): 149-170. Doi: 10.1046/j.1365-2613.2001.iep0082-0149-x.
18. Chan JY, Wong ST. The role of plasma Epstein-Barr virus DNA in the management of recurrent nasopharyngeal carcinoma. Laryngoscope, 2014; 124(1): 126-130. DOI: 10.1002/lary.24193.
19. Thompson MP, Kurzrock R. Epstein-Barr virus and cancer. Clin Cancer Res. 2004; 10(3): 803-821. Doi: 10.1158/1078-0432.ccr-0670-3.
20. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2006; 15(10): 1765-1777. DOI: 10.1158/1055-9965.EPI-06-0353.
21. Wilson JB, Manet E, Gruffat H, Busson P, Blondel M, Fahraeus R. EBNA1: Oncogenic Activity, Immune Evasion, and Biochemical Functions Provide Targets for Novel Therapeutic Strategies against Epstein-Barr Virus- Associated Cancers. Cancers (Basel). 2018; 10(4):109. Doi: 10.3390/cancers10040109.
22. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009; 6(7): e1000097. DOI: 10.1371/journal.pmed.1000097.
23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21(11): 1539-1558. DOI: 10.1002/sim.1186.

24. DerSimonian R. Meta-analysis in the design and monitoring of clinical trials. Stat Med. 1996; 15(12): 1237-1248. DOI: 10.1002/(SICI)1097-0258(19960630)15:12<1237::AID-SIM301>3.0.CO;2-N.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327(7414): 557-560. DOI: 10.1136/bmj.327.7414.557.
26. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994; 50(4): 1088-1101. DOI: 10.2307/2533446.
27. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315(7109): 629-634. DOI: 10.1136/bmj.315.7109.629.
28. Krishna SM, James S, Kattoor J, Balaram P. Serum EBV DNA as a biomarker in primary nasopharyngeal carcinoma of Indian origin. Jpn J Clin Oncol. 2004; 34(6): 307-311. Doi: 10.1093/jjco/hyh055.
29. Stevens SJ, Verkuijlen SA, Hariwiyanto B, Harijadi, Paramita DK, Fachiroh J, et al. Noninvasive diagnosis of nasopharyngeal carcinoma: nasopharyngeal brushings reveal high Epstein-Barr virus DNA load and carcinoma-specific viral BARF1 mRNA. Int J Cancer. 2006; 119(3): 608-614. DOI: 10.1002/ijc.21914
30. Yap YY, Hassan S, Chan M, Choo PK, Ravichandran M. Epstein-Barr virus DNA detection in the diagnosis of nasopharyngeal carcinoma. Otolaryngol Head Neck Surg. 2007; 136(6): 986-991. Doi: 10.1016/j.otohns.2006.11.027
31. Zhang Z, Sun D, Hutajulu SH, Nawaz I, Nguyen Van D, Huang G, et al. Development of a non-invasive method, multiplex methylation-specific PCR (MMSP), for early diagnosis of nasopharyngeal carcinoma. PLoS One. 2012; 7(11): e45908. DOI: 10.1371/journal.pone.0045908
32. Lourembam DS, Singh AR, Sharma TD, Singh TS, Singh TR, Singh LS. Evaluation of Risk Factors for Nasopharyngeal Carcinoma in a High-risk Area of India, the Northeastern Region. Asian Pac J Cancer Prev. 2015; 16(12): 4927-4935. Doi: 10.7314/apjcp.2015.16.12.4927
33. Nawaz I, Moumad K, Martorelli D, Ennaji MM, Zhou X, Zhang Z, et al. Detection of nasopharyngeal carcinoma in Morocco (North Africa) using a multiplex methylation-specific PCR biomarker assay. Clin Epigenetics. 2015; 7(1): 89. DOI: 10.1186/s13148-015-0119-8.