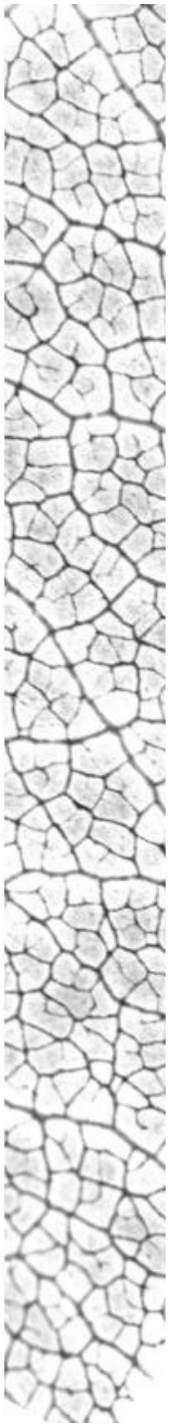

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The analysis of antibody serum titer of Iga anti-viral capsid antigens and anti-epstein-Barr nuclear antigens in nasopharyngeal carcinoma patients

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Abstract

Calloselasma rhodostoma (Kuhl, 1824) is one species of Indonesian medically important snake that distributed in many Indonesia regions. With th **Background:** The serological test as an effort to diagnose early Nasopharyngeal Carcinoma (NPC) in several countries, especially China, has succeeded in finding cases of early NPC that remain asymptomatic. In Indonesia, the serological test is rarely used, causing the value of anti-viral capsid antigen IgA titer (VCA) and anti-Epstein-Barr nuclear antigen virus (EBNA) for NPC patients remain unknown. **Purpose:** This study aims to obtain the value of anti-VCA IgA and anti-EBNA IgA in NPC based on the type of histopathology and stage to determine the specificity value and sensitivity of the anti-VCA IgA titer and anti-EBNA IgA titer in NPC. **Method:** This study used a cross-sectional study with a case-control study design. A total of 35 patients who met both case and control samples were taken for venous blood in the median cubital vein using a 5 ml syringe for material inspection. Then, serological tests were carried out in the form of serum analysis of anti-VCA IgA and anti-EBNA IgA antibody titer using the Elisa Test method. **Result:** The sensitivity of anti-VCA IgA titer reached 91.14%, whereas its specificity amounted to 97.14%. On the other hand, the sensitivity of anti-EBNA IgA titer reached 91.14% whereas its specificity amounted to 94.30%. **Conclusion:** The examination of both types of titers is excellent for diagnosing hidden NPC and detecting disease recurrence after treatment.

Recent snakebite cases of this snake and warrant of further improvement on anti-venin in Indonesia, the information on storage conditions for its venom is important for developing an anti-venin. The genetic identity that possibly holds a cryptic diversity that has not been resolved, could impact the future of anti-venin development of this species. We analyze the molecular data based on the ND4 gene to resolve the genetic relations of this snake from Java, Kangean, and Borneo population with the addition of the Thailand population by Bayesian Inference phylogenetic reconstruction. To evaluate the storage conditions, venom collection from six living specimens from the Java population was used to analyze the effect of svPLA$_2$ activity under different storage conditions for 14 days long. The phylogenetic results show a polytomy tree, with a low p-distance value between populations. Only the storage at 37ºC affects the performance of svPLA$_2$ significantly. The phylogenetic indicating a single species even though divided by geographical barriers, more genes need to be compared to resolve the genetic relationship. More samples are needed to compare the venom properties throughout *C. rhodostoma* wide distribution, to enlight the anti-venin future development.

Keywords: Nasopharyngeal Carcinoma, serology, anti-VCA IgA titer, anti-EBNA IgA titer

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INTRODUCTION

In Indonesia, Nasopharyngeal Carcinoma (NPC) is included in the top five malignant tumors with a high number of sufferers. According to the Ministry of Health of the Republic of Indonesia in 1977-1979, NPC was at the fourth level after cervical, breast and skin cancers. Meanwhile, for malignancy in the head and neck, NPC ranks first with a frequency of approximately 60% (Sibuea, et al. 2000. Wildeman, et al. 2012. Adham, et al. 2012. Susilo, 1995; Saliu, 2016) The main treatment for NPC is radiotherapy, but the results are less than optimal because most NPC patients (77.10%) who come for treatment have advanced stages (Suwondo, Surarso, & Kristyono, 2019. Zhao, et al. 2016. Lee, 2010. Tsukagoshi, et al. 2010). Therefore, it is

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necessary to conduct an effort to find the NPC case as early as possible. Finding cases of early NPC is difficult due to the hidden location of the nasopharynx and the NPC symptoms that resemble rhinitis or sinusitis. Another problem that arises is the difficulty of detecting recurrence. Although radiological tests such as CT scan or MRI can indicate the presence of tumors in the nasopharynx, they cannot be conducted routinely (Tsukagoshi, et al. 2010. Lee, 2010. Rhomdhoni, Kurniawan, & Hidayati, 2019).

The discovery of the Epstein-Barr virus as an etiological factor of NPC has opened the way towards early diagnosis of NPC through the serological test. With the highest number of NPC patients in the world, China used this test for mass screening and has been proven to find early NPC cases, even with asymptomatic cases (Wang, et al. 2010. Hutajulu, et al. 2014. Wang, et al. 2014). The serologic tests include anti-viral capsid antigen (VCA) IgA titers, anti-early antigen (EA) IgA, and anti-Epstein-Barr nuclear antigen IgA virus (EBNA). However, anti-EA IgA antibodies are less sensitive for early detection of NPC. Anti-EA IgA antibodies are permanently in the serum of NPC patients, only the titer changes according to the progression of the disease, making it more suitable for determining the course of the disease and prognosis (Muharani, 2016. Muniret al. 2007. Hutajulu, et al. 2014).

Antibody titer toward Epstein-Barr virus in NPC patients shows different results, compared to non-NPC. The increase of anti-VCA IgA titer in 97.2% of NPC patients, 8% were not NPC patients, and 5% in benign head and neck tumors, using the immunofluorescence test method. In stages III and IV of NPC patients, titers range from 10–1280 u/dl with an average titer of 160 u/dl. Based on the notion where the test is considered positive if the titer value is above or equal to 1:10, this test method indicates a 97.2% sensitivity and 93.3% of specificity .(Kresno, et al. 1993).

Based on the case, the serological test is required to detect the early stage of NPC cases by obtaining serum antibody titer values. This study aims to obtain the value of anti-VCA IgA and anti-EBNA IgA of NPC based on the type of histopathology and stage to determine the specificity and sensitivity value of anti-VCA IgA titer and anti-EBNA IgA titer in NPC.

METHOD

This research utilized a cross-sectional study with case-control study design. This research was conducted at outpatient installation laboratory/SMF ENT Pathology Faculty of Medicine of Universitas Airlangga/Dr. Soetomo Regional Public Hospital Division of Clinical Immunology Lab/Functional Medicine Staff of Clinical Pathology Faculty of Medicine/Dr. Soetomo Regional Public Hospital, Surabaya. This study used a total of 35 sample subjects. The case samples in this study were stage I to IV NPC patients, where all types of histopathology were based on WHO classification (1987). In addition, all patients had never received prior therapy either radiation or chemotherapy, and they came from all ages and genders.

The control samples in this study were non-NPC patients who met the criteria of malignancy in the non-NPC head and neck which was another type of carcinoma. All patients had never received prior radiotherapy nor chemotherapy. In this study, the independent variables consisted of the NPC stage and the type of histopathology, while the dependent variables included the anti-VCA IgA antibody titer and anti-EBNA IgA.

The data were obtained from the history, physical test, and histopathology test in the medical record. Patients who met both case and control sample requirement underwent blood sampling, using a 5 ml syringe through the median cubital vein. Furthermore, the blood specimens were centrifuged in less than one hour after blood sampling to take the serum components. This serum underwent the serological test, i.e., the analysis of IgA antibody titers anti-VCA IgA and anti-EBNA IgA using the Elisa Test method.

This research involved subjects in the process of using a questionnaire that was in accordance with ethical research principles based on research ethics regulations. This research applies the basic principles of ethics of respect, goodness, non-crime, and justice.

All data collected were compiled and went through a descriptive-analytic process using the t-test for independent groups. The homogeneity test was carried out on the basic data, which included the distribution of age, gender, ethnicity, education, and occupation. T-test was used for the age, while for gender, ethnicity, education, and occupation the Chi-Square test was conducted.

Table 1. The Result of Sample Homogeneity Tests

Data Type	Statistic Test	P-value
Age	T-test	0.205
Gender	Chi-Square	0.445
Ethnicity	Chi-Square	0.218
Education	Chi-Square	0.106
Profession	Chi-Square	0.104

RESULT

The homogeneity of basic data from case groups (NPC) and control (non-NPC) is known through statistical tests. The results of the statistical analysis of the basic data in **Table 1** suggested that all p-values were greater than 0.05. Therefore, the samples of the case and control groups came from populations with homogeneous conditions. The most common main complaint experienced by 22 NPC patients (62.86%) included a lump in the neck, while the least complaint was double vision, reported by 1 patient (2.86%).

Table 2. The Occurrence Distribution of 35 NPC Cases Based on Main Complaints

Main complaint	Total	%
Lump on the Neck	22	62.86
Hearing disorders	3	8.57
Headache	3	8.57
Influenza	2	5.71
Nasal Congestion	2	5.71
Nosebleed	2	5.70
Double vision	1	2.86
Total	35	100.00

NPC: Nasopharyngeal Carcinoma

Table 3. The Occurrence Distribution of 35 NPC Cases Based on Histopathological Descriptions

Histopathology Type	Total	%
Type 1 WHO	2	5.71
Type 2 WHO	4	11.43
Type 3 WHO	29	82.86
Total	35	100.00

NPC: Nasopharyngeal Carcinoma

Table 4. The Occurrence Distribution of 35 NPC Cases Based on the Stages

Stage	Total	%
Stage I	0	0
Stage II	1	2.86
Stage III	3	8.57
Stage IV	31	88.57
Total	35	100

NPC: Nasopharyngeal Carcinoma

Table 5. The Occurrence Distribution of 35 Non-NPC Cases Based on Diagnosis

	Case Type	Total	%
a.	Head and Neck Malignancies:		
	Nasal cavity carcinoma	2	5.71
	Sinonasal Carcinoma	3	8.57
	Tonsillar carcinoma	2	5.71
	Malignant Ameloblastoma	1	2.86
	Malignant lymphoma	2	5.7
	Palate carcinoma	1	2.86
	Tongue Carcinoma	3	8.57
	Olfactory Neuroblastoma of the nasal cavity	1	2.86
b.	Non-Malignancies:		
	Nasal Polyps	1	2.86
	Chronic Pharyngitis	2	5.71
	Chronic maxillary sinusitis	2	5.71
	Allergic Rhinitis	6	17.14
	Healthy	9	25.71
	Total	35	100

NPC: Nasopharyngeal Carcinoma

Based on the histopathology description, most of the NPCs were in the form of type 3 WHO classification, amounting to patients (82.86%), followed by type 2 WHO classification by 4 patients (11.43%), and last type 1 WHO classification by 2 patients (5.71%). The distribution of NPC, based on the stages, was mostly on NPC patients who were already in stage IV as many as 31 patients (88.57%). Meanwhile, no stage 1patients were identified. The aforemention results were presented in **Table 4**. From the 35 non-NPC cases, 15 cases were a malignant cases in the head and neck. The other cases included nasal polyps, chronic pharyngitis, chronic maxillary sinusitis, allergic rhinitis, and even on healthy people.

The cut-off point in this research for anti-VCA IgA and anti-EBNA IgA titer between NPC and non-NPC reached

Table 6. Seropositive and Mean anti-VCA and anti-EBNA IgA titers in NPC and non-NPC

Case Type	Group	
	NPC (n = 35)	Non-NPC (n = 35)
anti-VCA IgA		
% positive	91.4	2.9
Average	53.74	7.32
Standard deviation	35.85	12.68
Range	4.36-119.4	2.4-79.4
anti-EBNA IgA		
% positive	91.4	5.71
Average	66.14	5
Standard deviation	36.04	7.09
Range	3.2-130.2	0.1-40.8

NPC: Nasopharyngeal Carcinoma
VCA: Viral Capsid Antigen
EBNA: Epstein-Barr Nuclear Antigen Virus

Table 7. Seropositive and Mean anti-VCA and anti-EBNA IgA titers in NPC, Head and Neck Malignancy, and Non-Malignancy

Antibody	Group		
	NPC (n = 35)	Non-NPC (n = 15)	Non-Malignancy (n = 20)
anti-VCA IgA			
% positive	91.4	6.7	0
Average	53.74	10.73	5.03
Standard deviation	35.85	19.20	1.8
Range	4.36-119.4	2.44-79.42	2.62-9.24
anti-EBNA IgA			
% positive	91.4	13.3	0
Average	66.14	7.11	3.44
Standard deviation	36.04	10.46	1.8
Range	3.2-130.2	1-40	0.1-6.2

NPC: Nasopharyngeal Carcinoma
VCA: Viral Capsid Antigen
EBNA: Epstein-Barr Nuclear Antigen Virus

10 and 15 PanBio Units (PU), respectively. The patients with anti-VCA IgA titers above 10 PU and anti-EBNA IgA above 15 were cpnsidered NPC positive. Based on these results , the seropositive of anti-VCA IgA titer obtained in NPC amounted to 32 patients (91.4%) with 53.74 PU in average whereas one patient (2.9%) was declared non-NPC with an average of 7.32 PU. The seropositive IgA anti-EBNA titer in NPC amounted to 32 patients (91.4%) with an average of 66.14 PU whereastwo patients (5.7) were declared to be non-NPC with an average of 5 PU as shown in **Table 6**.

Out of 35 non-NPC cases, there were 15 cases of malignancy in the head-neck (KL) and 20 non-malignancy cases. In the case of KL malignancy, one patient acquired seropositive anti-VCA IgA titer (6.7%) with an average titer of 10.37 PU. Two patients gained anti-EBNA titer (13.3%) with an average titration of 7.15 PU. Meanwhile, in the case of non-malignancy both anti-VCA IgA titer and anti-EBNA IgA, there were no positive results with an average of anti-VCA IgA 5.03 PU and anti-EBNA IgA 3.44 PU, as shown in **Table 7**.

The statistical calculations that utilized Oneway ANOVA obtained a p-value of 0.001 for anti-VCA IgA titer and anti-EBNA IgA between NPC with KL malignancy, and 0.001 for NPC with a non-malignancy. Thus, there is a significant difference between NPC with both KL and non-malignancy as the p-value was greater

Table 8. The Sensitivity and Specificity of anti-VCA and anti-EBNA IgA titer

Antibody	NPC (n = 35)	Non-NPC (n = 35)	
anti-VCA IgA:			
Positive	32	1	Sensitivity: 91.14%
Negative	3	34	Specificity: 97.14%
anti-EBNA IgA			
Positive	32	2	Sensitivity: 91.14%
Negative	3	33	Specificity: 94.3%

NPC: Nasopharyngeal Carcinoma
VCA: Viral Capsid Antigen
EBNA: Epstein-Barr Nuclear Antigen Virus

Table 9. Seropositive and Mean anti-VCA IgA titer and anti-EBNA IgA by Histopathology Type

Antibody	Histopathology Type		
	WHO 1 (n = 2)	WHO 2 (n = 4)	WHO 3 (n = 29)
anti-VCA IgA			
% positive	100	100	89.66
Average	35.4	52.47	55.17
Standard deviation	30.26	40.79	36.38
Range	14-56.8	26.7-112.6	4.4-119.4
anti-EBNA IgA			
% positive	100	75	93.10
Average	67.7	39.5	69.9
Standard deviation	7.5	39.85	35.91
Range	59.4-70	3.2-93.2	12.8-130.2

VCA: Viral Capsid Antigen
EBNA: Epstein-Barr Nuclear Antigen Virus

than 0.05. The KL malignancy and non-malignancy obtained a p-value of 0.565 for anti-VCA IgA titer and a p-value of 0.682 for anti-EBNA IgA titer. Hence, there was no significant difference between a KL malignancy and non-malignancy.

Table 8 contains the diagnosis of NPCs for the examination of anti-VCA IgA titer, which indicated 91.14% of sensitivity (32 positive cases of 35 NPCs) and 91.14% of specificity (34 negative cases of 35 non-NPCs). Meanwhile, the anti-EBNA IgA titer obtained a sensitivity of 91.14% (positive 32 of 35 NPC) and a specificity of 94.30% (negative 33 of 35 non-NPC).

The statistical calculations used the ANOVA test for an average of anti-VCA IgA filter between type 1 WHO classification and type 2 WHO classification obtained a p-value of 0.470. Meanwhile, the statistical calculations between type 1 WHO classification and type 3 WHO classification obtained a p-value of 0.322. Next, the statistical calculations between type 2 WHO classification and type 3 WHO classification obtained a p-value of 0.852.

The anti-EBNA IgA titers between type 1 WHO classification and type 2 WHO classification reached a p-value of 0.257. Meanwhile, the anti-EBNA IgA titers between type 1 anti-EBNA IgA titers and type 3 anti-EBNA IgA titers gained a p-value of 0.780. Last, the anti-EBNA IgA titers between type 2 anti-EBNA IgA titers and type 3 anti-EBNA IgA titers obtained a p-value of 0.028. Since the p-value obtained was less than 0.05, there was no significant difference between anti-VCA IgA titer and anti-EBNA IgA among type 1, type 2, and type 3 WHO Classifications.

Table 10. Seropositive and Mean anti-VCA IgA titer and anti-EBNA IgA by Histopathology Type

Antibody	Stage			
	I (n = 0)	II (n = 1)	III (n = 3)	IV (n = 31)
anti-VCA IgA				
% positive	-	100	100	90.3
Average	-	14	48.8	56.2
Range	-	-	10.99-92.2	4.4-119.4
anti-EBNA IgA				
% positive	-	100	66.66	93.54
Average	- 14	70	44.3	68.1
Range	-	-	3.2-110.2	12.8-130.2

VCA: Viral Capsid Antigen
EBNA: Epstein-Barr Nuclear Antigen Virus

In NPC stadium, stage II seropositive anti-VCA IgA reached 100% with an average of 14 Pus. stage III seropositive anti-VCA IgA reached 100% with an average PU of 48.8%. Next, stage IV seropositive anti-VCA IgA amounted to 90.3% with an average PU of 56.2%. Next, seropositive for anti-EBNA IgA stage II reached 100% with an average PU of 79 %, whereas in stage III the number reached 66.66% with an average PU of 44.3%. Last, in stage IV, the number reached 93.54% with an average PU of 68.%. There were no stage I patients in the data, meaning that, the differences in titer values between the various stages of NPC in **Table 10** cannot be statistically analysed.

DISCUSSION

In this study, the NPC titer value for anti-VCA IgA titer reached 10 PU, whereas the anti-EBNA IgA obtained 15 PU. These figures were obtained by finding the cut-off point between the titer values of the NPC and non-NPC. Then, the patients with anti-VCA IgA and anti-EBNA IgA that obtained above 10 and 15 PU, respectively, were declared NPC positive. Based on the result, the anti-VCA IgA seropositive titer in in 32 NPC patients (91.4%) and one non-NPC patient (2.9%). Anti-EBNA seropositive titer IgA reached 32 NPC patients (91.14%) and 2 non-NPC patients (5.7%). In 35 non-NPC cases, then, grouped into 15 cases of KL malignancy and 20 cases of non-malignancy, seropositive case was found in one patient (6.7%) of anti-VCA IgA malignancy,whereas positive results were not found in non-malignancy cases. In anti-EBNA IgA titers, there were two cases of malignancy (13.3%) found whereas, in non-malignancy cases, no positive results were found. The difference in the average value of anti-VCA and anti-EBNA IgA titer between NPC and non-NPC, both in KL malignancy and non-malignancy, statistically obtained a significant p-value of 0.001 (greater than 0.05). On the other hand, no significance were found between the KL malignancy and non-malignancy since the p-value reached 0.565 for anti-VCA IgA titers, and 0.682 for anti-EBNA IgA titers (p-value greater than 0.05). Therefore, the the results obtained were in accordance with similar studies (Kresno, et al.1993. Foo, et al. 2002).

In this study, it was revealed that the lump on the neck was the main complaint of 22 patients (68.86%)

during their treatment. In various studies about NPC, the lump on the neck is also the main complaint of NPC patients when the patients carried out a treatment (Susilo, 1995. Munir, et al. 2007. Tambunan, Aryati, & Nafika, 2018. Labrecque, et al. 1995). Based on the description of NPC histopathology, type 3 WHO classification was found in 29 patients (82.86%), type 2 WHO classification in 4 patients (11.43%), and type 1 WHO classification in 2 patients (5.71%). Based on the results on the NPC histopathology, type 3 WHO classification was the most commonly experienced by NPC patients. This finding indicated that, based on the histopathology, the majority of people with NPC currently belong to type 3 WHO classification (2,15,18–20). Meanwhile, based on the stages, it was identified that NPC patients whose treatment data were already in stages III and IV reached 8.57% and 88.75%, respectively, while those in stage II reached 2.86%. No patients were found in stage I. This NPC study also indicated the results that most NPC patients were in advanced stage (Susilo, 1995. Kresno, et al. 1993. Sastrowijoto, Losin, Setiamika, 1995. Soetjipto, 1993). Through this distribution, it can be seen that NPC patients who came for treatment had reached severe level of sickness.

The levels of sensitivity and specificity obtained in this study showed that serological methods with anti-VCA IgA and anti-EBNA IgA titers were best to detect NPC early. The sensitivity of anti-VCA IgA titer reached 91.14%, whereas the specificity reached 97.14%. The sensitivity anti-EBNA IgA reached of 91.14% and the specificity reached of 94.3%. The sensitivity of anti-VCA IgA and anti-EBNA IgA had the same value, but anti-VCA IgA was more specific for the supporting diagnosis of NPC. In this study, the Elisa Test method was used with a relatively high sensitivity and specificity results, which was similar to previous studies that used the Immunofluorescence Test method which showed high sensitivity and specificity results as well(Kresno, et al. 1993. Foo, et al. 2002).

Out of 35 NPC patients, based on the type of histopathology, the average titer of the two antibodies used was not statistically significant among the three types of NPC histopathology with a p-value grater than 0.05. The results obtained have some differences and similarities with similar studies. Previous studies showed the the result of Type 1 WHO classification was 100%, Thpe 3 WHO classification reached 97.1%, while type II WHO classification was not found. Another studies showed that high antibody titers anti-Epstein-Barr virus were found in type 2 and type 3 WHO classifications, whereas type 1 WHO classification was found in a very low titer. The difference was caused by the imbalance in the total samples between type 1, type 2, and type 3 WHO classification, with a ratio of 2:4:29. Based on the results obtained, there were no stage I patients. Therefore, the difference of titers cannot be statistically assessed among various NPC stages. These results are similar to previous studies in the same field, where there were no stage II patients, causing it statistically unassessable.

CONCLUSION

Based on the research results, the NPC titer values were obtained for anti-VCA IgA with a threshold above 10 PU and anti-EBNA IgA with a threshold above 15 PU. There was a significant difference between NPC and non-NPC in the examination of the two types of titers. Based on the results of the titer values, anti-VCA IgA specialization obtained the NPC value of 91.14% and specialization of 97.14%. Therefore, the sensitivity of anti-EBNA IgA reachees 91.14% and its specificity reaches 94.3%.

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