PROFILE IGA (VCA-P18+EBNA1) AND VIRAL LOAD EBV DNA AS A RISK FACTOR AMONG RELATIVES OF NASOPHARYNX CARCINOMA PATIENT WITH EBV POSITIVE AT DR. WAHIDIN SUDIROHUSODO HOSPITAL IN MAKASSAR

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Abstract

Introduction: Nasopharyngeal carcinoma (NPC) is closely associated to viral infection, i.e. Epstein-Barr virus (EBV), about 90 % of the worldwide are infected with EBV at same point of their lives. This virus is highly oncogenic and easily transmitted via saliva. The high level of biomarker for EBV infection can become a risk factor for NPC in relative's NPC patients. Therefore, EBV related biomarkers is needed to enable screening and detecting early diagnosis of NPC in endemic area.

Objective: This study aims to analyze the role of IgA (VCA-p18+EBNA1) levels and viral load (VL) as screening tools for early detection in relatives of positive EBV NPC patients. Therefore, it can be used as predicted risk factor for NPC.

Method: The method used in this study is cross-sectional with consecutive sampling of 50 relatives of NPC patients treated at the Dr. Wahidin Sudirohusodo Hospital from August 2012 to May 2013. Univariate and bivariate analysis and t-test are used to measure and to show the correlation of IgA (VCA-p18+EBNA1) level and VL.

Result: Results showed that 28 % of the NPC patients are female with an average age of 31.12±11.91. Sixty-eight percent of the relatives are siblings, and most of them are Buginese tribe (58%). According to the risk distribution of NPC, the sampling is grouped in 3 categories i.e. high risk, intermediate risk, and low risk to NPC. There is 50% of the relatives of the NPC patients categorized to the intermediate risk. The IgA (VCA-p18+EBNA1) level is significant ($p=0.000$) compared with the VL level ($p=0.337$). Sixteen percent of the high-risk group have a normal pattern, but it still needs high awareness. Statistically, the Pearson correlation analysis showed a relationship but not significant.

Conclusion: It could be concluded that IgA (VCA-p18+EBNA1) and VL level could be used as a screening method and early detection in relatives of NPC patients and become a predicted risk factor for NPC.

Article Info

Keywords:

NPC relatives, IgA (VCA-p18+EBNA1), viral load

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1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy which is the most common neoplasm in the upper aerodigestive tract. NPC is one of the malignancies in the field of Ear Nose Health Science (ENT) that gets a lot of attention because the mortality rate is still relatively high. There were approximately 65,000 new cases and 38,000 deaths per year worldwide. Epidemiologically the NPC shows an interesting pattern of spread in various parts of the world, due to the presence of distinct geographic variations. NPC is most commonly found in the Mongol race, in addition to the Mediterranean, and in several races in northern Africa with groupings in high-risk groups (Canton, China), intermedia (North Africa) and low-risk groups (Western countries) (Guy de The, 2005). The incidence of NPC shows geographical characteristics as the highest incidence of NPC is in South China, where NPC is endemic malignancy in Cantonese people in Guangdong province of China, with an incidence of 10 to 150 per 100,000 population per year, with an average age of 40-50 years [1].

In Indonesia, NPC ranks 4th among all cancers after uterine, breast, and skin cancer, with an incidence of around 4.7 per 100,000 inhabitants (SK Menkes, 2007). However, all parts of ENT in Indonesia place the NPC in the first rank of cancer in the head and neck area with a ratio between men and women is 2-3:1 [2]. In Yogyakarta, NPC is relatively higher, reaching 5.7 per 100,000 population. Dr. Dadi Hospital also reported the male and female ratio incidence in Makassar, South Sulawesi province, was about 2.6:1 [3]. The relationship between EBV and NPC was first investigated in 1966, a rise in serum antibodies to cells infected with EBV. From several studies, It was obtained that there was an increase in levels of IgG and IgA antibodies to VCA and the increased levels of IgA antibodies did not occur in head and neck tumors other than NPC. Increased VCA can be seen 8-30 months before the occurrence of NPC, so it is very important for screening and early detection [4].

Serological tests have been shown to be used as early detection and follow-up of NPC patients. In addition, EBV viral load has the power to show the progression of the disease [5]. EBV infects in a latent mode and it will remain dormant in human B cell lymphocytes for a very long time. If the immune system condition decreases (immunocompromised) then the episome can experience reactivation and then it will spur replication in individuals. Therefore, viral load in the circulation is expected to be used as an indicator in clinical interests. Viral load is the best indicator for determining NPC prognostics and is an important marker for progressive monitoring of recurrence of NPC patients [5].

NPC tends to occur more in certain races (Mongoloid) and is more common in men than women (2-3:1). This raises the suspicion of genetic factors that play a role in the etiology of this disease. The risk of NPC increases significantly in the first generation, the incidence is 6 times higher than the general population. Even NPC is also found in the second and third generations [6, 7] The incidence of NPC in relatives of NPC patients is 15.5%. From the family relationship, 71% of siblings.

Realizing the importance of early detection for the relatives of NPC patients to improve treatment outcome and life expectancy in Indonesia, the study analyzed the possibility of EBV related biomarker as the risk factor of NPC and its ability to be used as screening test for NPC. The author conducted this research to analyze the role of IgA antibody responses (VCA-p18+EBNA1) and viral load in relatives of patients with NPC. This study has never been done in South Sulawesi, especially in Makassar. This study aims to analyze the role of IgA antibody responses (VCA-p18+EBNA1) and viral load in relatives of positive EBV NPC patients.

2. MATERIAL AND METHODS

The study used cross-sectional design. The study was conducted at Dr. Wahidin Sudirohusodo Hospital in Makassar and the UGM Medical Biomolecular Laboratory for 10 months, from August 2012 until May 2013. The study population was families of NPC patients including biological children, siblings, biological parents, who came to the ENT outpatient's clinic Dr. Wahidin Sudirohusodo Hospital. The sample is a first or second degree relatives. Sampling was done by consecutive sampling based on Gaus distribution of 50 people. The criteria of the sample into two, they are inclusion criteria and exclusion criteria. Inclusion criteria are NPC patients' families, including children, and/or siblings and/or biological parents in part or all without complaints related to NPC, not limited to age, sex, ethnicity and race, at home with patients at least the last 1-2 years, do not have a history of disturbances hemostatic and willing to be a research sample. Exclusion criteria are NPC patients who have been diagnosed, blood lysis samples, but the patients are not cooperative.

IgA (VCA-p18+EBNA1) examination of blood serum levels was analyzed by enzyme-linked immunosorbent assay (ELISA) method using synthetic peptide immunodominant epitope protein VCA-p18 and EBNA1. Examination of viral load through DNA isolation by polymerase chain reaction (PCR) with Boom method, the results of concentration will be obtained after light cyclers PCR runs 45 minutes.

3. RESULT

During the study period from August 2012 to May 2013, a research was conducted to look at IgA antibody responses (VCA-p18+EBNA1) and viral load of 50 family members of nasopharyngeal carcinoma patients. Blood tests (whole blood) were used to assess IgA (VCA-p18+EBNA1) and plasma examination to assess viral load.

3.1 Sample General Characteristics

Table 1. Characteristics of Research Samples

Characteristics	n	%
Gender		
Male	22	44.0
Female	28	56.0
Age		
<20 years old	8	16.0
20-29 years old	17	34.0
30-39 years old	13	26.0
40-49 years old	8	16.0
≥50 years old	4	8.0
Family relationship with NPC patients		
Child	34	68.0
Sibling	13	26.0
Mother	2	4.0
Grandchild	1	2.0
Ethnic Group		
Bugis	29	58.0
Toraja	9	18.0
Makassar	10	20.0
Raha	1	2.0
Bali	1	2.0

Out of 58 patients, we found 56% of the NPC patients were female (n=28), the average age of families with NPC patients was 31.12±11.91 where the youngest was 13 years old, and the oldest was 66 years old. 34 of the samples are biological children of the patients (68%), 13 samples are the siblings (26%), and 2 samples are biological mother of the patients (4%), and 1 of them is the biological grandchild of the patient (2%). While most tribes are Bugis, 29 people (58%), followed by Makassar tribe, 10 people (20%), Toraja tribe, 9 people (18%), and lastly Raha and Bali, 1 person (2%) who have long resided due to migration on Sulawesi.

Table 2. Grouping of Families of NPC Patients Based on histopathological overview of NPC Patients

Histopathology View	n	%
Type II	6	12.0
Type III	44	88.0
Total	50	100.0

In this study we found that 88% of the samples (n=44) were relatives of WHO type III NPC patients then followed by WHO type II (n=6).

The distribution of families of NPC patients based on the stage of NPC patients according to TNM-UICC 2010 had the most stage IVB 16 people (32%), then stage III 13 people (26%), stage IIB 7 people (14%), stage IVA 6 people (12%), stage II A 4 people (8%) and the last same number stadium I and IVC which is 2 people (4%) can be seen in table 3.

Table 3. Distribution of NPC patients based on the NPC stage

Staging of NPC	Total	%
Stage I	2	4.0
Stage IIA	4	8.0
Stage IIB	7	14.0
Stage III	13	26.0
Stage IVA	6	12.0
Stage IVB	16	32.0
Stage IVC	2	4.0
Total	50	100.0

3.2 IgA Examination Results (VCA-p18+EBNA1)

From the results of examination of levels of IgA (VCA-p18+EBNA1) using the ELISA method in 50 family members of NPC patients found in families of NPC patients above 28 people Cut Cut Value (CoV) (56%) can be seen in table 4.

Table 4. IgA (VCA-p18+EBNA1) levels in families with NPC patients

IgA (VCA-p18+EBNA1)	n	%
>CoV	28	56.0
≤CoV	22	44.0
Total	22	100.0

*Cut-off Value=0.32

Graphic 1. Level of IgA (VCA-p18+EBNA1) in a family with NPC

Based on graph 1 there are 26 samples showing positive results for checking IgA (VCA-p18+EBNA1), 2 samples are in the, and 22 samples show results below Cut-off Value (CoV).

Table 5. Relationship of age, sex and family status of people with NPC with IgA (VCA-p18+EBNA1)

Variable	IgA (VCA-p18+EBNA1)			
	>CoV		≤CoV	
	n	%	n	%
Age				
<20 years old	3	10.71	5	22.7
20-29 years old	9	32.1	8	36.4
30-39 years old	7	25.0	6	27.3
40-49 years old	6	21.4	2	9.1
≥50 years old	3	10.7	1	4.5
Gender				
Male	10	35.7	12	54.5
Female	18	64.3	10	45.5
Family Relation				
Child	17	60.7	17	77.3
Sibling	8	28.6	5	22.7
Mother	2	7.1	0	0.0
Grandchild	1	3.6	0	0.0

Table 5 shows that in the age group of 20-29 years the most shows above CoV there are 9 people (32.1%) while the highest sex above CoV is female 18 people (64.3%). Based on the most family relationships above, CoV is 17 biological children (60.7%).

3.3 Viral Load Check

From the results of viral load examination in 50 family members of NPC patients, 13 people showed results above the Cut-off Value (CoV). In table 6, there are 5 family members (4 biological and 1 sibling) with results above CoV, but when compared with the results of IgA (VCA-p18+EBNA1) the results are below CoV.

Table 6. Number of EBV DNA and IgA Levels (VCA-p18+EBNA1) in families with NPC patients

No	Sample Code	Family Relation with NPC patients	Total of EBV DNA per ml of blood	Level of IgA (VCA-p18+EBNA1)
1	NWB 08	Child	247.600	0.3260
2	NWB 09	Child	7.678.000	0.6520
3	NWB 12	Child	405.600	2.3759
4	NWB 31	Child	263.000	0.1145
5	NWB 32	Child	135.100	0.0580
6	NWB 38	Sibling	125.180.000.000	0.0205
7	NWB 42	Child	17.092.000	0.1710
8	NWB 48	Child	334.200	0.1030
9	NWB 57	Sibling	408.000	2.4065
10	NWB 58	Sibling	421.800	2.3910
11	NWB 59	Child	278.400	0.5335
12	NWB 60	Child	6.732	2.1365
13	NWB 70	Mother	21.400	0.8680

Graphic 2. Amount of EBV DNA per ml of blood

The range of EBV DNA detected in the sample is 6.732×10^3 - 1.25×10^{10} . In 13 patients whose EBV DNA was detected all were above CoV (2,000 EBV DNA per ml of blood).

3.4 IgA (VCA-p18 + EBNA1) and Viral Load Examination Results

Table 7. Risk distribution of NPC based on IgA (VCA-p18+EBNA1) and VL

Category	Total	%
High IgA (VCA-p18+EBNA1) and VL	8	16.0
High IgA (VCA-p18+EBNA1) low VL	20	40.0
Low IgA (VCA-p18+EBNA1) high VL	5	10.0
Low IgA (VCA-p18+EBNA1) and VL	17	34.0
Total	50	100.0

Table 7 showed that the group with high IgA (VCA-p18+EBNA1) and low viral load was the group with highest number with 20 people (16%). It then followed respectively by the group with low IgA (VCA-p18+EBNA1) and viral load with 17 people (34%), high IgA group (VCA-p18+EBNA1) and viral load with 8 people (16%) and the last group was low Ig A (VCA-p18+EBNA1) and high viral load with 5 people (10%).

Table 8. Risk distribution of NPC based on IgA (VCA-p18+EBNA1) and VL

Category	n	%
High-risk NPC	8	16.0
Intermediate risk NPC	25	50.0
Low-risk NPC	17	34.0
Total	50	100.0

Table 8 group categories are reduced to 3 groups, namely the high risk group NPC, intermediate risk NPC and low risk NPC where the highest in the intermediate risk group of the NPC as many as was 25 people (50%), then the low risk group NPC was 17 people (34%) and the last high risk group NPC was 8 people (16%).

Table 9. Risk of NPC according to gender in the relatives of NPC patients

Gender	High Risk		Intermediate risk		Low risk		Total	
	n	%	n	%	n	%	n	%
Male	4	50.0	10	40.0	8	47.1	22	44.0
Female	4	50.0	15	60.0	9	52.9	28	56.0
Total	8	100.0	25	100.0	17	100.0	50	100.0

Table 9 shows that female was found higher in the intermediate and low-risk NPC group (60% and 52.9%, respectively), while male and female were found to be equally in the high-risk NPC group.

Table 10. Distribution of NPC risk according to age

Age	High Risk		Intermediate risk		Low risk		Total	
	n	%	n	%	n	%	n	%
<20 y.o	1	12.5	3	12.0	4	23.5	8	16.0
20-29 y.o	1	12.5	10	40.0	6	35.3	17	34.0
30-39 y.o	1	12.5	8	32.0	4	23.5	13	26.0
40-49 y.o	4	50.0	2	8.0	2	11.8	8	16.0
≥50 y.o	1	12.5	2	8.0	1	5.9	4	8.0
Total	8	100.0	25	100.0	17	100.0	50	100.0

Table 10 shows the distribution of NPC risk according to patient's age. It was found that intermediate-risk group was found to be the most commonly found (n=10; 40%) between the ages of 20 to 29 years old, followed by low-risk group (35.3%) within the same age range.

Table 11. Risk distribution of NPC based on family relationships

Family Relation	High Risk		Intermediate risk		Low risk		Total	
	n	%	n	%	n	%	n	%
Child	5	62.5	16	64.0	13	76.5	34	68.0
Sibling	2	25.0	7	28.0	4	23.5	13	26.0
Mother	1	12.5	1	4.0	0	0.0	2	4.0
Grandchild	0	0.0	1	4.0	0	0.0	1	2.0
Total	8	100.0	25	100.0	17	100.0	50	100.0

In table 11 the most in the three risk groups of NPC were biological children with the most intermediate risk group with 16 people (64%) followed by the low-risk group 13 people (76.5%) and the last high-risk group 5 people (62.5 %).

Table 12. IgA (VCA-p18+EBNA1) and Viral Load levels in families with NPC patients

Family with NPC patients	n	Average ±SD	P
IgA (VCA-p18+EBNA1)	50	0.9261 ± 0.9557	0.000
Viral Load	13	9.631.330.141 ± 34.718.054.846.927	0.337

*CoV IgA=0.32, CoV EBV Viral Load=2000 EBV DNA per ml blood

Table 12 shows that the levels of IgA (VCA-p18+EBNA1) in the families of NPC patients were very significant (p=0.000), whereas with viral load levels there were not have significant differences (p=0.337).

Table 13. Results of Pearson correlation analysis between IgA (VCA-p18+EBNA1) and EBV Viral Load in families with NPC patients

EBV Viral Load		
Ig A (VCA-p18+EBNA1)	R	-2.75
	P	0.363
	N	13

From the results above, a significant value of 0.363 which indicates that there is no correlation between IgA (VCA-p18+EBNA1) and the family viral load of NPC patients with Pearson correlation value of -2.75 shows a negative correlation with weak correlation strength. There is a correlation but not significant or statistically not meaningful, but there is a relationship.

4. DISCUSSION

Based on data from various world populations, the highest incidence rate of NPC is in Guangdong, China where (150/100,000 population). The incidence rate in Indonesia is 4.7/100,000 population, especially in Makassar 5/100,000 population and usually occurs more in men with age productive within the productive age between 40-50 years. This study took a family sample of NPC patients because in the previous study it was said that the incidence of the risk of NPC was quite high (6 times higher than the general population) in the first generation [7, 9]. Based on the characteristics of the average age of the families of NPC patients, 31.12 ± 11.91 showed a trend below the productive age which was found in the age range of 20-29 years as many as 17 people (34%) with the youngest age of 13 years and the oldest 66 years. The most incidence based on gender was female with a ratio of 1.2:1, this is slightly different from previous studies where more men were obtained with a ratio of 1.3:1 and 0.8:1 [10]. This could be caused by the samples were dominated by female. The majority of the tribe is Bugis,

29 people (58%), almost the same as previous studies about patients and families of people with NPC in Makassar [11, 12].

Based on family relations with NPC patients in this study, the majority were 34 children (68%) followed by 13 siblings (26%) and biological parents in this case the mother contained 2 people (4%). This is almost the same as previous studies regarding families of NPC patients where most of the biological children were 48.6%, siblings 34.3% and biological parents 17.1%. However, in contrast to previous research [10], the highest family relationships in a row were 54.8% siblings, biological parents 49.3% and biological children 6.7% from a total sample of 929 people.

The families of NPC patients based on histopathological sensation of NPC patients according to WHO 1979 were the most WHO type III 44 people (88%) followed by WHO type II as many as 6 people (12%) and could not be found at all WHO type I, according to those issued by [11, 13]. WHO type II and III histopathologies are almost 100% associated with Epstein-Barr virus infection [14-16].

In the distribution of families of NPC patients based on the stage of NPC patients according to TNM-UICC 2010 most were stage IV B 16 people (32%) followed by stage III 13 people (26%), inversely with previous studies [12, 17] where most stage III was obtained 48.6%, then stage IV 40%. Thus, it indicates that almost the majority of NPC patients come to treatment in an advanced stage, which causes a prognosis of treatment and poor life expectancy. Therefore, early screening and detection of NPC are needed through a pattern of biomolecular approaches to Epstein-Barr virus.

From the risk distribution of NPC based on examination of IgA (VCA-p18+EBNA1) and viral load, we grouped it into 3 categories of groups. They are high-risk NPC where IgA (VCA-p18+EBNA1) and viral load are high, intermediate risk NPC with IgA (VCA-p18+EBNA1) is high and viral load is low, and IgA (VCA-p18+EBNA1) is low and the last high-risk viral load is low-risk KNF where IgA (VCA-p18+EBNA1) and viral load are low. The most intermediate risk group is 25 people (50%) with the highest female gender in both the intermediate risk and low-risk groups (60% and 52.9%). While the age range was the most at the age of 20-29 years in the intermediate risk group (40%) and biological children held the highest rank in the intermediate risk group (64%). In this intermediate risk group, there is high IgA (VCA-p18+EBNA1) and low viral load where the possibility of the virus is not reactivated in latent infection. So that this group is a carrier where the virus does not appear and is not expressed whereas low IgA (VCA-p18+EBNA1) and high viral load are not likely to be NPC because it is not IgG, so it is not a risk factor.

4.1 Levels of IgA (VCA-p18+EBNA1) and Viral Load levels in families with NPC patients in Makassar

The results of the examination of IgA (VCA-p18+EBNA1) levels by the ELISA method in 50 family members of NPC patients were found above 28 people in CoV (56%) and 13 people in the viral load above CoV with an average of 26%. 0.9261 ± 0.9557 at levels of IgA (VCA-p18+EBNA1) and at an average viral load level of $9,631,330,141 \pm 34,718,054,846,927$. The results of the levels of IgA (VCA-p18+EBNA1) turned out to be very significant with a value of $p=0.000$ ($p<0.05$) compared to the results of viral load levels which turned out to be not significant with $p=0.337$ ($p>0.05$).

Of the 50 samples, there were 8 family members (16%) who were high both IgA (VCA-p18+EBNA1) and viral load levels (high-risk category of KNF). In accordance with the table of possible z-values of observations, they are still within the normal distribution limit but need to be vigilant for those in the high-risk category because they tend to have a higher risk and require a shorter time to become KNF. Because of that more rigorous follow-up with careful clinical examination is needed because most sufferers in countries with high NF incidence such as Indonesia only come to hospitals for treatment in stage III or IV. Looking at Wai's research, Tong Ng et al. (2005) examined 929 samples from families of NPC patients who obtained positive IgA VCA in 84 people and carried out a follow-up period of 6-32 months which turned out to be 9 people (10.7%) [10]. Lo's research, S et al. (2004) found that 66 normal people with positive Ig VCA were followed for <4 years, only 1 person (2%) developed NPC [18]. In Xiaohong's research (Rose) Yang et al. (2005) in Taiwan from 100 samples of families of NPC patients only 1 was detected by NPC [6]. While research by Tiwawech, Danai (2008) in Thailand, there are 122 control samples obtained positive IgA/EBNA1 in 5 people (4.10%). Data from Malaysia found that 2 patients

with stage IIB found high viral load DNA, 2 other patients with high stage IVB and IIB persistent IgA VCA titers but low viral load DNA.

Pearson correlation analysis between IgA (VCA-p18+EBNA1) levels and EBV viral load in families with NPC patients despite showing a negative correlation with weak correlation strength but statistically showed a relationship although not significant. This can be caused by differences in gene factors, differences in environmental factors [19] and endurance.

Differences in the profile of IgA (VCA-p18+EBNA1) and viral load are likely caused by genetic variation of hosts and viruses. Host variations can be caused by variations in MHC class I/HLA, while variations in viruses are LMP. Another host aspect that might play a role besides genetics is the difference in eating habits.

Viewed from the whole data indicate that the family of NPC patients is a high-risk group for the occurrence of NPC, and this finding supports the genetic factor hypothesis, so it is important to do early screening and detection for family members of NPC patients.

5. CONCLUSION

The conclusion of the study is IgA (VCA-p18+EBNA1) levels were statistically more significant than viral load levels. Although it shows a negative correlation with a weak correlation between IgA (VCA-p18+EBNA1) and viral load statistically there is a relationship even though it is not significant. IgA (VCA-p18+EBNA1) levels with viral load can be a method of screening and detection for family members of NPC patients. Sample for screening and early detection of patient's families is first-degree relatives only, and addition form of more detailed data patient's family. Periodic follow-up family members with IgA levels (VCA-p18+EBNA1) and viral load above the cut-off value. Further research are is need longitudinally with a larger number of samples in order to get better results.

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