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HLA-A01 Gene in Nasopharyngeal Carcinoma Patients and Controls in Makassar

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Abstract

The aim of this study was to determine the presence of HLA- A01 gene in Nasopharyngeal Carcinoma (NPC) patients and control's variables in Makassar. This study applied a cross sectional study in 20 controls and 21 patients with NPC who came in Dr. Wahidin Sudirohusodoho Hospital by using consecutive sampling were tested with Fischer Exact. The examination was conducted by DNA isolation with BOOM's method followed by PCR with forward and reverse primer of HLA-A01. Results revealed that HLA-A01 gene in nasopharyngeal cytobrush samples were also found in the venous blood of NPC patients with P <0.05. Positive HLA-A01 in control variable was 81.3%, while HLA-A01 positive control was 70% it's not significant (P> 0.05). Based on the assumption of case-control study, we found that OR = 1, 857. so that it can be said that the HLA-A01 is a risk factor of NPC but not significantly. To sum up that there is no role of HLA-A01gene as the cause of NPC in Makassar.

***Keywords*:** HLA-A01 Gene, Nasopharingeal Carcinoma, Epstein Barr Virus

1. Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy is the most common malignant tumor with an incident auto digestive upper tract. NPC is one of malignancy in the field of Health Sciences Ear Nose Throat (ENT), which received much attention because the mortality rate is still relatively high.

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Globally, approximately 65,000 new cases and 38,000 deaths per year. [1,2] Incidents in Makassar in South Sulawesi province, [3] reported on the Dadi Hospital and Dr. Wahidin Sudirohusodo Hospiotal over a period of 10 years (1990-1999) found 274 (47.98%) cases of malignant tumors of NPC head and neck with a comparison between men and women is 2.6: 1 [3]. Then the period January 2004 through June 2007 found 33% of malignancies in the ear, nose and throat [4]. Next [5] reported that during the period of 10 years (2000-2009) found 362 cases (57.28%) cases of NPC of malignant tumors of the head and neck [5]. NPC is a disease due to a multifactorial causes. The incidence and geographic distribution depends on several factors, such as genetic susceptibility, environmental factors, diet and personal habits. Many theories etiologic factors have been proposed, but the exact cause is still not found. Numerous studies show that the etiology of NPC is multifactorial, including genetic, environmental and viruses [6]. Results of the latest research indicating the role of genetic factors in disease progression and viral [7]. Various studies have found an association between HLA genes with NPC, good relationship and protective HLA vulnerable to the NPC. In Tunisia found the relationship between the NPC with the HLA-DRB1 * 03 and HLA DRB1 * 15. In Taiwan associated with the HLA A * 0207, in Thailand with the HLA-B * 4601 and in Morocco HLA-B18 [8,9,10].

The expression of HLA class I HLA A * 0101 proved to increase the risk of EBV-positive Hodgkin lymphoma. HLA class I molecules presenting viral peptides for recognition by T lymphocytes and because the CD 8 T cells known to play a role in the control of EBV infection. In Hodgkin's lymphoma (LH) with EBV + cell response is relatively weak so that HLA A * 01 can expressed in EBV-related diseases [11]. Hafez et. al., reported the frequency of HLA-A01 were significantly higher in patients with Hodgkin lymphoma (53.8%) compared with controls (16.2%) in the population relative risk Mesir [12] shows that people who have HLA-A01 six times more vulnerable than people who do not carry the HLA-A01. These data are supported by Falk and osoba, which also reported an increased incidence of Hodgkin lymphoma in patients with HLA-A01 gene in HLA-A01 Kanada [13] more susceptible to EBV^+ Hodgkin's lymphoma while HLA A * 02 is the protection of EBV + Hodgkin lymphoma [14]. In populations China HLA-A * 0207 is a risk factor for nasopharyngeal carcinoma but is a protective factor in LH EBV^+ [15]. Hodgkin's lymphoma is EBV associated malignancies and has a latency that is identical to the type of NPC that latency type II with a restricted expression pattern for LMP1, LMP2 and EBNA [16]. This study aims to determine the presence of the gene allele HLA- A01 in patients with nasopharyngeal cancer and control in Makassar

2. Materials and Methods

This research was carried out for 6 months, from February to July 2014 in the Hospital of Dr. Wahidin Sudirohusodo Makassar applied analytic observational study design with cross sectional design. The study population was all nasopharyngeal cancer patients who seek treatment in the ENT clinic of Dr. Wahidin Sudirohusodo hospital Makassar during the study period. The study sample were 21 patients with NPC based on histopathology and 20 controls (healthy people) who are willing to join the study and signed a consent form after receiving an explanation (informed consent). Data collection was done after getting approval from the ethics committee recommendation biomedical research in humans Faculty of Medicine Hasanuddin University, Makassar No. 0275 / H4.8.4.5.31 / PP36-KOMETIK / 2014.

Sampling was done by taking 3 ml of venous blood in patients with NPC and control and brushing of the carcinoma nasopharynx patients using a brush with the guidance of endoscopic visualization. HLA-A01 examination conducted in the laboratory Biomolecular Engineering, Gadjah Mada University BOOM'S method and the PCR with the forward primer and reverse HLA-A01. Data results are recorded and collected in the observation sheet. The data obtained were processed and the results are displayed in the form of narrative, tables or images. Tested with Fischer's Exact test.

3. Results

Results of this study are elaborated in term of table and figure, then described narrative, these data are presented as follow;

3.1 Sample Characteristics

Table 1 describe that of 21 samples of NPC cases and 20 controls the characteristics of the study sample obtained based on sex, more men suffer from NPC are 16 people (76.2%) than women 5 (23.8%) with a ratio of 3.2 : 1, where the age of NPC patients at most in the range of 8 people 40-49 years 38.1% with most parts distribution is Bugis with 9 people (42.9%).

Table 1. Characteristics of respondents

Group	Case	%	Control	%
Sex				
Male	16	76,2	10	50
Female	5	23,8	10	50
Total	21	100	20	100
Age (yr)				
20-29	2	9,5	15	75
30-39	1	4,8	4	20
40-49	8	38,1	1	5
50-59	5	23,8	0	0
≥ 60	5	23,8	0	0
Total	21	100	20	100
Tribes				
Bugis	9	42,9	11	55
Makassar	5	23,8	5	25
Toraja	1	4,8	0	0
Other than South Sulawesi	6	28,6	4	20
Total	21	100	20	100

Based on Table 2. Distribution histopathology of NPC patients according to the WHO in 1979 obtained the highest is WHO type III, 17 people (80.9%), then WHO type II by 4 people (19.1%) and not WHO type I obtained in this study . Distribution of patients based on the 2010 AJCC Stage KNF highest stage IV were 11 people (52.4%), stage II with 6 people (28.6%) and Stage III of 4 people (19.0%), [17].

Table 2. Sample Characteristics base on picture of Histopathology and Stadium KNF

Group	Case	%
Histopathology		
Picture		
WHO Type I	0	0
WHO Type II	4	19,1
WHO Type III	17	80,9
Total	21	100
Stadium		
I	0	0
II	6	28,6
III	4	19,0
IV A	6	28,6
IV B	4	19,0
IV C	1	4,7
Total	21	100

3.2 Results PCR HLA-A01

On examination of the venous blood sample control on the image 1, it was viewed of 20 samples were amplified in accordance with the results of previous PCR optimization with the amount of 101 bp product, seems to be emerging DNA bands on several samples with varying thicknesses and bring 13 samples (65%). In the examination of cases of venous blood sample in Figure 2, of the 16 samples were amplified in accordance with the results of previous PCR optimization, DNA bands seem to have emerged in several samples with varying thicknesses and bring 13 samples (81.25%). In the case of nasopharyngeal examination cytobrush samples in Figure 3 of 21 samples were amplified in accordance with the results of previous PCR optimization seems to be emerging DNA bands in several samples with varying thicknesses and bring 16 samples (76.19%).

Based on statistical tests Fischer exact test in Table 3 shows that in 16 paired samples of HLA-A01 contained in the nasopharynx 100% cytobrush samples were also found in the venous blood of patients with NPC and showed significant results with P = 0.00 (P <0 , 05). Based on the assumption of case-control study in Table 4

obtained OR = 1, 857 (OR> 1) so that it can be said that HLA-A01 is a risk factor for NPC but not statistically significant (P> 0.05)

Table 3. Comparison of brushing and venous blood case samples KNF (n=16)

		HLA-A01 vena blood				
		Positive	%	negative	%	Total
HLA-A01 Brushing	Positive	13	81,3	0	0	13
	Negative	0	0	3	18,8	3

Table 4. Comparison of venous blood samples of cases and controls

HLA-A01	Group				P
	Case	%	Control	%	
Positive	13	81,3	14	70	0,45
Negative	3	18,8	6	30	

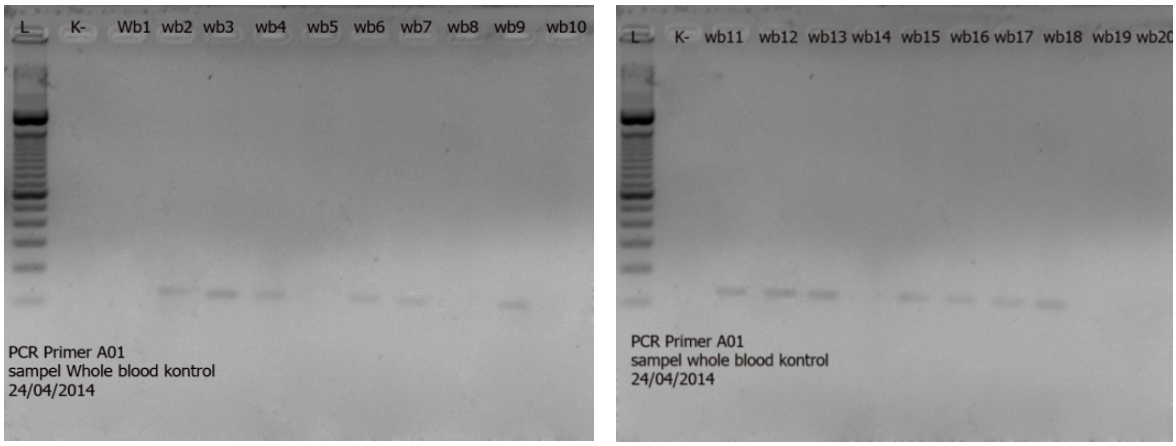

Figure 1. Examination result of PCR of vena blood control sample (17)

4. Discussion

In this study, venous blood samples were collected 30 and cytobrush nasopharynx and 20 control venous blood samples were sent to the Laboratory Biomolecular Engineering, Gadjah Mada University, but with non-

technical reasons of the overall sample to do DNA isolation 16 venous blood samples, 21 samples cytobrush nasopharynx and 20 whole venous blood sample control.

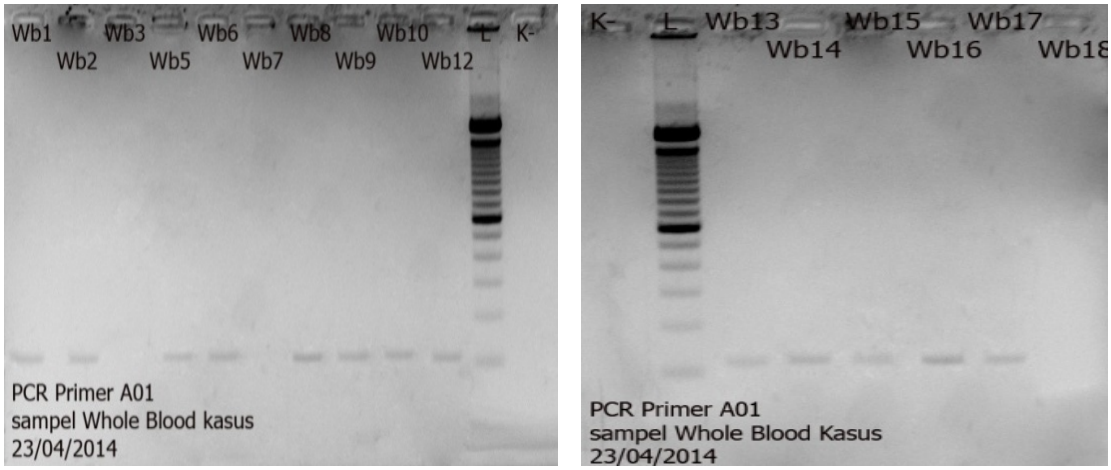

Figure 2. Examination result of PCR HLA-A01 of vena blood casus sample (17)

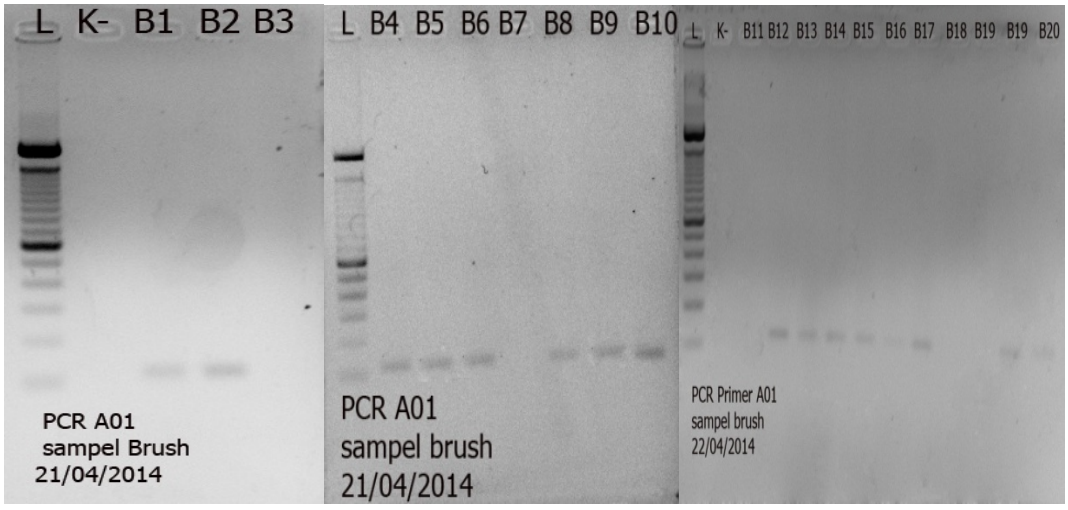

Figure 3. Examination result of PCR HLA-A01 of brushing cases sample (17)

Based on Gender, more men than women suffer KNF 3.2: 1. This is consistent with research conducted by [16] found more men with a ratio of 2.04: 1:16 Similarly Munir research [17] and Savitri [18] that more men than women suffer from NPC.

The age group most NPC patients in this study in the range of 8 people 40-49 years 38.1%. The youngest patient age was 22 years old and the oldest 66 years. The average age of patients in this study was 48.5 years. [17] found the highest incidence of NPC at the age of 50-59 years with an average age of 48.8 . [18] found that the average age of patients with NPC in Makassar was 44.8 ± 13,8.18. [16] reported the highest incidence of NPC patients at the age of 30-49 years with a mean age of 43.9 tahun.16 tribe most is the Bugis were 9 people (42.9%). Similar to the study Savitri [18] who get most tribes are Bugis ethnic group (41%). 18 Similarly [16] study also reported that most tribes namely Bugis 44.3%. 16.

Distribution histopathology of NPC patients according to the WHO in 1979 obtained the highest is WHO type III is 80.9%. In accordance with research [16] and [18] earlier in Makassar, obtained the highest histopathology is III.16,18,19 KNF WHO type III type is the most common type, especially in Southeast Asia. According to research [19], in this type was found 100% EBV. WHO KNF Type 1 predominantly found in Caucasian ethnicity as in Europe, while in Japan the WHO KNF Type 2 is the type most widely ditemukan.20 Based on the AJCC 2010 staging of NPC by most established stage IV 52.4%. This is consistent with the results of research [18] and [20] also obtain the highest stage is stage IV. 19 This is because the diagnosis is often delayed KNF enforced, because the nasopharynx lies hidden behind the nasal cavity. Fault diagnosis can also be caused by a lack of knowledge about the propagation of tumors and misinterpretation on histopathologic examination. In addition, early symptoms are not typical of this tumor is sometimes just tinnitus (noises) in the ear so often neglected patients.

Based on statistical tests on 16 samples of cases in pairs, suggesting that HLA-A01 contained in nasopharyngeal cytobrush samples were also found in the venous blood of NPC cases. This suggests that HLA-A01 examination with venous blood samples (non-invasive) can replace HLA-A01 examination with cytobrush nasopharynx (invasive). This is similar to the research conducted by Savitri and his colleagues [21] in Makassar, the research found the presence of HLA-A24 at the same cytobrush samples with venous blood samples of patients KNF..21.This study also obtain the result that the HLA-A01 based on the assumption of case-control study found OR 1.857. This suggests that clinically HLA-A01 will increase the incidence of NPC by 1,857 times than that do not have the HLA-A01 but not statistically significant (P> 0.05). So DAPT is said that the HLA-A01 is a risk factor for NPC. It is difficult to see the incidence of NPC is used because the process is very complex events. Only one of the parts of the body's immune system.

According to [22] that the HLA alleles are associated with certain diseases can also be found in healthy individuals, and if all individuals were followed prospectively, most of them never get sick, so that a particular HLA gene expression alone is not enough as the cause of disease. HLA genes are only one of several factors that contribute to the occurrence of the disease despite an important factor [22].

5. Conclusions and Recommendations

Examination of HLA-A01 with venous blood samples can replace cytobrush examination of the nasopharynx in patients with NPC. In a healthy person can be found also HLA-A01. HLA-01 can be a risk factor for NPC. Cannot be used to view the events of NPC. Need to do further tests in the control group (healthy people) with

HLA-A01 positive as screening. We Suggest Sequence epitope further examination to see whether the HLA-A01 immunogenic or not.

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