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Association of HLA DRB-1*11 and HLA DRB-1*03 Alleles with Nasopharyngeal Carcinoma in Minangkabau Ethnic Group

Sukri Rahman[1*], Eti Yerizel[2], Daan Khambri[3], Djong Hon Tjong[4]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine Andalas University / Dr. M. Djamil Hospital, Padang, Indonesia.
[2]Department of Biochemistry, Faculty of Medicine Andalas University, Padang, Indonesia.
[3]Department of Surgery, Faculty of Medicine Andalas University /Dr. M. Djamil Hospital, Padang, Indonesia.
[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.

Abstract

The etiology of NPC is multifactorial, involving an interaction between Epstein-Barr virus (EBV) infection, genetic susceptibility, and environmental factors. The host's immune response mainly determines the growth of malignancy caused by the virus. Human leukocyte antigen (HLA) plays a crucial role in presenting viral antigens, essential in determining the host's immune response impact against viral infections. Inhibition of HLA expression may facilitate the evasion of tumor cells from host immunosurveillance. This study analyzed the association of the HLA-DRB1*11 and HLA-DRB1*03 alleles with nasopharyngeal carcinoma in the Minangkabau. Genotyping of the HLA-DRB1*11 alleles and DRB1*03 alleles was carried out using the polymerase chain reaction sequence-specific primer (SSP-PCR) method. The frequency of HLA-DRB1*11 alleles in the control group (43.5%) was significantly higher than in the NPC group (4.3%) ($p < 0.05$). The frequency of the HLA-DRB1*03 allele was also higher in the control (26.1%) compared to the NPC case (4.3%), but statistically, there was no significant association between NPC and the HLA-DRB1*03 allele ($p > 0.05$). This study found the HLA-DRB1*11 alleles were protective against the development of nasopharyngeal carcinoma. There was no association between the HLA-DRB1*03 allele and nasopharyngeal carcinoma in the Minangkabau ethnic group.

Keywords: HLA DRB-1*11, HLA-DRB1*03, Alleles, Nasopharyngeal Carcinoma.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor of the nasopharyngeal epithelium. The incidence of NPC, compared to other malignancies globally, is rare. The World Health Organization (WHO) estimates that there were 129,079 new cases of NPC in 2018, only 0.6% of all malignancies diagnosed worldwide in that year [1-5]. However, these malignancies have a unique geographic distribution pattern. Nasopharyngeal carcinoma is a malignancy and a frequent cause of death in certain areas, such as in southern China and Southeast Asia, including Indonesia. Nasopharyngeal carcinoma is the most common malignancy of the head and neck in Indonesia, with an incidence rate of 6.2/100,000. Cancer registry data based on the Dharmais National Cancer Center Hospital in 2007 reported that NPC was the most common cancer in men. In West Sumatra, based on data at RSUP Dr. M. Djamil also reported that NPC is the most common malignant tumor in the head and neck region [6-8].

The cause of NPC is an interaction of multiple factors. The main etiologic factors include environmental, genetic susceptibility, and EBV infection. The host's immune response largely determines malignancy growth due to the virus. Human leukocyte antigen plays a significant role in presenting viral antigens, which is crucial to determining the impact of the host immune response against this viral infection. Inhibition of HLA expression may facilitate the evasion of tumor cells from host immunosurveillance. Various studies have found a relationship between the HLA complex and the immune response to EBV. Several studies support the hypothesis that EBV can down-regulate the expression of HLA alleles, which will cause cancer cells to evade the immune response by reducing the expression of EBV antigens from cancer cells [9,10]. Individuals with

particular HLA alleles have a decreased capacity to present virus antigens. They may be less efficient in triggering an immunological response against EBV-infected cells, thus increasing their susceptibility to Nasopharyngeal Carcinoma [11,12]. Various studies have reported the association of the HLA class I allele with NPC in areas of high incidence of NPC [13,14]. However, studies on the relationship between HLA class II and the incidence of NPC are still limited. Various studies of HLA-DRB1 in NPC have obtained inconsistent results. One meta-analysis that examined the association of the HLA-DRB1 allele with the incidence of NPC found that the HLA-DRB1*03, *08, *09, and *10 alleles contributed to susceptibility to NPC. In contrast, the HLA-DRB1*11 and *12 alleles were protective factors against NPC, especially in Asian populations [15]. The HLA-DQB1 study in Batak tribes found no alleles associated with NPC susceptibility [16].

Some ethnic groups tend to suffer from NPC, such as the Bidayuh in Borneo, the Nagas in northern India, and the Inuits in the Arctic, where the incidence is reported to be more than 16 per 100,000 people/year. In China alone, this incidence is only high in southern China, namely in Guangdong, Hainan, Guangxi, Hunan, Fujian, and Hong Kong. Suppose the overall incidence of NPC in China is calculated. In that case, the incidence is only 1.9/100,000, which raises the suspicion that genetic factors in one ethnicity and environment play a role in the incidence of NPC [2,17]. This study analyzed the association of the HLA-DRB1*11 allele and the HLA-DRB1*03 allele with the incidence of nasopharyngeal carcinoma in the Minangkabau ethnic group.

2. Materials and methods

The subjects of this study were NPC patients of Minangkabau, and the controls were healthy people who were also Minangkabau. Venous blood was taken from patients with nasopharyngeal carcinoma and control to examine the HLA-DRB1*11 allele and HLA-DRB1*03 allele. The NPC cases were staged according to the 8th edition AJCC staging criteria. DNA was isolated from 200 µl whole blood samples using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Genotyping of the HLA-DRB1*11 allele and HLA-DRB1*03 allele was carried out using the polymerase chain reaction sequence-specific primer (SSP-PCR) method. The primers used for the amplification of the DRB1*11 allele (Olerup et al., 1992) were primer forward: 5'- GTTTCTTGGAGTACTCTACGT C - 3'; Reverse primer: 5' - CTGGCTGTTCCAGTACTCCT - 3'. The primers used for the amplification of the HLA-DRB1*03 allele were: Primer forward: 5'- TACTTCCATAACCAGGAGGAGA - 3'; Reverse primer: 5'- AGTAGTTGTCCACCCGGC - 3'. This study has received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Andalas University No.422 / KEP / FK / 2018. The ethical implications of this study follow the provisions of the Declaration of Helsinki. All medical records relating to this study are confidential.

3. Results

23 NPC patients and 23 non-NPC controls were recruited in this study. All the patients and controls were related to the Minangkabau people, and All the patients were recruited from Dr. M. Djamil Hospital, Padang, Indonesia. Pathologically confirmed NPC diagnosis was the only selection criterion for patients. The case group (15 males and eight females) had a mean age of 46.6 ±12.89 years. The control group consisted of 23 healthy volunteers. The mean age of the control group (15 males and eight females) was 46,2±13,72years. There was no significant difference between patients and control. The majority of cases of NPC (91.3%) were advanced stages, consisting of stage III (13.0%) and stage IV (78.3%). The undifferentiated carcinoma subtype was the most common (69.6%).

*3.1 HLA-DRB1*11 allele in Nasopharyngeal Carcinoma*

Polymerase chain reaction (PCR) for HLA-DRB1*11 allele of the NPC and control groups can be seen in Figures 1 and 2. Based on Figure 2, the positive HLA-DRB1*11 alleles were found in 10 samples, namely in sample numbers S08, S09, S14, S17, S18, S19, S21, S22, S23, and S24. In the NPC group, the HLA-DRB1*11 alleles were found in 4.3%, while in the control group, they were found in 43.5%, as shown in Table 1.

*3.2 HLA-DRB1*03 allele in Nasopharyngeal Carcinoma*

The method used to recognize and amplify the HLA-DRB1*03 allele is SSP PCR. PCR results for the NPC and control groups can be seen in Figures 3 and 4. Based on Figure 2, positive HLA-DRB1*03 allele was found in 6 samples; on sample numbers S06, S07, S08, S09, S10 and S11. Positive samples for the HLA-DRB1*03 (S06) allele were sequenced to confirm the PCR results. Based on Table 2, it was found that the percentage of samples that were positive for the HLA-DRB1*03 allele was higher in control (26.1%) than in the NPC case, which was 4.3%; statistically, there was no significant relationship between the incidence of NPC and the HLA-DRB1*03allele ($p > 0.05$).

4. Discussion

*4.1 Association of HLA-DRB1*11 Alleles with NPC*

Specific HLA alleles besides causing susceptibility to NPC growth, some HLA alleles have been reported to be associated with a reduced risk of NPC or are protective/resistant. Several studies have consistently reported that the HLA-DRB1*11 allele is protective against the incidence of NPC [18-20]. This study also found a protective relationship between HLA-DRB1*11 and the incidence of NPC. In this study, the HLA-DRB1*11 alleles were higher in healthy controls, namely 43.5% and only 4.3% in NPC cases, and this association was statistically significant (OR= 0.059; 95% CI 0.007-0.516). These results conclude the protective nature of the HLA-DRB1*11 allele against the risk of NPC in the Minangkabau ethnic group.

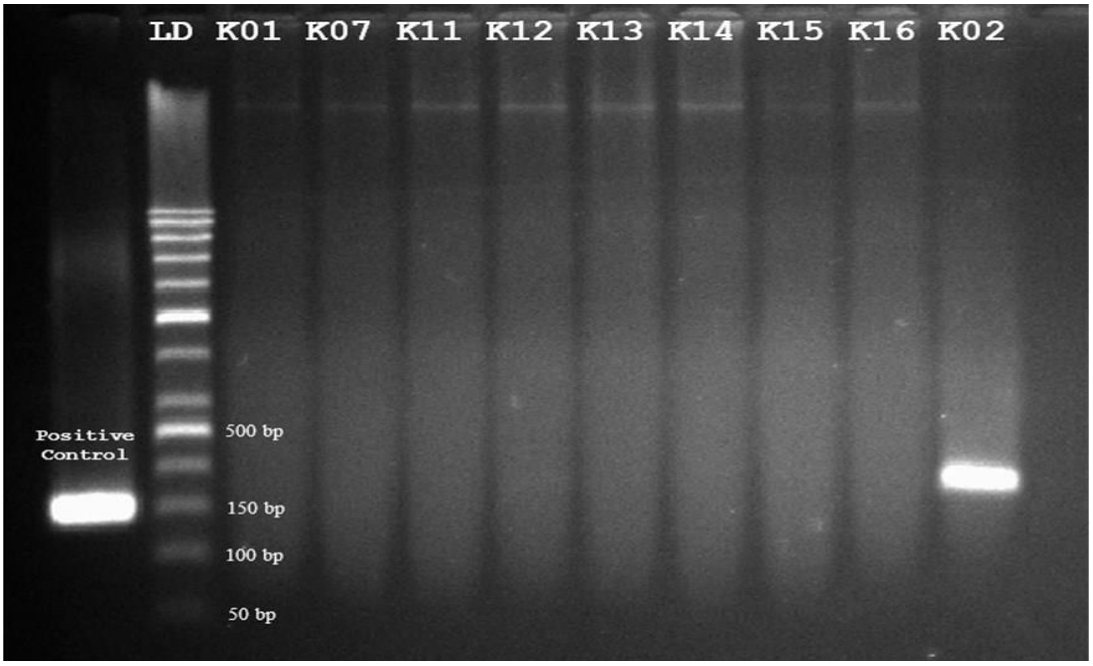

Figure 1. The HLA-DRB1*11 allele in the NPC group was positive in 1 sample (K02).

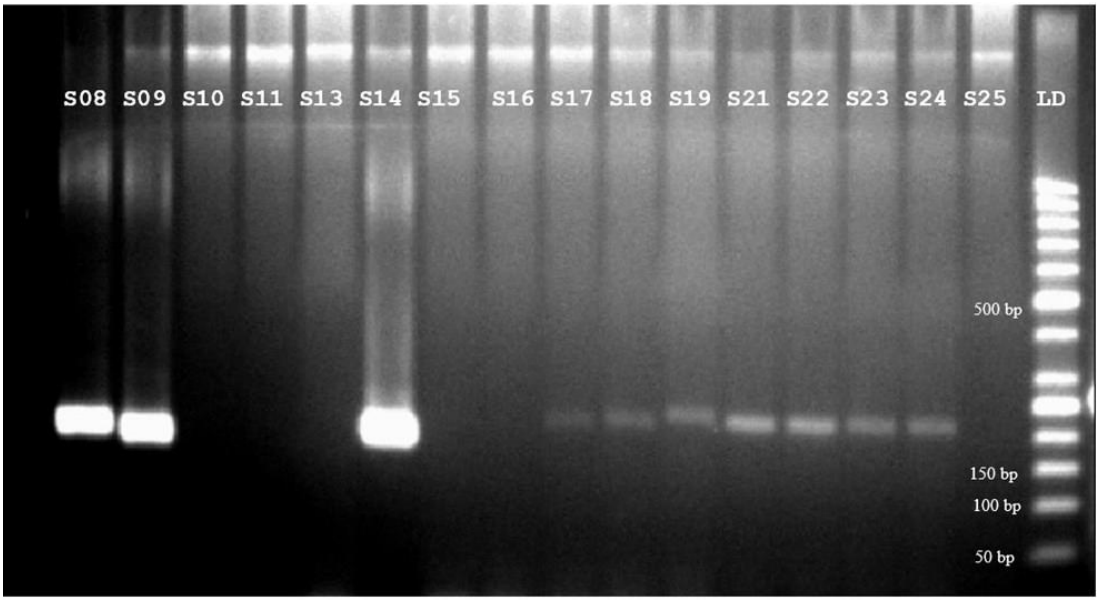

Figure 2. The HLA-DRB1*11 allele in the control group was positive in 10 samples.

Table 1: Association of HLA-DRB1*11 Allele with NPC

Allele HLA-DRB1*11	Group		p
	NPC f (%)	Control f (%)	
Negative	22 (95.7)	13 (56.5)	
Positive	1 (4.3)	10 (43.5)	0.006
Total	23 (100)	23 (100)	

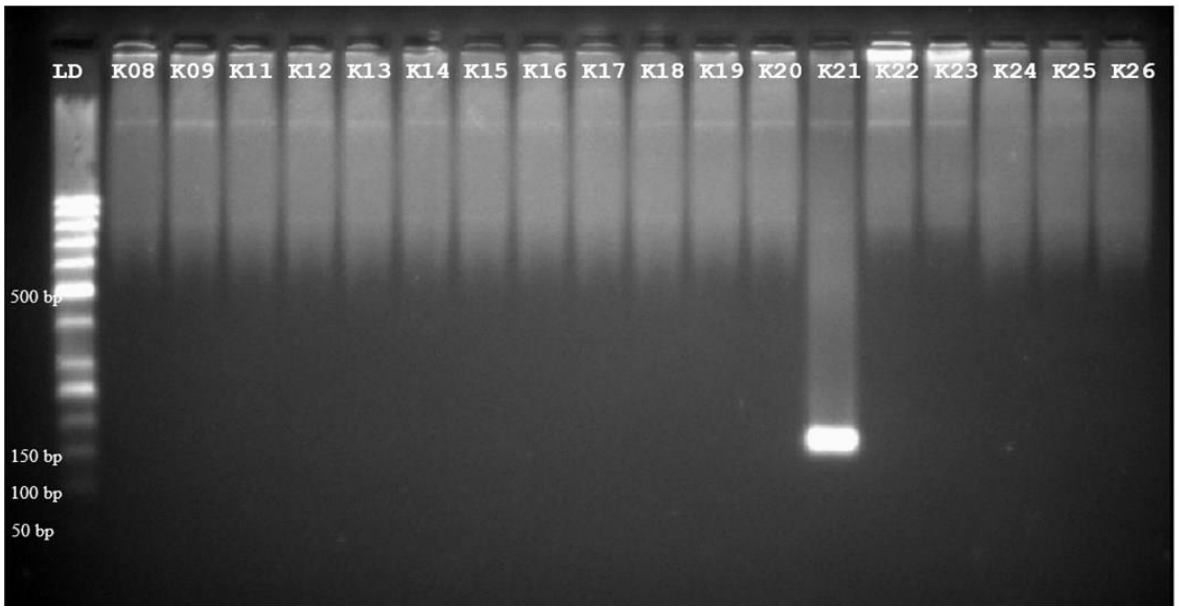

Figure 3. The HLA-DRB1*03 allele in the NPC group was positive in 1 sample (K21).

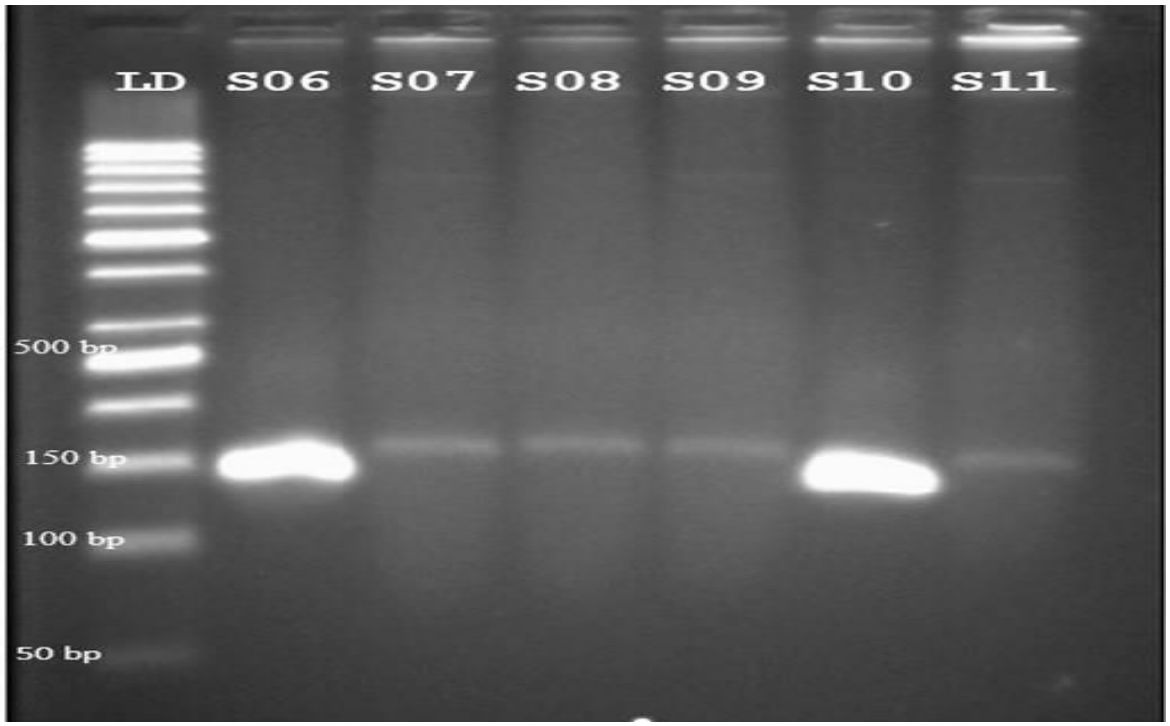

Figure 4. The HLA-DRB1*03 allele in the control group was positive in 6 samples.

Table 2: Association of HLA-DRB1*03 Allele with NPC

HLA-DRB1*03 allele	Group		p
	NPC f (%)	Control f (%)	
Negative	22 (95.7)	17 (73.9)	
Positive	1 (4.3)	6 (26.1)	0.09
Total	23 (100)	23 (100)	

Mokni-Baizig [21], who conducted a study in Tunisia, also reported that the HLA-DRB1*11 alleles were associated with a reduced risk of NPC, where the allele frequency in cases was 8.33% and in controls 27.69%, and there was a significant relationship between allele frequency and a decrease in the incidence of NPC (OR= 0.3; p=0.027). The HLA-DRB1*11 allele enhances protection against NPC, likely due to its ability to efficiently represent multiple EBV epitopes, mainly derived from EBV latency proteins. This level of protection can be further increased by combining it with other alleles [22].

*4.2 Association of HLA-DRB1*03 Alleles with NPC.*

Genetics is a significant risk factor in NPC. Numerous studies have consistently reported the role of HLA, including HLA-DRB1, in the pathogenesis of NPC in various ethnicities, where specific HLA-DRB1 alleles cause susceptibility to NPC growth [20,23]. The HLA gene is the most polymorphic gene complex in humans, consisting of more than two hundred genes on chromosome 6 [24]. In this study, the HLA-DRB1*03 allele obtained different results from the available reports in the literature. In this study, the HLA-DRB1*03 allele percentage in NPC cases was lower than in healthy controls, 4.3% in NPC, and 26.1% in control, even though there was no statistically significant difference between cases and controls ($p > 0.05$). This result differs from the meta-analysis by Yao et al. [15], which found a significant relationship between the HLA-DRB1*03 allele and an increased incidence of NPC (OR=1.55;95% CI 1, 3-1.8). This relationship was also seen in Asians and Tunisians when grouped by ethnicity but not in Caucasians. Another meta-analysis study by Yang et al. [20] also found that the HLA-DRB1*03 allele increased the incidence of NPC (OR=1.70; 95% CI 1.04-2.76), and, when grouped, also found associations in Asian and Tunisian ethnicities, but also not in Caucasians. Studies from areas of intermediate incidence of NPC, such as Tunisia, also reported that the HLA-DRB1*03 allele was associated with an increased risk of NPC, including the Mokni-Baizig [21] report, where the allele frequency was 41.66% in cases and 21.53% in controls, there was a significant relationship between allele frequency and an increase in the incidence of NPC.

The same thing was also reported by Wang et al. [19], who conducted a study on a population in Northwest China where the risk factors were different from the people in southern China. Wang et al. [19] reported that the HLA-DRB1*03 allele frequency was higher in healthy controls (11.3%) than NPC (6.8%), and there was also no significant relationship (OR=0.872; 95% CI 0.361-2.108). Reports from areas with a high incidence of NPC generally get the HLA-DRB1*03 alleles, increasing the risk of NPC, but in certain ethnic groups, there is no association. Susceptibility to NPC varies by ethnicity [20,23].

5. Conclusions

The HLA-DRB1*11 alleles were protective against developing nasopharyngeal carcinoma in the Minangkabau ethnic group. There is no association between the HLA-DRB1*03 allele and the incidence of nasopharyngeal carcinoma in the Minangkabau ethnic group.

Conflict of Interest
The author reports no conflicts of interest in this work.

Acknowledgments
None

Ethical statement

The study was approved by the Faculty of Medicine Andalas University ethics committee (No.422 / KEP / FK / 2018). The ethical implications of this study follow the provisions of the Declaration of Helsinki. All medical records relating to this study are confidential, and written informed consent was obtained from participants in both NPC and control groups.

Availability of data and material
We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Code availability
Not applicable

Consent to participate
All authors participated in this research study.

Consent for publication
All authors submitted consent to publish this article research in IJCBS.

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