Association between IL-1RN VNTR polymorphism and head and neck cancer in Indonesian population

A V Rudijono¹, A W Suhartono¹, Y H Midoen², C Talbot³ and E I Auerkari¹*

¹Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, 10430, Indonesia
²Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia
³Department of Genetics, University of Leicester, Leicester LE1 7RH, England

*E-mail: eiauerkari@yahoo.com

Abstract. The interleukin-1 receptor antagonist (IL-1RN) is a natural antagonist of IL-1 receptors, and its 86-bp variable number tandem repeat (VNTR) polymorphism within intron 2 has been associated with the development of several cancers. This study aimed to evaluate the IL-1RN polymorphism for its potential association with head and neck cancer in Indonesia population. Polymerase chain reaction analysis was performed on stored blood-derived DNA samples from 110 control participants and 90 patients with head and neck cancer for the IL-1RN VNTR polymorphism. Chi-square and odds ratio were used for the statistical analysis. This study showed no significant difference in the frequencies of genotypes or alleles in either the control or the cancer groups. However, a larger sample size could reveal a more significant difference between the groups. The IL-1RN VNTR polymorphism was not associated with head and neck cancer in the studied Indonesian population.

1. Introduction

Head and neck cancer (HNC) is the seventh most common cancer in the world and the ninth most common in America. In 2014, more than 54,000 new cases were found in America, and approximately 12,000 deaths occurred [1]. In Indonesia, HNC is the fourth most common malignancy found both in men and women and the second most common in men, with a prevalence of 4.7/100,000 population [2]. HNC is a cancer of the upper aerodigestive tract, typically arising from the mucosal lining surface, comprising squamous cells inside the head and neck; therefore, 90% of HNC is squamous cell carcinoma. Although the most common sites of this cancer are the lower lips and tongue, it can begin at the lips, oral cavity, nose, pharynx, larynx, paranasal sinus, or thyroid gland [3]. HNC evolves through various stages, which are influenced by environmental factors such as smoking or other tobacco use, alcohol use, ultraviolet exposure, and genetic changes [4,5].

Interleukin-1 receptor antagonist (IL-1RN) belongs to the interleukin-1 (IL-1) family, along with IL-1β and IL-1α, which are potent proinflammatory cytokines. IL-1RN competes with the other two polymorphic genes to prevent the IL-1 cell receptor from receiving proinflammatory signals [6]. A genetic polymorphism of the IL-1RN gene has been found to potentially affect human susceptibility to various diseases, including malignancies [7]. A variable number of tandem repeats (VNTR) of 86 bp (base pairs) in length is found in intron 2 of the IL-1RN gene (at 2q14.2). Variations in IL-1RN repeats
between individuals is between 2 and 6, and the number of alleles exhibits variation among ethnic and geographic populations [8,9].

Several previous studies have reported significant associations between the IL-1RN gene and certain cancers, including various malignancies in the head and neck [6-9]. The proinflammatory immune response of individuals with the II allele of IL-1RN (IL-1RN*2 allele), the short allele that corresponds to two repeats, compared with the other genotypes of IL-1RN (long alleles), has been considered to be prolonged and more severe [8]. A significant association with oral squamous cell carcinoma has been reported for this gene polymorphism in North India [9]. This IL-1RN gene polymorphism has also been significantly associated with nasopharyngeal carcinoma in Portugal [6]. The information regarding the effect of this polymorphism on HNC in the world is limited, and there is nothing published in relation to Indonesia. The present study aimed to investigate the association between the IL-1RN gene and HNC in the Indonesian population.

2. Materials and Methods

The materials used in this study consisted of previously extracted DNA samples that were stored at −20 °C. The samples for this study were originally extracted from the blood serum of 90 patients with HNC and 110 healthy controls, using the methods of a previous study for sampling and isolation [10]. This study was approved by the Dental Research Ethics Committee of the Faculty of Dentistry of the Universitas Indonesia.

Genotyping of the IL-1RN VNTR polymorphism was performed using polymerase chain reaction (PCR) analysis. The 20 µL PCR mixture contained 10 µL mastermix (Bioline, UK), 0.75 µL of each primer, 8.2 µL ddH2O, and 0.3 µL DNA template. The forward primer sequences for amplification were 5’-CCCCTCAGCAACA CACTCC-3’, and the reverse primer sequences were 5’-GGTCAGAGGGGCAGAG-3’. The amplification started with an initialization step at 94 °C for 4 minutes. The next steps were repeated for 35 cycles, consisting of a denaturation step at 94 °C (30 seconds), annealing at 57 °C (30 seconds), and elongation at 72 °C (45 seconds). The last step was a final elongation step at 72 °C (5 minutes). The PCR products were then analyzed by 2.5% agarose gel electrophoresis (stained with GelRed) and documented in a Geldoc system.

The expected PCR amplification products were 410 bp (IL-1RN*1), 240 bp (IL-1RN*2), 325 bp (IL-1RN*3), 500 bp (IL-1RN*4), and 595 bp (IL-1RN*5). The IL-1RN*1 allele was a wild-type allele that corresponded to four copies of the 86 bp repeat and are classified as long alleles, along with IL-1RN*3, *4, and *5, which have more than two copies of the 86 bp repeat. The short allele was IL-1RN*2, which corresponded to two copies [6,8]. These alleles will be referred to as allele I, II, III, IV, and V.

Data analysis for the study was performed using the statistics program SPSS 22. Chi-square test was used to analyze the frequency differences of the genotypes and alleles between the cancer and control groups, and to assess concordance with the Hardy-Weinberg equilibrium. Odds ratios (ORs) were also determined to assess the risk of HNC and its association with the IL-1RN VNTR gene polymorphism. In all cases, significance was generally assumed at \( p < 0.05 \).

3. Results

Examples of the amplified PCR products visualized using the Geldoc system are shown in Figure 1, in which the I/I genotype (wild-type genotype) is represented by one band at 410 bp, the II/II genotype by one band at 240 bp, the I/III genotype by two bands at 410 bp and 325 bp, and the I/II genotype by two bands at 410 bp and 240 bp.

This study on the Indonesian population observed various combinations of three alleles (I, II, and III) of the IL-1RN VNTR gene polymorphism in intron 2. The observed genotype and allele distributions are shown in Table 1.
Figure 1. PCR product visualization on agarose gel (2.5%) showing genotypes of various gene polymorphisms.

Table 1. Genotype and allele distributions of the IL-1RN VNTR gene polymorphism in healthy controls (n = 110) and in patients with HNC (n = 90).

|                | Controls (n = 110) | HNC (n = 90) | P-value |
|----------------|--------------------|--------------|---------|
| **Genotype**   |                    |              |         |
| Nonpolymorphic | I/I                | 77 (70%)     | 69 (76.7%) | 0.347  |
|                | III/III            | 1 (0.9%)     | 0 (0%)   | |
|                | I/III              | 0 (0%)       | 1 (1.1%) | |
| Polymorphic    | II/II              | 1 (0.9%)     | 0 (0%)   | |
|                | I/II               | 29 (26.4%)   | 20 (22.2%) | |
|                | II/III             | 2 (1.8%)     | 0 (0%)   | |
| **Allele**     |                    |              |         |
| Nonpolymorphic | I                  | 183 (83.2%)  | 159 (88.3%) | 0.321  |
|                | III                | 4 (1.8%)     | 1 (0.6%) | |
| Polymorphic    | II                 | 33 (15%)     | 20 (11.1%) | |

Of 110 healthy controls, 77 had I/I (70%), 1 had III/III (0.9%), 1 had II/II (0.9%), 29 had I/II (26.4%), and 2 had II/III (1.8%); of 90 patients with HNC, 69 were I/I (76.7%), 1 was I/III (1.1%), and 20 were I/II (22.2%). The allele distributions in the healthy controls were 183 (83.2%) with the I allele, 33 (15%) with the II allele, and 4 (1.8%) with the III allele; in the patients with HNC, 159 (88.3%) had the I allele, 20 (11.1%) had the II allele, and 1 (0.6%) had the III allele. The wild-type I/I genotype and the I allele were the most frequent in both observed groups. The genotype distributions in both the control and cancer groups were consistent with the Hardy-Weinberg equilibrium (P > 0.001). The results of chi-square test, summarized in Table 1, indicate no significant association between the genotype and allele of the IL-1RN VNTR gene polymorphism and HNC in the studied Indonesian population (P = 0.347 and P = 0.321). It was also observed that this polymorphism might not be a risk factor for HNC (OR = 0.708, 95%).

4. Discussion
IL-1RN presents naturally as a specific cytokine receptor antagonist in the human body. However, studies have revealed that IL-1RN concentrations are elevated during the final stages of the inflammatory response [11-13]. High levels of IL-1RN have been considered to be associated with the
development of some cancers, including oral squamous cell carcinoma, gastric cancer, and breast cancer [13-15]. In some studies, however, low levels of this gene have been associated with other human malignancies, such as colorectal and prostate cancer. The biological mechanisms underlying IL-1RN alterations in the development of tumors have not yet been determined, given its properties are likely due to changes in the IL-1 pathways. Therefore, it is suggested that IL-1RN plays an important role in maintaining the normal condition of cells, and a loss of protein expression could result in an increased risk of the development of HNC [11-16].

The II allele of the IL-1RN VNTR polymorphism has been revealed to be significantly associated with various diseases. Human IL-1RN II allele homozygosity results in a proinflammatory immune response that is more severe and more prolonged than that of the long alleles of IL-1RN [13]. The present study showed no significant association between the IL-1RN VNTR polymorphism and the II/II genotype (II allele homozygosity) because this genotype did not present in the HNC samples (0%) and was found in only 1 out of 110 healthy controls (0.9%); although II alleles do present in the heterozygote genotypes.

Two previous studies on the IL-1RN VNTR polymorphism’s association with nasopharyngeal cancer and oral squamous cell carcinoma were compared with this study, as shown in Table 2. The difference in significance could be partially due to the difference in ethnicity of the studied populations (Portuguese, North Indian, and Indonesian), which could also influence the genetic risk factors. Individuals’ susceptibility to cancer could be different depending on their ethnicity and other environmental factors, such as smoking and alcohol consumption [17]. The total samples of the Portuguese and North Indian studies were also larger than that of our study: 657 samples [6], 270 samples [9], and 200 samples, respectively. These sample sizes could be another critical point to revealing a more significant association between otherwise similar patients with cancer and controls. Any studies related to HNC and the IL-1RN VNTR polymorphism are advised to extend and count the sample size statistically. Determining a more specific type of cancer could also affect the significance of the study, given the IL-1RN VNTR gene polymorphisms in various types of HNC could be different from one another.

Table 2. Comparison of studies on the association between the IL-1RN VNTR gene polymorphism and head and neck cancer.

| Population       | Controls (n) | Cases (n) | Significance    | HNC                          | Ref.   |
|------------------|--------------|-----------|-----------------|------------------------------|--------|
| Portuguese       | 433          | 224       | Significant     | Nasopharyngeal cancer        | [7]    |
| North Indian     | 140          | 130       | Significant     | Oral squamous cell carcinoma | [10]   |
| Indonesian       | 110          | 90        | Not significant | Various types of head and neck cancer | This study |

In the studied Indonesian population samples, no significant association was found between the tested healthy controls and the patients with HNC.

5. Conclusion
The IL-1RN VNTR polymorphism was not associated with head and neck cancer in the studied Indonesian population.

Abbreviations
HNC : Head and neck cancer
OR : Odds ratios
PCR : Polymerase chain reaction
VNTR : Variable number tandem repeat
References
[1] Rettig E M 2015 Epidemiology of head and neck cancer Surg. Oncol. Clin. N. Am. 24 379-96.
[2] Kurniasari F N, Surono A and Retno Pangastuti I 2015 Status Gizi sebagai Prediktor Kualitas Hidup Pasien Kanker Kepala dan Leher J. Hum. Nutr. 2 60-67.
[3] Ridge J A, Glisson B S, Horwitz E M and Meyers M O 2008 Head and neck tumors. In: Richard P, Kevin A C, Lawrence D W, William J H. 11th ed. Cancer Management: A Multidisciplinary Approach book (United Kingdom: Cmp United Business Media) p 39-89.
[4] Toporcov T N, et al. 2015 Risk factors for head and neck cancer in young adults: a pooled analysis in the INHANCE consortium. Int. J. Epidemiol. 44 169-85.
[5] Cognetti D M, Weber R S and Lai S Y 2008 Head and neck cancer: an evolving treatment paradigm. Cancer 113 1911-32.
[6] Sousa H, et al. 2013 IL-1RN VNTR polymorphism as a susceptibility marker for nasopharyngeal carcinoma in Portugal. Arch. Oral Biol. 58 1040-6.
[7] Lee K M, et al. 2006 Genetic polymorphisms of interleukin-1 beta (IL-1B) and IL-1 receptor antagonist (IL-1RN) and breast cancer risk in Korean women. Breast Cancer Res. Treat. 96 197-202.
[8] Hu Z, et al. 2006 Allele 2 of the interleukin-1 receptor antagonist gene (IL1RN*2) is associated with a decreased risk of primary lung cancer. Cancer Lett. 236 269–275.
[9] Gupta M K, Sagar N, Pant R and Banerjee M 2016 Cytokine gene polymorphisms and their association with oral Squamous cell carcinoma (oscc): a north Indian study. Eijmr. 3 550-558.
[10] Auerkari E I, Suryandari D A, Umami S S, Kusdhany L S, Siregar T W, Rahardjo T B, Talbot C and Hogervorst E 2014 Gene promoter polymorphism of RUNX2 and risk of osteoporosis in postmenopausal Indonesian women Sage Open Med. 2 2050312114531571.
[11] Witkin S S, Gerber S and Ledger W J 2002 Differential characterization of women with vulvar vestibulitis syndrome. Am. J. Obstet. Gynecol. 187 589–594.
[12] Zhang Y, Liu C, Peng H, Zhang J and Feng Q 2012 IL1 receptor antagonist gene IL1-RN variable number of tandem repeats polymorphism and cancer risk: a literature review and meta-analysis Plos One 7 e46017.
[13] Shiiba M, et al. 2015 Interleukin-1 receptor antagonist (IL1RN) is associated with suppression of early carcinogenic events in human oral malignancies. Int. J. Oncol. 46 1978-84.
[14] Mclean M H and El-omar E M 2014 Genetics of gastric cancer. Nat Rev Gastroenterol Hepatol Nat. Publ. Gr. 11 664-674.
[15] Xu H, Ding Q and Jiang H 2014 Genetic polymorphism of interleukin-1A (IL-1A), IL-1B, and IL-1 receptor antagonist (IL-1RN) and prostate cancer risk Asian Pac. J. Cancer Prev. 15 8741-7.
[16] Arend W P, Malyak M and Guthridge C J 1998 Interleukin-1 receptor antagonist: role in biology Annu. Rev. Immunol. 16 27-55.
[17] Garte S 1998 The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1 Carcinoqenesis 19 1329-32.