Distribution of Interleukin-6 Gene Polymorphism (-174 G/C) in Head and Neck Cancer Patients in an Indonesian Population: A Preliminary Study

Frisky Amanda Putri[1], Yurnadi Hanafi Midoen[2], Niniarty Djamal[1], Nurtami Soedarsono[1],Winoto Soehartono[1], Elza Ibrahim Auerkari[1*]

1. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
2. Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

Abstract

The aim of this study was to investigate whether the IL-6 (-174G/C) polymorphism is also associated with the susceptibility to head and neck cancer (HNC) in an Indonesian population. The study was conducted on Indonesian samples, with 50 samples of healthy controls and 50 samples of head and neck cancer (HNC) patients. The -174G/C polymorphism of the IL-6 gene in HNC patients and healthy controls was genotyped by the PCR-RFLP method. The genotype and allele distributions were analyzed by the chi-square test for potential association with HNC susceptibility. The GG genotype was the most common variant in the HNC and control groups, with only 2 GC genotypes found in the HNC group. No significant difference regarding the IL-6 (-174G/C) polymorphism was found between HNC patients and healthy controls. The genotype distribution of the IL-6 (-174G/C) promoter gene polymorphism showed no significant differences between the groups of HNC patients and healthy controls.

Clinical article (J Int Dent Med Res 2017; 10(Special Issue): pp. 551-554)

Keywords: Interleukin 6; Polymorphism; Distribution; Head and Neck cancer; Indonesia

Received date: 14 August 2017 **Accept date:** 16 September 2017

Introduction

Head and neck cancer is a common type of cancer worldwide, with an incidence of this cancer reaching 550,000 cases per year with 300,000 deaths. Overall, 90% of the cases of this cancer are squamous cell carcinoma types. This type of carcinoma is the 6th most common cancer worldwide.[1]

Head and neck squamous cell carcinoma can occur in the oral cavity, pharynx, larynx, sinus, paranasal cavity, nasal cavity, and salivary gland. The malignancy originates from proliferation of squamous cells from the epithelial cells. It is counted as a common cancer type worldwide, with a higher risk in males, and it usually attacks individuals in their 50s and older.[1]

In Indonesia, this type of cancer is not common. However, people suffering from this type are usually in the latest stages of the disease. This late diagnosis means that more than 14,000 people are suffering from this type of cancer in Indonesia.[2]

Head and neck squamous cell carcinoma is a multi factorial disease that involves some factors in the process of its progression. These factors make changes in the DNA of the original cell. The factors that are involved in cancer pathogenesis are divided to internal and external factors, with genetics and growth included in the internal list and viruses, drugs, radiation, trauma, and diet included in the external ones. These factors can affect a cell individually or in combination with one another.[3]

Cellular activity contributes greatly to cancer progression, beginning with genetic or epigenetic abnormalities in a cell. Genetic abnormalities are abnormalities that affect the DNA sequence, which can change the instructions within the cell or the expression shown. The epigenetic abnormalities are the changes that happen in gene expression without any changes found in the DNA sequence. The most common abnormalities are mutation and polymorphism.[4]

Genetic abnormalities, especially polymorphisms, can progress to cancer, as

Corresponding author:
Elza Ibrahim Auerkari
Department of Oral Biology
Faculty of Dentistry, Universitas Indonesia
E-mail: eauerkari@yahoo.com

confirmed by many studies. This process is believed to have a tight relation with the changes in the cell cycle affected by polymorphisms in the DNA sequence. The cell might be grow uncontrollably, be unable to repair itself, or may not die at the time it should because the cell has received a changed internal instruction due to the polymorphism. These changes will later create more dangerous abnormalities that usually show up as unstoppable proliferation that is later known as cancer.[5,6]

Interleukin 6 (IL-6) is believed to play a contributory role in the progression and severity of head and neck cancer. Some studies performed to confirm the occurrence of the polymorphism in the promoter of this gene have shown a significant association with cancer. Some have reported that polymorphism of IL-6 promoter -174G/C has a significant association with cancer susceptibilities.[7]

This study was conducted to determine the possible association between the IL-6 promoter -174G/C polymorphism and head and neck cancer in an Indonesian population.

Materials and Methods

Samples

This study used stored DNA samples extracted from blood serum and paraffin embedded specimens of 50 head and neck cancer patients and 50 healthy subjects. These samples were stored at -20°C in the oral biology laboratory of the Faculty of Dentistry, Universitas Indonesia. This study has been approved by the institutional review boards at the Universitas Indonesia.

Genotyping

Genotyping of IL-6 -174G/C (rs1800795) was performed by the polymerase chain reaction (PCR) and the restriction fragment length polymorphism (RFLP) method. The primers used in this study were F: 5'–TTGTCAAGACATGCCAAAGTG-3' and R: 5'–TCAGACATCTCCAGTCCTATA-3'.

The PCR reaction was carried out in 25µl of a reaction mixture containing 100 ng/µL genomic DNA, 12.5 µL Taq polymerase (KAPA Mastermix), 5 µmol forward primer, 5 µmol reverse primer, and 11 µL ddH_2O and was performed using standard PCR. The conditions used were: initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min. The 300 base pair (bp) PCR product obtained by this method was visualized using Geldoc to check the products after electrophoresis for 30 minutes at 80V in a 1.5% agarose gel.[8-10]

The 300 base pair PCR product was then digested overnight with 2.5 units of Nla III (Thermo science) restriction enzyme at 37°C and inactivated at 65°C for 20 min. to yield DNA fragments of 13, 54, and 233 bp in the case of the G allele and 13, 54, 111, and 122 base pairs in case of the C allele. The restriction products were electrophoresed in a 3% agarose gel (1 × TAE buffer, voltage 70 V, 40 min) gel stained with GelRed.

The distribution of genotypes and alleles were then analyzed for Hardy Weinberg equilibrium and Fisher's test with the SPSS program to determine the significance of the genotype/allele distributions among the two groups in the population.

Results

The PCR reaction produced a 300 bp product (figure 1) and digestion by the Nla III enzyme and agarose gel electrophoresis identified 233, 54, and 13 bp bands for the wild-type G allele and 13, 54, 111, and 122 bp bands for the C allele (Figure 2). The genotype variations in this population are shown in table 1.

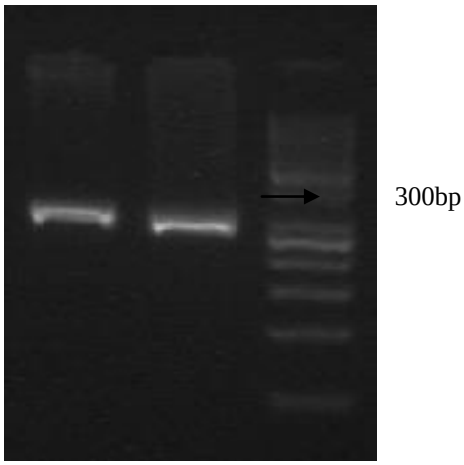

Figure 1. PCR analysis of *IL-6* gene
Lane 1 and 2 represent the visualised PCR product.

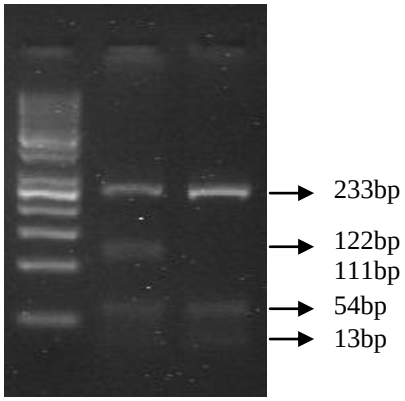

Figure 2. RFLP analysis of *IL-6* -174G/C polymorphism.

Lane 1 represents the GC genotype with (233, 122, 111, 54, 13) bp fragment length and lane 2 represents the GG genotype with (233, 54, 13) bp fragment length.

Table 1: Genotypes and Alleles Distribution of Indonesian Population.

	Healthy Controls N=66	Head and Neck Cancer N= 131	TOTAL
Genotype Homozygote GG	50	48	98
Heterozygote GC	-	2	2
Homozygote mutant CC	-	-	-
Allele			
Allele G	100	98	198
Allele C	-	2	2

Discussion

This study was conducted to determine the association between the IL-6 -174G/C promoter gene polymorphism and head and neck cancer in Indonesia. The C alleles were so rare in the Indonesian population that they showed up in only 2 patients. A low frequency of C alleles was also reported in some studies done in Asian populations. The reason for the C allele rarity remains unclear.[7]

The association between the IL-6 -174G/C promoter gene polymorphism and cancer could indicate a protective effect against this cancer or the risk of it. However, what actually was observed from this genetic abnormality was the IL-6 serum level. The C allele carriers are believed to have decreased serum levels, while the G allele carriers (the most common) have an increased IL-6 serum level. The polymorphic allele was suspected to increase inflammation in the affected cells while down regulating the anti-inflammatory activity.[11-13]

This study supports some studies and contradicts others conducted on other populations. The previous studies show varied results, even though some were performed on the same populations. Vairaktaris et al. examined a Caucasian population and concluded that IL-6 had a strong association with the risk of oral cancer.

Another study done by Singh et al in India also found a significant association between IL-6 -174G/C promoter gene polymorphism and oral cancer. By contrast, and in support of the results of the present study, Xu et al., Yu et al., and Tian et al., found no significant association between IL-6 -174G/C promoter gene polymorphism and various cancers in the Asian population.[7,11] For comparison, the data of some studies done are shown in table II. Various results of studies were affected by other factors that may attach the polymorphism, such as ethnicity.[7,11]

Conclusion

No significant association was found between the IL-6 -174G/C promoter gene polymorphism and head and neck cancer in an Indonesian population. This finding suggests that the wide distribution of the G allele exhibited by the Indonesia population means that the IL-6 serum in this population is quite high on average and no malignancy development is expected from this promoter gene.

Acknowledgement

The authors wish to gratefully acknowledge the financial support from the Indonesian Ministry of Education through the Universitas Indonesia (EIA). The publication of this manuscript is supported by Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest.

References

1. Union for International Cancer Control. 2014 Review of Cancer Medicines on the WHO List of Essential Medicines; 2014:1-8.
2. Kurniasari FN, Surono A, Pangastuti R. Status Gizi sebagai Prediktor Kualitas Hidup Pasien Kanker Kepala dan Leher. Indonesian Journal of Human Nutrition2015;2(1):60–7.
3. Mehrotra R, Yadav S. Oral Squamous Cell Carcinoma: Etiology, Pathogenesis and Prognostic Value of Genomic Alterations. Indian J Cancer 2006;43(2):60–6.
4. The Center for Ecogenetics & Environmental Health. Fast Facts about Epigenetic; 2011. Available at: http://depts.washington.edu/ceeh/downloads/FF_Epigenetics.pdf
5. da Silva SD, Ferlito A, Takes RP, et al. Advances and Applications of Oral Cancer Basic Research. Oral Oncol 2011;47(9):783–91.
6. Nogueira GA, Lourenço GJ, Oliveira CB, et al. Association between Genetic Polymorphisms in DNA MismatchRepair-Related Genes with Risk and Prognosis of Head and NeckSquamous Cell Carcinoma. Int J Cancer 2015;137(4):810-8.
7. Tian G, Mi J, Wei X, et al. Circulating Interleukin-6 and Cancer: A Meta-Analysis Using Mendelian Randomization. Sci Rep 2015;5:11394.
8. Gupta S,Nigam K, Bhatt MLB, Abbas Ali Mahdi. The Interleukin-6-174 G/C and 572 G/C Promoter Polymorphism Associated with High Risk of Oral Pre Cancer and Cancer in North Indian Population. International Journal of Innovations in Biological and Chemical Sciences 2016;8:13-24.
9. Du Y,Gao L, Zhang K, Wang J. Association Of The IL6 Polymorphism rs1800796 with Cancer Risk: A Meta-Analysis. Genet Mol Res 2015;14(4):13236-46.
10. Tanjaya J, Auerkari EI. IL-1 β Genetic Polimorphism in Menopause Women as Periodontal Disease Risk Factor. J Dent Indones 2011;18(1):1–5.
11. Singh PK, Chandra G, Bogra J, et al. Association of Interleukin-6 Genetic Polymorphisms with Risk of OSCC In Indian Population. Meta Gene 2015;4:142–51.
12. Zhao K, Xu J, Tian H. Correlation Analysis between an IL-6 Genetic Polymorphism and Non-Small Cell Lung Cancer Prognosis. Genet Mol Res 2016;15(1):15017021.
13. Yeh KY, Li YY, Hsieh LL, Chen JR, Tang RP. The -174 G/C Polymorphism in Interleukin-6 (IL-6) Promoter Region is Associated with Serum IL-6 and Carcinoembryonic Antigen Levels in Patients with Colorectal Cancers in Taiwan. J Clin Immunol 2010;30(1):53–9.