**MDM2 SNP309 T>G Gene Polymorphism in Head and Neck Cancer in an Indonesian Population**

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**Abstract**

Murine double minute 2 (MDM2) is a negative regulator of the tumor suppressor p53. Studies have reported that the MDM2 SNP309 T>G polymorphism increases the susceptibility to head and neck cancer (HNC). In particular, the GG genotype of SNP309 in the MDM2 promoter region was found to increase the affinity of the Sp1 transcription factor, leading to higher levels of mRNA and MDM2 protein, thereby reducing the activity of p53 and potentially promoting the development of cancer.

This study was conducted to compare the status of the polymorphism in 50 patients with HNC and 50 healthy individuals among an Indonesian population using the polymerase chain reaction-restriction fragment length polymorphism technique. Statistical analysis using Fisher's exact test was performed to analyze the significance of the observed differences in frequencies between the two groups.

Results demonstrated the presence of all genotype variants of the polymorphism, but there were no significant differences in the genotype or allele frequencies between patients with HNC and healthy individuals among the tested Indonesian population.


**Keywords:** Genetic polymorphism, MDM2 SNP309 T>G, head and neck cancer.

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**Introduction**

Cancer is one of the most common causes of death in the world, with approximately 14 million cases and 8.2 million deaths reported every year.¹ The incidence of cancer has been estimated to increase by up to 70% in the next two decades.² In Indonesia, the prevalence of cancer has been reported to be 1.4% among all age groups.³

Head and neck cancer (HNC) generally occurs in the upper aerodigestive tract, such as the oral cavity (40%), the pharynx (15%), the larynx (25%), and the adjoining tissues such as the salivary glands (20%).⁴ HNC has been found to be more frequent in men than in women (2:1 to 4:1). Its prevalence in Indonesia is high, ranking the fourth position among all malignancies. The most frequent type is squamous cell carcinoma, representing >90% of the cases.⁵ Although smoking and drinking are the primary carcinogenic risk factors associated with HNC, there is also a genetic component in the etiology.⁶-⁸ Single nucleotide polymorphisms (SNPs) represent the most common type of human genetic variation and affect a single nucleotide, with a frequency >1% in humans. Some SNPs have been found to be risk factors for cancer.⁹,¹⁰ Yu et al. (2011) reported that the GG genotype causes an increase in the levels of the protein murine double minute 2 (MDM2), a decrease in p53 levels, and a decrease in apoptosis (which depends on the p53 command) in response to DNA damage. Overexpression of MDM2 can also be involved in the downregulation of other important cellular proteins that can promote carcinogenesis.¹¹

Yu et al. (2011) conducted a study on HNC using 1083 samples of patients with squamous cell carcinoma head and neck (SSCHN) and 1090 cancer-free control subjects and found no significant difference in the

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genotype distribution of polymorphisms of MDM2 and p53 codon 72 between patients with SSCHN and control subjects.\(^\text{11}\)

In the present study, we particularly considered the \textit{MDM2} SNP309 T>G polymorphism and its potential association with HNC. The expected impact of the polymorphism is its influence on the level and function of p53 that protects genome integrity by promoting cell cycle arrest and apoptosis in cells that suffer carcinogenic damage. In particular, the GG genotype of \textit{MDM2} SNP309 T>G in the promoter region is expected to increase the affinity of the Sp1 transcription factor, thereby overexpressing MDM2, reducing the level and function of p53, and potentially enhancing carcinogenesis.\(^\text{10,11}\)

Therefore, the aim of this study was to compare the status of \textit{MDM2} SNP309 T>G polymorphism in patients with HNC and healthy individuals among an Indonesian population.

\textbf{Materials and methods}

This study included 50 samples from patients with HNC and 50 samples from subjects without a history of cancer. DNA suspensions extracted from blood samples\(^\text{12-14}\) and stored at \(-20^\circ\text{C}\) in the Laboratory of Oral Biology, Faculty of Dentistry, University of Indonesia, were used in this study. This study was approved by the ethical committee of the Faculty of Dentistry, Universitas Indonesia.

Genotyping of \textit{MDM2} SNP309 T>G polymorphism was conducted by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The specific primers used were forward 5′-CGG GAG TTC AGG GTA AAG GT-3′ and reverse 5′-AGC AAG TCG GTG CTT ACC TG-3′. The resulting 352-bp fragment was amplified using a 25-μl reaction mixture containing 12.5 μl KAPA Taq Polymerase, 0.3 μl (10 μmol) forward primer, 0.3 μl (10 μmol) reverse primer, 0.3 μl (1 ng/μl) DNA template, and 11.6 μl ddH\(_2\)O. The PCR protocol included an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 53.1°C for 45 s, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR product was separated by electrophoresis on a 1.5% agarose gel at 50 V and 400 mA for 45 min.

For RFLP, the product was digested using an 18-μl reaction mixture containing 10 μl of the PCR product, 1 U of \textit{MspA1} I restriction enzyme in 2 μl of 1 × CutSmart Buffer, and 5.9 μl ddH\(_2\)O. The digested products were separated by electrophoresis on a 3% agarose gel at 50 V and 400 mA for 45 min. The wild-type homozygote genotype (TT) produced three bands (233, 187, and 31 bp), the variant heterozygote genotype (TG) produced five bands (233, 187, 88, 46, and 31 bp), and the variant homozygote genotype (GG) produced four bands (187, 88, 46, and 31 bp).

Statistical analysis using Fisher’s exact test in SPSS v.23 was conducted to compare the distribution of genotypes and alleles in the HNC group and the healthy control group, assuming a significance at \(p < 0.05\). Chi-square test was conducted to assess the compatibility of the genotypes with the Hardy–Weinberg equilibrium.

\textbf{Results}

Examples of the PCR products and the RFLP results are depicted in Figures 1 and 2, respectively.

\textbf{Figure 1.} Visualised PCR products of \textit{MDM2} SNP309 T>G: lanes 1, 2, 3, and 5 show bands at 352 bp, and lane 4 is a 50-bp DNA ladder marker.

\textbf{Figure 2.} PCR-RFLP patterns of polymorphisms of MDM2 SNP309 T>G with MspA1 I enzyme showed using Gel Doc. MDM2 PCR products TT (233, 88, and 31 bp); TG (233, 187, 88, 46, and 31 bp); GG (187, 88, 46, and 31 bp).
Results of the Fisher’s exact test revealed no significant differences in the distribution of genotypes (p = 0.356) or alleles (p = 0.396) between the HNC and control groups. In addition, the genotype distributions were consistent with the Hardy–Weinberg equilibrium.

Table 1. Frequency Distribution of MDM2 SNP309 Gene Polymorphism.

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>HNC (n = 50)</th>
<th>Control (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (wild-type homozygote)</td>
<td>15 (30%)</td>
<td>10 (20%)</td>
<td>0.356</td>
</tr>
<tr>
<td>TG (variant heterozygote)</td>
<td>45 (45%)</td>
<td>52 (52%)</td>
<td>0.396</td>
</tr>
<tr>
<td>GG (variant homozygote)</td>
<td>55 (55%)</td>
<td>48 (48%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Genotype Distribution and MDM2 SNP309 Gene Allele in Patients with HNC and Healthy Individuals.

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>15</td>
</tr>
<tr>
<td>TG</td>
<td>22</td>
</tr>
<tr>
<td>GG</td>
<td>13</td>
</tr>
<tr>
<td>Allele T</td>
<td>52</td>
</tr>
<tr>
<td>Allele G</td>
<td>48</td>
</tr>
</tbody>
</table>

As shown in Tables 1 and 2, the polymorphic variants occurred in both subject groups, with no significant differences. The genotype frequencies for HNC patients were 30% TT, 44% TG and 26% GG, and for healthy individuals 20% TT, 50% TG and 30% GG.

**Discussion**

This study was conducted to assess the genotype and allele distribution of MDM2 SNP309 T>G polymorphism in patients with HNC and healthy individuals among an Indonesian population. Single nucleotide polymorphisms (SNPs) in the p53 pathway such as p53, MDM2 and p21, were reported to be associated with cancer risk and pathogenesis, because they play a crucial role in DNA damage and genomic instability.15

In general, the polymorphic variant introducing the G allele is considered to be associated with an early onset of cancer, including HNC, through increased affinity of the Sp1 transcriptional factor, elevated levels of MDM2 mRNA and protein, and reduced p53 activity.10 Furthermore, overexpression of the polymorphic variant could be involved in the downregulation of pRb, E2F1, and p19ARF, further promoting carcinogenesis.16

Some previous studies found significant differences in the polymorphic genotype frequencies between the HNC and healthy control groups among some populations. On the other hand, Yu et al. (2011) found no significant differences in a Mexican population, similar to the present study. Nevertheless, they suggested that the G allele could be associated with the early onset of carcinogenesis.11 Another study conducted by Xiao et al. (2010) demonstrated significant differences between the HNC group and healthy individuals.17 Furthermore, Alhadyan et al. (2012) reported that the heterozygous and homozygous variants of the polymorphism were less frequent in patients with HNC than in healthy individuals among a Saudi Arabian population. They suggested that the variant has a protective effect.18 In the present study, we also found that the polymorphic variants were less frequent in patients with HNC than in healthy individuals, which indicates the protective effect of the variant in the Indonesian population. The reasons for the inconsistent results between the various studies are unknown; however, the frequencies of the polymorphic variants vary between different ethnic groups. Ethnic differences may well have affected the variable results in the studies.17-18

**Conclusions**

This study demonstrates the presence of all genotype variants of the MDM2 SNP309 T>G polymorphism, but there were no significant differences in the genotype or allele frequencies between patients with HNC and healthy individuals among the tested Indonesian population.

**Acknowledgments**

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Declaration of Interest

The authors report no conflict of interest.

References