The chemical changes in the total antioxidant status and biological activity of GSTP1 polymorphism on nasopharyngeal carcinoma patients

F Farhat¹*, M I Sari², J Chrestella³ and R P Syari⁴

¹ Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
² Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
³ Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
⁴ Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

*E-mail: farhat@usu.ac.id

Abstract. Oxidative stress is associated with cancer, including NPC, which is developed by the relation of genetic alteration, EBV infection, and environmental risk. Ile/Val genotype of GSTP1 polymorphism decrease enzyme functions and result in lower total antioxidant status. This study aimed to identify the association of GSTP1 polymorphism and the TAS level. This was a cross-sectional design study. There were 29 NPC patients with their blood as the samples of the study. Blood as the samples for identifying GSTP1 polymorphism by PCR-RFLP-electrophoresis method and measurement of TAS using the ELISA method. There were 21 (72.4%) NPC patients with Ile/Val polymorphism and lower TAS. We found there was no significant association of GSTP1 polymorphism and TAS. Future research with larger samples and adding data about environmental risk such as smoking, alcohol consumption, and toxic substances exposure or multivitamin consumption of the patients can reveal more definitive results. The study may be used as references for determining antioxidant therapy in NPC as well as prevention. The identifying genetic risk factor may help the prevention of NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) has a unique geographic distribution. It is rare in most regions of the world but common in South China, Southeast Asia, North America, and Arctic [1]. In 2018, the prevalence of NPC in the world was 4.75%, with 129,079 new cases and 72,987 deaths [2]. The highest incidence and mortality were found in Asia, about 84.6%, followed by Africa, about 7.4% [3]. Indonesia was the first rank for the highest NPC incidence among Southeast Asia region in 2018, with the incidence rate of 51.88% [4].

Cancer is known to be associated with oxidative stress. Several etiology of many types of malignant diseases share similar mechanisms, which is oxidative stress [5]. Oxidative stress occurs due to the imbalance of reactive oxygen species (ROS) and the antioxidant system [6]. Polymorphism of enzyme works in oxidative stress inhibition, which is glutathione S-transferases (GSTs), had been known to be associated with NPC [7]. GSTP1 is part of GSTs included in the antioxidant defense system by eliminating reactive oxygen species (ROS), which inhibit oxidative stress [5,6].

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There was some research about the association of GST1 polymorphism with cancer [8]. The polymorphism of GSTP1 decreased its function due to the alteration of enzyme conjugating activity leading to dysfunction in reducing ROS [9]. However, carcinogenesis of NPC is a multifactorial process that includes other factors such as dietary habits, smoking, and also Epstein Barr virus (EBV) infection [10]. This environment risk and EBV infection also generate oxidative stress [11-13]. Total antioxidant status (TAS) is one marker for antioxidant defense. Decreased TAS had been found in cancer patients [14,15]. The association of GSTP1 polymorphism, which means dysfunction of antioxidant defense with TAS, had not yet been explored. We did the study to analyze the relation of GSTP1 polymorphism and TAS. The study may be used as references for using antioxidants in preventing and therapy of NPC, especially with individuals carrying GSTP1 polymorphism.

2. Materials and Method

2.1. Patients and Samples

The samples of this study were the blood from 29 NPC patients. We did an analytic study with a cross-sectional design and applied a consecutive sampling method. NPC patients were diagnosed by history taking, physical examination, and nasopharynx biopsy with histopathological examination. Histopathological examination was done in the Laboratory of Pathologic Anatomy of the Faculty of Medicine of Universitas Sumatera Utara. The patients were agreed to be the participant and have been informed about the study. Patients with other malignancies were not included in this study. This study's ethical clearance was given by the Health Research Ethical Committee, Faculty of Medicine, Universitas Sumatera Utara/Adam Malik General Hospital.

The blood was taken from the NPC patients collected in two tubes, one tube with EDTA for analyzing GSTP1 polymorphism and another tube without anticoagulant for measuring TAS. We made the identification of GSTP1 polymorphism and TAS in the Integrated Laboratory of Faculty of Medicine, Universitas Sumatera Utara. Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and electrophoresis were done to identify GSTP1 polymorphism. The blood was undergone DNA extraction before performing PCR. ELISA method was done for measuring TAS.

2.2. PCR analysis and ELISA

Forward primer and reverse primer in the study were 5′GTAGTTTGCCCAAGGTCAAG3′ and 5′AGCCACCTGAGGGTGTAAG3′. Volume reaction for performing PCR was 25 μl that was consisted by 1 μl of forwarding GSTP1 primer, 1 μl of reverse GSTP1 primer, 12.5 μl GoTaq® Green Master, 2 μl of DNA template and 8.5 μl nuclease-free water. The thermal conditions followed the method by Yaghmaei et al., which were 5 min initial denaturing step at 95°C for 30 s, 64°C for 1 min annealing, and 72°C for 6 min elongation[16]. The PCR product then underwent RFLP with BsmA1 as a digestive enzyme. GSTP1 polymorphism was later identified using a UV light transilluminator.

The level of TAS was measured by the ELISA method using total antioxidant status assay kit Sigma Aldrich®. The samples were 10 μl serum that added in samples well of the microplate. Then, 20 μl myoglobin, 150 μl ABTS substrate working solution and 10 ml ABTS substrate solution were added into samples well. Trolox standard and 150 μl ABTS substrate working solution were added into standard wells. Then, the microplate was incubated for 5 minutes at room temperature, and 100 μl stop solution was inserted into the wells. The absorbance was read with a microplate reader.

2.3. Statistical Analysis

The association of GSTP1 polymorphism and TAS was analyzed using SPSS with Fisher's exact test. A significant association means if the p-value is less than 0.05.
3. Results

The characteristics of NPC patients in this study were showed Table 1. There were 29 NPC patients with a common age in the age group of 41-60 years old with 16 (55.2\%) patients. It then was followed by the age group of 21-40 years old, with 11 (37.9\%) patients and the age group of more than 60 years old with 2 (6.9\%) patients. Patients with age under 20 years old was not found in this study. Male was common than females with a ratio of 1.4:1. Based on the histopathological result, non-keratinizing squamous cell carcinoma had a higher frequency with 21 (72.4\%) patients. Keratinizing squamous cell carcinoma was 5 (17.2\%) patients, and undifferentiated carcinoma was 3 (10.4\%).

Table 1. Characterization of NPC patients According to Age, Sex, Histopathological Type and TAS level

| Characteristic                      | n   | %    |
|-------------------------------------|-----|------|
| Age (years)                         |     |      |
| ≤ 20                                | 0   | 0.0  |
| 21 – 40                             | 11  | 37.9 |
| 41 – 60                             | 16  | 55.2 |
| > 60                                | 2   | 6.9  |
| Gender                              |     |      |
| Male                                | 17  | 58.6 |
| Female                              | 12  | 41.4 |
| Histopathological Type              |     |      |
| Keratinizing Squamous Cell Carcinoma| 5   | 17.2 |
| Non-Keratinizing Squamous Cell Carcinoma | 21 | 72.4 |
| Undifferentiated carcinoma          | 3   | 10.4 |
| GSTP1 Polymorphism                  |     |      |
| Ile/Val                             | 21  | 72.4 |
| Ile/Ile                             | 6   | 20.7 |
| Val/Val                             | 2   | 6.9  |
| TAS                                 |     |      |
| <1.535 ± 0.235 mM                   | 21  | 72.4 |
| 1.535 ± 0.235 mM                    | 8   | 27.6 |

Figure 1. Band of GSTP1 polymorphism.
We associated GSTP1 polymorphism with TAS in this study. There were three types of GSTP1 polymorphism: Ile/Val, Ile/Ile, and Val/Val. There were 21 (72.4%) patients with Ile/Val genotype that showed by the presence of a band in 104bp, 222bp and 329bp fragment in this study. Ile/Ile genotype was 6 (20.7%) patients who showed by the presence of a band in 104bp and 329bp fragment. Val/Val genotype was 2 (6.9%) patients, and it was identified by the presence of a band in 104p and 222bp fragment. The band of GSTP1 polymorphism could be seen in Figure 1. TAS levels were mostly reduced in NPC patients in the study. There were 21 (72.4%) patients with lower TAS and 8 (27.6%) with normal TAS. The reference of the normal range for TAS by the TAS assay kit we used was 1.30-1.77 mM. Fisher's exact test resulted in no significant association between GSTP1 polymorphism and TAS in this study with p-value > 0.05. This could be seen in Table 2.

Table 2. Polymorphism of GSTP1 and TAS in NPC

| GSTP1    | TAS          | p-value |
|----------|--------------|---------|
|          | <1.535 ± 0.235 mM | 1.535 ± 0.235 mM | 1.000a |
| Ile/Val  | 15 (71.4%)   | 6 (75.0%) |         |
| Ile/Ile  | 4 (19.0%)    | 2 (25.0%) | 1.000a  |
| Val/Val  | 2 (9.5%)     | 0        |         |

4. Discussion

NPC is developed by the association of genetic alteration, EBV infection, and environment risk [17]. In this study, we analyze the relation of GSTP1 polymorphism and the TAS level. GSTP1 polymorphism is known to be associated with several cancers [8]. One of the GSTP1 polymorphism genotypes is Ile/Val genotype causes reduced enzyme activity and changes substrate specificity [18]. The substitution of isoleucine with valin lower the conjugating activity of GSTP1 [19]. Reduced enzyme activity of GSTP1 affects its function in the detoxification of xenobiots, environmental substances, and carcinogenic compounds [20]. We found 20 (72.4%) NPC patients with Ile/Val genotype, 6 (20.7%) NPC patients with Ile/Ile genotype and 2 (6.9%) NPC patients with Val/Val genotype. The most common GSTP1 polymorphism in this study was Ile/Val genotype. There had been several studies analyzing GSTP1 polymorphism in NPC. Yan et al. found 113 (62.1%) patients with Ile/Val genotype, 55 (30.2%) patients with Ile/Ile genotype and 14 (7.69%) patients with Val/Val genotype [21]. The study by Guo et al. and Cheng et al. also found that Ile/Ile was the most common genotype of GSTP1 polymorphism. All of those studies found there was no significant association of GSTP1 polymorphism and NPC [22,23].

TAS is one of the oxidative stress biomarkers [24]. Oxidative stress occurs due to the imbalance of ROS production and the defense ability of the antioxidant system. The increased ROS and reduced antioxidants are found in several cancers, including head and neck cancer. ROS has several mechanisms in promoting tumorigenesis. Elevated ROS, together with decreased antioxidant inactivate tumor suppressor genes such as p53, activates oncogenes, including Akt, extracellular-regulated kinase (ERK), and c-MYC [25]. Increased ROS also associate with angiogenesis and epithelial-mesenchymal transition (EMT). ROS can be produced by internal factors such as mitochondria, cytochrome P450 metabolism, peroxisomes, and activation of inflammatory cell or external factors such as environmental factors, including non-genotoxic carcinogens, xenobiots, and viruses [6, 26, 27].

There had not been a study about the TAS level in NPC patients. However, the TAS level had been found lower in other cancers, such as breast cancer [14, 15]. In our study, there were 21 (72.4%) NPC patients with lower TAS, and 8 (27.6%) NPC patients with normal TAS. Mahdavi et al. found higher TAS in cancer, including gastrointestinal cancer, head and neck cancer, and lung cancer. This is thought of as the result of antioxidant upregulation as the response of ROS elevation [28]. Patel et al.
also found a higher TAS level in oral cancer patients with squamous cell carcinoma. It also had thought to be associated with multivitamin consumption of the patients[15].

Elevation of ROS increases the antioxidant level and antioxidant defense enzymes. However, ROS itself can also alter the antioxidant function[25]. Dysfunction of the antioxidant system due to ROS add with Ile/Val polymorphism can result in lower TAS of NPC patients. In this study, we found no significant association of GSTP1 polymorphism and TAS with p-value > 0.05. There was no information on environmental risk, which can affect the role of GSTP1 polymorphism and the antioxidant intake of the patients.

5. Conclusions

Mostly NPC patients had lower TAS level and carrying Ile/Val genotype of GSTP1 polymorphism in this study. There was no significant association between GSTP1 polymorphism and TAS. Future research with larger samples and adding data about environmental risk such as smoking, alcohol consumption, and toxic substances exposure or multivitamin consumption of the patients can reveal more definitive results. The study may be used as references for determining antioxidant therapy in NPC as well as prevention. Identifying genetic risk factors may help the prevention of NPC.

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