The genetic variant of GSTP1 and immune response of immunoglobulin A (IgA) on nasopharyngeal carcinoma patients

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Abstract. GSTP1 polymorphism is one of the genetic factors contributed to (NPC). The polymorphism alters its function in detoxifying carcinogen and inhibits oxidative stress. Oxidative stress induces EBV lytic reactivation and enhances IgA response. This study was aimed to analyze the association of GSTP1 polymorphism and serum IgA levels in NPC patients. The study was analytic research with a cross-sectional design. The samples were the blood of NPC patients who underwent PCR-RFLP-electrophoresis and ELISA method to identify GSTP1 polymorphism and IgA level. We found 21 (72.4%) patients with Ile/Val genotype, 6 (20.7%) patients with Ile/Ile, and 2 (6.9%) patients with Val/Val. Patients with elevated IgA levels were 21 (72.4%) patients. In this study, there was no significant association of GSTP1 polymorphism and serum IgA level. We found the GSTP1 polymorphism and serum IgA elevation in nasopharyngeal carcinoma patients, but there was no significant association between them. Future research with the inclusion of environmental risk data is needed as well as larger samples. The study may help to determine effective prevention, treatment, and monitoring therapy of nasopharyngeal carcinoma.

1. Introduction

Indonesia is part of Southeast Asia known as an endemic region for nasopharyngeal carcinoma (NPC). The 5-year prevalence of NPC in Indonesia, according to GLOBOCAN 2018, was 18.4%. There were 17,992 new cases of NPC in Indonesia in 2018. This cancer was the 5th rank for the latest cases and the 6th rank for most death due to cancer in Indonesia. This is the most frequent head and neck cancer [1,2].

NPC has a clear geographic distribution that associated with its etiology. The disease is caused by genetic alteration, environment risk, and Epstein Barr Virus infection [1]. Genetic factor as the risk for NPC such as chromosomal abnormalities, human leukocyte antigen (HLA) polymorphism, and polymorphism of enzymes responsible for carcinogen metabolisms such as Glutathione S-transferases (GSTs) and CYP2E1 [2]. GSTs are the enzyme of phase II metabolism[3]. This enzyme acts in detoxifying carcinogens and oxidative stress inhibition [4,5]. GSTP1 is a type of GSTs, and the
polymorphism of this enzyme had been known to be related to cancer, including breast cancer, head and neck cancer, and hepatocellular carcinoma. The polymorphism, which is Ile/Val genotype, causes enzyme dysfunction leading to cancer development. GSTP1 polymorphism had found to be associated with environmental risk factors such as smoking and tobacco chewing [6].

EBV infection had thought to be the main etiologic factor of NPC. Most populations in the world had been infected with this virus [7]. EBV persists in the human body in the form of latent infection and expresses some latent genes that responsible for carcinogenesis in NPC. However, lytic genes of EBV lytic reactivation is also thought to be associated with NPC [8]. Lytic infection of EBV can trigger immunoglobulin A secretion of B cell in the mucosa-associated lymphoid tissue present in the salivary glands and upper-respiratory and gastrointestinal tracts. It explains the elevated IgA level found in nasopharyngeal carcinoma compared with normal nosapharynx [9,10].

Polymorphism of GSTP1 causes dysfunction of GSTP1 in inhibiting oxidative stress leading to the accumulation of reactive oxygen species (ROS). Besides enhancing carcinogenesis, ROS can act as an inducer of EBV lytic reactivation [5,11]. GSTP1 polymorphism can be indirectly related to elevated IgA levels with ROS as the intermediate variable. There was no study about the association of GSTP1 polymorphism with the IgA level. This study was done to reveal the relation of GSTP1 polymorphism with the IgA level. The understanding of the association of GSTP1 polymorphism with IgA level induced by ROS can help for determining effective prevention, treatment, and monitoring therapy of NPC as well as used to determine prognostic factors of nasopharyngeal carcinoma.

2. Materials and Method

2.1. Patients and samples
This was analytical research with a cross-sectional design. There were 29 of nasopharyngeal carcinoma patients in this study. Samples were blood by nasopharyngeal carcinoma patients, and we used consecutive sampling for sampling technique. The patients were diagnosed by history taking, physical examination, and biopsy. The inclusion criteria in this study were nasopharyngeal carcinoma patients who agreed to participate after being explained about the study and whether the exclusion criteria were patients with other malignancies. Histopathological examination was done in the Laboratory of Pathologic Anatomy of the Faculty of Medicine of Universitas Sumatera Utara. GSTP1 polymorphism and IgA level were identified and measured in the Integrated Laboratory of Faculty of Medicine, Universitas Sumatera Utara.

We used two blood tubes for this study that filled with about 2 ccs of blood. One tube was a blood tube with EDTA for GSTP1 polymorphism analyzing by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method and then electrophoresis. Before, the blood was undergone DNA extraction to get the DNA. Another tube was a blood tube without anticoagulant for measuring IgA level by ELISA method. We used blood serum for the ELISA collected with centrifugation and separation of serum from the plasma. This study was approved by the Health Research Ethical Committee, Medical Faculty of Universitas Sumatera Utara/Adam Malik General Hospital.

2.2. PCR analysis and ELISA
We used the method by Yaghmaei et al. for the identification of GSTP1 polymorphism. The forward primer and reverse primer used in the study were 5’GTAGTTTGGCCCAAGGTCAAG3’ and 5’AGCCACCTGAGGGGTTAGAG3’. There was 12.5 μl GoTaq® Green Master used as the Taq polymerase in this study. This enzyme together with 1 μl of forwarding GSTP1 primer, 1 μl of reverse GSTP1 primer, 2 μl of DNA template and 8.5 μl nuclease-free water were the reaction mix of PCR for each sample. The samples then underwent PCR reaction with thermal conditions 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[12]. We continued the step with the RFLP method, which PCR products were digested by BsmA1 enzyme and resulted in the fragmentation of DNA. Electrophoresis that used 2% of gel agarose was then done, and
the RFLP products were read under the UV light. The genetic variance of GSTP1 was homozygous mutant (val/val) with the presence of a band in fragment 222bp and 104bp, heterozygous genotype (ile/val) with the presence of a band in 329bp, 222bp and 104bp and the homozygous wild type (ile/ile) with the presence of a band in 329 and 104 bp. IgA level was measured by the ELISA method using nasopharyngeal carcinoma patients serum that was added into sample wells of microplate and with standard into standard wells. The microplate was covered with sealed and incubated at 370°C for 30 minutes. The microplate was washed with buffer, and then 100μl HRP Conjugate Reagent was added into standard and sample wells. Then, it was incubated and washed with a buffer then continued with adding 50μl TMB substrate into each well. It was shaken with ELISA shaker and then had been incubated before adding Stop Solution into each sample. A microplate reader used to read the absorbances.

2.3. Statistical Analysis
Fisher's exact test was used to analyze the association of GSTP1 polymorphism and IgA level. The association is significant if the p-value is less then 0.05.

3. Results
This study was done to found the association of GSTP1 polymorphism and IgA level in nasopharyngeal carcinoma patients. In this study, the patients were 29 patients with some characteristics that showed in Table 1. Patients with an age range of 41-60 years old were more common than others, with 16 (55.2%) patients. The age group of 21-40 years old was 11 (37.9%) patients, and the age group of more than 60 years old were 2 (6.9%) patients. There were no patients with age under 20 years old in this study. According to gender, the number of males was dominant over females, with 17 (58.6%) males and 12 (41.4%) females. There were 21 (72.4%) patients who were non-keratinizing squamous cell carcinoma types based on histopathological examination.

| Table 1. Characterization of NPC patients According to Age, Sex, and Histopathological Type |
|---------------------------------|---|---|
| **Characteristics**             | **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 |
| **IgA level**                  |     |      |
| >200 ± 61 mg/ml                | 21   | 72.4|
| <200 ± 61 mg/ml                | 8    | 27.6|
The GSTP1 polymorphism has three types of genotype, which are Ile/Val, Ile/Ile, and Val/Val. Ile/Val genotype showed by the presence of a band in 104bp, 222bp, and 329bp fragment. In this study, there were 21 (72.4%) patients with Ile/Val genotype. It then followed by Ile/Ile genotype, which showed by the presence of a band in 104bp and 329bp fragment with 6 (20.7%) patients. The last was Val/Val genotype with 2 (6.9%) patients. Val/Val genotype showed by the presence of a band in 104 and 222 bp fragments. Figure 1 showed the band of GSTP1 polymorphism under UV light transillumination.

Table 2. Polymorphism of GSTP1 and IgA in NPC

| IgA level       | GSTP1     |          |          | p-value  |
|-----------------|-----------|----------|----------|----------|
| >1.535 ± 0.235 mM | Ile/Val   | 16 (76.2%) | 5 (62.5%) |          |
| ≤1.535 ± 0.235 mM | Ile/Ile   | 3 (14.3%)  | 3 (37.5%) | 0.354a   |
|                 | Val/Val   | 2 (9.5%) | 0        |          |

According to the IgA level, nasopharyngeal carcinoma patients in this study mostly had elevated IgA. The normal value of the IgA level in this study followed the study by Wara et al. [10], which was 200 ± 61 mg/100ml. There were 21 (72.4%) nasopharyngeal carcinoma patients with elevated IgA and 8 (27.6%) patients with normal IgA. We analyze the association of GSTP1 polymorphism and IgA level of nasopharyngeal carcinoma patients in the study, and the result was there was no significant association between those two with p-value = 0.354. This can be seen in Table 2.

4. Discussion

The etiology of nasopharyngeal carcinoma is multifactorial, which are genetic such as chromosomal alteration, epigenetic alteration, HLA class 1 gene, and polymorphism of enzyme for nitrosamine metabolism; EBV infection and environmental risks such as smoking and dietary habit [13,14]. In this study, we analyzed the association of GSTP1 polymorphism and IgA level in nasopharyngeal carcinoma patients. GSTP1 is part of the GSTs enzyme that responsible for biotransformation of toxic xenobiotic, including carcinogen into excretable forms and also inhibits
stress oxidative [15,16]. GSTs are phase II enzymes that have a role in detoxification of activated metabolites of procarcinogens produced by phase I metabolism [17]. GSTP1 gene is located on chromosome 11q13 and its polymorphism had been known to be associated with cancer [6,16].

There were several studies analyze the association of GSTP1 polymorphism with cancer, but it is rare in nasopharyngeal carcinoma. Polymorphism of GSTP1 were classified into three genotype including homozygous for GSTP1a/a (ile105/ile105), heterozygous for GSTP1a/b (ile105/val105), or homozygous for GSTP1b/b (val105/val105)[18]. In this study, the most common type of GSTP1 polymorphism was Ile/Val genotype with 21 (72.4%) patients. The substitution of isoleucine for valine at amino acid codon 105 (Ile105Val) in GSTP1 gene lead to reduced activity of the enzyme. Valine is less stable than isoleucine and the presence of valine result of decrease GSH-binding affinity thus lead to GSTP1 dysnfunction in detoxifying carcinogen [6,17]. Guo et al. in their study found majority of the cases were ile/ile with 172 (65.4%) cases and then followed by ile/val with 79 (30%) cases and 12 (4.6%) cases. Previous study revealed no association of GSTP1 polymorphism with nasopharyngeal carcinoma [19]. The result was similar with Yan et al. and Cheng et al. that found there were no significant different of the risk for nasopharyngeal carcinoma compare with normal persons. Ile/Ile was also the most common genotype of GSTP1 polymorphism in their study [20].

EBV infection is major etiologic factor of nasopharyngeal carcinoma [7]. EBV infects about 90% of adults worldwide. The prevalence of the infection depends on age, geographic location and race/ethnicity [21]. EBV infects human body in two types of infection, lytic and latent infection that help the persistent and lifelong infection of virus in human. Both latent and lytic EBV infection express latent genes such as LMP1, there are EBNA1, EBNA2, LMP2, BARTs, miR-BARTs and lytic genes such as BALF1/BCRF1/BHRF1 that are associated with nasopharyngeal carcinoma [22-24]. EBV lytic infection induce response of immunoglobulin A secretion of B cell in the mucosa-associated lymphoid tissue present in upper-respiratory tracts [9,10]. Previous study by Wara et al. found the elevation of serum IgA in 18 nasopharyngeal carcinoma patients [10]. Similar result was found by Khan et al. which showed elevation of serum IgA in 15 nasopharyngeal carcinoma patients. Elevation of IgA in nasopharyngeal carcinoma patients was thought to be the sign of EBV involvement in nasophryngeal carcinoma development [25]. In our study, we also found serum IgA elevation in 21 (72.4%) nasopharyngeal carcinoma patients.

GSTP1 is part of GSTs that is included in antioxidant enzyme group. Polymorphism of the enzyme affects its defense mechanism against oxidative stress[26]. Cellular stress including oxidative stress is contributed to EBV lytic reactivation [24]. This can be told that GSTP1 polymorphism has indirect association with IgA level. We analyzed the association of GSTP1 polymorphism and IgA level and found there was no significant association between both of them. This can be explained that environmental factors such as smoking, diet and alcohol consumption which are factors that can stimulate oxidative stress were not analyzed in this study whereas these factors are related to the function of GSTP1. Larger samples also may be needed to get definitive conclusion.

5. Conclusion
We found the GSTP1 polymorphism and serum IgA elevation in nasopharyngeal carcinoma patients but there was no significant association between both of them. Future research with inclusion of environment risk data are needed as well as larger samples. The research may be help to determine effective prevention, treatment and monitoring therapy of nasopharyngeal carcinoma.

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