Distribution of stromal cell-derived factor-1 genetic polymorphism in head and neck cancer patients of Indonesian population

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Abstract. Head and neck cancer (HNC), the fourth most common cancer in Indonesia, is associated with several risk factors, including genetic ones. The chemokine Stromal Cell-Derived Factor-1 (SDF-1) contributes to tumor growth, angiogenesis, and metastasis of cancer. Recent studies suggest the G801A genetic polymorphism of SDF-1 as a factor increasing susceptibility to HNC. The aim of this study was to investigate whether the G801A polymorphism of SDF-1 is associated with the susceptibility of HNC in the Indonesian population. Samples from 50 head and neck cancer patients and 50 healthy controls were genotyped by PCR-RFLP method. The distributions of genotypes and alleles were analyzed for the Hardy-Weinberg Equilibrium (HWE) and for the potential association with the head and neck cancer susceptibility by Fisher’s exact test. The study showed no statistically significant difference in the frequencies of SDF-1 G801A polymorphism between the control and case groups. The homozygous variant genotype occurred at low frequency in both cancer and control groups, while the wild type was not less common in the cancer group than in the control group. Unlike in some studies on other Asian populations, the polymorphism was not found to be significantly associated with HNC susceptibility in the Indonesian population.

Keywords: head and neck cancer, SDF-1, polymorphism

1. Introduction

Head and neck cancer (HNC) encompasses different forms of malignancy developing in the head and neck region of the body. This includes cancers of the mouth, salivary gland, nose, sinuses, pharynx, and larynx. In Indonesia, HNC is the fourth most common cancer in men and women, and the second most common cancer in men, with prevalence of 4.7 cases in 100,000 people [1]. There are many risk factors of HNC, and they include a genetic component [2].

Chemokines are a group of chemotactic cytokines that bind to certain G-protein-coupled receptors [3]. As signaling proteins, chemokines secreted by cells are capable in triggering
directed chemotaxis in surrounding responsive cells. It is chemokines’ work that result in the migration of hematopoietic and immune cells to sites of differentiation and inflammation [4]. In cancer cells, chemokines stimulate tumor cell growth, angiogenesis, and metastasis [5 - 7]. The Stromal Cell-Derived Factor-1 (SDF-1) or C-X-C motif Chemokine Ligand 12 (CXCL12) is a C-X-C subfamily of chemokine responsible for chemo attraction of T cells and monocytes [4, 8]. It is expressed in various organs and in cancer [3, 9, 10]. It regulates leukocytes and hematopoiesis trafficking and many essential biological processes, including cardiac and neural development, stem cell motility, neovascularization, and tumorigenesis [3, 11].

The SDF-1 gene is in the chromosomal location 10q11.1 and has a single nucleotide polymorphism (SNP), a Guanine to Adenine (G → A) transition at a position 801 in the 3' untranslated region (UTR). The G801A polymorphism (rs1801157) may stimulate more SDF-1 protein formation, and it has been associated with many diseases such as diabetes, HIV-1 infection, inflammatory diseases, oral cancer, breast cancer, lung cancer, non-Hodgkin’s lymphoma, leukemia, and prostate cancer [3, 6, 9, 12 – 14].

The frequencies of the G801A polymorphism of SDF-1 in the Indonesian HNC patients have not been previously determined. The present study had the following objectives: i) to assess the G801A polymorph frequencies of HNC cases in comparison to those of healthy control cases, and ii) to assess any association between the polymorphism and the HNC susceptibility in an Indonesian population.

2. Materials and methods

2.1. Study population
This study was a laboratory study with descriptive analysis of a total of 100 DNA samples, extracted from blood serum of 50 head and neck cancer patients and 50 healthy subjects. The DNA isolation procedures were supported by previous studies [15, 16]. Samples were stored at -20°C in the Oral Biology Laboratory of the Faculty of Dentistry, University of Indonesia. The ethical committee of the Faculty approved the study and the applied methods.

2.2. Genotyping
Genotyping of SDF-1 G801A polymorphism was performed by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) methods. The primers used in this study were F: 5’-CAGTCAACCTGGGCAAAGCC-3’ and R: 5’-CCTGAGAGTCCTTTTGCGGG-3’ [17]. The PCR reaction was carried out in 25 µl reaction containing 0.3 U genomic DNA, 12.5 µl Taq polymerase (KAPA Mastermix, KAPA Biosystem), 18,75 nM forward primers (IDT), 18,75 nM reverse primers (IDT), and 10.7 µL ddH2O. The PCR conditions involved initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, at 60°C for 1 min, at 72°C for 2 min, and final extension at 72°C for 20 min. The PCR products were electrophoresed in 1.5% Agarose Gel (Thermoscientific) set at 70V, 400mA, for 40 minutes with 50 bp DNA ladder and 6 µL of each PCR product, then visualized using Gel Doc.

For RFLP, the resulting 293 bp PCR product (10 µL) was then digested by 1 U of HpaII (Thermoscientific) in 2 µL of 10x Buffer Tango and 5.9 µL ddH2O, then incubated at 37°C for 16 hours and inactivated at 80°C for 20 minutes. The RFLP products were electrophoresed in 3% Agarose Gel (Thermoscientific) set at 70V, 400mA, for 40 minutes with 50 bp DNA ladder and 6 µL of each RFLP product, then visualized using Gel Doc.

2.3. Statistical analysis
The statistical analyses were performed through IBM SPSS Statistics software version 22 (IBM, NY, USA). The distribution of genotypes and alleles were analyzed for Hardy-Weinberg equilibrium (HWE) using chi-square testing, while potential association between genetic polymorphism and the susceptibility to head and neck cancer was evaluated using the Fisher’s exact test. A p value of less than 0.05 was regarded to indicate statistical significance [18].
3. Results

3.1. PCR-RFLP

PCR-RFLP results (the same for cancer and control groups), the wild type homozygous variant (GG) yielded 100 and 193bp product fragments, the heterozygous genotype (GA) yielded 100, 193 and 293bp product fragments, and the homozygous mutant type (AA) a 293bp product fragment (Fig 1).

![Figure 1. Results of PCR-RFLP: Lane 1- variant homozygote (AA), lane 2-variant heterozygote (GA), lane 3- wild type (GG); on left a 50 bp ladder.]

3.2. Frequency of genotypes and alleles in case groups and control groups

The most common genotypes were wild type homozygous (GG) in the HNC group and heterozygous variant type (GA) in the healthy control group. The most common allele was G allele in both HNC and control groups. The genotype distribution was consistent with the Hardy-Weinberg equilibrium (HWE, p=0.723) for the HNC group, but the same was not the case for the control group (p=0.010). From the Fisher’s exact test, p=0.055 for genotypes was slightly outside the adopted limit of significance, and much more clearly so for alleles with p=0.305, indicating no significant difference in the G801A polymorphism of SDF-1 between the HNC patients and the control group as summarized in Table 1.

| Genotype/Allele | HNC    | Control | P value |
|-----------------|--------|---------|---------|
| GG              | 23 (46%) | 13 (26%) |         |
| GA              | 21 (42%) | 33 (66%) | 0.055   |
| AA              | 6 (12%)  | 4 (8%)   |         |
| A               | 33 (33%) | 41 (41%) |         |
| G               | 67 (67%) | 59 (59%) | 0.305   |

4. Discussion

The observed frequency of the AA genotype appeared quite low in comparison to that of GA genotype, particularly for the control group that in this case was not consistent with HWE. As the deviation from HWE did not apply to the cancer group, it may have been spurious and related to the relatively small sample size. In the present study the AA genotype appeared at a higher frequency in the HNC group than in the control group, but the same was not true for the GA genotype nor for the A allele (Table 1). Also, the wild type GG appeared at higher frequency in the cancer group than in the control group, and this would not be generally expected if the A allele carried elevated cancer risk. Nevertheless, the differences in the genotype or allele frequencies were not statistically significant.

To the knowledge of the present authors, no previous studies have indicated the status of G801A polymorphism of SDF-1 for Indonesian HNC patients or healthy control subjects. Elsewhere, the SDF-1 (G801A) AA and GA genotypes have been previously implicated in the up-regulation of SDF-1 protein to bind its exclusive receptor, CXCR4, and to promote carcinogenesis, neoplastic transformation and metastasis [6, 19, 20]. However, also
conflicting results have been reported, for example suggesting that such cancer-promoting
effect of A allele could be more prominent in Asians than in Europeans, and more significant
in certain cancers (such as breast and lung cancers) than in e.g. oral cancer [21 – 25].

Lack of significant differences in genotype and allele frequencies of SDF-1 G801A
polymorphs between controls and HNC (oral cancer) cases has been reported previously for
Greek and German populations [6]. In contrast, studies on the same genetic polymorphism in
Polish laryngeal cancer patients and Taiwanese oral cancer patients indicated that individuals
with at least one mutated A allele had higher risk in developing cancer compared to those
with the wild type GG genotype [4, 17]. The conflicting results of the studies may have been
due to the differences in the details of sampling, as ethnic origin, type and status of cancer,
and other features including the sample size could have been of importance [21 -25]. In
particular, there have still been relatively few studies on the G801A polymorphism in HNC or
specific cancers within the head and neck region. Cancers within the HNC regime could have
varied in the associated risk not only according to the G801A polymorphism but also with the
specific anatomical site and the status of cancerous growth. For example, increased risk of
benign but not malignant salivary gland tumors has been reported for Chinese population in
association with SDF-1 G801A polymorphism [22]. In case of nasopharyngeal carcinomas,
the AA genotype has been found to be associated with reduced progression-free and reduced
distant metastasis-free survival in the Chinese population [23]. In recent meta-analyses, the
risk-promoting influence of G801A polymorphism appears to have been clear for lung cancer,
but much less so for many other cancers including HNC [21, 24, 25]. While some conflicts
may be solved with larger samples, a fuller picture possibly only by considering the status of
other interacting genetic (and possibly epigenetic) factors. On a positive note, a potential
therapeutic target in HNC could be on offer if the signaling pathway of SDF-1 or
CXCR4/CXCL12 were important in the cancer progression [23].

The interpretation of the present preliminary study was limited by the small sample size
and lacking information about factors such as age, sex, diagnostic details and progression of
cancer. Therefore, it was concluded that larger studies are needed with more attention to these
variables. In comparison, a call for expanded sampling has been a relatively common
conclusion in many other studies on the effect of G801A polymorphism of SDF-1, including
in meta-analyses encompassing HNC [21 -25].

5. Conclusion
This study evaluated the frequencies of the G801A polymorphism of Stromal Cell-Derived
Factor-1 (SDF-1), a candidate indicator on cancer risk, in head and neck cancer patients and
healthy control subjects of the Indonesian population. The homozygous variant genotype
occurred at similarly low frequency in both cancer and control groups, and the wild type
homozygous genotype was not appearing at lower frequency in the cancer group than in the
control group. The results indicated no significant association between G801A polymorphism
of SDF-1 and the susceptibility to head and neck cancer. However, the sample was small and
lacked information on potentially influential factors such as age, sex, diagnosis and details of
cancer progress. It was suggested to extend the study to a wider population sample with more
details on background information.

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References
[1] Kurniasari FN, Surono A and Pangastuti R 2015 Status gizi sebagai prediktor kualitas hidup pasien kanker kepala dan leher *Indones. J. Hum. Nutr.* 2 60–7
[2] Zhang Y, Wang R, Miao L, Zhu L, Jiang H and Yuan H 2015 Different levels in alcohol and tobacco consumption in head and neck cancer patients from 1957 to 2013 *PLoS. One* 10 1–13
[3] de Oliveira KB, Guembarovski R, Oda JMM, Mantovani M, Carrera CM, Reiche E, Voltarelli JC, Herrera ACSA and Watanabe M 2009 CXCL12 rs1801157 polymorphism in patients with breast cancer, Hodgkin’s lymphoma, and non-Hodgkin’s lymphoma *J. Clin. Lab. Anal.* 23 387–93
[4] Kruszyna Ł, Lianeri M, Rydzanicz M, Szyfter K and Jagodziński P 2010 SDF1-3’A gene polymorphism is associated with laryngeal cancer *Pathol. Oncol.* 16 223–7
[5] Sarvaiya PJ, Guo D, Ulasov I, Gabikian P and Lesniak MS 2013 Chemokines in tumor progression and metastasis *Oncotarget* 4 2171–85
[6] Vairaktaris E, Vyliotis A, Spyridonodou S, Derka S, Vassiliou E, Yapijakis C, Serefoglou Z, Neukam F and Patsouris E 2008 A DNA polymorphism of stromal-derived factor-1 is associated with advanced stages of oral cancer *Anticancer Res.* 28 271–6
[7] Wang Y, Chen L, Pan L, Xue J and Yu H 2015 The association between SDF-1 G801A polymorphism and non-small cell lung cancer risk in a Chinese Han population *Int. J. Clin. Exp. Med.* 8 8153–7
[8] Kryczek I, Wei S, Keller E, Liu R and Zou W 2007 SDF-1/CXCL12 and human tumor pathogenesis *Am. J. Physiol. Cell. Physiol.* 292 C987–95
[9] Umapathy D, Krishnamoorthy E, Muthukumaran P and Rajaram R 2013 Stromal cell-derived factor (SDF-1β) gene single nucleotide polymorphism at position G801A is associated with type 2 diabetes mellitus in a South Indian population *Int. J. Genet. Eng.* 3 1–5
[10] Allami RH, Graf C, Martchenko K, Voss B, Becker M, Berger MR, Galle PR, Theobald M, Wehler TC and Schimanski CC 2016 Analysis of the expression of SDF-1 splicing variants in human colorectal cancer and normal mucosa tissues *Oncol. Lett.* 11 1873–8
[11] Zhu K, Jiang B, Hu R, Yang Y, Miao M, Li Y and Liu Z 2014 The CXCL12 G801A polymorphism is associated with cancer risk: a meta-analysis *PLoS. One* 9 1–8
[12] Razmkhah M, Doroudchi M, Ghayumi SMA, Erfani N and Ghaderi A 2016 Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer *Lung Cancer* 49 311–5
[13] Razmkhah M, Talei AR, Doroudchi M, Khalili-Azad T and Ghaderi A 2005 Stromal cell-derived factor-1 (SDF-1) alleles and susceptibility to breast carcinoma *Cancer Lett.* 225 261–6
[14] Hirata H, Hinoda Y, Kikuno N, Kawamoto K, Dahiya A V, Suehiro Y, Tanaka Y and Dahiya R 2007 CXCL12 G801A polymorphism is a risk factor for sporadic prostate cancer susceptibility *Clin. Cancer. Res.* 13 5056–62
[15] Tanjaya J and Auerkari EI 2011 IL-1 β genetic polymorphism in menopausal women as periodontal disease risk factor *J. Dent. Indones.* 18 1–5
[16] Auerkari EI, Suhartono AW, Djamal NZ, Verisqa F, Suryandari DA, Kusdhany LS, Masulili SLC and Talbot C 2013 CRP and IL-1B gene polymorphisms and CRP in blood in periodontal disease *Open. Dent. J.* 7 88-93
[17] Teng YH, Liu TH, Tseng HC, Chung TT, Yeh CM, Li YC, Ou YH, Lin LY, Tsai HAT and Yang SF 2009 Contribution of genetic polymorphisms of stromal cell-derived factor-1 and its receptor, CXCR4, to the susceptibility and clinicopathologic development of oral cancer *Head. Neck.* 31 1282–8
[18] Zar JH 1996 *Biostatistical Analysis* (New Jersey: Prentice-Hall International) p 662
[19] da Silva JM, Soave DF, Santos TPM, Battista AC, Russo RC, Teixeira MM and da Silva TA 2016 Significance of chemokine and chemokine receptors in head and
neck squamous cell carcinoma: a critical review Oral Oncol. 56 8–16
[20] de Oliveira CEC, Cavassin GG de O, Perim A de L, Nasser TF, de Oliveira KB, Fungaro MHP, Carneiro JL do V and Watanabe MAE 2017 Stromal cell-derived factor-1 chemokine gene variant in blood donors and chronic myelogenous leukemia patients J. Clin. Lab. Anal. 21 49–54
[21] Gong H, Tan M, Wang Y, Shen B, Liu Z, Zhang F, Liu Y, Qiu J, Bao E and Fan Y 2012 The CXCL12 G801A polymorphism and cancer risk: evidence from 17 case-control studies Gene 509 228-31
[22] Liu W, Zhu E, Wang R, Wang L and Liu T 2012 CXCL12 G801A polymorphism is associated with an increased risk of benign salivary gland tumors in the Chinese population Med. Oncol. 29 677-81
[23] Chen R et al 2015 CXCL12 genetic variants as prognostic markers in nasopharyngeal carcinoma OncoTargets Ther. 8 2835-42
[24] Meng D, Wu Y, Heerah V, Peng S, Chu M, Xu Y, Xiong W and Xu S 2015 CXCL12 G801A polymorphism and cancer risk: an updated meta-analysis J. Huazhong Univ. Sci. Technol. 35 319-26
[25] Tong X, Ma Y, Deng H, Wang X, Liu S, Yan Z, Peng S and Fan H 2016 The SDF-1 rs1801157 polymorphism is associated with cancer risk: an update pooled analysis and FPRP test of 17876 participants Sci. Rep. 6 27466