The Role of CYP2A6 Genetic Polymorphism in Nicotine Dependence and Tobacco Consumption among Bataknese Male Smokers

Noni Novisari Soeroso¹, Rozaimah Zain-Hamid², Bintang Y. M. Sinaga¹, Ahmad Hamim Sadewa³, Tamsil Syafiuddin¹, Elsina Syahruddin¹, Gino Tann¹, Erna Mutiara³

¹Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Sumatera Utara, Jl. Dr Mansyur No.5 Medan 20155, Indonesia; ²Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Sumatera Utara, Jl. Dr Mansyur No.5 Medan 20155, Indonesia; ³Department of Biochemistry, Faculty of Medicine, Gadjah Mada University, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia; ⁴Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Indonesia, Jl. Persahabatan Raya No.1, Jakarta 13230, Indonesia; ⁵Department of Clinical Pathology, Faculty of Medicine, University of Sumatera Utara, Jl. Dr Mansyur No.5 Medan 20155, Indonesia; ⁶Department of Biostatistics, Faculty of Public Health, University of Sumatera Utara, Jl. Dr Mansyur No.5 Medan 20155, Indonesia

Introduction

Cigarettes are one of the causes of public health problems with an estimated mortality rate of 5 million every year [1]. Nicotine is one of the components of cigarettes which has an important role regarding physical dependence mediated through neuronal nicotinic acetylcholine receptors (nAChRs) [2]. This basic mechanism of physical dependence has been known for a long time. However, there are several other factors which have a role in the pathophysiology of physical dependence on nicotine but cannot be explained in detail as it can be influenced by several multifactorial factors. There are two factors which might influence individuals' physical dependence on cigarettes, namely environmental and genetic factors [3].

The CYP2A6 gene is a gene encoding the P450 2A6 cytochrome which has a role in the physical dependence on nicotine. Furthermore, it is also responsible for 70-90% of nicotine metabolism in the blood into cotinine; thus, it can eliminate or decrease the effect of nicotine to stimulate the brain reward system [4]. The nicotine dependence will be further
assessed using Fagerstrom Tolerance Questionnaire (mFTQ) [5]. On the other hand, the Brinkman Index is used to identify the cumulative number of smoking habits.

In our previous report, the study about the relationship between genetic polymorphism of CYP2A6 and nicotine metabolism in male Batak men smokers with lung cancer was explained. Batak men smokers, which have pure genetic inheritance, used as participants due to their tradition on smoking [6], it can give a proper model for the study related to the genetic factor and the smoking habit.

Thus, this study intends to analyse the relationship between smoking habits of male Batakmen smokers with the Brinkman Index.

Material and Methods

The subjects of this research were 140 Batak men male with a history of smoking, active smokers, and the age > 20 years. The participants involved were recruited from Haji Adam Malik Hospital, USU Hospital, and Elizabeth Hospital in Medan, North Sumatra, Indonesia. The nicotine dependence was measured using seven items of questionnaire modified according to Fagerstrom Tolerance Questionnaire (mFTQ) with its scoring ratings. The interpretation of this questionnaire was as follows: (1) very low nicotine dependence indicated with a score of <4; (2) low nicotine dependence indicated with a score of 5-7; (3) moderate nicotine dependence indicated with a score of 8-9; (4) high nicotine dependence indicated with a score of 10-14; and (5) very high nicotine dependence indicated with a score of ≥15. Also, the smoking status was documented through interviews. The subject can be categorised as an active smoker if he has a smoking history ≥ 100 cigarettes throughout his life [7]. The severity level of smoking can be assessed using the Brinkman Index. The Brinkman Index value was obtained from the multiplication of the average number of cigarettes smoked a day and multiplied by the duration of smoking (years). The value of Brinkman Index (IB) is mild if 0-199, moderate if 200-599, and severe if > 600 [8].

Genotyping of CYP2A6 was conducted using the following primer: 2Aex7F (5’-GRCCAAAGATGCCCTACATG-3’) and 2A6R2 (5’-AAAAATGGGCATGAACGCC-3’) [9]. The blood sample from the subject (0.5 µg), which obtained by employing Puregene DNA Isolation Kit (Promega), was added with PCR mixtures (25 µl) (It contained 1 PCR buffer, 1.5 mM MgCl2, 0.4 µM of each primer, 250 µM dNTPs, and 1 U of Taq DNA polymerase). The initial denaturation was then carried out at 95°C (1 minute). After that, the application was applied with denaturation at 95°C (15 seconds), annealing at 60°C (20 seconds), and extension at 72°C (3 minutes for 35 cycles), followed by a final extension at 72°C (7 minutes). The triple-digestion with restriction enzymes, namely EcoRⅠ, Accl, and StuⅠ, was done on the PCR product. The analysis using electrophoresis at 2% of agarose gel was then applied to the product [10]. Data analysis was performed by using Epi Info-7 software.

Results

Based on the data collected, which also was reported in our previous report, there were 106 subjects aged <65 (75.7%) and 34 subjects aged ≥65 (24.3%) involved in the study. The Brinkman Index obtained was 9.3% for mild, 37.9% for moderate, and 52.9% for severe. Therefore, it was discovered that the average age <65 years was the most commonly found with a severe degree of Brinkman Index value. The nicotine dependence was assessed based on the Fagerstrom score using a special questionnaire. Also, the results showed that 91 people (65%) had a very high Fagerstrom score, 31 people (22.1%) had a high Fagerstrom score, and 18 people (12.9%) had low-moderate Fagerstrom score.

Table 1 showed that individuals with the *1A wild-type alleles were 1.13 times more likely to have very-high high nicotine dependence than the variant alleles (*1B and *4A) although this relationship was not statistically significant.

| Table 1: The Relationship between CYP2A6 Genetic Polymorphism and Nicotine Dependence |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CYP2A6 allele type *1A | Wild type | Variant *1B and *4A | Total | p-value | OR | 95% CI |
| Wild type (109) | 109 | 44.7 | 15 | 41.7 | 0.73 | 1.13 | 0.55-2.29 |
| Variant (135) | 135 | 55.3 | 21 | 58.3 | 0.73 | 0.73 | 0.38-1.44 |
| Total (244) | 244 | 100 | 36 | 100 | | | |

*Logistic Regression Test.

Table 2 showed that there was a significant relationship between the nicotine dependence level and the number of cigarettes consumed (p = 0.015). It can be seen that the higher the level of nicotine dependence, the more the number of cigarettes consumed.

Table 2: The Relationship between Nicotine Dependence and Brinkman Index

| Table 2: The Relationship between Nicotine Dependence and Brinkman Index |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Nicotine Dependence | Severe IB | Moderate IB | Mild IB | p-value |
| High – Very High | 66 | 89.2 | 48 | 90.6 | 8 | 61.5 | 0.015 |
| Low – Moderate | 8 | 10.8 | 5 | 9.4 | 5 | 38.5 | |
| Total | 74 | 100 | 13 | 100 | 53 | 150 | |

*Chi-square test.
Discussion

The results of this study also indicated that there was a significant relationship between CYP2A6 genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of Brinkman Index.

Based on above results, using cigarette smoking as a paradigmatic substance-use problem, these findings suggest that the pathway to dependence is complex. Both genetic and sociocultural factors play a significant aetiological role at the stages of initiation and dependence. For example, social, environmental factors play a major role in the smoking behaviour of Batak people because smoking becomes an important element in various cultural activities and as a treat that must be provided with food and beverages in each series of customary activities.

In conclusion, the results of this study showed that individuals with the *1A wild-type alleles had 1.13 times greater risk of severe-very severe nicotine dependence compared to the variant alleles (*1B and *4A) although this relationship was not statistically significant. Furthermore, there was a significant relationship found between CYP2A6 genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of the Brinkman Index.

References

1. World Health Organization: The Facts About Smoking and Health, May 30, 2006. http://www.wpro.who.int/media_centre/fact_sheets/fs_20060530.htm.
2. Hukkanen J, Jacob P, Benowitz N. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005; 57(1):79-115. https://doi.org/10.1124/pr.57.1.3 PMid:15734728
3. Tyndale RF, Sellers EM. Genetic variation in CYP2A6-mediated nicotine metabolism alters smoking behaviour. Therapeutic Drug Monitoring. 2002; 24:163–171. https://doi.org/10.1097/00007691-200202000-00006
4. O'Brian CP. Drug Addiction and Drug Abuse’. In Brunton LL, Lazo JS & Parker KL, Goodman & Gilman’s. 11th Ed. The Pharmacological Basis of Therapeutics, 2006:607-672.
5. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom test for nicotine dependence, a revision of The Fagerstrom Tolerance Questionnaire. Br J Addict. 1991; 86:1119-27. https://doi.org/10.1111/j.1360-0443.1991.tb01879.x PMid:1932883
6. Siregar AP. Determinan perilaku merokok siswa sekolah dasar di Desa Simatahari Kecamatan Kota Pinang Kabupaten Labuhan Batu Selatan [Tesis]. Universitas Sumatera Utara, 2015.
7. Ryan H, Trosclear A, Groerer J. Adult current smoking: Differences in definitions and prevalence estimates-NHIS and NSDUH 2008. Journal of Environmental and Public Health. 2012; 2012.
8. Perhimpunan Dokter Paru Indonesia (PDPI). Penyakit Paru Obstruktif Kronik (PPOK): diagnosis dan penatalaksanaan. PPOK Books, 2011.
9. Benowitz NL. Cotinine is a biomarker of environmental tobacco smoke exposure. Epidemiol Rev. 1996; 18(2):188-204. https://doi.org/10.1093/oxfordjournals.epirev.a017925 PMid:9021312
10. Nakajima M, Yoshida R, Fukami T, McLeod HL, Yokoi T. Novel human CYP2A6 alleles confound gene deletion analysis. FEBS Lett. 2004; 569(1-3):75-81. https://doi.org/10.1016/j.febslet.2004.05.053 PMid:15225612