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Quit Attempts Among Smokers and its Association with CYP2A6 (rs56113850) Polymorphisms

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Abstract **Introduction:** Nicotine serves as the primary addictive component of tobacco. While it is not a carcinogen itself, it plays an important role in encouraging smoking and ongoing exposure to numerous carcinogenic substances found in tobacco. The half-life of nicotine tends to be extended in individuals with reduced P450 2A6 activity, and smokers with lower enzyme activity frequently reduce either the intensity of their smoking or the number of cigarettes they consume when compared to individuals with 'normal' enzyme activity. Hence, a study assessed CYP2A6 polymorphisms and quit attempts among smokers. **Materials and methods:** 5ml of salivary samples were collected from 100 smokers and 100 nonsmokers who had visited Saveetha Dental College in April- May 2023. DNA isolation was done, followed by PCR analysis and Amplification with RFLP. Quit attempts, duration of quit attempts, and methods employed for smoking abstention were assessed using a questionnaire. The statistical association between quit attempts and CYP2A6 (rs56113850) polymorphism was done using the Chi-square test. **Results:** About 27.9% of smokers had an attempt to quit smoking previously; the rest of smokers (72.1%) did not make an attempt to quit the habit. About 74% smokers have the normal allele (T), the rest 26% have a variant allele (C), about 73% of non-smokers have a normal allele, and the rest 27% have a variant allele. Association between CYP2A6 allele variants and interest to quit scale showed that the normal genotype has a higher scale of interest in quitting tobacco, and the homozygous variant genotype shows the least interest towards quitting tobacco usage, suggesting that there is an association between gene polymorphism and quit attempts. **Conclusion:** In the present study, there is an association between quit attempts and CYP2A6 polymorphisms among the smokers who had visited the dental hospital. However, the association is not statistically significant.

Key Words CYP2A6 gene, Quit attempts, Human well being, Smokers, DNA, Allele, Saliva, Health, Tobacco control

1. Introduction

Tobacco use is a significant global public health concern which is related to respiratory and cardiovascular problems. Tobacco consumption, either direct use of tobacco or passive smoking, is responsible for nearly six million deaths [1]. Tobacco consumption is a significant contributor to preventable fatalities on a global scale, and in India, tobacco use is characterized by the use of both smoking and smokeless tobacco [2], [3]. The nicotine levels in the blood increase quickly after smoking when compared with nicotine absorption after smokeless tobacco use. The quantity of nicotine in the bloodstream is influenced by variables such as the product's pH, the size of tobacco particles, and various other factors [4]. Nicotine dependence depends on the quantity of nicotine administered and the method of delivery. The quicker the delivery, absorption rate, and attainment of elevated nicotine concentrations, the higher the likelihood of addiction. There is variability in the nicotine levels in cigarettes, and the metabolism of nicotine exhibits a role in nicotine dependence. The rate of nicotine metabolism should be related to smoking behaviors, and faster elimination of nicotine is associated with increased smoking and nicotine dependence, [5]. The cytochrome P450 (CYP) enzymes are essential for metabolizing pharmaceuticals and other foreign substances. According to estimates, CYP enzymes play a vital role in clearing the body of nearly half of the drugs that are commonly used [6]. The tobacco ingredient nicotine is principally accountable for the emergence and continuation

of smoking dependency. Nicotine raises dopamine levels by stimulating dopamine release, but cocaine inhibits dopamine absorption, and heroin activates opioid receptors despite both substances raising dopamine levels [7]. Nicotine and its oxidized metabolite, cotinine, are highly metabolized by CYP2A6. Metabolism of Nicotine by CYP2A6 gene is a two-step reaction. The enzyme cytosolic aldehyde oxidase changes nicotine to nicotine iminium ion and then to cotinine. The rate-limiting step in this reaction is the creation of the nicotine iminium ion by CYP2A6, and up to 80% of nicotine is converted to cotinine during this process [8]. This polymorphism is particularly intriguing because CYP2A6 may metabolize nicotine and numerous procarcinogens found in cigarette smokers. Recent research has demonstrated that people with the mutant CYP2A6 allele have different nicotine metabolic kinetics [9]. The cloning and sequencing of the human CYP2A6 gene occurred in 1990. This gene is situated over a 6 kb region comprising nine exons and has been precisely located on the long arm of chromosome 19, specifically between 19q12 and 19q13.2. It resides within a gene cluster spanning 350 kbp alongside the CYP2A7 and CYP2A13 genes, exhibiting significant sequence similarity to the CYP2A6 gene. CYP2A13 is primarily expressed in the nasal mucosa, lungs, and trachea [10]. The half-life of nicotine in human serum spans several hours, while cotinine has a half-life of approximately one day. Many of the symptoms associated with nicotine withdrawal resemble those seen in other drug withdrawal syndromes, including anxiety, disrupted sleep patterns, depression, impaired concentration, impatience, irritability or anger, and restlessness. For most smokers, these withdrawal symptoms typically manifest within the first day of attempting smoking cessation. Typically, the majority of smokers do not succeed in quitting smoking within the initial two weeks of starting a smoking cessation program. Interestingly, the rates of smoking cessation at both 30 days and 90 days were nearly identical in a non-treatment, non-pharmacological group (NTNPG), suggesting that the first week of attempting smoking cessation plays a critical role in achieving success in quitting smoking [11].

Previous research on CYP2A6 alleles aimed to investigate any association between this gene and smoking frequency, smoking habits, and behavioral changes. The findings indicated that neither the daily cigarette consumption nor the age of initial experimentation with smoking showed any significant associations with CYP2A6 genotypes. Furthermore, the CYP2A6 genotypes were not linked to passive smoking exposure among nonsmokers in home or workplace settings. The CYP2A6 polymorphism has only a limited influence on public health since no protective effect was discovered in participants who were heterozygous for the deletion allele, despite the possibility of protection against active smoking habit in those homozygous for the deletion allele. In another study, it was found that heavy smokers had a lower occurrence of the CYP2A6 deletion allele (genotype D) compared to light smokers. At the same time, ex-smokers exhibited a lower prevalence as well [12].

Though CYP2A6 gene polymorphisms were associated with smoking behaviors, depression, and anxiety levels, there is a lack of evidence for quit attempts among smokers. So, the study aims to find the association between quit attempts among smokers and CYP2A6 gene polymorphisms.

2. Materials and Methods

Sample collection

5ml of salivary samples were collected from 100 smokers and 100 non-smokers who had visited Saveetha Dental college in the month of April- May, 2023.

DNA isolation

The collected Samples were centrifuged at 6000 RPM for 5 minutes. The cell collection was done by dissolving the pellet (PBS buffer) and adding a lysis buffer 400 μl, 100 μl 5M of NaCl with 600μl of chloroform. The lysate solution centrifuged at 7000PM for 3 minutes. After centrifugation, the aqueous layer in a fresh tube was collected and ice-cold ethanol was added to equal volume of the aqueous layer for precipitation. The sample was centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded and Pellet was found at the bottom. The pellet was washed with 70% ethanol and centrifuge at the same condition. After the air-drying sample, the elution buffer was added to it. Eluted DNA was checked with Nanodrop plus at A260/280 for quantity and purity.

Genotyping Analysis

Polymerase Chain Reaction: The PCR (Polymerase Chain Reaction) and RFLP (Restriction fragmentation length Polymorphism) methods were used for genotyping analysis. The CYP2A6 primers Forward 5' GCTCCAATCCAGCCTCGTTTAA 3' and reverse 5' TCCAGCCCTTGCAGCAACTG 3' amplify the DNA. The PCR was performed in 20 μl containing 50 ng of genomic DNA, 5 pmol/ul of each primer, and PCR master mix [Takara, Shiga, Japan]. PCR is carried out in a thermal cycler with a condition that includes an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 35 seconds, primer annealing at 58°C for 35 seconds, extension at 72°C for 35 seconds and a final extension at 72°C for 5 min. The Amplicon size of PCR was 344bp. The amplified PCR product was checked on a 1% agarose gel electrophoresis.

RFLP (Restriction Fragmentation Length Polymorphism)

The PCR product was digested with MboII restriction enzyme [New England Biolabs, UK] at 37°C. The digested product was electrophoresed in 2 % agarose with 0.5 μg/ml ethidium bromide and photographed using a gel documentation system.

Statistical analysis

Descriptive statistics and Chi square analysis was done, p-value$>$0.05 is considered as a statistically insignificant dif-

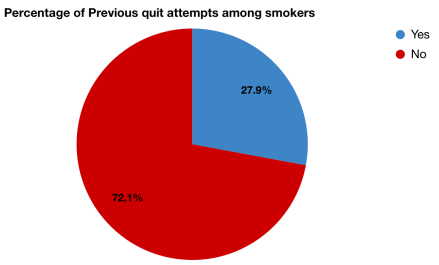

Figure 1: Percentage of previous attempts among smokers who visited the dental hospital.

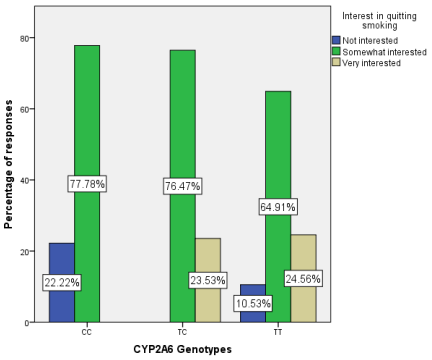

Figure 2: Bar graph showing association between CYP2A6 allele variants and interest to quit among the sample population.

ference.

3. Results

Quit attempts of smoking, interest in quit attempts, and reason for not quitting the smoking habit were assessed among 100 smokers. Among 100 smokers, 25.6% were very interested in quitting their smoking habit, 65.1% were somewhat interested, and about 9.3% were not interested in abstaining from tobacco smoking. Figure 1 shows a pie chart representing the percentage of previous quit attempts among smokers who had visited the dental hospital. About 27.9% of smokers had an attempt to quit smoking previously; the rest of smokers (72.1%) did not make an attempt to quit the smoking habit. Among those who stopped using tobacco, nearly one-fourth of them (26.6%) reported that they gradually stopped the usage of tobacco, 2.2% of them said that they stopped the habit with the support from their family members, 4.4% stopped due to group merge, and 33.3 % stated self-help programs.

Table 1 shows the distribution of the alleles among the study population. About 74% smokers have the normal allele (T), the rest 26% have a variant allele (C), about 73% of non-smokers have a normal allele, and the rest 27% have a variant allele. No difference exists in the distribution of normal and variant alleles of CYP2A6 (rs56113850) among smokers and non-smokers.

Figure 2 shows a bar graph representing the association between CYP2A6 allele variants and responses about their interest in quitting smoking among smokers. The x-axis shows the CYP2A6 gene polymorphism and the y-axis shows the interest in quitting tobacco. A chi-square analysis was done, with a p-value>0.05, meaning no significant difference exists between them. It shows that smokers with the normal genotype have a higher interest in quitting tobacco, and the homozygous variant genotype shows the least interest in quitting tobacco usage, suggesting that there is an association between gene polymorphism and quit attempts. However, this difference is not statistically significant. (p-value - 0.08, chi-square value - 8.128)

4. Discussion

Numerous methods are used to quit the smoking habit, and information on the number of quit attempts made by an individual helps to gain an insight into a person's addictive behavior [13].

Vulnerability to nicotine addiction differs between individuals, and these differences are multidimensional due to social factors, psychological and genetic factors [14]. Among all the factors, genetic factors contribute to nearly half of the variability in nicotine addiction and have a role in the initiation of smoking initiation, nicotine dependence development, and quitting the smoking habit. Allelic variation in highly polymorphic CYP2A6 decreases the metabolism of nicotine, which in turn results in reduced intake of cigarettes and also increase in cessation of smoking habit [15].

The previous study was done to assess how genetic variations in CYP2A6 influence smoking habits and the effectiveness of smoking cessation treatments within a Turkish population; it was concluded that genetic variations of CYP2A6 were not associated with smoking habits or rates of cessation [16]. Among smokers of European descent who are not under pharmacotherapy, there is a higher likelihood of quitting smoking in individuals with CYP2A6 genotypes associated with reduced metabolism compared to those with genotypes linked to normal metabolism [17]. Polymorphisms within the CYP2A6 gene can impact smoking cessation behaviors, leading to increased attempts to quit and greater success in individuals with slower metabolizing genotypes [18].

Nicotine metabolism varies significantly between individuals, and it has been discovered that genetic variants in the CYP2A6 gene cause these variations [19]. It has been observed that Asians exhibit a relatively elevated allele frequency for the deletion variant of the CYP2A6 gene, which often leads to reduced enzymatic activity in this population. Due to the substantial role played by CYP2A6 in nicotine metabolism, there is a hypothesis suggesting that an individual's CYP2A6 genotype may influence their susceptibility to lung cancer as well as their smoking habits [20].

Genes that undergo mutations play a causal role in the development of cancer. Carcinogenesis involves a series of steps where various genetic events occur, disrupting normal functions in oncogenes and tumor suppressor genes [21]–

Groups	TT (n)	TC (n)	CC (n)	T	C	P value
Smokers	54	33	13	0.74	0.26	0.80
Non smokers	58	28	14	0.73	0.27	

Table 1: showing the distribution of alleles among the sample population.

[23]. In a study, 380 control smokers and 370 lung cancer patients were compared for the prevalence of the CYP2A6*4 variations. The absence of the *4/*4 genotype in patients with small or squamous cell carcinoma implies that individuals with the *4/*4 genotypes have a reduced likelihood of developing lung cancer, particularly among older individuals [24]. From the results of the present study, it can be concluded that there is no significant association between quit attempts and CYP2A6 gene polymorphism. The gene polymorphism becomes a tool in identifying the relationship between quit attempts and smokers and the future studies to be conducted on a larger population and check for the association between them.

5. Conclusion

In the study, there is no significant association between quit attempts among smokers and CYP2A6 genetic polymorphiam who had visited the dental hospital.

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Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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