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PLASMA LEVELS OF CETIRIZINE AND LEVOCETIRIZINE IN SMOKERS AND NON-SMOKERS: A PHARMACOGENETIC PERSPECTIVE IN PASHTUN POPULATION OF KHYBER PAKHTUNKHWA

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Abstract

Pharmacogenetic studies uses genetic information to determine right drug for the right patient in right dose hence maximize drug efficacy and minimize adverse drug reactions. The present Pharmacogenetic study aimed to analyze the difference in plasma levels of Cetirizine and Levocetirizine in smokers and non-smokers from with different polymorphic forms of CYP1A2 variants (wild type AA, heterozygous AC/CA and variant CC) belonging from Pashtun ethnic population Khyber Pakhtunkhwa, Pakistan. Volunteers with history of heavy smoking were enrolled for study in the age range of 15-35 years. Blood level of Cetirizine and Levocetirizine were determined on their respective T_{max} using High Performance Liquid Chromatography (HPLC). Restriction Fragment Length Polymorphism was used for Genotyping. The Extraction and amplification of Genomic DNA was done using DNA extraction kit (Thermo Scientific, UK). Restriction enzyme Bsp120I was used for digestion of PCR products. The plasma levels of subject population was genotyped for CYP1A2 variants; wild type AA, heterozygous AC/CA and variant CC to get an inference for optimal therapeutic response in genetic perspective. Variation and frequency of alleles was calculated using one-way ANOVA and Hardy Weinberg Equation among different genotypes in smokers and non-smokers. The plasma concentration of Cetirizine was 83.80-±7.7 ng /ml and 98.54-±2.85 ng/ml in smokers and non-smokers respectively. The difference in plasma concentration of Cetirizine in smokers and non-smokers was significant with p-value of 0.027 ($p < 0.05$). The plasma concentration of Levocetirizine was 97.50 ±14.79 ng /ml and 99.40 ± 6.55 ng/ml in smokers and non-smokers respectively. The difference in plasma concentration of Levocetirizine in smokers and non-smokers was not significant with p- value of 0.069 ($p < 0.05$). The frequency of CYP1A2 variants (wild type AA, heterozygous AC/CA and variant CC) was 78.22%, 19.56% and 2.22% in Pashtun population respectively. The metabolism of Levocetirizine was observed at a higher rate in volunteers having heterozygous genotypes (AC/CA) of CYP1A2 gene. Smokers with different polymorphic forms of CYP1A2 gene showed significant difference in plasma concentration of Levocetirizine with p-value of 0.0003 ($p<0.05$). The findings of the present study suggests that in smokers polymorphic forms of CYP1A2 affects plasma levels of Levocetirizine while the plasma levels of Cetirizine is not affected.

Keywords: Cetirizine, Cytochrome CYP1A2, Levocetirizine, Non-Smokers, Smokers.

1. INTRODUCTION

Since early 5000 BC, Tobacco use in various forms has been prevalent. Tobacco use accounts for majority of preventable deaths all over the world. From smoking, 5 million

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deaths per year are reported and on average one in ten person dies every other day. According to WHO report, the frequency of adult male and female population of Pakistan using various forms of tobacco is 31.8% and 5.8% respectively [1], [2], [3], [4].Tobacco smoking influences drug metabolism. CYP1A2 , a Cytochrome P450 enzyme, is involved metabolism of drug and neurotoxins[5]. CYP1A2 also activates aromatic amines and is the key enzyme causing chemical carcinogenesis [6]. The aromatic hydrocarbons in tobacco smoke increases enzymatic activity of cytochrome CYP1A2 after binding to the Ah-receptor. This phenomenon varies in different individuals. Cytochrome CYP2C19 polymorphism also alters the activity of cytochrome CYP1A2 in human [7], [8].Poly modal distribution was displayed by the enzymatic activity [9], [10]. The study does not focus on Cytochrome enzymes CYP 1A1, CYP1A2 and CYP2E1. CYP1A1 present in lungs and placenta as many components of Tobacco smoke may cause their induction [11], [12], [13]. CYP1A2 Allelic variants found in various ethnicities have shown to alter drug metabolism providing us with the concept of fast and slow metabolizers as reported in various studies. Alteration in ethnicity that are linked with genetics, can alter drug action. Hence, the data of one ethnic group may differs from another. This study focuses on the Pashtun ethnic group of Pakistan's Khyber Pakhtunkhwa province and the different aspects of CYP1A2 gene polymorphism.

Anti-Histamine Drugs (AHDs) Cetirizine (C) and Levocetirizine (LC) are commonly prescribed for the treatment of allergic symptoms that may be caused by smoking. Genetic variations is the key factor that might be responsible for the variable response to drug therapy in individuals. Considerable variation in plasma levels of Cetirizine and Levocetirizine in individuals is linked to genetics in different ethnic groups resulting in varying pharmacokinetics and pharmacodynamics [14]. Previous studies have shown Cytochrome CYP1A2 polymorphism in sequencing at the intron 1, exons 2 and 7 regions [15], [16], [17]. Intron 1 polymorphism affects cytochrome CYP1A2 induction [18]. The functional relevance of CYP1A2 has been proven in healthy Caucasian volunteer[19], [20]. The current study determines the possible shift (increase or decrease) in plasma levels of Cetirizine and Levocetirizine and correlating it in smoker and non-smoker groups. This study targets the genetic polymorphism of CYP1A2 and its relationship with plasma levels of Cetirizine and Levocetirizine in the target population.

2. MATERIAL AND METHODS

2.1. Study Design and Protocol

A total of 496 volunteers were enrolled in the study using statistical tools for clinical research [21] The volunteers were from Peshawar, Swat, Kohat and Abbotabad. The study period was January 2017 to December 2019. Inclusion criteria for study were: young adult volunteers (male/female) of Pashtun ethnicity, volunteers having allergic symptoms without chronic illness (Heart diseases, Diabetes), volunteers with and without history of smoking (fifteen or more cigarettes /day) with at least six months history of smoking [22] and volunteers who gave consent to participate in the study. Exclusion criteria were: volunteers of the target population who refused to give consent, volunteers

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with chronic illness (Heart diseases, Diabetes), smoker volunteers who consumed less than fifteen cigarettes per day for less than six months history of smoking. Ethical approval (05/EC-16/Pharm) was taken from the Pharmacy Department's Committee for Research Ethics, University of Peshawar. The safety concerns, aims and procedures of the study were explained to volunteers in the context of Pashto language. The written consent was taken from the willing volunteers.

Research work is illustrated in a schematic view shown in **Figure 1.** Subjects were divided into two main treatment groups; receiving Cetirizine (10mg) and Levocetirizine (5mg) for treatment of allergic symptoms. The subjects were grouped as mentioned in **Figure 2.** The treatment groups were further classified as Group A (smoker), Group B (non-smoker) receiving cetirizine (10mg). Similarly, Group C (smoker) and Group D (non-smoker) receiving levocetirizine (5mg).

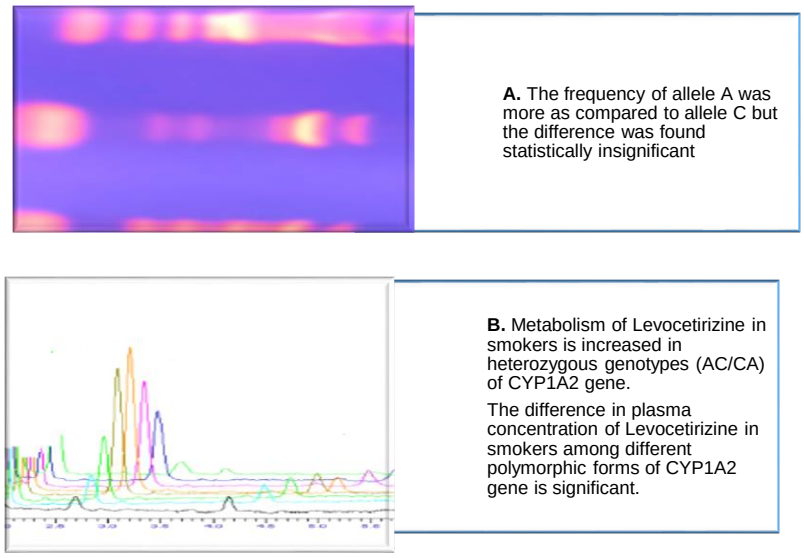

Figure 1: Research work illustrated in a schematic view. **A**. Portion of work analyzed by HPLC. **B**. Portion of work analyzed by Genotyping.

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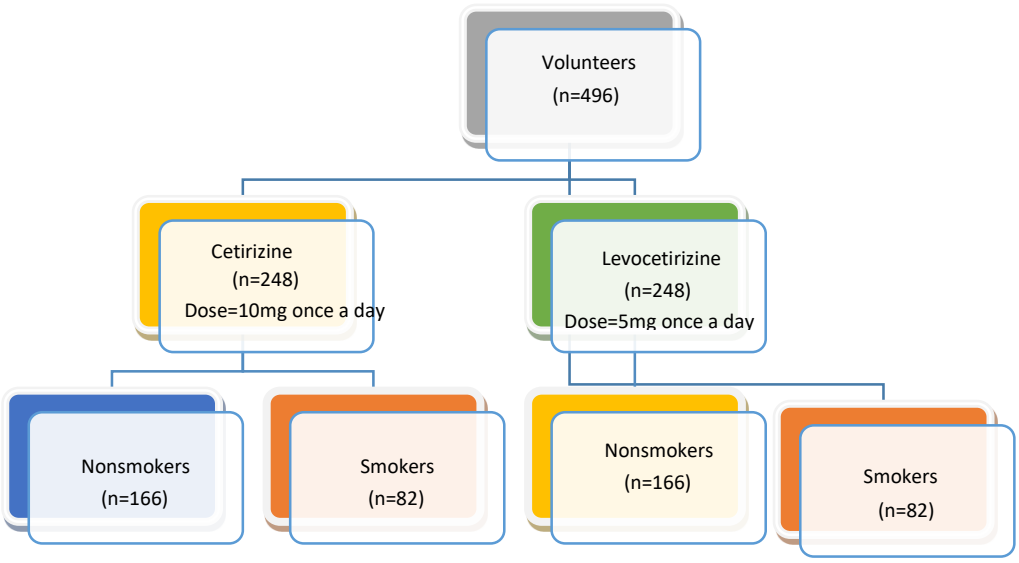

Figure 2. Flowchart representing design of study and administration of drugs to volunteers.

2.2. Blood Sampling

Blood from the volunteers was collected in gel tubes on their respective T_{max} of Cetirizine and Levocetirizine. The plasma was analyzed for levels of Cetirizine and Levocetirizine using HPLC. Samples were taken in EDTA tubes for genotyping and DNA extraction.

2.3. Analysis of Plasma Levels of Cetirizine and Levocetirizine

Plasma levels of Cetirizine and Levocetirizine were determined on their respective Tmax using RP- HPLC/UV (Perkin Elmer series system 200, Norwalk, USA). The method was developed and optimized using, ACE Generix100-5C18 RP of 250 × 4.6 mm, 5 µm column, mobile phase of Acetonitrile and Distilled water pH 7.2 in ratio of 59:41v/v, with a flow rate of 1.5 mL/min, detection wavelength was 230 nm and column oven temperature was set at 25°C.

2.4. Genotyping of CYP1A2 Gene

A Kit method (Thermo Scientific, UK) was used for extraction of Genomic DNA. In first phase, the extraction of DNA was carried out through a number of reactions including deproteination of blood (proteinase K), lysis of blood cells (lysis buffer), purification of DNA (washing buffer) and finally the elution of DNA (through Elution Buffer). Standard protocols provided by Thermo Scientific were used. For further analysis, the extracted DNA was stored on -20 °C. The genes were amplified using gradient thermo cycler PCR. Amplification was followed by assessment of PCR products using a100 bp ladder and 2% agarose gel. Intron 1 of CYP1A2 was amplified. Forward primer P1f

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AAGAGTCCCTGCCAGTGCTGGC and reverse primer GGAACTCCTGGTCCCTTGGGTA were used.

The primers were designed using primer blast software (PRIMIER Biosoft). The PCR products were digested using restriction enzyme Bsp120I. The assessment of PCR products were done on 5% agarose gel and a 100 bp ladder [23].

2.5. Statistical Analysis

Data is presented in the form of tables and figures using Graph Pad Prism 6 and Microsoft Excel. Differences in plasma levels of smokers and non-smokers were determined using the student "z" test. Allele frequencies were expressed in graphs. Variation among different genotypic alleles in smokers and non-smokers was calculated using one-way ANOVA. Hardy Weinberg equation was used to calculate allelic frequency distribution.

3. RESULTS

3.1. Socioeconomic and cultural features of the volunteers enrolled

The socioeconomic and sociocultural status of volunteers revealed that most of the volunteers were male. The female were reluctant to give consent. The reason was cultural restriction. Thus excluded from the study. The volunteers were mostly from Peshawar area. The frequency of volunteers in 21-25 years of age was high. Marital status of volunteers was noticed. The frequency of smoking in volunteers of low income was high. Socioeconomic and cultural features are expressed in Table 1. The findings complies with data reported from Helsinki Health Study which showed that smoking was common in uneducated and low income volunteers [24].

Table 1. Socioeconomic and cultural features of the volunteers enrolled.

Variables	Case n (%)		Control n (%)	*p*-value
	Cetirizine	Levocetirizine		
Gender				
Male	82(100%)	82(100%)	332(100%)	1.00
Female	0(0%)	0(0%)	0(0%)	1.00
Mean Age				
15-20 years	22(26.83%)	21(25.61%)	86(25.90%)	0.79
21-25years	51(62.19)	52(63.41%)	208(62.65%)	0.866
26-30 years	7(8.54%)	6(7.32%)	27(8.13%)	0.663
31-35years	2(2.44%)	3(3.66%)	11(3.31%)	0.561
Mean Weight	69	67	69.5	0.768
Occupation				
Labour	28(34.15%)	29(35.37%)	116(34.94%)	0.822
Farmer	6(7.32%)	5(6.09%)	24(7.23%)	0.323
Housewife	24(29.27%)	25(30.49%)	100(30.12%)	0.404
Driver	9(10.98%)	8(9.76%)	32(9.64%)	0.696
Government Employee	14(17.07%)	16(19.5%)	60(18.07%)	0.758

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Marital status				
Married	25(30.48%)	27(32.93%)	111(33.43%)	0.639
Unmarried	57(69.51%)	55(67.07%)	221(66.56%)	0.746
Cousin marriage	51(62.19%)	50(60.97%)	134(40.36%)	0.864
Non cousin marriage	31(37.80%)	32(39.02%)	198(59.64%)	0.830
Area				
Urban	47(57.32%)	48(58.54%)	187(56.32%)	0.430
Rural	35(42.68%)	34(41.46%)	145(43.67%)	0.837
Geographical Location				
Peshawar	60(73.17%)	65(79.26%)	260(78.31%)	0.443
Swat	15(18.29%)	10(12.19%)	40(12.05%)	0.109
Kohat	7(8.54%)	9(10.97%)	30(9.04%)	0.427
Abbotabad	3(3.66%)	1(1.22%)	2(0.60%)	0.521
Smoking Profile				
Smokers	82(100%)	82(100%)	0(0%)	1.00
Non-smokers	0	0	332(100%)	1.12
Cigarettes consumption equal and less than 15/day	2(100%)	2(100%)	0(0%)	1.00
Cigarettes consumption greater than 15/day	82(100%)	82(100%)	0(0%)	1.00

3.2. Analysis of Plasma levels for Cetirizine and Levocetirizine

The plasma concentration of Cetirizine was 83.80-±7.7 ng /ml and 98.54-±2.85 ng/ml in smokers and non-smokers respectively. The difference in plasma concentration of Cetirizine in smokers and non-smokers was significant with p-value of 0.027 ($p < 0.05$). The plasma concentration of Levocetirizine was 77.50 ±14.79 ng /ml and 99.40 ± 6.55 ng/ml in smokers and non-smokers respectively. The difference in plasma concentration of Levocetirizine in smokers and non-smokers was not significant with p- value of 0.069 ($p < 0.05$). Plasma levels of Cetirizine and Levocetirizine in smokers and non-smokers is shown in Table 2.

Table 2. Results for comparison of mean plasma levels of Cetirizine and Levocetirizine in the smokers and nonsmokers.

Drug	Smokers (Mean in ng/ml with ± SD)	Non-smokers (Mean in ng/ml with ± SD)	*p*-value
C_{max} of Cetirizine	83.750±7.7245	98.5543±2.855	0.027652979
Cmax of Levocetirizine	77.507±14.79	99.402±6.558	0.069467929

3.2. Frequency of Genotypes of CYP1A2 Gene

The frequency of CYP1A2 variants (wild type AA, heterozygous AC/CA and variant CC) was 78.22%, 19.56% and 2.22% in Pashtun population respectively. The frequency of

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Allele A was high than allele C. In smokers and non-smokers Pashtun population, the frequency of wild genotype (AA) was greater as compared to mutant heterozygous genotype (AC/CA) and homozygous mutant genotype (CC). The frequency of CYP1A2 variants (wild type AA, heterozygous AC/CA and variant CC) in smokers and non-smokers Pashtun population are shown in Figure 3 and Figure 4 respectively.

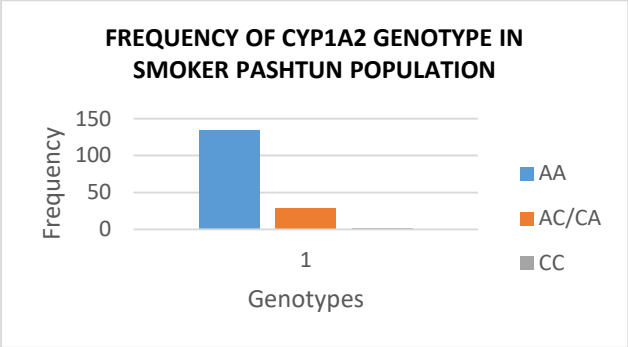

Figure 3: Frequency of CYP1A2 genotype in smoker Pashtun population.

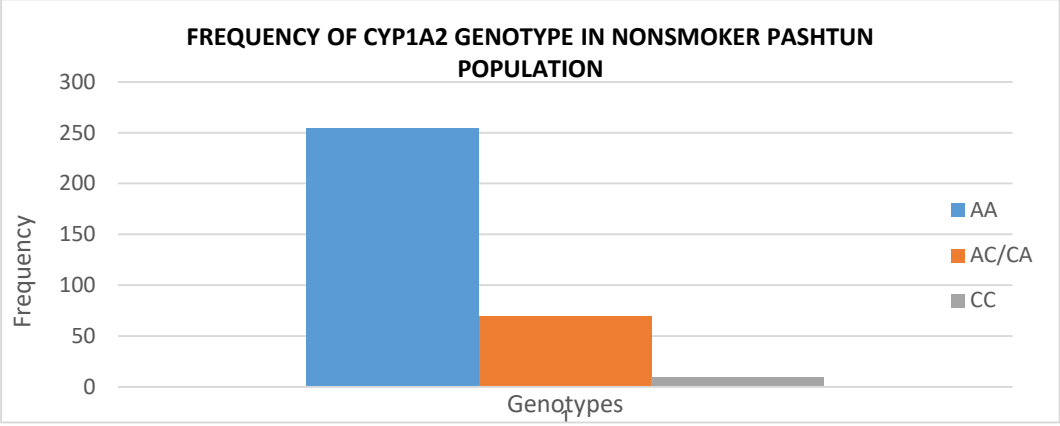

Figure Error! No text of specified style in document.**4:** Frequency of CYP1A2 genotype in nonsmoker Pashtun population.

Genotypic distribution of CYP1A2 gene polymorphism was determined by using Hardy Weinberg equation and chi-square test to find p-value in smoker and nonsmoker volunteers of Khyber Pakhtunkhwa, shown in Table 3.

Table 3. Distribution of CYP1A2 gene polymorphism in Pashtun population.

Target population (n=496)	Genotype			Allele	
	AA(%)	AC/CA(%)	CC(%)	A%	C%
Non-Smoker Pashtun population	254(51.21%)	69(13.91%)	9(1.81%)	0.766	0.027
Smoker Pashtun population	134(27.02%)	28(5.6%)	2(0.40%)	0.817	0.012
n=496	388(78.22%)	97(19.56%)	11(2.22%)	0.792	0.03

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3.3. Plasma Levels of Cetirizine and Levocetirizine

The difference in plasma concentration of Levocetirizine in smokers among different polymorphic forms of CYP1A2 gene was statistically significant with p-value of 0.0003 ($p<0.05$). The difference in plasma concentration of Levocetirizine in non-smokers was statistically insignificant with p-value of 0.3836.The difference in plasma concentration of Cetirizine in smokers and non-smokers among different polymorphic forms of CYP1A2 gene was statistically insignificant with p-value of 0.792 and 0.1497 respectively. Mean plasma levels for Cetirizine and Levocetirizine were compared with polymorphic forms of CYP1A2 gene and are expressed in Figure 5 & 6 respectively.

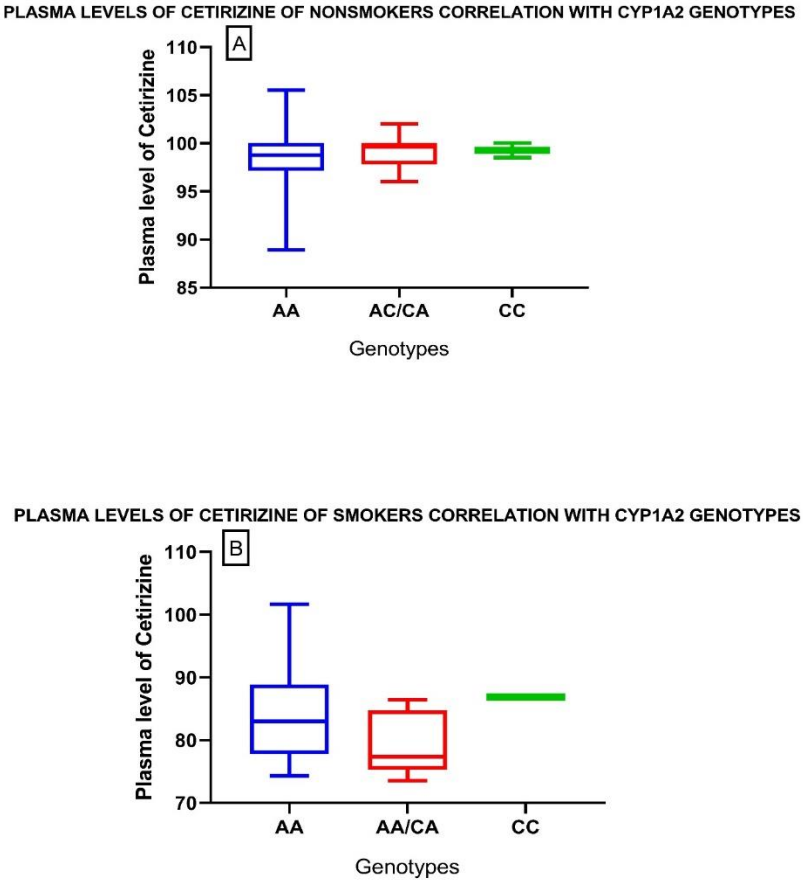

Figure 5: A. Mean plasma levels (ng/ml) of Cetirizine in non-smokers versus genotypes AA, AC/CA and CC with *p*-value of 0.792 *(p>0.05).***B.** Mean plasma levels (ng/ml) of Cetirizine in smokers versus genotypes AA, AC/CA and CC with *p*-value of 0.1497 *(p>0.05).*

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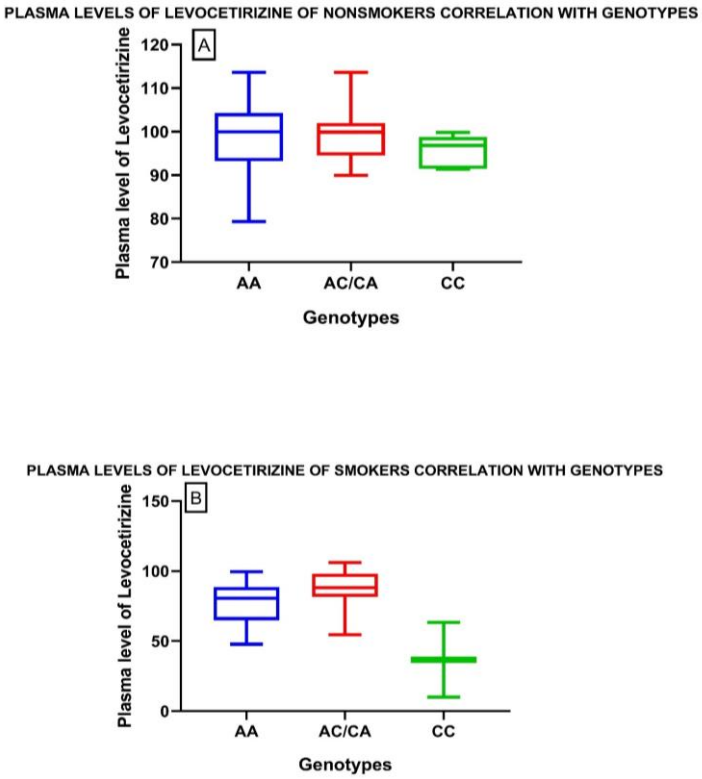

Figure 6: A. Mean plasma levels (ng/ml) of Levocetirizine for non-smokers versus genotypes AA, AC/CA, and CC with *p*-value of 0.3836 ($p>0.05$).
B. Mean plasma levels (ng/ml) of Levocetirizine for smokers versus genotypes AA, AC/CA, and CC with *p*-value of 0.0003 ($p<0.05$).

4. DISCUSSION

Polymorphism at intron 1 region of the CYP1A2 that is responsible for various isoform of CYP1A2 in different ethnic groups [25]. CYP1A2 polymorphism affects metabolism of a number of a drugs. However, the Pharmacogenetic role of CYP1A2 is every much less explore [26]. This comparative study of its kind in Pashtun ethnic population investigate plasma levels of Cetirizine and Levocetirizine in smokers and non-smokers with different isoforms of CYP1A2.

The plasma level of Cetirizine and Levocetirizine in smokers and non-smokers were determind by were determined on their respective T max using High Performance Liquid Chromatography (HPLC). The plasma concentration of Cetirizine was 83.80-±7.7 ng /ml and 98.54-±2.85 ng/ml in smokers and non-smokers respectively. The difference in plasma concentration of Cetirizine in smokers and non-smokers was significant with p-value of 0.027 ($p < 0.05$). It was reported for the first time in Pashtun population. In a study conducted at Brookdale University Hospital and Medical Center Emergency

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Department, there was no statistical difference in efficacy of Cetirizine in male, female and smoker groups [27]. However, findings of the study were similar to the findings reported in a research study conducted in Lahore, where difference was statistically significant among variant alleles of CYP450 1A2 in healthy smokers and non-smokers. The plasma levels of subject population was genotyped for CYP1A2 variants; wild type AA, heterozygous AC/CA and variant CC to get an inference for optimal therapeutic response in genetic perspective.

Upon Genotyping the frequency of CYP1A2 variants (wild type AA, heterozygous AC/CA and variant CC) was 78.22%, 19.56% and 2.22% in Pashtun population respectively. In pharmacokinetics studies, Caffeine test conducted on 205 Japanese volunteers showed that 86% of Japanese were AA allele of CYP1A2 [16]. An allelic frequency of 68% for CA allele was found in the Caucasian population [28].

Levocetirizine was observed at a higher rate in volunteers having heterozygous genotypes (AC/CA) of CYP1A2 gene. The difference in plasma concentration of Levocetirizine in smokers among different polymorphic forms of CYP1A2 gene was statistically significant with p-value of 0.0003 ($p<0.05$). Induction of CYP1A2 by cigarette smoking might have occurred [29], [30], [31]. Hence a decrease in plasma levels of levocetirizine in the heavy smokers may affects its therapeutic role, which requires further work up in a clinical trial.

5. CONCLUSION

The findings of the present study suggests that in smokers, polymorphic forms of CYP1A2 affects plasma levels of Levocetirizine while the plasma levels of Cetirizine is not affected in smokers and non-smokers. The metabolism of Levocetirizine was observed at a higher rate in volunteers having heterozygous genotypes (AC/CA) of CYP1A2 gene. To the best of our knowledge the present study is first kind in Pashtun population of Khyber Pakhtunkhwa, Pakistan. Similar projects on large scale should be designed to promote the concept of personalized medicine and individualization of therapy. This study can be used as a base to calculate dose of drugs in smokers and specified ethnic group.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors have no conflict of interest.

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