

Influence of Leukotriene Pathway Polymorphisms (Arachidonate 5-lipoxygenase ALOX5, Cysteinyl Leukotriene Receptor CysLTR1) On Response to Montelukast in A sample of Asthmatic Iraqi Patients

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Abstract

Background: Montelukast, a cysteinyl leukotriene receptor 1 antagonist, is safe drug used in asthmatic patients. The mechanisms underlying the variation in response to montelukast are thought to be due to genetic variability partly.

Objective: examine the genotype and allele frequencies of LT pathway candidate gene polymorphisms in Iraqi general population and asthmatic patients and determine associations between the genetic variants with outcomes in Iraqi patients with asthma receiving montelukast.

Method: This study conducted in a study group of Iraqi patients and healthy subjects from Baghdad. (200) participants (men, women) recruited as healthy subjects (80) and patients with bronchial asthma (120) fulfill entry criteria and classified into three groups, first group contained 40 asthmatic patients taking montelukast for 4 weeks at a dose of 10 mg once a day before bed time, second group contained 40 asthmatic patients taking inhaled short acting beta agonist salbutamol at a dose of four puffs daily (0.1mg/dose) for 4 weeks, third group contain 40 asthmatic patients taking budesonide/formoterol inhalation powder (160/4.5mcg/dose) at a dose of two puffs daily for 4 weeks.

Results: The allelic variants of ALOX5 and CysLTR1 were found in Iraqi population. G allele frequency of ALOX5(rs2115819) was (0.40), C allele frequency of CysLTR1 (rs320995) was (0.28) among the studied patients. C allele frequency of CysLTR1 for healthy individuals was (0.28) and G allele frequency of ALOX5(rs2115819) was (0.21) for healthy individuals. For ALOX5 SNP and CysLTR1 SNP in patients used montelukast, there was non-significant difference in percentage change in % predicted FEV1 over baseline and percentage change in % predicted PEF over baseline in patients taking montelukast. For salbutamol patients group, there was non-significant difference in percentage change in % predicted FEV1 over baseline for ALOX5 SNP and CysLTR1 SNP. For budesonide/formoterol inhalation powder patients group, non-significant difference in percentage change in % predicted FEV1 over baseline for ALOX5 SNP and CysLTR1 SNP.

Conclusion: Our study results concluded that genetic variation in leukotriene pathway candidate genes may not contributed to variability in clinical responses to montelukast in Iraqi asthmatic patients from Baghdad who received montelukast for one month.

Keywords: Asthma, montelukast, ALOX5 SNP, CysLTR1 SNP, HWE (Hardy–Weinberg equilibrium), MAF (minor allele frequency), LT

INTRODUCTION

Asthma defines as a chronic inflammatory disease of the airways in which many types of cells and cellular elements play a role especially

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mast cells, neutrophils, eosinophils, macrophages, T lymphocytes and epithelial cells. In vulnerable individuals, this inflammation causes recurrent episodes of coughing (especially at night or early in the morning), shortness of breath, wheezing and chest tightness. (GINA, 2021). Montelukast is safe and effective drug used in asthma treatment. Interpatient variability in response to montelukast in both children and adults with asthma is significant, with more than 80% of patients receiving montelukast considered as non-responders (Szeffler SJ, et al., 2005). The mechanisms explain interpatient variability in response to montelukast are thought to be due to genetic variability (Silverman ES et al., 2001). Several studies have mentioned that polymorphisms in the ALOX5 promoter, LTC4S, LTA4H and MRP genes contribute to variability in response to LT selective antagonists (Drazen JM et al., 1999; Szczeklik A et al., 2001; Lima JJ et al., 2006). CysLTs are strong mediators of asthma inflammation and are synthesized from arachidonic acid located in the cell membrane by phospholipase A2 (PLA2) in response to stimulation (Kanaoka Y and Boyce JA, 2004).

Arachidonic acid is converted to 5-hydroperoxyeicosatetraenoic acid and LTA4 by ALOX5 and 5-lipoxygenase activating protein (19). LTA4 is converted to LTB4 by LTA4 hydrolase (LTA4H), or is conjugated with reduced glutathione by LTC4 synthase to form LTC4 (20, 21). LTC4 is converted to LTD4 and LTE4 by glutamyltransferase and dipeptidase (Lewis RA et al., 1990; Woods JW et al., 1993). Symptoms of asthma caused by cysLTs and mediated by the cysLT1 receptor (Drazen JM and Austen KF, 1987).

The present study needed to conclude associations between polymorphisms in Leukotriene pathway (LT) candidate genes with outcomes in individuals administering montelukast. The basic aim of this pharmacogenetic study is that patients with asthma carrying polymorphisms that increase the LT activity will respond better to montelukast compared with polymorphisms that have no effect or that down regulate LT activity.

MATERIALS AND METHODS

Chemicals

Primers, 100bp DNA ladder, Loading dye, Free nuclease water, Absolute ethyl alcohol (99.9), TAE Buffer (50x)

Study design and patient studies

The study is cross sectional study compared the efficacy of montelukast tablet 10 mg one tablet daily, salbutamol inhaler (0.1 mg/dose) 4 puffs/day and budesonide/formoterol inhalation powder (160/4.5 mcg/dose) at a dose of two puffs daily for 4 weeks. In addition, healthy subjects for allele frequencies participated in this study. The participants enrolled from Baghdad hospitals. Before beginning the study, all patients completed a questionnaire that included

questions about demographic characteristics, smoking history, age at onset of asthma, medical history and the patients has not used anti-leukotriene drugs or long acting beta agonists in the, intra venous or oral corticosteroid 2 months before to lung function testing. Ethical approval of this study obtained from Institute Review Board of the Medical College / Al-Nahrain University after approval of scientific committee of the Department of Pharmacology in the College of Medicine/ Al-Nahrain University/Iraq.

Genotyping of the single nucleotide polymorphisms

In this study, we targeted rs2115819 on the ALOX5 gene and rs2660845 on the LTA4H gene. DNA was collected from all participants who participated for this pharmacogenetic study. PCR product sent for via Sanger sequencing by Macrogen Corporation – South Korea.

Outcomes

Associations between genetic variants analyzed according to the following outcomes, as follows:

Percentage change in % predicted PEF after 4 weeks of montelukast

treatment compared with % predicted FEV1 recorded at baseline. Percentage change in % predicted FEV1 after 4 weeks of montelukast treatment compared with % predicted FEV1 recorded at baseline. percentage change in % predicted FEV1 after 4 weeks of salbutamol inhaler compared with % predicted FEV1 recorded at baseline. percentage change in % predicted FEV1 after 4 weeks of budesonide/formoterol inhalation powder compared with % predicted FEV1 recorded at baseline.

Statistics

Descriptive and analytic statistics done using SPSS V24 software statistical program. It were as follow: The descriptive statistics included mean \pm standard deviation (S.D) for measurable variables. One-way Analysis of Variance test (ANOVA-test with tukey's multiple comparison test used to analyze the data. Unpaired t-test used to compare between two different groups. Dependent t-test of two means (paired) applied for the differences in parameters within each group. MAF, minor allele frequency; HWP, P-values for Hardy-Weinberg equilibrium. Statistical significance was determined using the chi-squared test and unpaired Student's t-test. P-values <0.05 were taken to indicate statistically significant differences.

RESULTS

The genotype and allele frequencies of SNPs in ALOX5 and LTA4H genes shown in Table (1). Table 1 also lists HWP values. There were significant differences between members of the general population and patients of whole study for each genotype frequency ($p=0.000$) and they were deviated from Hardy-Weinberg equilibrium.

Table 1. Genotype distribution

Gene SNP (M>m)	Total NO. of alleles of BA(patients)	Genotype of BA patients (%)	Genotype of healthy (%)	Total NO. of alleles of healthy	MAF Of BA patients	MAF of healthy	HWP of BA	HWP of healthy	P-value
ALOX5 (A>G)	111	AA=28 AG=74 GG=9	AA= 34 AG=46 GG=0	80	0.41	0.21	0.000	0.054	0.000
CysLTR1 (T>C)	114	TT=70 CT=24 CC=20(17.7)	TT=46 CT=20 CC=10(8.8%)	76	0.28	0.72	0.000	0.056	0.000

Association between outcomes and the single nucleotide polymorphisms

In bronchial asthma group received montelukast, we compared clinical lung function outcomes between GG homozygotes (n =1) and A allele carriers (AA+AG, n =34) for the ALOX5 (rs2115819), and between T allele carriers (TT+CT, n=32) and CC homozygotes (n =7) for the CysLTR1(rs320995) among montelukast patients group.

In asthmatic patients on salbutamol inhaler, we compared some lung function test outcomes with GG homozygotes (n =5) and A allele carriers (A/A+A/G, n= 35) of the ALOX5 SNP (rs2115819). In addition, we compared some lung function test outcomes between CC homozygotes (n =7) and T allele carriers (TT+CT, n=32) for the CysLTR1(rs320995) among salbutamol inhaler patients group.

In patients with bronchial asthma and used budesonide/formoterol inhalation powder, we compared some lung function test outcomes with GG homozygotes (n =3) and A allele carriers (AA+AG, n =33) for the ALOX5 (rs2115819). In addition, we compared lung function test outcomes with CC homozygotes (n =6) and T allele carriers (TT+CT, n=30) for the CysLTR1(rs320995) among budesonide/formoterol inhalation powder group.

Percentage change in % predicted PEF after 4weeks of montelukast treatment compared with % predicted PEF recorded at baseline were recorded with genotypes of ALOX5 and CysLTR1 and shown in Fig.1 There was no statically significant difference in the % change in % predicted PEF between GG homozygote and A allele carriers (AA+ AG) of the ALOX5 SNP. There was no statically significant difference in the % change in % predicted PEF between T allele carriers (TT+CT, n=30) and CC homozygotes for the CysLTR1(rs320995) SNP .

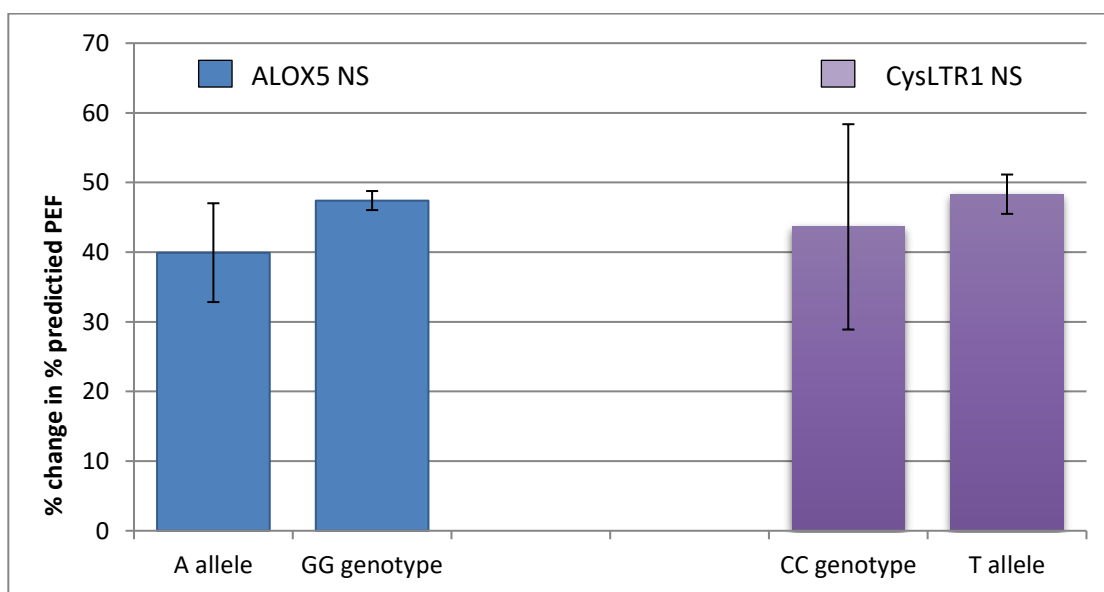


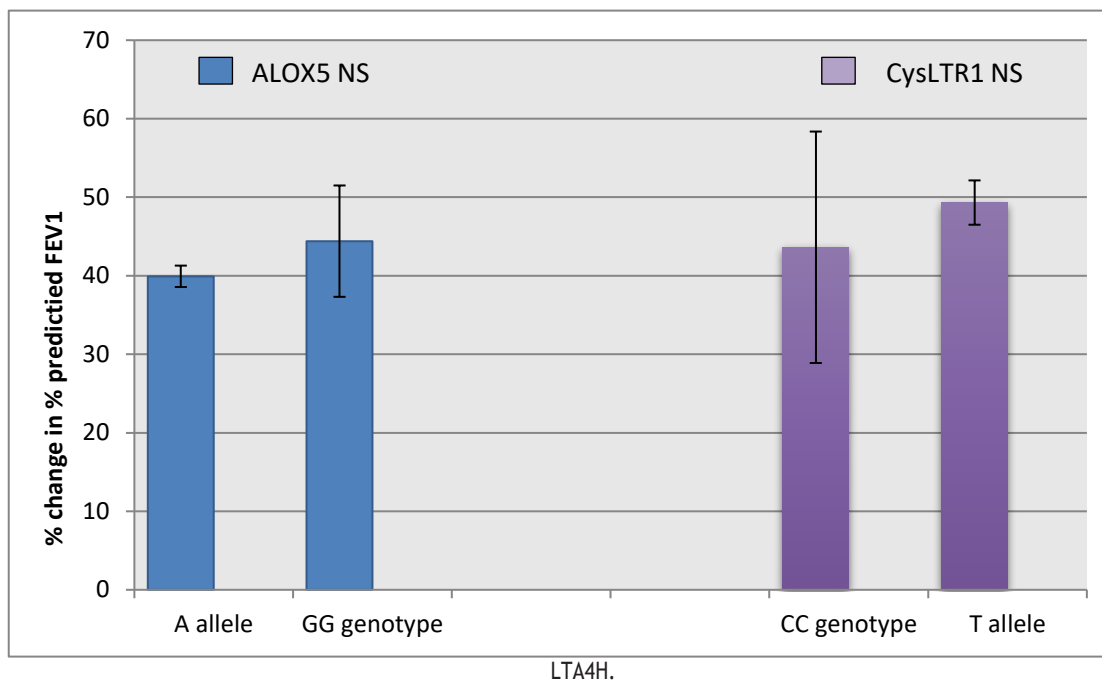
Fig.1.Percentage change in percentage predicted PEF over baseline after 4 weeks of montelukast treatment compared by genotypes of ALOX5, LTA4H , NS=non significant.

Percentage change in % predicted FEV1 after 4weeks of montelukast

treatment compared with % predicted FEV1 recorded at baseline were recorded with genotypes of *ALOX5* and *CysLTR1* and shown in Fig.2. For the *ALOX5* (rs2115819)

SNP, There was no statically significant difference in the % change in % predicted FEV1 and GG homozygote and A allele carriers (AA+ AG) . For the *CysLTR1*, there was no difference statically in % changes in % predicted FEV1 with T allele carriers (TT+CT, n=30) and CC homozygotes.

Fig.2. Percentage change in percentage predicted FEV1 over baseline after 4 weeks of montelukast treatment compared by genotypes of ALOX5,



Percentage change in % predicted FEV1 after 4weeks of salbutamol inhaler compared with % predicted FEV1 recorded at baseline and compared by genotypes of *ALOX5*, *LTA4H*. There was no difference between GG homozygotes of the *ALOX5* SNP and A allele carriers as regard to

percentage change in % predicted FEV1. There was no statically significant difference in the % change in % predicted FEV1 between T allele carriers (TT+CT, n=30) and CC homozygotes for the *CysLTR1*(rs320995) SNP as showed in Fig.3.

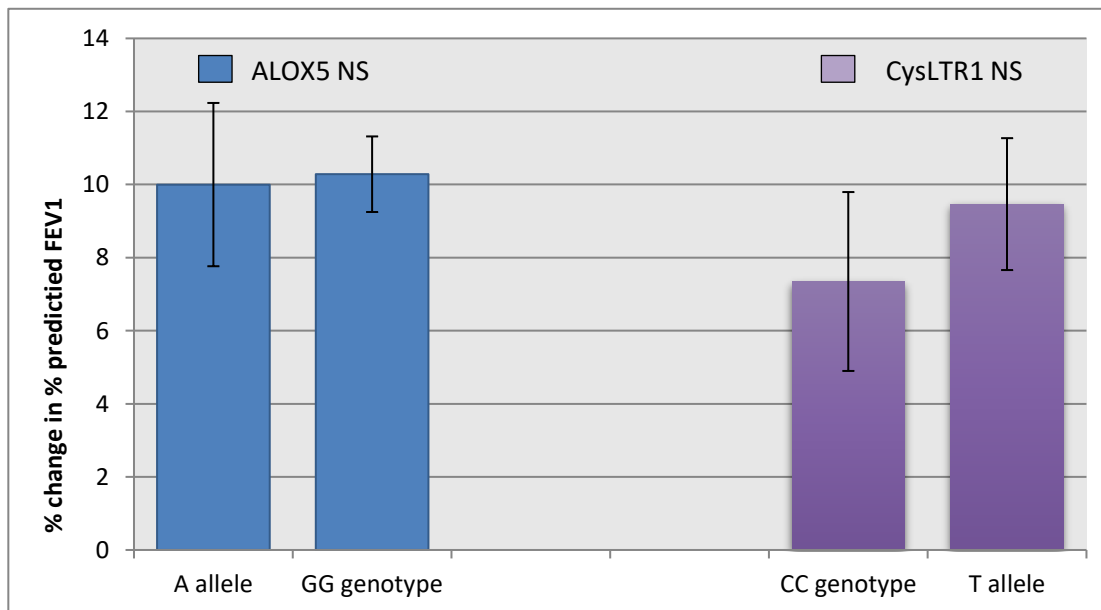


Fig.3. Association between ALOX5, LTA4H, genotypes and % change in % predicted FEV1 over baseline in patients taking inhaled salbutamol, NS=non significant

Percentage change in % predicted FEV1 after 4 weeks of budesonide/formoterol inhalation powder compared with % predicted FEV1 recorded at baseline and association with ALOX5, LTA4H. Non-significant difference present between GG genotype carriers and A allele carriers (AA+ AG) of

ALOX5 with percentage change in % predicted FEV1. There was no statically significant difference in the % change in % predicted FEV1 between T allele carriers (TT+CT, n=30) and CC homozygotes for the CysLTR1(rs320995) SNP as showed in Fig.4.

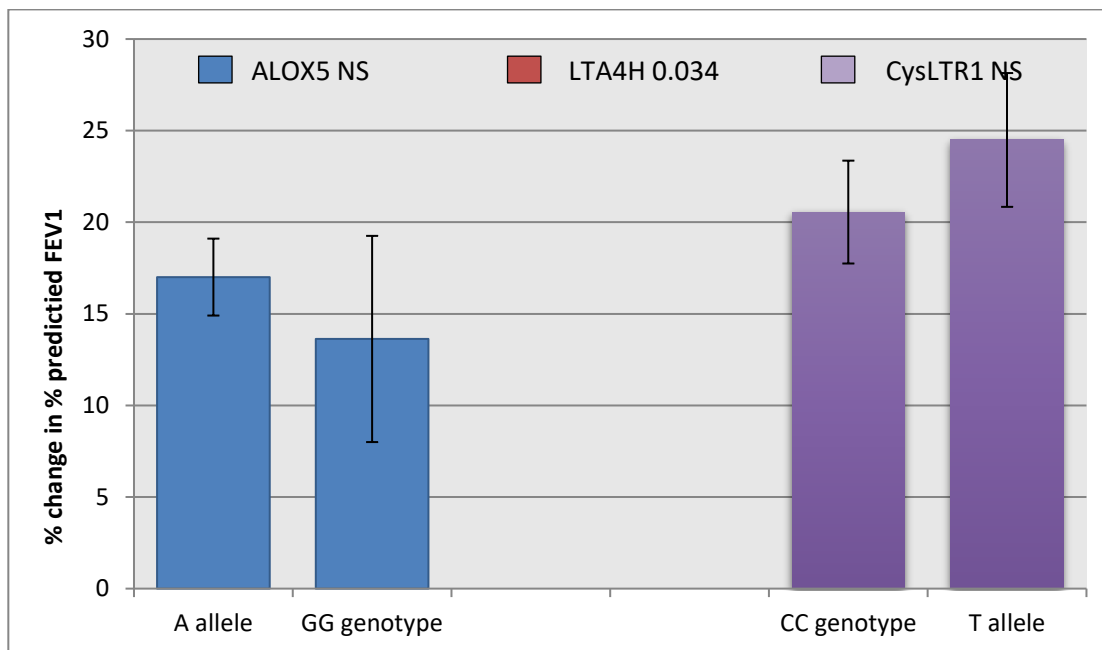


Fig.4. Association between ALOX5, LTA4H and % change in % predicted FEV1 over baseline in patients taking budesonide/formoterol inhalation powder, NS=non significant

DISCUSSION

Montelukast given as an alternative to low dose inhaled corticosteroids (ICS) for patients with mild persistent asthma and as add on therapy to ICS treatment in patients with moderate persistent and severe persistent asthma. Responsiveness to montelukast is highly variable among patients, which is thought to be due to genetic variation. Several studies revealed that the polymorphism in ALOX5 and cysLTR1 contributes to the variability in response to montelukast (Lima JJ *et al.*, 2006). The present study reported associations between polymorphisms in candidate genes encoding key proteins in the LT pathway with response in patients administered montelukast treatment. In the present study for ALOX5, cysLTR1 SNPs of leukotriene pathway candidate genes, there were significant differences in each genotype and allele frequencies on comparison of individuals from the general population and patients with asthma ($p=0.000$). SNPs failing to meet Hardy-Weinberg equilibrium (HWE) ($P = 0.000$), so these SNPs associated with risk factors for development of asthma.

For the ALOX5 (rs2115819) and cysLTR1 SNPs among montelukast patients group, there was no difference in values of FEV1 and PEF, while study performed by Lima JJ *et al.*, 2006 reported that there was significant improvement in values of FEV1 among ALOX5 (rs2115819) SNP carriers.

For the ALOX5 (rs2115819) and cysLTR1 SNPs among

budesonide/formoterol inhalation powder patients group and salbutamol inhaler patients group there was no difference in values of FEV1. This may be positive results because these drug had different receptors targets and also the samples of both groups were small.

CONCLUSION

The present study gave evidence that genetic variation in a candidate gene in the leukotriene pathway, cysLTR1(rs320995) SNP and ALOX5(rs2115819) SNP show novel associations with variability in clinical responses to montelukast in Iraqi patients with asthma. Further studies are required to replicate our associations.

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