Influence of Leukotriene Pathway Polymorphisms (Arachidonate 5-Lipoxygenase ALOX5, Leukotriene A4hydrolase LTA4H) on Response to Montelukast in A Sample of Asthmatic Iraqi Patients

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Abstract

Background: Montelukast, which is safe, and efficacious cysteinyl leukotriene receptor 1 antagonist used in asthma, which is a chronic inflammatory disease, imposes a large burden on society since large proportion of morbidity, mortality and healthcare costs associated with asthma. objective: To determine associations between polymorphisms in leukotriene pathway candidate gene with lung function outcomes in Iraqi patients with asthma receiving montelukast for one month in order to determine the interpatient variability in response to montelukast. Method: This study conducted in a study group of Iraqi patients and healthy subjects from Baghdad. Two hundred 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 buffs 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 buffs daily for 4 weeks. Results: The allelic varients of ALOX5 and LT4H were found in Iraqi population. A allele frequency of ALOX5(rs2115819) was (0.59), G allele frequency was (0.40). The G allele frequency of LT4H(rs2660845) was (0.58), A allele frequency was (0.42). G allele frequency of ALOX5 for healthy individuals was (0.21%) while A allele frequency was (0.79). A allele frequency of LTA4H for healthy individuals was (0.71) while G allele frequency was (0.29). For ALOX5 SNP, there was non-significant difference in percentage change in % predicted FEV1 over baseline and percentage change in % predicted PEF over baseline in patients montelukast intake. For LTA4H (rs2660845) SNP of our study, the values of % change in % predicted PEF in patients with the AA genotype were improved after montelukast intake compared with those who carried the G allele, while percentage change in % predicted FEV1 over baseline showed significance improvement in patients with the G allele carriers compared with those who carried AA genotype. For salbutamol patients' group, A significant association observed in the carriers of G allele of LTA4H (rs2660845) SNP compared to AA genotype carriers who were on salbutamol treatment and the % change in % predicted FEV1 showed non-significant associations between GG genotype and A allele carriers of ALOX5 (rs2115819) in participants taking inhaled salbutamol.

For budesonide/formoterol inhalation powder patients' group, A significant association observed in the carriers of AA genotype of LTA4H (rs2660845) SNP compared to G allele carriers with the % change in % predicted FEV1 with non-significant associations were observed between % change in % predicted FEV1 with ALOX5 (rs2115819). Conclusion: Our study results concluded that genetic variation in leukotriene pathway candidate genes 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, LTA4H SNP, HWE (Hardy–Weinberg equilibrium), MAF (minor allele frequency).

1. Introduction

Asthma defines as a chronic inflammatory disease of the airways in which many types of cells and cellular elements play a role especially 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. These episodes are usually associated with widespread but changeable airflow obstruction and bronchospasm that is often reversible either spontaneously or with treatment (National Asthma Education and Prevention Program Expert Panel Report 3, 2007, Qiu et al, 2014, GINA,2021). Leukotrienes (LTs) are derived from

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arachidonic acid (AA) which is one of the constituents of phospholipids fatty acid found within the phospholipids and considered an important component of cell membranes. Leukotriene (LTs) are inflammatory mediators that play important role in both normal host defense and in inflammatory diseases like asthma, these cells, in turn, cause the smooth muscle contraction and airway tightening in asthma (Gauvreau GM et al., 2015). AA in the first step of th Leukotriene synthesis process oxidized by ALOX5 enzyme and form leukotriene A4 (LTA4). LTA4 is either converted to LTB4 by LTA4 hydrolase (LTA4H) or conjugated to glutathione by leukotriene C4 synthase (LTC4S) and producing leukotriene C4 (LTC4) (Radmark O et al.,2015).

2. 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(0.1mg/dose) 4 puffs/day and budesonide/formoterol inhalation powder(160/4.5mcg/dose) at a dose of two buffs daily for 4 weeks. In addition, control 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.

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 were analyzed according
to the following outcomes, as follows:
Percentage change in % predicted PEF after 4weeks of montelukast
treatment compared with % predicted FEV1 recorded at baseline, percentage change in % predicted FEV1 after 4weeks of montelukast treatment compared with % predicted FEV1 recorded at baseline, percentage change in % predicted FEV1 after 4weeks of salbutamol inhaler compared with % predicted FEV1 recorded at baseline, percentage change in % predicted FEV1 after 4weeks of budesonide/formoterol inhalation powder compared with % predicted FEV1 recorded at baseline.

Statistics

Descriptive and analytic statistics done using SPSS V24software statistical program. It were as follow: The descriptive statistics included mean ± 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.

3. 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
LTA4H (G>A)	110	GG=27 GA=74 AA=9	GG=38 GA=20 AA=10	68	0.42	0.71	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 AA homozygotes (n = 2) and G allele carriers (GG+GA, n =33) for the LTA4H SNP (rs2660845).

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 with AA homozygotes (n =3) and G allele carriers (G/G+G/A, n =36) of the LTA4H SNP (rs2660845).

In patients with bronchial asthma and used budesonide/formoterol inhalation powder, 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 lung function test outcomes with G allele carriers (GG+GA, n =33) and AA genotypes group(n=4) for the LTA4H SNP (rs2660845).

Percentage change in % predicted PEF after 4weeks of montelukast treatment compared with % predicted FEV1 recorded at baseline were recorded with genotypes of ALOX5 and LTA4H 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. Compared with G allele carriers (GG+GA), AA genotype carriers for the LTA4H (rs2660845) SNP had a higher % change in % predicted PEF after 4 weeks of montelukast treatment

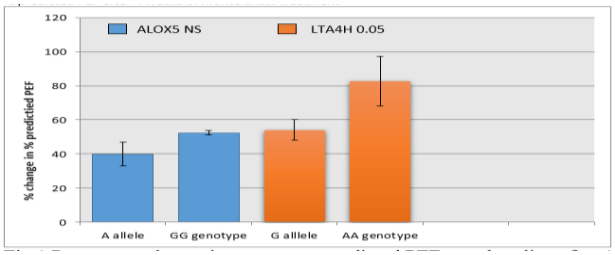

Fig 1. Percentage change in percentage predicted PEF over baseline after 4 weeks of montelukast treatment compared by genotypes of ALOX5, LTA4H, NS=non-significant.

Percentage change in % predicted FEV1 after 4weeks of montelukast

treatment compared with % predicted FEV1 recorded at baseline were recorded with genotypes of ALOX5and LTA4H and shown in Fig.2. For the ALOX5 (rs2115819) SNP, there was no difference statically between GG genotypes and A allele carriers (AA+ AG) in % changes in % predicted FEV1. Compared with AA genotype, G allele carriers (GG+GA) for the LTA4H (rs2660845) SNP had significantly higher % changes in % predicted FEV1 as showed in Fig.2.

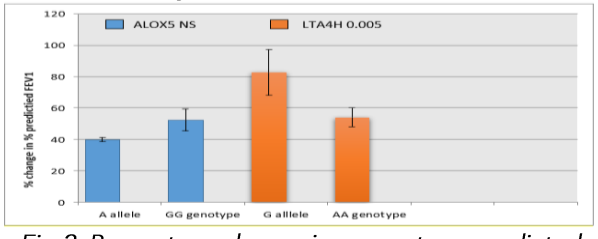

Fig 2. Percentage change in percentage predicted FEV1 over baseline after 4 weeks of montelukast treatment compared by genotypes of ALOX5, LTA4H.

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 G/G homozygotes of the ALOX5 SNP and A allele carriers as regard to percentage change in % predicted FEV1. Compared with AA genotype, G allele carriers (GG+GA) for the LTA4H (rs2660845) SNP had a higher % change in % predicted FEV1 versus AA genotype carriers with significant difference present between them as showed in Fig.3.

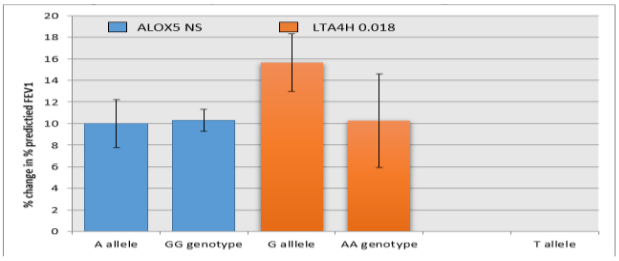

Figure 3. Association between ALOX5, LTA4H, genotypes and% change in % predicted FEV1 over baseline in patients taking inhaled salbutamol, NS=non-significant

Percentage change in % predicted FEV1 after 4weeks 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. Compared with AA genotype, G allele carriers (GG+GA) for the LTA4H (rs2660845) SNP had a lower % change in % predicted FEV1 versus AA genotype carriers as showed in Fig.4.

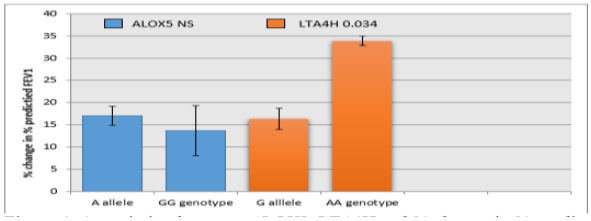

Figure.4. Association between ALOX5, LTA4H and % change in % predicted FEV1 over baseline in patients taking budesonide/formoterol inhalation powder, NS=non-significant

4. Discussion

Montelukast is 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 LTA4H 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, LTA4H SNPs of leukotriene pathway candidate genes, there were a significant difference 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.
In the present study for ALOX5 SNP, % change in % predicted PEF and % changes in % predicted FEV1 values showed non-significant differences between GG homozygotes of the ALOX5 SNP and A allele carriers among montelukast patients' group while for LTA4H SNP, the % change in % predicted PEF significantly improved in AA genotype carriers compared with those who carried the G allele after montelukast intake. % Changes in % predicted FEV1 significantly improved in G allele carriers compared to AA genotype carriers. Our findings are consistent with the report by Kotani H et al, 2012 which reported that clinical responses to montelukast were better in the group with the AA genotype than in the G allele carriers for LTA4H SNP while no
significant association between the ALOX5 SNP (rs2115819) and
changes PEV1 values.

Among budesonide/formoterol inhalation powder patients' group, % changes in % predicted FEV1 significantly improved in AA genotype carriers compared to G allele carriers for LTA4H SNP while no significant differences between GG homozygotes of the ALOX5 SNP and A allele carriers. Our sample size was small, and it is possible that the associations we observed between LT pathway SNPs and responsiveness to budesonide/formoterol inhalation powder could represent false positive results.
Among salbutamol inhaler users, % changes in % predicted FEV1 significantly improved in G allele carriers compared to AA genotype carriers with no significant differences between GG homozygotes of the ALOX5 SNP and A allele carriers. Our sample size was small, and it is possible that the associations we observed between LT pathway SNPs and responsiveness to salbutamol inhaler represent false positive results.

5. Conclusion

The present study gave evidence that genetic variation in a candidate gene in the leukotriene pathway, LTA4H (rs2660845) SNP and ALOX5(rs2115819) SNP contributes to variability in clinical responses to montelukast in Iraqi patients with asthma.

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