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Effect of Genetic Polymorphism of CYP2C8 Enzyme on the Montelukast Therapy Responses in Iraqi Asthmatic Children

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Abstract

Background: Some reports show that the CYP2C8 enzyme plays an influential role in montelukast metabolism, and genetic polymorphism of CYP2C8 genes may influence therapeutic responses of asthmatic children to montelukast treatment.

Aim of the study: The current study aimed to detect the effects of genetic polymorphism of the CYP2C8*1B (rs7909236) gene on montelukast response in asthmatic children.

Methods and Patients: An observational cross-sectional study was carried out in the respiratory clinic center in the Kerbala Teaching Hospital of Children from early October 2022 to late September 2023.

The trial included one hundred children older than six years old with asthma who had been previously diagnosed and were taking montelukast every day for at least one month. Alle-specific PCR patients out following DNA extraction to determine each patient's genotype to determine each patient's genotype. Total serum Ig E levels, Asthma Control Test (ACT), FEV1, and PEF of Pulmonary Function Tests were also measured.

Results: The distribution of CYP2C8*1B (rs 7909236) genetics polymorphism was found to be 74% for CC wild homozygous, 22% for CA mutants heterozygous, and 4% for mutants homozygous AA, according to the results of genetic amplification. Patients with wild-type and mutant genes do not significantly differ in serum total IgE, FEV1, PEF values, or ACT scores. Therefore, this polymorphism and montelukast responsiveness is predicted to be not significantly related (p value<0.05).

Conclusions: The montelukast respond and the CYP2C8*1B g.-271 G>A (rs7909236) genetic variation did not significantly associate.

تأثير تعدد الأشكال الجيني لإنزيم CYP2C8 على استجابات العلاج بمونتلوكاست لدى الأطفال العراقيين المصابين بالربو

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الخلاصة

المقدمة: خلفية البحث تبين في بعض الدراسات دور مؤثر لأنزيم CYP2C8 في ايض عقار المونتيلوكاست والتغييرات الجينية في جين هذا الانزيم من الممكن تؤثر على استجابات العلاجية لعقار المونتيلوكاست لمرضى الربو.

هدف الدراسة: هدفت الدراسة لمعرفة العالقة بين تعدد الأشكال الجيني، CYP2C8*1B (rs7909236) لدي استجابة الأطفال الذين يتناولون عقار المونتيلوكاست يوميًا.

الطرق: أجريت هذه الدراسة المقطعية من تشرين الثاني ٢٠٢٢ الى نهاية أيلول ٢٠٢٣ وقد ساهم بها ١٠٠ طفل يعانون من مرض الربو ويتناولون عقار المونتيلوكاست بشكل يومي لمدة لا تقل عن شهر قبل المشاركة في الدراسة وسحب عينة من الدم منهم. تم فحص اختبار وظائف الرئة، اختبار السيطرة على الربو وتحليل مستويات مضادات الامينوغلوبين نوع E لدي كل المشاركين في الدراسة. تم استخلاص الحامض النووي وعمل اختبار PCR خاص بنوع الاليل لتحديد الأنواع الجينية.

النتائج

ظهرت الدراسة وجود طفرات في جين CYP2C8*1B (rs7909236) وكانت بنسبة ٧٤٪ لنمط الوراثي المتجانس (CC) وبنسبة ٢٢٪ لنمط الوراثي المتغاير (CA) وبنسبة ٤٪ للنمط الوراثي (AA).

لم يكن هنالك ارتباط كبير بين استجابة المرضى لعقار المونتيلوكاست و تعدد الجيني لانزيم CYP2C8*1B g.-271 C>A (rs7909236) .

1. Introduction

Asthma prevalence and severity remain a primary global health concern in all age groups. Asthma, which affects both adults and children, has a high morbidity rate but a low mortality rate in comparison to other chronic illnesses. According to available data, asthma is a complicated disorder whose etiology is increasingly thought to result from interactions between environmental exposures, host characteristics, and genetic susceptibility factors (genetic loci associated with asthma). (Air pollution, mold, other aeroallergens, weather, obesity, dietary variables, infections, and hypersensitivity to allergies) (Kim et al., 2017).

Cysteinyl leukotriene antagonists, such as montelukast, have been approved to treat asthma in children and adults (Davidson et al., 2010). In many in vitro studies and on the montelukast label, the key P450 enzymes involved in montelukast metabolism are identified as CYP2C9 and CYP3A4. More recent in vitro research, however, links CYP2C8 to the 36-hydroxylation of montelukast. More proof of the possible role of CYP2C8 comes from monmontelukast'sility to bind firmly into the CYP2C8 enzenzyme's site and to significantly and competitively suppress this enzyme in vitro (Sánchez and Buitrago, 2018).

Currently, the CYP2C8 gene has about 100 non-single nucleotide polymorphisms (SNPs). Among these, CYP2C8*2, CYP2C8*3, and CYP2C8*4 are the most common. It’It'sP2C8*2(Ile269Phe within exon 5) allele, with an allelic frequency of 18%, is predominantly observed in Africans. CYP2C8*3 (Arg139 Lys with Lys399Arg in exons 3 and 8; an allelic frequency of 10-23%) and CYP2C8*4 (Ile264 Met in exon 5; an allelic frequency of 7.5-11%) are primarily carried by Caucasians. In Asians, both of these alleles are comparatively rare (0.5%). Very few additional mutations exist that alter the amino acids. Additionally, SNPs (*1B and *1C) within the CYP2C8 regulatory areas have been found (De Oliveira Cardoso et al., 2015; Sun and Liu, 2019). Compared to Caucasians, Chinese people have a lower percentage of alleles for CYP2C8*1B(rs7909236), only 8% (Sun and Liu, 2019). Previous research has indicated that CYP2C8 is involved with the metabolism of the montelukast (De Oliveira Cardoso et al., 2015).

This study aimed to explore the relationship between the genetic polymorphism in CYP2C8*1B (rs7909236) and the montelukast responses for Iraqi children (De Oliveira Cardoso et al., 2015; Sánchez and Buitrago, 2018; Sun and Liu, 2019).

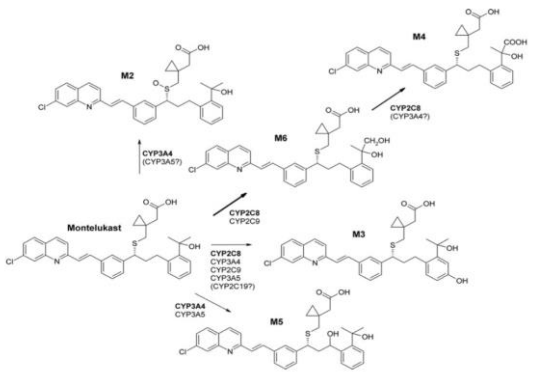

Figure 1: Oxidative Metabolism of Montelukast. The P450 Isoforms Responsible for The Forma- Tion Of the Metabolites M2, M3, M4, M5, And M6 Are Shown According to Chiba Et Al. And the Present Work (Filppula et al., 2011)

2. Material, Patients and Method

2.1. Study Design

This observational cross-sectional study was conducted in the respiratory clinic center of the Kerbala Teaching Hospital of Children between early October 2022 and late September 2023. Children with mild to moderate asthma who exhibited persistent symptoms were included in the trial. One hundred children who were frequent visitors to the hospital's asthma center and had mild to moderate persistent asthma were evacuated, and their responses to Montelukast therapy were evaluated by using ACT (Asthma Control Test), lung function test (PEF, FEV1), and serum total Ig E level.

2.2. Inclusion Criteria Applied for All Patients

1. Children are suffering from mild and modulated asthma.
2. The age range of 6–15.
3. Used montelukast mono-therapy for at least one month regularly.

2.3. Exclusion Criteria Applied for Enrolled Patients

1. A patient experiencing severe acute attacks of asthma.
2. A patient with a history of diabetic mellitus, chronic lung disease, ongoing renal failure, or chronic heart illness. A chest x-ray can diagnose pneumonia and other respiratory conditions (CXR).
3. The patient is using recognized inducers or inhibitors of Montelukast.

2.4. Ethical Approval

The protocol for the study was approved by the research and ethical committee at the University of Kerbala's College of Pharmacy. Before proceeding, the leadership of Kabala Teaching Hospital on Children and the Karbala Health Directorate were consulted. The parents of each patient were requested to obtain permission after being informed about the purpose and nature of the study.

2.5. Samples Collection

Each volunteer's four milliliters of venous blood for the study were divided into two tubes: two milliliters for the total serum Ig E evaluation and two milliliters for genetic testing.

2.6. Childhood Asthma Control Test C-ACT

According to the ACT score, the patients were divided into two groups: controlled asthma (≥20 score) and uncontrolled asthma (≤19 score).

2.7. Assessment **of Lung Function (Pulmonary Function Test)**

The American Thoracic Society (ATS) evaluated lung function through a disposable turbine and Spirometer (Spiro lab I).

2.8. IgE **assessment**

ELISA was performed using the Uroimmun kit to find the total serum IgE level.

2.9. Chemicals

Primers, ethidium bromide, TAE Buffer, absolute Ethanol, Agarose powder, nuclease-free water, DNA extraction kit, Premix kit, and DNA ladder.

2.10. Genotyping of the single nucleotide polymorphisms

In children with asthma, the current study focuses on the CYP2C8*1B gene (rs7909236). Following DNA extraction, allele-specific Polymerase Chain Reactions (AS-PCR) were performed. After that, PCR products were run across a gel electrophoresis enabled via a UV-trans illuminator.

2.11. Primers

The primers were designed by Professor Dr. Hassan Mahmood using Primer Blast software (http:www.ncbi.nih.gov/tools/primer-blast) and sent to Macrogen company for further production. The forward and reverse primers' sequences and product sizes are listed in Table 1

Table 1: Primer Sequence of CYP2C8*1B Enzyme Gene G. -271 C> A

rs7909236	**Sequence (5'->3')**
Forward primer	CAGCACCAGGACCACAAAAG
Allele G	ATCATCACAGCACATTGGAAC
Allele T	ATCATCACAGCACATTGGAAA
Product length 318	

2.12. Optimization of Polymerase Chain Reaction (PCR)Conditions

The desired conditions for CYP2C8*1B (rs7909236) can be achieved using:

1. 6 µL of DNA sample
2. 1 µL of sense primer
3. 1 µL of antisense primer
4. 12 µL of nuclease free water

Table 2: Conditions for CYP2C8*1B Enzyme Gene Optimization

Steps	**Temperature**	**Time**	**Cycles**
Initial Denaturation	95	5 min	1
Denaturation	95	20 sec	35
Annealing	54.1	20 sec	35
Extension	72	35 sec	40
Final extension	72	5 min	1

2.13. Statistical Analysis

IBM's Statistical Programmer for Social Sciences (SPSS) version 24 was used to maintain, process, and analyze the data after it was transferred from research participant data into an electronic database and error and consistency

checked. The results included standard percentage, mean, and standard deviation (SD). The analysis of variance (ANOVA) test was used to assess group differences. The total IgE level was evaluated using the student t-test. Fisher's exact values were calculated for cell counts greater than five. Pearson's correlation was utilized to assess the relationship between clinical parameters (IgE, FEV1, and PEF) and genotype groups in asthmatic children. Only a p-value of less than 0.05 is deemed significant for all statistical tests.

3. Results

One hundred children with mild to moderate asthma who received montelukast medication regularly for at least four weeks before the trial began were enrolled. Table 3 display the outcomes of genetic amplification, Serum Total IgE, and other parameters.

Table 3: Distribution of Different Genotypes of CYP2C8*1B Enzyme G.-271C>A (Rs 7909236) Gene Polymorphism in Asthmatic Children

Patient Genotypes	**N (%)**
Wild homozygous (CC)	74(74%)
Mutant heterozygous (CA)	22(22%)
Mutant homozygous (AA)	4(4%)
Total	100(100%)

As shown in the Table 4 there was no significant association (p value <0.05) between demographic characteristics and different genotypes of CYP2C8*1B enzyme gene g.-271C>A (rs 7909236) polymorphism in asthmatic children.

Table 4*: Association of Some Demographic Characteristics with Different Genotype of CYP2C8*1B Enzyme Gene G.-271C>A (Rs 7909236) Polymorphism in Asthmatic Children

Data express as percent (%), N=100					
Demographic characteristics		**Patient's genotypes**			**P value**
		CC	**CA**	**AA**	
BMI (kg/m^2)	**Underweight**	12(75%)	3(18.8%)	1 (6.2%)	0.62
	Healthy weight	44(71%)	15 (24.2%)	3 (4.8%)	
	Overweight	5(100%)	0 (0)	0 (0%)	
	Obesity	13(76%)	4(23.5%)	0(0%)	
Gender	**Male (n=64)**	43(67%)	18 (28.1%)	3(4.7%)	0.083
	Female (n=36)	31(86%)	4(11.1%)	1(2.8%)	
*Fisher's Exact test was used with a significant P value of less than 0.05.					

The Table 5 Illustrate the Association Between ACT Score and Different Genotypes of CYP2C8*1B Enzyme Gene G.-271C> A Polymorphism in Asthmatic Children. There Was Also No Significant Association (P Value <0.05) Between These Two Parameters.

Table 5: Association of Asthma Control Test with Different Genotypes of CYP2C8*1B Enzyme Gene G.-271C> A Polymorphism in Asthmatic Children

Asthma Control Test (ACT) Score	Patient Genotype (N=100)			P Value
	CC	CA	AA	
Controlled asthma (≥20)	16(76.2%)	5(23.8%)	0(0%)	0.81
Uncontrolled asthma (≤19)	58 (73.4%)	17 (21.5%)	4 (5.1%)	

The Table 6 Show the Mean Distribution of Serum Total Ige In Different Genotypes Of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism In Asthmatic Children. There Was No Significant Difference of Serum Total Ige Between Each Genotype Groups.

Table 6: Mean Distribution of Serum Total Ige Levels Among Different Genotypes of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism in Asthmatic Children

Patients Groups	Genotypes of Group 1, N=46			P value
	CC N=33	CA N=11	AA N=2	
Group1, (age6-9) N= 46	176.21±37.39	189.18±40.57	172.5±24.74	0.33
	Genotypes of Groups 2, N=54			P value
	CC N=41	CA N=11	AA N=2	
Group2, (age10-15) N=54	217.46±45.59	205.81±53.13	215±21.21	0.46
Results express as mean ± SD, *The ANOVA test with significant difference (p value <0.05) was used				

As seen in the Table 7 there was no significant difference in mean distribution of PFT parameters among different genotypes of CYP2C8*1B enzymes gene g.-271C>A polymorphism in asthmatic children

Table 7: Distribution of Pulmonary Function Test Among Different Genotypes of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism in Asthmatic Children

Pulmonary function test Parameters	Patients Genotype N=100			P value
	CC N=74	CA N=22	AA N=4	
FEV1 (L)	85.16±7.98	83.4±6.16	86.25±7.93	0.34
PEF(L/S)	83.28±8.89	81.81±6.17	84±5.77	0. 76
Results were express as mean± SD *ANOVA test with significant p < 0.05 was used				

The Pearson's correlation was done between CYP2C8 enzyme gene g.-271 C>A polymorphism and clinical parameters in asthmatic patients and the result show no significant correlation (p value <0.05) between CYP2C8 enzyme gene g.-271 C>A polymorphism and clinical parameters as described in the Table 8.

Table 8: Pearson's Correlation Between CYP2C8 Enzyme Gene G.-271 C>A Polymorphism and Clinical Parameters in Asthmatic Patients

Clinical parameters	Pearson correlation	P value
Serum total IgE level in group 1	0.089	0.55
Serum total IgE level in group 1	-0.078	0.57
FEV1	-0.48	0.63
PEF	-0.4	0.69

4. Discussion

This study was done to find the correlation between the resistance of ESA and the genetic polymorphism in the SOCs gene (ORAI1). The results showed that TT and TC genotypes were close in percentage, 39.3% and 38.4%, respectively, while the CC genotype represented 22.3%; by comparing these results with previous studies, some similarities presented, for example, the Taiwanese study in which 290 normal controls were included also found that the two prominent groups were TT and TC (41.72% and 43.45% respectively). The CC group was the lowest in percent (14.83%) (Chang et al., 2014). Another Taiwanese study of 579 chronic kidney disease patients showed the following genetic predisposition: TT genotype 40.7%, TC genotype 47.0%, and CC genotype 12.3% (Zhuang et al., 2023). Depending on the results of this study, there is no association between gender differences Genetic variations and neither the duration of the dialysis nor the duration of the treatment has a statistically significant effect on the patients' response to ESA. And there is no considerable variation in erythropoietin levels between the genetic groups.

The Hardy-Weinberg equilibrium test represented that the expected results will be a decrease in CC and TT genotypes with an obvious increase in the TC genotype, which is expected to be the prominent group and is statistically significant.

In this study, the CC genotype showed a statistically significant elevation in hemoglobin level over the TT group, which indicates better response; on the other hand, the CC group has a higher BU level than the other groups, representing poor clinical outcomes. This differs from the results of a previous study that was done in 2021, which showed that the CC/TC genotype has a high risk of erythropoietin resistance (Chang et al., 2014; Zhuang et al., 2023).

5. Conclusion

In ORAI1 genetic polymorphism (rs6486795), the CC genotype may represent the lowest percentage. Furthermore, this group may have a higher Hb level than other groups but may also have negative outcomes. On the other hand, ORAI1 genetic polymorphism has no significant association with erythropoietin resistance in Iraqi patients with CRF on hemodialysis. Despite the presence of the CYP2C8*1B g.-271C> genes in Iraqi asthmatic patients, a genetic polymorphism was non-significantly linked to montelukast responses. More research and a bigger sample size are required to confirm its effects on the montelukast response.

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