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Evaluation the Serum Levels of ORMDL3 among Iraqi Asthmatic Patients and Controls

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Annotation: The aim of this study was to evaluating the serum levels of ORMDL3among Iraqi asthmatic patients.Forty-five asthmatic patients (23 males and 22 females), their age 19-70 years and 35 healthy controls (18 males and 17 females) ,their age 18-71 years were selected from Wasit Province using a convenient sampling method. Serum levels of ORMDL3 was performed using by enzyme-linked immunosorbent assay technique (ELISA) using Human- ORMDL3 kit .The results of the present study demonstrate highly significant differences, the ORMDL3 levels in the asthmatic patients were lower than that of controls (219.15264±7.02402) vs. controls (597.17106±105.99455), P= 0.0001. It is noteworthy that the ORMDL3 levels in the current study were lower significantly among male and female patients with asthma compared to the control group, (219.81270± 9.48480 versus 682.41033±.183.86672), P =0.0070, 218.46259±10.62402versus 506.91771±100.64629), P= 0.0025respectively. ORMDL3 levels revealed non-significant difference when comparing male patients with female patients 219.81270±9.48480 versus218.46259± 10.62402with a non-

significant difference P=0. 9248. Based on the polymorphism of Orosomucoid-like 3 ORMDL3 rs4795405 C/T,serum levels of ORMDL3 showed asthmatic patients with homozygous CC genotypes appeared non-significant difference when compared with the healthy individuals with CC genotype 244.06078+12.84876 vs. 244.06078+12.84876 ,P= 0.5022.The patients carrying the heterozygous CT genotype revealed significant decline in ORMDL3 serum levels 212.04050+9.05509 incomparison with controls carrying this genotype618.98526+132.17806,P=0.0009. the asthmatic patients with recessive TT genotype showed non-significant decrease in ORMDL3 levels 217.35117+15.64638 versus 573.41457+281.05874 in controls ,P=0.2690. These results indicate that ORMDL3 levels in the sera of asthmatic patients for all genotypes CC,CT and TT have decreased compared to their counterparts in the control group. There were no significant differences between genotypes of ORMDL3 and its level in asthmatic patients. In conclusion, there is a potential association of ORMDL3rs4795405 C/T polymorphism and lower ORMDL3 serum levels with asthma susceptibility.

Introduction

Asthma is a complex respiratory condition characterized by airway inflammation and hyperresponsiveness, influenced by both environmental and genetic factors. Among the various genetic loci associated with asthma, the *ORMDL3* gene, located on chromosome 17q21, has emerged as a significant contributor to the disease's pathogenesis. The SNP rs4795405, a C>T polymorphism within the *ORMDL3* gene, has been particularly highlighted in recent studies for its association with asthma susceptibility and severity. Research indicates that this polymorphism may alter the expression of *ORMDL3*, thereby influencing immune responses and airway inflammation, which are critical in asthma development and exacerbation (Balantic *et al*.,2013;Guo *et al*.,2022).

The association between the rs4795405 variant and asthma has been supported by multiple genome-

wide association studies (GWAS), demonstrating that this SNP is linked to increased risk of asthma, particularly in pediatric populations (Schedel *et al*.,2015).

The T allele of rs4795405 has been shown to correlate with higher *ORMDL3* expression levels, suggesting a potential mechanism through which genetic predisposition can affect disease outcomes(Moffatt *et al* .,2007) . Furthermore, functional analyses have indicated that *ORMDL3* plays a role in regulating cellular processes such as autophagy and calcium homeostasis, which are vital for maintaining airway epithelial integrity and function(Guo *et al*.,2022).

Given the substantial evidence linking *ORMDL3* and asthma, the current study aims to evaluate serum levels of ORMDL3 in asthmatic patients. By examining the frequency of this variant in different populations and its relationship with clinical phenotypes, this will elucidate its role in asthma pathogenesis and contribute to the understanding of genetic factors influencing this prevalent respiratory disease.

Materials and Methods

The current study is a case-control study. The study was carried out from 1st October 2023 to 1th May2024. This study was performed at the Department of Biology College of Education for the Pure Sciences University of Wasit

A total of 80 participants (45 confirmed asthmatic patients and 35 healthy individuals as controls were selected by using a convenient sampling method.

1- Asthmatic patients group: Forty-five asthmatic patients (23 males and 22 females), and their age range was between 19–70 years (40 ± 12.51 years, median= 40 years).

2- Control group: The control group comprised of 35 healthy individuals (18 males and 17 females) with an age range between 18-71 years (32.38 ± 13.68 years, median=28 years). All patients were diagnosed according to global criteria by the physician. The data recorded for all participants included: name, gender, age, other diseases, smoking, treatment, weight, height, body mass index, residence, profession, the patient's disease history, inheriting the disease in the family, and date of sample collection. All samples were collected from Alzahraa Teaching Hospital, Chest Diseases Centre, and Blood Bank in Kut, Iraq .Five ml of venous blood was placed in a plain tube to collect serum through centrifugation at 3000 revolutions per minute (rpm) for15 min. Sera were dispensed into Eppendorf tubes and preserved at -20Co.

Determination of Orosomucoid-like3(ORMDL3)concentrations in sera from patients and controls

An enzyme-linked immunosorbent assay (ELISA) is what this kit is for. Human ORMDL3 has been pre-coated on the plate (Bioassay Technology Laboratory).

Results

Serum levels of ORMDL3 with asthma patients and controls groups

Determination of Orosomucoid-like 3(ORMDL3) in sera of patients with asthma and controls was done by using an Enzyme-linked immunosorbent assay (ELISA). The results are shown in Table (1). The results reveal highly significant differences, the ORMDL3 levels in the asthmatic patients were lower than that of controls (219.15264±7.02402) Vs. Controls (597.17106±105.99455), *P*= 0.0001.

Table (1) : Mean levels of ORMDL3 in asthmatic patients and control groups

Parameters Groups	ng/ml Mean+SE
Control	597.17106 +105.99455*
Patients	219.15264+7.02402
P-value	0.0001
Significance level	Sig.[1]

***Data was shown as Mean ± SE.**

SE: Standard error

ng: nanogram

Sig.[1]:Significant P≤0.01

Serum ORMDL3 levels among males and females of studied groups

The ORMDL3 levels were lower significantly among male and female patients with asthma compared to the control group, (219.81270± 9.48480 versus 682.41033±.183.86672), *P* =0.0070, 218.46259±10.62402versus 506.91771±100.64629), *P*= 0.0025respectively. ORMDL3 levels revealed non-significant difference when comparing male patients with female patients 219.81270±9.48480 versus218.46259± 10.62402with a non-significant difference *P*=0. 9248. Although there were significant differences Table (2).

Table (2): Mean comparison of ORMDL3 in male versus female in the study group

Parameters Groups	ng/ml Mean+SE			
	Male	Female	P-value	Significance level
Control	682.41033+183.86672*	506.91771+100.64629	0.4161	Ns.
Patients	219.81270+9.48480	218.46259+10.62402	0.9248	Ns.
P-value	0.007	0.0025		
Significance level	Sig.[1]	Sig.[1]		

***Data was shown as Mean ± SE.'**

SE: Standard error

ng: nanogram

Sig.[1]:Significant p< 0.01

Ns.: Nonsignificant p > 0.05

Relationship of *ORMDL3* gene polymorphism with ORMDL3 level in the study groups

Serum levels of ORMDL3 according to *ORMDL3* gene *C/G*enotypes are shown in Tables (3) . Asthmatic patients with homozygous CC genotypes appeared non-significant difference when compared with the healthy individuals with CC genotype 244.06078+12.84876 vs. 244.06078+12.84876 ,*P*= 0.5022.The patients carrying the heterozygous CT genotype revealed significant decline in ORMDL3 serum levels 212.04050+9.05509 in comparison with controls carrying this genotype618.98526+132.17806,*P*=0.0009.The asthmatic patients with recessive TT genotype showed non-significant decrease in ORMDL3 levels 217.35117+15.64638 versus 573.41457+281.05874 in controls ,*P*=0.2690. These results indicate that ORMDL3 levels in the sera of asthmatic patients for all genotypes CC,CT and TT have decreased compared to their counterparts in the control group. There were no significant differences between genotypes of ORMDL3 and its level in asthmatic patients.

Table (3): Mean levels of ORMDL3 according to *Orosomucoid-like3 ORMDL3* rs4795405 genotypes in patients and control groups

Parameters Groups	ng/mL Mean+SE		
	CC	CT	TT
Control	273.10260+52.94784*	618.98526+132.17806	573.41457+281.05874
Patients	244.06078+12.848	212.04050+9	217.35117+15.646

	76	**.05509**	**38**
P-value	**0.5022**	**0.0009**	**0.2690**
Significance level	**Ns.**	**Sig.[1]**	**Ns.**

SE: Standard erro

ng: nanogram

Sig.[1]:Significant p< 0.01

Ns.:Nonsignificant p > 0.05

***Data was shown as Mean ± SE.**

Discussion

ORMDL3 levels among asthmatic patients and controls

A complicated illness, asthma affects a large number of people in the industrialised world. In recent times, scientists have turned to genome-wide association studies in their quest to unravel the genetic roots of these complex diseases. Because it has been linked to immune-mediated pathology, asthma, inflammatory bowel disease (IBD), and Type I diabetes, one specific gene, ORMDL3, is of interest (Moffatt,2007;Moffatt,2010;McGovern,2010;Less,2009;Saleh,2011). By impacting several asthma-related biochemical and cellular processes, such as sphingolipid metabolism (Breslow et al., 2010) and the UPR (Cantero-Recasens et al., 2010), ORMDL3 may contribute to these symptoms.

The lungs are among the tissues that express ORMDL3. Its expression levels can be modulated by environmental factors such as allergens or infections, potentially influencing asthma development or exacerbation.

The precise mechanisms by which ORMDL3 contributes to asthma are still being elucidated, but several key pathways have been proposed:

Endoplasmic reticulum (ER) stress and inflammation: ORMDL3 may influence asthma by modulating ER stress responses, which in turn affects inflammation and immune responses. Increased ER stress can lead to the production of inflammatory cytokines and contribute to airway inflammation in asthma.

Sphingolipid Metabolism: ORMDL3 may affect immune response and inflammatory cell signalling pathways by controlling sphingolipid metabolism. Sphingolipids have an impact on immune cell activity, particularly that of T cells and macrophages, which are crucial in the pathophysiology of asthma.

Th2-helper type 2 (Th2) Immune Response: It has been suggested that ORMDL3 has a role in controlling Th2-helper type 2 immune responses. Th2 cells are essential for the development of asthma because they release cytokines that lead to airway inflammation and hyperreactivity, including IL-4, IL-5, and IL-13 (Moffatt et al.,2007;Torgerson et al.,2008; Gon et al.,2016; Rangasamy et al., 2012). Jiad and ahmed ,2022 indicated that cf-mt DNA down regulated significantly in Iraqi asthmatic patients(Jiad and ahmed ,2022) .

Abdulmutaleb and Ahmed, 2023 investigate the association of the LTC4S rs730012 C/A polymorphism with asthma susceptibility in Iraqi patients. These results suggested that the C allele might play a risk factor for asthma whereas the A allele might consider a protective role against asthma(Abdulmutaleb and Ahmed, 2023).

The results of the present study demonstrate that highly significant differences, the ORMDL3 levels in the asthmatic patients were lower than that of controls (219.15264±7.02402), Controls (597.17106±105.99455), *P*= 0.0001.Also the ORMDL3 levels were lower significantly among male and female patients with asthma compared to the control group, (219.81270± 9.48480 versus 682.41033±.183.86672), *P* =0.0070, 218.46259±10.62402versus 506.91771±100.64629), *P*=

0.0025respectively. These results indicate that ORMDL3 levels in the sera of asthmatic patients for all genotypes CC,CT and TT have decreased compared to their counterparts in the control group. The association between asthma and polymorphisms in the ORMDL gene was demonstrated by Toncheva et al. in 2015. Increased ORMDL levels in asthmatics indicate that ORMDLs may be a factor in asthma (Toncheva *et al*.,2015).

Nowakowska *et al*.,2023 revealed that ORMDL3 expression was increased in allergic asthma (Nowakowska *et al*.,2023).

Serum levels of ORMDL3 according to ORMDL3 gene C/Genotypes demonstrated that ORMDL3 levels in the sera of asthmatic patients for all genotypes CC, CT and TT have decreased compared to their counterparts in the control group. These results suggest that low protein levels are not associated with genotype. There are no previous studies to evaluate protein levels in the sera of asthma patients, and there are no studies to evaluate the relationship between protein levels and genotyping.

Conclusions

1. A potential association between lower ORMDL3 levels and asthma.
2. The reduction in ORMDL3 levels is a common feature in asthma, regardless of gender.
3. A potential association between the CT genotype and lower ORMDL3 levels in asthma.

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