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THE ROLE OF BETA-2 ADRENERGIC RECEPTOR GENE POLYMORPHISM IN THE PATHOPHYSIOLOGY OF ASTHMA ASSOCIATED WITH OBESITY

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ABSTRACT:

Background and objectives: Several mechanisms have been suggested to explain the association between asthma and obesity, one of them is the presence of common genetic predictors as β2 adrenergic receptor gene polymorphism. To test this suggestion, the present study was carried out.Subjects: 60 asthmatic patients (50 females and10 males) and 60 normal subjects (46 females and 14 males) were included in this study. Methodology: measurement of body weight and height, waist circumference and waist to hip ratio. Asthma severity was assessed by disease severity score. Pulmonary function tests and bronchial reactivity by methacholine inhalation challenge ($PD_{20\text{-}FEV1}$) were performed and DNA extraction, Polymerase chain reaction (PCR)and restriction digestion was performed at codons 16 and 27of β2AR to determine β2 adrenergic receptor gene polymorphism. Results: No significant difference was detected between the asthmatic and control subjects as regards allelic or genotype frequencies of β2AR gene at codon 16 and codon 27 and no significant difference was found as regards genotype frequencies of β2AR gene at codon 16 and codon 27 between obese and non-obese asthmatics. A significantly increased frequency of Gly/Gly at codon 16 was found among severe asthmatics in comparison with mild and moderate asthmatics. A significantly higher frequency of genotype Glu/Glu at codon 27 was detected among obese subjects when compared to non-obese subjects. There was a significant difference in FEV1% predicted and in FEF25-75% predicted in non- obese asthmatics when distributed according to genotypes at codon 16. No significant difference in the distribution of the asthmatics according to the value of PD20-FEV1 among genotypes of B2AR gene at codon 16, codon 27 was found.

Conclusion: Arg16Arg was found to be protective from development of severe asthmaGlu27Gluwassignificantly associated with obesity. However, there was no detectable specific genotype for the association of asthma and obesity.

Keywords: *Asthma; β2 adrenergic receptor gene polymorphism; Obesity.*

INTRODUCTION

Asthma and obesity are prevalent disorders, each with a significant public health impact. [(1)] Epidemiologic data indicate that obesity increases the prevalence and Incidence of asthma and reduces asthma control.[(2)]

Several mechanisms have been suggested to explain the association between asthma and obesity including mechanical effects of obesity on pulmonary physiology, systemic inflammation, co-morbidities of obesity, prenatal diet and nutrition, hormonal factors and common genetic predictors. [(3-7)]

Some studies [(8, 9)] that have focused on either asthma or obesity have identified genes, including angiotensin I–converting enzyme (ACE), adrenergic receptor β-2 (ADRB2), and vitamin D (1,25 dihydroxyvitamin D3) receptor (VDR) that might influence both diseases. Genes such as leptin(LEP); protein kinase C alpha (PRKCA); and tumor necrosis factor (TNF) have also been evaluated for pleiotropic effects that influence both asthma and obesity simultaneously. [(10-12)]

Comparative linkage analyses of asthma and obesity show an overlap of chromosomal regions (chromosomes 5q, 6p, 11q and 12q) linked to both conditions.[(13)] Chromosome 5q contains the gene ADRB2, which codes for the β-2-adrenergic receptor that exerts effects on the sympathetic nervous system and in baseline metabolism; its pharmacological activation leads to bronchodilation of the airways.[(14)]

The ADRB2 is expressed on airway smooth muscle (ASM), inflammatory cells, and adipose tissue, where endogenous catecholamines influence energy expenditure, airway tone, and airway inflammation.[(15)] Furthermore, insulin levels, which are altered in obesity, can influence β2-adrenergic receptor sensitivity. [(16)] The ADRB2 is involved in lipid mobilization, as a major lipolytic receptor in human fat cells, and genetic variation in this receptor gene could theoretically reduce lipolysis and predispose to obesity. [(17)]

The most frequent single-nucleotide polymorphisms occur at codon 16 and codon 27. [(18)] Accordingly,

the aim of the present work is to study the possible role of beta2 adrenergic receptor gene polymorphism in the pathophysiology of the association between obesity and asthma.

SUBJECTS AND METHODS

Study Design

The study was carried out in 3 sessions on three separate days:
During the first session full history taking was done with anthropometric measurements (BMI, waist circum-ference and waist/hip ratio)and exclusion criteria were applied according to ATS guidelines for Spirometry and Methacholine challenge testing. Training on Spirometry was carried out.

During the second session Spirometry, Methacholine, questionnaire to assess severity and sampling were carried out .

During the third session molecular studies were done.

SUBJECTS

The study included 120 adults. 60 asthmatic patients (50 females and10 males) as defined by the American Thoracic Society [19] and 60 normal subjects (46 females and 14 males) as controls were included in this study in the period from January 2010 to April 2011. Both patients and control subjects were selected and grouped according to their Body Mass Index (BMI) into nonobese group, BMI ≤ 25.00 kg/m2 and obese group, BMI ≥ 30.00 kg/m2 .

The four groups (each group includes 30 subjects)are:

- Obese asthmatics
- Non-obese asthmatics
- Obese control subjects
- Non-obese control subjects

METHODS

1. Assessment for asthma severity. [20]
2. Asthma severity was assessed by Disease Severity Score (DSS). [20]
3. Physiological studies

The following parameters were assessed for both patients and normal subjects:

a) Pulmonary function tests including Forced vital capacity (FVC),Forced expiratory volume in one second (FEV1), FEV1/FVC% and Forced expiratory flow rate between 25% and 75% ($FEF_{25\%-75\%}$) were measured using computerized dry spirometer (Jaeger, Germany) with automatic dosimeter for methacholine inhalation challenge. All measurements were performed according to the European Respiratory Society and American Thoracic Society protocol for spirometry standardization. [21]

b) Determination of bronchial reactivity by methacholine inhalation challenge (only in asthmatic subjects) using the five-breath dosimeter protocol according to the guidelines of The American Thoracic Society for methacholine inhalation challenge test. [22]

4. Molecular studies

A) DNA extraction:
Two milliliter of whole venous blood were collected from each subject on vaccutainer EDTA tube under complete aseptic technique. Genomic DNA was extracted from whole blood by DNA salting out technique. [23]

B) Polymerase Chain Reaction(PCR)
The Arg16Gly and Gln27Glu in the β2AR gene were amplified using the following gene-specific primers: [24-26]
The forward primer:5-GGC-CCA-TGA-CCA-GAT-CAG–CA-3
The reverse primer: 5-GAA-TGA-GGC-TTC-CAG-GCG-TC-3
The forward primer: CTT-CTT-GCT-GGC-ACG-CAA-T-3
The reverse primer:CCA-GTG-AAG-TGA-TGA-AGT-AGT-TGG-3
The forward primer: 5-GCC-TTC-TTG-CTG-GGC-ACC-CAT-3
The antisense primer:5-CAT-ACG-CTC-GAA-CTT-GGC-CAT-C-3

DNA amplification was performed in a Thermohybraid PCR Express Thermacycler, with a total reaction volume of 25 ul containing:
Milli Q water: 12.5 ul
DNA: 300 ng
Forward primer (Bioron): 20 Pico moles
Reverse primer (Bioron): 20 Pico moles
PCR master mix: 12.5 ul Amplification was performed using initial denaturation at 95°C for 3 minutes
followed by 35 cycles of 94°C for 30 seconds, 66°C for 30 seconds and 72°C for 30 seconds with a final extension of 70°C for 6 minutes.
Following amplification, 9 ul of the PCR product were mixed with 2 ul of 6 x loading buffer (0.09 % bromophenol blue, 0.09 % xylene cyanol, 60 Mm EDTA in 60 % glycerol) and loaded on agarose gel 2 % (containing ethidium bromide 20 ng/ul).

5 ul of 50 bp ready to use DNA ladder (MBI Fermentas) was loaded in a separate lane. Products were visualized on a UV Transilluminator.

C) Restriction digestion:
Restriction digestion was performed at codons 16 and 27 of β2AR using Ncol, BsrDI, Bbvl and ItaI restriction enzymes. [24-26] Digestion was performed under the following conditions:
Restriction enzyme: 1 unit
PCR water: 6 ul
Buffer: 3 ul
PCR product: 10 ul
Overnight incubation at 37°C was followed by gel electrophoresis on 3% agarose gel. Figure1-3.

STATISTICAL ANALYSIS

The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test and revealed

that most of the data were normally distributed, so parametric statistics were applied. Quantitative data were described using mean and standard deviation. Qualitative data as genotypes were described using number and percent.

Association between 2 qualitative variables (2x2) done using Chi square test. This was used for genotypes.

Comparing quantitative variables in 2 groups were conducted using independent sample t test .This was used for pulmonary function parameters. Kruskal Wallis test was used to study the distribution of pulmonary function parameters among the genotype groups.

Correlation between 2 quantitative variables done using pearson correlation test.

In all statistical tests, level of significance of .05 was used, below which the results considered to be statistically significant

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA)

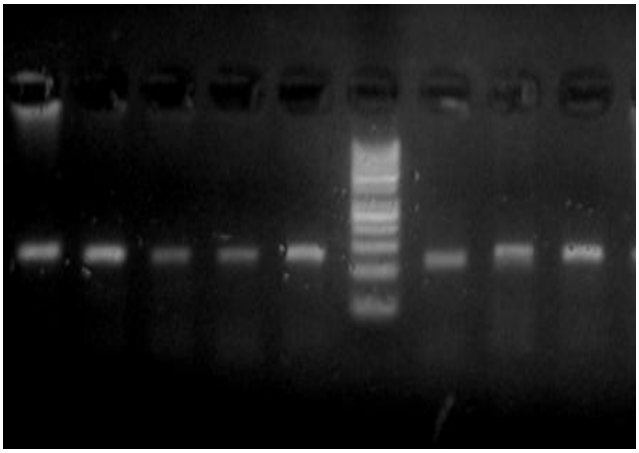


Fig. (1): Lanes 1, 2, 3, 4 and 5: Arg / Arg genotype, Lane 6 : 50 bp DNA ladder, Lane 7 : Gly /Gly genotype, Lanes 8 and 9 : Arg / Arg genotype

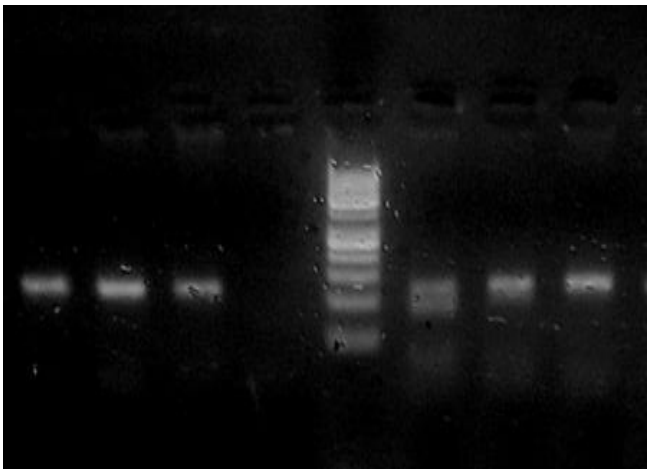


Fig. (2): Lanes 1, 2 and 3: Arg /Arg genotype, Lane 5: 50 bp DNA ladder, Lane 6: Gly /Arg genotype, Lanes 7 and 8: Arg /Arg genotype.

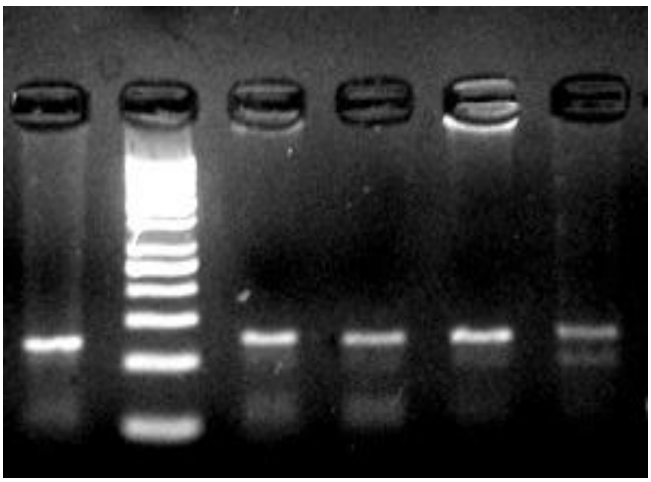


Fig. (3): Lanes 1, 3, 4 and 5: Arg /Arg genotype. Lane 2 : 50 bp DNA ladder, Lane 6 : Gly / Arg genotype

RESULTS

The characteristics of asthmatic patients and controls are presented in table (1). There were no significant difference between patients and control groups as regards the age, weight, and height.Pulmonary function parameters in asthmatic and control subjects are presented in table (1) as actual and percent predicted values. There was a significant difference between the asthmatic and the control subjects in all pulmonary function parameters measured.

The asthmatics were classified according to severity into mild, moderate and severe asthmatics: 24 (40.0%), 30 (50.0%), 6 (10.0%) respectively.

There was no significant correlation between asthma severity or airway hyper-responsiveness ($PD_{20\text{-}FEV1}$) and parameters of obesity in the asthmatics Table (2). The Comparison between obese (n=30) and non-obese asthmatics (n=30) according to AHR ($PD_{20\text{-}FEV1}$) showed no significant difference between the two groups.

Molecular studies: The Distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27 and codon 16 - codon 27 among obese and non-obese asthmatics [3] and obese and non-obese subjects is illustrated in table (4) which showed a significant association only between the group categories and combined genotype Gly16Arg –Glu27Glu [p =0.044, 95% C.I.=0.040-0.048]

The distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27 and codon 16 /codon 27 among mild, moderate and severe asthmatics are illustrated in table (5)which revealed a significantly increased frequency of Gly16Gly among severe asthmatics (66.7%) [p = 0.021] in comparison with the mild (20.8%) and the moderate (13.3%) asthmatics and a significantly higher frequency of Gly16Gly-Gln27Gln in severe asthmatics (33.3%) [p = 0.012] in comparison to mild (4.2%) and moderate (0%) asthmatics.

The pulmonary function parameters in the four studied groups distributed according to genotypes of β_2AR gene at codon 16 in table (6) revealed a significant difference in FEV1%pred ($p = 0.017$) and in $FEF_{25-75\%}$ percent predicted ($p = 0.034$) in non- obese asthmatics when distributed according to genotypes at codon 16.

The pulmonary function parameters in the four studied groups distributed according to genotypes of β_2AR gene at codon 27 showed no significant difference. The pulmonary function parameters in the four studied groups distributed according to combined genotypes of β_2AR gene at codon 16-codon 27 showed no significant difference. There was no significant difference in the distribution of the asthmatics according to the value of $PD_{20\text{-}FEV1}$ (provocational dose that resulted in 20% drop in FEV_1) among genotypes of β_2AR gene at codon 16, codon 27, and codon 16 - codon 27.

Table (1): The characteristics and the Pulmonary function parameters for asthmatic and control subjects

	Asthmatics (n = 60)	Controls (n = 60)	p
Sex Male Female	10 (16.7%) 50 (83.3%)	14 (23.3%) 46 (76.7%)	x2p = 0.361
Male : female ratio	1 : 5	1 : 3.3	
Age Mean ± SD	33.0 ± 8.0	34.0 ± 10.0	0.819
Weight (kg) Mean ± SD	80.0 ± 21.0	82.0 ± 20.0	0.554
Height (cm) Mean ± SD	164.0 – 7.0	165.0 ± 9.0	0.606
B.M.I. (kg/m2) Mean ± SD	30.0 ± 8.0	30.04 ± 6.65	0.774
Waist circumference (cm) Female Mean ± SD	N = 50, 90.72 ± 15.65	N = 46, 90.24 ± 13.45	0.873
Waist circumference (cm) Male Mean ± SD	N = 10. 92.50 ± 13.04	N = 14, 99.86 ± 18.18	0.287
Waist / Hip ratio Female Mean ± SD	N = 50, 0.83 ± 0.08	N = 46, 0.84 ± 0.06	0.302
Waist / Hip ratio Male Mean ± SD	N = 10, 0.89 ± 0.12	N = 14, 0.90 ± 0.10	0.858
FVC (L) Mean ± SD	3.19 ± 0.64	3.52* ± 0.81	0.016
FVC %pred. Mean ± SD	89.0 ± 11	96.0** ± 12.0	0.001
FEV_1 (L) Mean ± SD	2.75 ± 0.54	3.24** ± 0.81	<0.001
FEV_1 % pred Mean ± SD	89.0 ± 12.0	102.0** ± 12.0	<0.001
FEV_1 / FVC % Mean ± SD	87.0 ± 9.0	92.0** ± 6.0	<0.001
$FEF_{25-75\%}$ (L/Sec) Mean ± SD	2.97 ± 0.94	4.17** ± 1.38	<0.001
FEF_{25-75}% pred. Mean ± SD	76.0 ± 22.0	105.0** ± 25.0	<0.001

FEV1: forced expiratory volume in one second
FVC: forced vital capacity
$FEF_{25-75\%}$: forced expiratory flow between 25 and 75 %
% pred.: percent predicted
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

Table (2): The Correlation between Parameters of asthma severity (clinical severity score, airway reactivity score and FEV_1%pred) and airway hyper-responsiveness ($PD_{20\text{-}FEV1}$) and the studied parameters of obesity (BMI, waist circumference and waist/hip ratio) in the asthmatics.

	$PD_{20\text{-}FEV1}$		Clinical severity score		Airway reactivity score		FEV_1%pred.	
	r_s	p	r	p	r	p	r	p
BMI (kg/m^2)	0.038	0.771	0.124	0.346	0.189	0.148	-0.011	0.933
Waist circumference (cm) (male)	-0.320	0.367	0.217	0.546	0.321	0.366	-0.574	0.083
Waist circumference (cm) (female)	0.162	0.261	0.045	0.754	0.172	0.232	-0.029	0.844
Waist /hip ratio (male)	-0.185	0.609	0.125	0.731	0.323	0.363	-0.522	0.122
Waist /hip ratio (female)	0.229	0.109	-0.072	0.619	0.113	0.435	0.085	0.555

r: Pearson coefficient rs: Spearman coefficient
BMI: Body mass index PD_{20FEV1}: Provocational dose of methacholine that resulted in 20% drop in FEV_1

Table (3): Distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27and codon 16 - codon 27 among obese and non-obese asthmatics.

Genotype	Asthmatics		Test of sig.	p	O.R.	95% C.I.
	Obese n = 30	Non-Obese n = 30				
Codon 16						
Gly / Gly	8(26.7%)	5(16.7%)	χ^2 = 0.884	0.347	0.550	0.157 – 1.931
Gly / Arg	19(63.3%)	21(70.0%)	χ^2 = 0.300	0.584	1.351	0.460 – 3.968
Arg / Arg	3(10.0%)	4(13.3%)	FE	1.000	1.385	0.282 – 6.796
Codon 27						
Gln / Gln	4(13.3%)	8(26.7%)	χ^2 = 1.667	0.197	2.364	0.627 – 8.917
Gln / Glu	18(60.0%)	19(63.3%)	χ^2 = 0.071	0.791	1.152	0.406 – 3.263
Glu / Glu	8(26.7%)	3(10.0%)	χ^2 = 2.783	0.095	0.306	0.072 – 1.291
Codon 16 - Codon 27						
Gly /Gly- Gln / Gln	2(6.7%)	1(3.3%)	FE	1.000	0.483	0.041 – 5.628
Gly / Gly- Gln / Glu	4(13.3%)	3(10.0%)	FE	1.000	0.722	0.147 – 3.545
Gly / Gly- Glu / Glu	2(6.7%)	1(3.3%)	FE	1.000	0.483	0.041 – 5.628
Gly / Arg- Gln / Gln	2(6.7%)	6(20.0%)	FE	0.254	3.500	0.645–18.980
Gly / Arg- Gln / Glu	13(43.3%)	14(46.7%)	χ^2 = 0.067	0.795	1.144	0.414 – 3.166
Gly /Arg- Glu / Glu	4(13.3%)	1(3.3%)	FE	0.353	0.224	0.024 – 2.136
Arg / Arg- Gln / Gln	0(0.0%)	1(3.3%)	FE	1.000	1.034	0.968 – 1.106
Arg / Arg- Gln / Glu	1(3.3%)	2(6.7%)	FE	1.000	2.071	0.178 –24.148
Arg / Arg-Glu / Glu	2(6.7%)	1(3.3%)	FE	1.000	0.483	0.041 – 5.628

p: p value for comparing between the two studied groups
χ^2: Chi square test
FE: Fisher Exact test
Gly : Glycine
Arg: Argnine
Gln: Glutamine
Glu: Glutamic acid
%= percentage
O.R: Odds ratio
95% C.I: 95% confidence interval

Table (4):Distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27 and codon 16 -codon 27 among obese and non-obese subjects (asthmatics and Non-asthmatics)

Genotype	Obese n = 66	Non-obese n = 54	Test of sig.	p	O.R.	95% C.I.
Codon 16						
Gly / Gly	15(22.7%)	9(16.7%)	χ^2 =0.682	0.409	0.680	0.271 – 1.704
Gly / Arg	45(68.2%)	37(68.5%)	χ^2 =0.002	0.969	1.016	0.469 – 2.201
Arg / Arg	6(9.1%)	8(14.8%)	χ^2 =0.944	0.331	1.739	0.564 – 5.362
Codon 27						
Gln / Gln	9(13.6%)	11(20.4%)	χ^2 =0.970	0.325	1.620	0.617 – 4.256
Gln / Glu	40(60.6%)	37(68.5%)	χ^2 =0.809	0.369	1.415	0.663 – 3.017
Glu / Glu	17(25.8*%)	6(11.1%)	χ^2 =4.112	0.043	0.360	0.131 – 0.991
Codon 16 - Codon 27						
Gly /Gly- Gln / Gln	3(4.5%)	1(1.9%)	FE	0.626	0.396	0.040 – 3.922
Gly / Gly- Gln / Glu	8(12.1%)	5(9.3%)	χ^2 =0.252	0.616	0.740	0.227 – 2.408
Gly / Gly- Glu / Glu	4(6.1%)	3(5.6%)	FE	1.000	0.912	0.195 – 4.262
Gly / Arg- Gln / Gln	5(7.6%)	9(16.7%)	χ^2 =2.382	0.123	2.440	0.766 – 7.776
Gly / Arg- Gln / Glu	29(43.9%)	27(50.0%)	χ^2 =0.438	0.508	1.276	0.620 – 2.626
Gly /Arg- Glu / Glu	11(16.7**%)	1(1.9%)	χ^2 =7.243	0.007	0.094	0.012 – 0.756
Arg / Arg- Gln / Gln	1(1.5%)	1(1.9%)	FE	1.000	1.226	0.075-20.076
Arg / Arg- Gln / Glu	3(4.5%)	5(9.3%)	FE	0.465	2.143	0.488 – 9.406
Arg / Arg-Glu / Glu	2(3.0%)	2(3.7%)	FE	1.000	1.231	0.168 – 9.038

χ^2: Chi square test
FE: Fisher Exact test
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
Gly : Glycine
Gln: Glutamine
%= percentage
95% C.I: 95% confidence interval
Arg: Argnine
Glu: Glutamic acid
O.R: Odds ratio

Table (5): Distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27and codon 16 - codon 27 among asthmatics classified into mild, moderate and severe according to clinical severity score

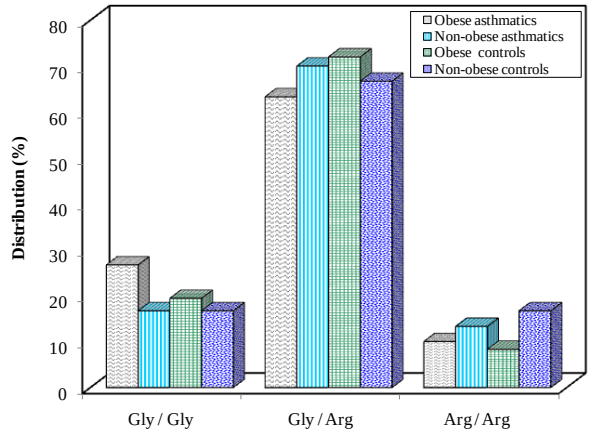
Genotype	Asthmatics			MCp	95% C.I.
	Mild n = 24	Moderate n = 30	Severe n = 6		
Codon 16					
Gly / Gly	5(20.8%)	4(13.3%)	4(66.7%)*	0.021	0.018 – 0.024
Gly / Arg	15(62.5%)	23(76.7%)	2(33.3%)	0.127	0.120 – 0.134
Arg / Arg	4(16.7%)	3(10.0%)	0(0.0%)	0.615	0.605 – 0.625
Codon 27					
Gln / Gln	6(25.0%)	4(13.3%)	2(33.3%)	0.429	0.420 – 0.439
Gln / Glu	15(62.5%)	19(63.3%)	3(50.0%)	0.862	0.855 – 0.868
Glu / Glu	3(12.5%)	7(23.3%)	1(16.7%)	0.714	0.705 – 0.723
Codon 16 - Codon 27					
Gly /Gly- Gln / Gln	1(4.2%)	0(0.0%)	2(33.3%)*	0.012	0.010 – 0.014
Gly / Gly- Gln / Glu	4(16.7%)	2(6.7%)	1(16.7%)	0.615	0.605 – 0.625
Gly / Gly- Glu / Glu	0(0.0%)	2(6.7%)	1(16.7%)	0.333	0.323 – 0.342
Gly / Arg- Gln / Gln	4(16.7%)	4(13.3%)	0(0.0%)	0.679	0.670 – 0.688
Gly / Arg- Gln / Glu	9(37.5%)	16(53.3%)	2(33.3%)	0.493	0.483 – 0.502
Gly /Arg- Glu / Glu	2(8.3%)	3(10.0%)	0(0.0%)	1.000	1.000 – 1.000
Arg / Arg- Gln / Gln	1(4.2%)	0(0.0%)	0(0.0%)	0.503	0.493 – 0.512
Arg / Arg- Gln / Glu	2(8.3%)	1(3.3%)	0(0.0%)	0.698	0.689 – 0.707
Arg / Arg-Glu / Glu	1(4.2%)	2(6.7%)	0(0.0%)	1.000	1.000 – 1.000

MC: Monte Carlo test
*: Statistically significant at $p \leq 0.05$ by Monte Carlo test
Gly : Glycine
Arg: Argnine
Gln: Glutamine
Glu: Glutamic acid
%= percentage
95% C.I: 95% confidence interval

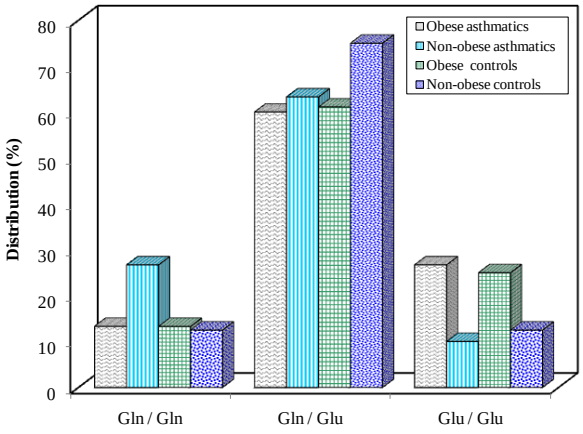
Table (6): The pulmonary function parameters in the four studied groups distributed according to genotypes of β_2AR gene at codon 16

			Codon 16			^{KW}p
			Gly/Gly	Gly / Arg	Arg/Arg	
$FVC_{(\%)}$ pred.	Asthmatics	Obese	88.0 (66.50 – 105.40)	91.50 (70.40 – 116.70)	85.20 (83.70 – 95.50)	0.98
		Non obese	86.70 (82.40 – 97.80)	88.40 (68.60 – 103.70)	101.25 (82.8 – 102.4)	0.20
	Controls	Obese	105.20 (82.7 – 116.6)	96.35 (59.1 – 111.2)	89.90 (87.1 – 103.7)	0.53
		Non Obese	100.25 (79.20 – 123.0)	93.15 (77.0 – 119.90)	91.75 (84.50 – 106.40)	0.78
$FEV_{1(\%)\ pred.}$	Asthmatics	Obese	88.90 (72.70 – 113.80)	84.80 (68.10 – 105.0)	79.90 (69.10 – 104.60)	0.61
		Non obese	81.20 (70.90 – 96.10)	87.40 (69.10 – 101.50)	108.85* (88.8 – 111.8)	0.017
	Controls	Obese	109.80 (75.10 – 128.8)	99.75 (70.9 – 122.3)	94.10 (85.2 – 119.1)	0.59
		Non obese	104.75 (90.40 – 123.30)	103.75 (88.40 – 123.50)	100.15 (88.2 – 112.6)	0.88
$FEF_{25-75\%}$ (%)pred.	Asthmatics	Obese	79.90 (62.20 – 104.30)	80.30 (41.30 – 105.50)	60.70 (27.70 – 92.10)	0.57
		Non obese	56.40 (44.20 – 86.90)	77.50 (39.40 – 115.0)	102.0* (73.80 –111.30)	0.03
	Controls	Obese	93.30 (56.90 – 145.50)	104.1 (63.6 – 152.3)	94.0 (69.30 – 135.8)	0.96
		Non obese	96.15 (91.0 – 102.8)	119.30 (72.39 – 155.5)	94.05 (70.0 – 142.7)	0.30

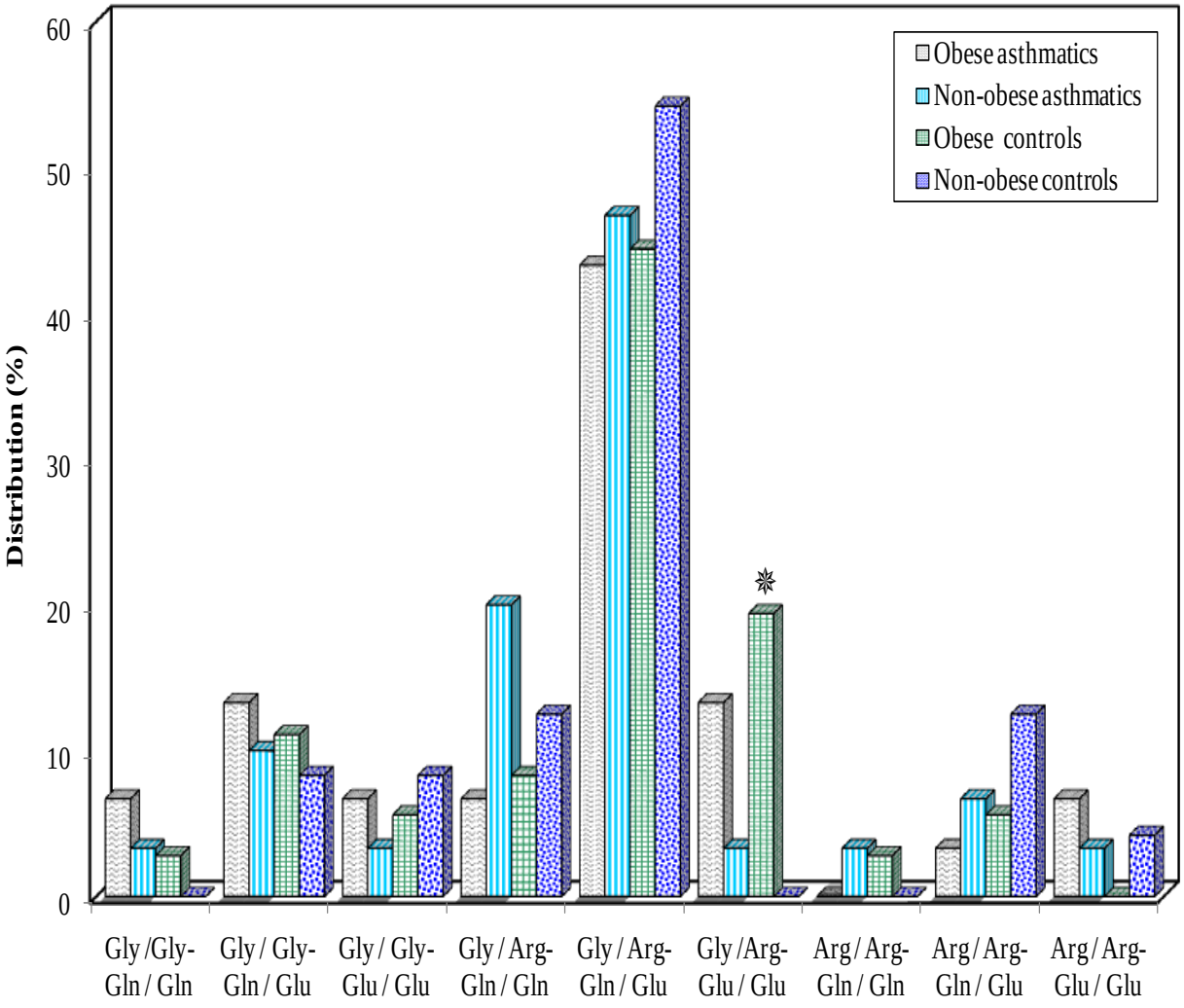
Values are expressed as median (Min. – Max.)
*: Statistically significant at $p \leq 0.05$
^{KW}p: p value for Kruskal Wallis test for comparing between the studied groups
n=30 in all groups



Codon 16



Codon 27



Codon 16 - Codon 27

Figure (4): Distribution of genotypes frequencies of β_2AR gene at codon 16, codon 27 and codon 16-codon 27 among obese and non-obese asthmatics and obese and non-obese control subjects

DISCUSSION

In the current study no significant difference could be detected among the asthmatic and control subjects in the distribution of allelic or genotypes frequencies of β2AR gene at codon 16 and codon 27. Also, there was no significant difference could be detected in the distribution of the asthmatics (according to the value of $PD_{20\text{-}FEV1}$) among genotypes of β2AR gene at codon 16 and codon 27. However, a significantly increased frequency of genotype Gly16Gly was observed among severe asthmatics in comparison with mild and moderate asthmatics. Yet, there was no significant genotype that associated asthma and obesity.

In the present study, there was no significant difference in the distribution of allelic or genotype frequencies of β2AR gene at codon16 and codon27 in asthmatics when compared to control subjects. This finding is in agreement with the findings of other investigators.[27-30]

However this is contradicted in a study by Salah K et al,2012 [31] which found higher frequencies of Arg16Gly and Gly16Gly genotypes in asthmatic children when compared with controls, and a study [32] that reported an association between β2AR-16 and β2AR-27 gene polymorphisms with asthma diagnosis and nocturnal symptoms among Mexican Mestizos, These results suggested that variation in the β2AR gene is associated with asthma pathogenesis and may act as a disease modifier in nocturnal asthma, and found an association among men with the "Gly16 allele without Glu27" and asthma diagnosis. They emphasize to the necessity of accounting for ethnic background in performing β2AR genotyping. Interethnic differences in the β2AR structure could explain differences in the incidence and prevalence of asthma, disease susceptibility, asthma severity, and treatment response. Assessment of the sex difference which could be of major importance in β2AR function and its role in the pathogenesis of asthma should be considered. The difference could also be explained by gene-environment interactions with β2AR gene polymorphisms. [32]

The present study showed that GLy16Gly genotype and the combined genotype Gly16Gly-Gln27Gln were significantly elevated in severe asthmatics in comparison with mild and moderate asthmatics. No significant association was found between polymorphism at codon 27 and asthma severity. This was in consistence with several studies [31,33-35] However, Palmer *et al.,*([36] revealed that the arginine-16 genotype of ADRB2 predisposes to exacerbations in asthmatic children and young adults. In addition Reihsaus *et al*.,[17] found no correlation between Gly16 homozygosity and hospital admissions, and Wier *et a*l.,[37] found no increase in frequency of Gly16 in fatal or no fatal asthma. So it seems that various host and environmental factors other than polymorphism can affect the severity of asthma; factors such as poor economic condition, lower level of education and concomitant diseases. [38] Allergy was found as another significant predictor, which proves that the positive history of allergy is a risk factor of asthma severity. [39]

Several studies [40-42] supported the current study whichdetected significant difference among non-obese asthmatic patients in FEV1 %predicted when distributed according to genotypes at codon 16 of the ADRB2; there was a significant increase in FEV1%predicted in Arg16 homozygotes. This is supported by In the present study there was a significant difference in proportion of combined genotype Gly16Arg-Glu27Glu among the four studied groups, however this was mainly due to theeffect of obesity rather than asthma.

Limitations:
Our findings have led us to expect that studies of gene-gene and gene-environment interactions and epigenetic mechanisms can help us understand the link between asthma and obesity. Because the β-receptor is only one element affecting β-agonist response, gene–gene interactions must be addressed to assess biological pathways that affect β- agonist response. The complex interrelationship between obesity and asthma is an example of how genes and environment interact. Through inflammatory mechanisms or lifestyle changes, obesity may give rise to asthmatic symptoms in susceptible individuals. The interaction between diet and genes may cause abnormalities in body growth patterns (leading to obesity) and/or alter airway tone (triggering asthma), and multiple interrelations between obesity and asthma may then take place.

Conclusion:
In conclusion Gly16Gly genotype was associated with increased asthma severity. Arg16Arg genotype was found to be protective from development of severe asthma. However, there was no detectable specific genotype for the association of asthma and obesity.

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