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INVESTIGATION THE ROLE OF MEPXH1 (HIS139ARG) POLYMORPHISM ON NUMBER OF EXACERBATIONS AND DISEASE SEVERITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN A SMOKER POPULATION

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ABSTRACT

The purpose of this study was to examine investigation the role of mEPXH1 (His139Arg) polymorphisms on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population. Chronic obstructive pulmonary disease (COPD) has become the fourth most common single cause of morbidity, and its prevalence is increasing worldwide. It is a syndrome composed of chronic bronchitis, small airways disease (bronchiolitis), and emphysema, in varying proportions between affected individuals. The study was performed cohort and prospectively. The population consist of 213 patients with COPD disease. Genotyping of mEPXH1 was performed using multiplex PCR. Data analysis included, Pearson's r correlations, regression analysis, ANOVA analyses, Tukey, test for comparison and SPSS software (package of Spss / pc + + ver18). The results showed that there is not relationship between polymorphisms of mEPXH1 and number of exacerbations. According the results, there is not significant relationship between polymorphisms of mEPXH1 and disease severity. Also there is not significant relationship between mEPXH1 and disease in COPD patients on basis parameters of spirometery and oxidative stress in COPD patients.

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KEY WORDS

mEPXH1,His139Arg Exacerbations,Disease severity Chronic Obstructive Pulmonary Disease (COPD)

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the sixth leading cause of morbidity and mortality in the western world and the prevalence of the disease is increasing as the population ages [1]. Cigarette smoking is the major environmental contributor to the disease in western societies yet only, 15% of cigarette smokers develop the disease, indicating that genetic factors play a part in determining susceptibility. Although cigarette smoking is considered the major environmental risk factor for the development of COPD, only 20–30% of chronic smokers develop severe impairment of lung function associated with this pathogenesis [2]. These individual differences in susceptibility to tobacco smoke injury may be related to genetic factors. During the last three decades, research studies reported that the imbalance of the protease–antiprotease and the oxidant–antioxidant systems is the major factor causing emphysema and COPD [3]. With the notable exception of severe α1-antitrypsin (α1-AT) deficiency, these genetic factors are poorly understood. Given that .95% of those who develop COPD are smokers and oxidative stress is thought to be important in the pathogenesis of the disease, genetic variation in enzymes that protect the lung against smoke-induced oxidative stress has been a significant focus of study. Microsomal epoxide hydrolase (EPHX1), an enzyme involved in the first-pass metabolism of epoxide intermediates, has received particular attention as two functional variants of the gene, which confer slow and fast metabolic activity, have been identified [4]. In order to replicate a subset of these previous genetic associations in COPD, a case–control study was performed in 492 Caucasian current or former smokers with and without COPD. The current authors chose single nucleotide polymorphisms (SNPs) in three relatively well-studied interesting candidate genes: tumor necrosis factor (TNF-α), b2-adrenoreceptor (ADRB2) and microsomal epoxide hydroxylase (EPHX1). A

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systematic literature review and meta-analysis of previous studies was also performed Tyr113His polymorphism with a recessive odds ratio (OR) of 3.5 [5], whereas subsequent studies failed to confirm this [6]. The aim of this study was to examine investigation the role of mEPXH1 (His139Arg) polymorphisms on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population.

MATERIALS AND METHODS

The study was performed cohort and prospectively. The population consist of 213 patients with COPD disease. In this study, patients candidate for COPD were selected in Beasat Clinic of Kerman in Iran. Inclusion criteria for COPD were chronic airway symptoms and signs such as coughing, breathlessness, wheezing, and chronic airway obstruction. COPD phenotype identification was based on chest radiographic and high-resolution computerized tomography density findings.

DNA preparation

For genotyping, 10 ml blood was drawn into an EDTA tube and stored at -20 C until DNA extraction was carried out. Genomic DNA was isolated from whole peripheral blood using the Salting out [7].

mEPXH1 Genotyping

mEPXH1 (His139Arg) polymorphism was detrmined as descripbed before by authors [8]. To examine the polymorphism of mEPXH1 a simultaneous amplification of genes of interest in the same reaction was performed using a multiplex polymerase chain reaction (PCR) as described in the literature [9,10,11].

Table: 1. The profile sequences and position-specific of primers mEPHX1

Primer	Sequence	GC%	Tm (°C)	Band size
Forward Common	5'-TGG CAG GAC TCA ATA TCT AGG CTC TG-3'	50	67.9	240 bp
Reverse Wild	5'-ATC AGC AAG GGC TTC GGG GTA T-3'	54.5	64	
Reverse Mutant	5'-ATC AGC AAG GGC TTC GGG GTA C-3'	59.1	65	

Statistical analysis

Data analysis included, pearson's r correlations, regression analysis, ANOVA analyses, Tukey' test for comparsion and SPSS software (package of Spss / pc + + ver18).

Demographics results

(1) Of the 213 patients enrolled in the study 162 were smoker and 51 were Nonsmoker (male:female ratio 163:50)
(2) The education level of 213 subjects were studied, 109 cases were lack education and 104 cases were Educated.

Determination mEPXH1 genotypes (Exon 4)

Figure- 1 shows different genotypes of mEPXH1 after the Multiplex PCR. Despite the band with primers forward common and Reverse G indicative GG genotype (Homozygote). Despite the band with primers forward common and Reverse A indicative AA genotype (Wild type) and band with primers Forward common, Reverse G and Reverse A indicative GA genotype (Heterozygote).

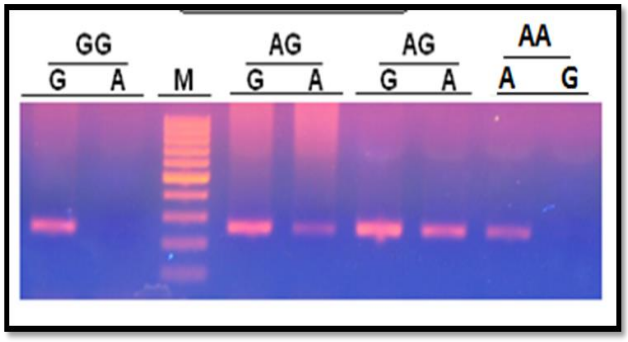

Fig: 1. The various genotypes of His139Arg polymorphisms (mEPXH1 gen) in Agarose gel

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Table: 2. Determination various genotype of His139Arg polymorphisms (mEPXH1 gen)

Genotype	Band Fragment (bp)	Phenotype (Gene expression)
GG (Homozygot)	240 (only G)	High
AG (Heterozygot)	240 (Both A ,G)	Intermediate
AA (Wild type)	240 (only A)	Low

RESULTS

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and number of exacerbations in COPD patients

Table- 3 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and number of exacerbations in COPD patients. There is not significant relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients.

Table: 3. The relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients

		mEPXH1 exon-4 genotype			
Exacerbation/yr.	Frequency	His/His	His/Arg	Arg/Arg	P-value
No	n (%)	48 (47)	42(41)	13(12)	0.857
1-2	n (%)	30(45)	29(43)	8(12)	
3-4	n (%)	23(54)	14(32)	6(14)	

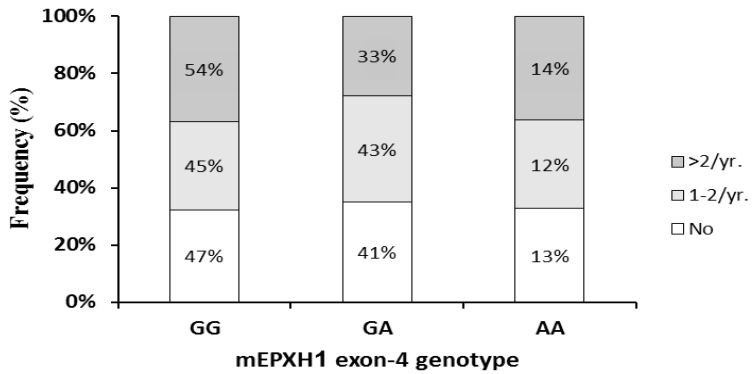

Fig: 2. The relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients

Table 4 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients. There is not significant relationship between polymorphisms of His139Arg and disease severity in COPD patients.

Table: 4. The relationship between polymorphisms of His139Arg and disease severity in COPD patients

mEPXH1 exon-4 genotype	FEV1<50 n(%)	FEV1>50 n(%)	OR (95% C.I)	P-value
His/His	50(46)	51(49)	-	ns
His/Arg	46(43)	39(37)	0.8 (0.5-1.5)	
Arg/Arg	12(11)	15(14)	1.2 (0.2-2.9)	

ns : Non-significant

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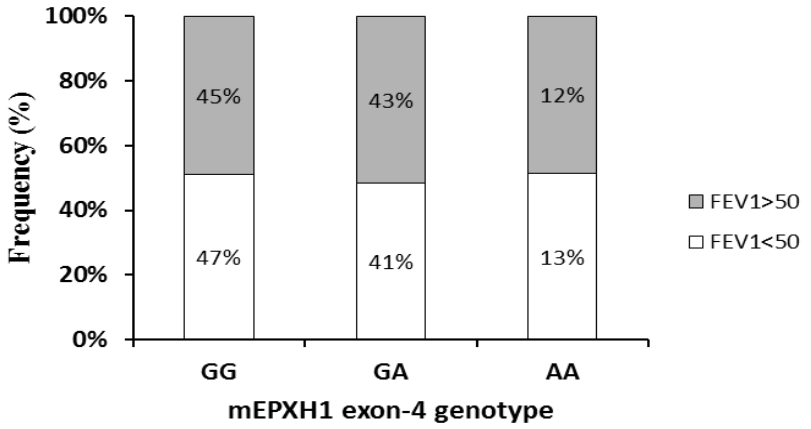

Fig: 3. The relationship between polymorphisms of His139Arg and disease severity in COPD patients

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in presence of confounding factors in COPD patients

Table- 5 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients in presence of confounding factors (sex, age, smoking and BMI). There is not significant relationship between polymorphisms of His139Arg and disease severity in presence of confounding factors in COPD patients.

Table: 5. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in presence of confounding factors in COPD patients.

Variable	B	P-value	OR (95% CI)
mEPXH 1 Exon4 genotype			
His/His		0.592	
His/Arg	-0.122	0.686	0.8 (0.5-1.6)
Arg/Arg	0.341	0.447	1.4 (0.6-3.4)
Sex	-0.594	0.135	0.5 (0.3-1.2)
Age	-0.001	0.92	0.99 (0.98-1.02)
Smoking Status	0.056	0.784	1.06 (0.71-1.58)
BMI	0.042	0.194	1.04 (0.98-1.11)

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameters of gender

Table -6 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on the basis parameters of gender. There is not significant relationship between polymorphisms of His139Arg and disease severity in COPD patients. But there is a protective effect against the disease, so that men with genotype AA low (Arg / Arg) show the severity high of the disease**.**

Table: 6. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameters of gender

Sex	COPD Stage	mEPXH1 exon-4 genotype			OR
		His/His	His/Arg	Arg/Arg	
Male	n (%)	40(51)	32(41)	6(8)	2.0
	n (%)	44(52)	29(34)	12(14)	
Female	n (%)	10(33)	14(47)	6(20)	0.2
	n (%)	7(35)	10(50)	3(15)	

OR : Odd Ratio

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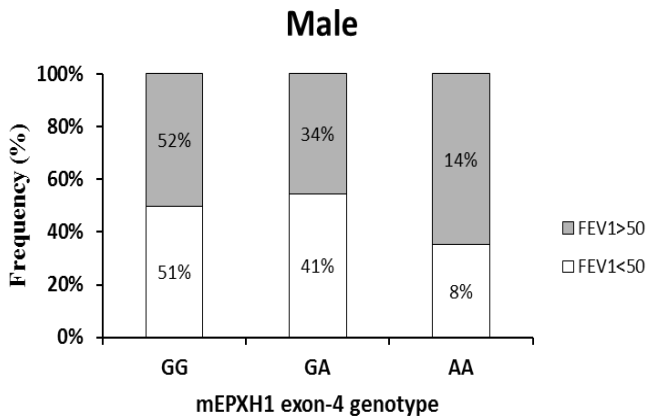

Fig: 4. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameter Male

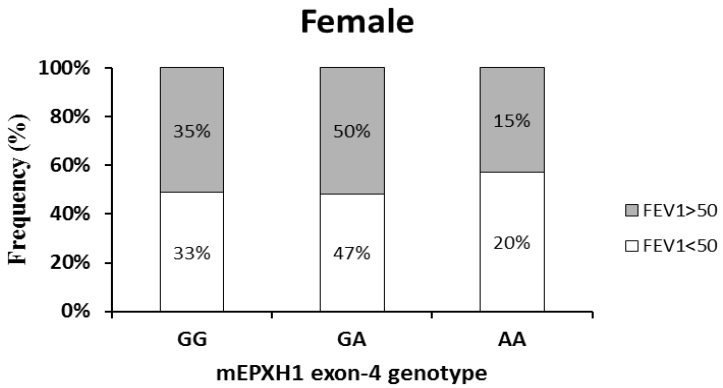

Fig: 5. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameter Female

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometery

Table- 7 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometery (FEV_1 ،FEV_1/FVC and FEF_{25-75}). There is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of spirometery.

Table: 7. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometery

mEPXH1 exon-4 genotype		N	Mean (95% CI)	P- value
FEV_1	Wilde	101	49.0(45.4-52.5)	-
	Hetro	85	50.7(46.9-54.4)	ns.
	Mutant	27	51.0(43.2-58.8)	ns.
FEV1/FVC	Wilde	101	73.5(71.6-75.3)	-
	Hetro	85	74.0(71.8-76.2)	ns.
	Mutant	27	76.6(73.2-79.9)	ns.
FEF_{25-75}	Wilde	100	37.5(32.2-42.7)	-
	Hetro	85	39.0(34.1-43.8)	ns.
	Mutant	26	38.6(30.8-46.3)	ns.

FEV_1: Forced Expiratory Volume in first second. FVC: Forced Vital Capacity
$FEF_{25-75\%}$: Mean forced Expiratory Flow during the middle half of FVC

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Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of oxidative stress

Table- 8 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of oxidative stress (LPO ،SOD و TAC). There is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of oxidative stress.

Table: 8. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on the basis parameters of oxidative stress

mEPXH1 exon-4 genotype		N	Mean (95% CI)	P- value
LPO	Wilde	101	0.2(0.15-0.23)	-
	Hetro	85	0.2(0.12-0.20)	ns.
	Mutant	26	0.2(0.13-0.27)	ns.
SOD	Wilde	60	2.6(2.50-2.76)	-
	Hetro	45	2.7(2.57-2.85)	ns.
	Mutant	18	2.7(2.45-2.88)	ns.
TAC	Wilde	101	1.4(1.35-1.50)	-
	Hetro	85	1.4(1.32-1.50)	ns.
	Mutant	26	1.4(1.25-1.57)	ns.

LPO: Lipid peroxidation SOD: Superoxide dismutase TAC: Total antioxidant capacity

DISCUSSION

The purpose of this study was investigation the role of mEPXH1 (His139Arg) polymorphism on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population. Of the 213 patients enrolled in the study 163 were male and 50 were female. The frequency of different genotypes mEPXH1 (His139Arg) was including 47.4 % of His/His, 40 %, His/Arg and 12.7% Arg/Arg. Erkisi et al (2010) reported in Turkey the abundance of His/His 53.7%, His/Arg 39% and Arg/Arg 7.3% [12]. Cheng et al (2004) reports the abundance of His/His 65.6%, His/Arg 31.3% and Arg/Arg 3.3% [9]. The results of this study show the there is not significant relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients. According the results, there is not significant relationship between Heterozygote genotype and FEV1 <50 (OR= 0.83; 95%CI: 0.77-1.5) and homozygous genotypes and FEV1 <50 (OR= 1.2; 95%CI: 0.22-2.9) in COPD patients. The results of this study show the there is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of spirometery (FEV_1 ،FEV_1/FVC and FEF_{25-75}) and oxidative stress (LPO ،SOD و TAC). These results are in compliant with result Cheng et al (2004), Smith and Harrison (1997) and Park (2007) [9, 13, 14]. Cheng et al reported the there is not significant relationship between polymorphisms of mEPXH1 His139Arg and risk of COPD in COPD patients (($OR_{His/Arg}$= 0.6; 95%CI: 0.3-1.1) and ($OR_{Arg/Arg}$=0.4; 95%CI: 0.1-2.6)) [9]. Sandford et al (2001) indicated the there is significant relationship between combining two polymorphisms mEPXH1 His139Arg, His113Tyr and reduced lung function in COPD patients on basis parameters of spirometery [15]. Yoshikawa et al (2000) finding the there is significant relationship between polymorphisms of His113Tyr and disease severity in COPD patients (OR=2.9; 95%CI: 1.1-7.4, p=0.025) [16]. Hu et al (2008) reported the there is significant relationship between Homozygous mEPXH1 and increased risk of COPD disease [17]. Although in some studies shown relationship between polymorphisms mEPXH1 (His139Arg) and COPD disease, but in this study there is not significant relationship between polymorphisms of His113Tyr and number of exacerbations, disease severity and susceptibility to disease in COPD patients.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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FINANCIAL DISCLOSURE

None.

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