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Association of Matrix Metalloproteinase 9 Polymorphism with Pulmonary Functions in South Indian Population - A cross sectional study

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ABSTRACT

Objectives: Matrix metalloproteinase (MMP) is an elastase released by activated macrophages. Increased activity of elatase can lead to extracellular matrix degradation.The study was aimed to estimate and correlate serum MMP-9 levels and Malondialdehyde (MDA) levels with pulmonary function test parameters.

Materials and Methods: In the current study, we have recruited healthy South Indian adults. After obtaining informed written consent, 5ml of venous blood was collected. Pulmonary function testing was done by spirometry. DNA extraction and Real time Polymerse Chain Reaction (RT PCR) was done. Serum MMP-9 and MDA levels were estimated.

Results: The genotype frequency of AA, AG, and GG was 26 (21%), 53 (42%), and 46 (37%), respectively. The frequency of the "A" allele was 0.42 and the "G" allele was 0.58. On comparison, serum MMP-9 levels were found to be elevated significantly in the AA genotype. Serum MDA levels were also elevated in the AA genotype but the difference was not statistically significant. On correlation, there was a significant negative correlation between serum MMP-9 levels and pulmonary function test (PFT) parameters in individuals with the AA genotype. A negative correlation was also found between MDA levels and PFT parameters in AA genotype but it was not statistically significant.

Conclusion: Hence, we conclude that the AA genotype individuals may be more prone to extracellular matrix degradation when they are exposed to oxidative stress and environmental stressors such as smoking and air pollution.

Keywords: *Gln279Arg*, Pulmonary functions, Matrix metalloproteinase-9 polymorphism, Malondialdehyde

INTRODUCTION

Matrix metalloproteinase (MMP) is an elastase released by the activated macrophages.[1] MMPs are involved in many physiological functions such as extracellular matrix remodeling, angiogenesis, bone growth, and neuritic growth. Over activity of MMPs can lead to extracellular matrix degradation. This over activity can be caused by inflammation, exposure to oxidative stress and also by over-expression polymorphism of genes coding MMPs.

It is hypothesized that the pathogenesis of chronic obstructive pulmonary disease (COPD) involves a triad of inflammation, elastase – anti-elastase imbalance, and oxidative stress.[2,3] The balance between elastin degrading enzymes and their antagonists should always be maintained

and it is the major factor governing the susceptibility of the lung parenchyma to destruction. Over activity of elastase will lead to degradation of elastin. Earlier studies had shown the association of single nucleotide polymorphisms of many of these identified MMPs such as MMP-9, MMP-12, and MMP-1 with COPD.[4,5] One such polymorphism of the MMP-9 gene is *Gln279Arg* (*rs17576*), which in the previous studies had proved to be an independent risk factor for the development and progression of COPD.[5,6]

On exposure to oxidative stress, the lung parenchymal epithelial cells and macrophages are activated. These activated cells release proteolytic and elastolytic enzymes that in turn lead to extracellular matrix degradation.[1] Serum malondialdehyde (MDA) is a marker of oxidative stress.

In the current study, we have established the normative frequency of COPD-related *Gln279Arg* in a healthy South Indian population and we have also estimated and compared serum levels of MMP-9 levels and MDA levels among different genotypes. Furthermore, we have assessed the lung functions in different genotypes of *Gln279Arg* polymorphism.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted to estimate the normative frequency of MMP-9 *Gln279Arg* (*rs17576*) polymorphism among healthy south Indian adults aged 18–45 years. The study was approved by the scientific advisory committee and ethics committee of the institute. The sample size was calculated to be 125 by Open Epi software. Genotype and allele frequency was estimated in125 healthy adults. However, serum MMP-9 and MDA were estimated only in 71 subjects because of financial constraints.

Inclusion criteria

Healthy volunteers aged 18–45 years, living in any one of the South Indian states (Puducherry, Tamil Nadu, Kerala, Andhra Pradesh, and Telangana) for at least three consecutive generations and speaking any one of the South Indian languages (Tamil, Telugu, Malayalam, and Kannada) as their mother tongue.

Exclusion criteria

Active smoking, hypertension, diabetes mellitus, alcoholics, endocrinological disorders, acute illness, valvular heart diseases, chronic respiratory illness, and subjects with abnormal pulmonary function tests (PFTs) were excluded from the study.

Procedure

Subjects were asked to report to the pulmonary function testing laboratory, department of physiology, at around 9 am, at least 1 h after a light breakfast, as the maximum forceful expiratory maneuver will be restricted when the subject is in full stomach. The procedure was clearly explained to the subjects and informed written consent was obtained. Following this, anthropometric parameters such as height, weight, and body mass index were recorded. PFTs of the subject such as forced vital capacity (FVC), forced expiratory volume at 1st second (FEV_1), FEV_1/FVC, peak expiratory flow rate (PEFR), and Forced expiratory $flow_{25–75\%}$ ($FEF_{25–75\%}$) were assessed by computerized Spirometry (SPIROLAB III). The test results were interpreted by comparing with the values predicted for height, weight, and ethnicity of each individual following the American Thoracic Society guidelines.[7] Subjects with normal pulmonary functions alone were included in the study while those with decreased pulmonary functions were excluded. Venous blood was collected under sterile aseptic precautions and DNA extraction was done using QIAamp DNA extraction mini kit from anti coagulated whole blood. *rs 17576* genotyping was done using the quantitative Real-Time Polymerase Chain Reaction (ABI 7300, Foster City, USA) using TaqMan SNP genotyping assay kit. The result of the genotyping was analyzed by 7300 sequence detection software version 1.4. Serum MMP-9 levels were estimated by RayBio Human MMP-9 ELISA kit. Serum MDA levels were estimated by Quantitative determination of Thiobarbituric acid Reactive Substances by QuantiChromTM TBARS Assay Kit (DTBA-100).

Statistical methods

The results were analyzed using IBM PASW Statistics Version – 19.0 (SPSS version 19.0). The normality of the parameters was tested using Kolmogorov–Smirnov test. Normally distributed parameters were expressed in Mean ± standard deviation (SD). Non normally distributed parameters were expressed as median with inter quartile range (IQR). The genotype distribution in the study population was expressed as frequencies and percentages. The allele frequency was calculated from the genotype frequencies obtained by the formula, Frequency (A) = (Frequency of AA ×2) + Frequency of AG/total number of alleles (250), Frequency (G) = (Frequency of GG ×2) + Frequency of AG/total number of alleles (250). The difference in serum MMP-9 levels and serum MDA levels among different genotypes in the study population was determined using Kruskal–Walis test. Correlation of serum MMP-9 levels and PFT parameters was done by Spearman's test and the correlation of serum MDA levels and PFT parameters was done by Pearson's test. $P < 0.05$ was considered statistically significant.

RESULTS

Out of 125 participants, 65 were males and 60 were females. The participants were from all South Indian states, namely, Pondicherry, Tamil Nadu, Andhra Pradesh, Telangana, Kerala, and Karnataka. State-wise distribution of study population was found to be more from Pondicherry (38%), Tamil Nadu (30%), Kerala (20%),and less form Andhra Pradesh (7%), Telangana (3%), and Karnataka (2%). The genotype frequency of AA was 26, AG was 53, and GG was 46. Percentage distribution of AA, AG, and GG was 21%, 42%, and 37%, respectively. The frequency of the "A" allele was 0.42 and the "G" allele was 0.58 in the study population. The observed genotype frequencies were found to be consistent with Hardy-Weinberg equilibrium with Chi-square test $P = 0.146$ (with 1 degree of freedom).

Baseline subject characteristics are expressed as Mean ± SD/ Median (IQR) in [Table 1].

Serum MMP-9 levels and MDA levels were estimated for 71 participants. On comparison of serum MMP-9 levels among different genotype groups, MMP-9 levels were found to be significantly elevated in AA genotype group, as shown in [Table 2].

On correlation by spearman's test, in AA genotype group serum MMP-9 levels were found to have significant negative correlation with PFT parameters as shown in [Table 3].

On comparison of serum MDA levels among different genotypes, it was also found to be elevated in AA genotype and MDA levels were also found to be negatively correlating with PFT parameters in AA genotype, though they are not statistically significant, as shown in [Tables 4 and 5].

Table 1: Mean±SD/Median (IQR) of the subject characteristics (*n*=71).

S. No.	Parameters	Mean±SD/Median (IQR)
1	Age (years)*	28 (20–35)
2	Body mass index (Kg/m^2)#	23.6±3.8
3	FVC (% predicted)#	86±14
4	FEV1(% predicted)#	94±15
5	FEV1/FVC (%)*	93.8 (91–98.2)
6	PEF (% predicted)*	82 (74–95)
7	MMP-9 (pg/ml)*	1087 (315–2567)
8	MDA (μM/L)#	2.7±1.9

*Values are expressed as median (IQR), #Values are expressed as mean±SD. FVC: Forced vital capacity, FEV1: Forced expiratory volume at first second, PEF: Peak expiratory flow, MMP-9: Matrix metalloproteinase-9, MDA: Malondialdehyde; IQR: Inter quartile range, SD: Standard deviation, μM/L: Micromole/litre

Table 2: Comparison of serum concentration of MMP-9 between different genotype groups (*n*=71).

Genotype	N	MMP-9 levels (pg/ml)	*P* value
AA	23	2188 (734.75–4888.5)	0.002*
AG	26	876.5 (336.5–3307)	(GG and AA)
GG	22	382 (182.25–1314.25)	(AG and AA)

MMP-9 levels are expressed as median (IQR), Analyzed by KruskalWallis test, *P<0.05 is considered statistically significant. MMP-9: Matrix metalloproteinase-9

Table 3: Correlation of serum MMP-9 levels and PFT parameters among different genotypes (*n*=71).

PFT parameters	AA genotype (*n*=23)		AG genotype (*n*=26)		GG genotype (*n*=22)	
	"r"	"*P*"	"r"	"*P*"	"r"	"*P*"
FVC (% predicted)	−0.485	0.06	−0.034	−0.9	−0.03	0.7
FEV1 (% predicted)	−0.528	0.006*	−0.016	−0.8	0.079	0.7
FEV1/FVC (%)	−0.112	0.4	0.187	0.38	−0.044	0.8
PEFR (% predicted)	−0.632	0.001*	0.026	0.8	−0.035	0.9
FEF_{25-75} (% predicted)	−0.481	0.018*	0.132	0.5	0.001	0.86

Analyzed by Spearman's test, *P<0.05 are considered statistically significant, r: correlation coefficient. PFT: Pulmonary function test, FVC: Forced vital capacity, FEV1: Forced expiratory volume at first second, PEF: Peak expiratory flow, FEF: Forced expiratory flow

DISCUSSION

This was a cross-sectional study conducted in healthy South Indian population. The median age of our study population was 28 years with IQR of 20–35 years. Since pulmonary functions have been reported to decline with age,[8] in the present study we have included individuals this age group.

MMP-9 is a multi-domain enzyme with elastase activity, which plays a major role in the development of COPD, and has been considered as a sensitive biomarker to assess the degree of lung dysfunction.[2,3] The activity of MMPs has to be very strictly regulated and even a minimal deviation from it can lead to tissue destruction.[2] Any modification at the level of gene expression can lead to dysregulation of MMP-9 levels. *Gln279Arg* (*rs17576*) is a missense mutation in the coding region of the gene at codon "279," exon-6 and it involves the substitution of nucleotide "G" for "A." In general, polymorphisms at promoter regions lead to either over expression or under-expression of the gene and polymorphisms at coding regions lead to defective protein-coding. Since *Gln279Arg* polymorphism involves coding region, this results in altered expression of amino acid. Normally, the middle nucleotide in codon 279 is "A," which codes for the amino acid Glutamine. In polymorphism, it is replaced by nucleotide "G" which encodes the amino

Table 4: Comparison of serum MDA levels between different genotypes (*n*=71).

Genotype	Serum MDA levels (μM/L)	*P* value
AA	2.98 (0.62–4.2)	0.12
AG	2.02 (0.59–3.35)	
GG	2.93 (0.9–3.19)	

MDA levels are expressed as median (IQR), Analysed by One-way ANOVA, Degree of freedom – 2. MDA: Malondialdehyde, μM/L: Micromole/litre

Table 5: Correlation of serum MDA levels and PFT parameters among different genotypes (*n*=71).

PFT parameters	AA Genotype (*n*=23)		AG Genotype (*n*=26)		GG Genotype (*n*=22)	
	"r"	"*P*"	"r"	"*P*"	"r"	"*P*"
FVC (% predicted)	−0.378	0.1	−0.141	0.5	0.005	0.9
FEV1 (% predicted)	−0.376	0.12	−0.159	0.38	0.053	0.8
FEV1/FVC (%)	0.248	0.2	0.003	0.9	−0.087	0.6
PEFR (% predicted)	−0.30	0.18	0.003	0.9	−0.179	0.5
FEF_{25-75}	−0.256	0.2	−0.199	0.2	−0.243	0.22

Analyzed by Pearson's test, r: Correlation coefficient.
MDA: Malondialdehyde, PFT: Pulmonary function test, FVC: Forced vital capacity, FEV1: Forced expiratory volume at first second, PEFR: Peak expiratory flow rate, FEF: Forced expiratory flow

acid Arginine. This substitution of uncharged amino acid glutamine by positively charged amino acid arginine in the substrate-binding site alters the ability of the enzyme to bind with its substrate elastin. This leads to prolonged enzyme activity predisposing to extracellular matrix destruction.[9-12]

In the present study, on comparison of serum MMP-9 levels between the three genotypes (AA, AG, and GG), the MMP-9 levels were found to be significantly elevated in AA genotype when compared to the other two genotypes [Table 2]. Elevated MMP-9 levels in AA genotype individuals can predispose these individuals to impaired lung function if they are exposed to inflammation and oxidative stress.

Furthermore, on the correlation of serum MMP-9 levels and PFT parameters, there was a statistically significant negative correlation between MMP-9 levels and pulmonary function parameters such as FEV_1, PEFR, and FEF_{25-75} in the AA genotype [Table 3]. In a study conducted in Swedish population, serum MMP-9 levels were found to be negatively correlated with FEV1%.[2] A study by Higashimoto *et al.* compared serum MMP-9 levels in two groups of COPD patients, the first group was rapid decliner and the other was non-decliner. The results showed significantly elevated MMP-9 levels in the rapid decliner group when compared to the non-decliner group.[13]

In the current study, on comparison of serum MDA levels among the different genotypes, MDA levels were found to be elevated in AA genotype when compared to the other genotypes, but the difference was not statistically significant [Table 4]. On the correlation of serum MDA levels and PFT parameters, there was a negative correlation between the above two parameters in the AA genotype individuals but the difference was not found to be statistically significant [Table 5]. A study conducted by Waseem *et al.* demonstrated significantly elevated MDA levels in COPD group when compared to the control group. The study also demonstrated a significant negative correlation between serum MDA levels and FEV1%.[15] This shows that the lung function could be associated with oxidant – anti-oxidant status as demonstrated by the correlation of MDA with lung functions in AA genotype individuals which was observed in previous studies. However, in the present study, we have not got a significant correlation which may be due to (i) less sample size; (ii) MDA level is less (there is no oxidative stress); and (iii) lung functions are normal as the study was conducted in healthy population.

Oxidative stress can lead to variety of effects and of this lipid peroxidation is the most frequently observed effect.[15] Lipid peroxidation of cell membrane alters permeability and leads to cellular damage. The end products of lipid peroxidation include various lipid hydroperoxides and aldehydes. MDA is one such lipid peroxidation end product.[16] Therefore, estimation of MDA levels should be a part of screening tests for assessing the health status of the vulnerable population.

CONCLUSION

In our study population, 21% of the individuals belong to the AA genotype. On the estimation of serum MMP-9 levels and MDA levels, it was found that the AA genotype individuals have increased metalloproteinase activity and oxidative stress when compared to the other two genotypes. MMP-9 and MDA levels in AA genotype group were also found to negatively correlate with PFT parameters.

Limitations

In our study, even though we have excluded active smokers we have not considered passive smoking in the study subjects. Due to financial constraints, serum MMP-9 levels and serum MD levels were estimated only in 71 subjects. And we have not estimated the anti-elastase levels in the blood.

Future perspectives

In the current study, we have included only healthy south Indian adults. Further studies should be directed towards assessing the genotype and allele frequencies of *Gln279Arg* polymorphism in the COPD group.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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