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Original Article

Gender Difference in the Link of MMP-9 Gene Polymorphism to Serum Matrix Metalloproteinase Level in the Determination of Lung Function in South Indian Population

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ABSTRACT

Background and Aim: Matrixins or matrix metalloproteinases (MMP) belong to metzincin superfamily and they are involved in many physiological functions like extracellular matrix remodeling, angiogenesis, bone growth and neuritic growth. Polymorphisms in MMP-9 gene will result in over expression of matrix metalloproteinases leading to excessive extracellular matrix degradation, which cannot be overcome by the anti-proteinases. One such polymorphism of MMP-9 is Gln279Arg. Degradation of pulmonary matrix can lead to chronic obstructive pulmonary disease. Studies in the past have demonstrated female preponderance in incidence of COPD among smokers. **Materials and Methods:** This was a cross sectional study conducted among healthy volunteers of age group 18 to 45 years. Pulmonary function tests of the subjects were recorded using computerised spirometry (SPIROLAB III). Frequency of Gln279Arg polymorphism was estimated by RT-PCR. Serum MMP-9 were estimated by Enzyme linked Immunosorbent Assay. **Results:** MMP-9 levels were found to be elevated in AA genotype group in females. **Conclusion:** The females with AA genotype, have increased MMP-9 levels and so they may be more prone for COPD if they are exposed to the environmental risk factors.

Keywords: Matrix metalloproteinase-9, Pulmonary functions, Gln279Arg, Extracellular matrix degradation in females, COPD related SN.

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INTRODUCTION

Matrixins or matrix metalloproteinases belong to metzincin superfamily and they are involved in many physiological functions like extracellular matrix remodeling, angiogenesis, bone growth and neuritic growth. About 25 human MMPs have been identified so far.[1] Earlier studies had shown association of Single Nucleotide polymorphisms of many of these identified MMPs like MMP-9, MMP-12, MMP-1 with COPD.[2] Polymorphisms will result in over expression of matrix metalloproteinases leading to excessive extracellular matrix degradation, which cannot be overcome by the anti-proteinases. One such polymorphism of MMP-9 is Gln279Arg. Studies in the past have shown significant association of Gln279Arg polymorphism in exon 6 of MMP-9 with Chronic Obstructive Pulmonary Disease (COPD).[3] Studies had also shown increased serum MMP-9 levels in COPD patients when compared to controls. Studies had also shown positive correlation of serum MMP-9 levels with the severity of the disease.[4] It is hypothesized that the pathogenesis of Chronic obstructive Pulmonary Disease (COPD) involves a triad of inflammation, elastase – anti elastase imbalance and oxidative stress.[1-2] 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.[5]

There are studies showing increased incidence of COPD in female smokers when compared to male smokers and also females are found to manifest more severe form of disease when compared to males.[6-7] In our previous studies we have proved the link of MMP-9 levels and MMP-9 related single nucleotide polymorphism (Gln279Arg) and lung functions in South Indian population. In the current study we aimed to determine the gender difference in the link of MMP-9 polymorphism and lung function.

MATERIALS AND METHODS

Study Design

This cross-sectional study was conducted among healthy south Indian adults aged 18 to 45 years. The study was approved by 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 were estimated in125 healthy adults. But, serum MMP-9 was estimated only in 71 subjects because of financial constraints.

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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, subjects with abnormal pulmonary function tests.

Procedure

Subjects were asked to report to pulmonary function testing laboratory, Department of Physiology, at around 9 am, at least 1 hr 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 like height, weight and body mass index (BMI) were recorded. Pulmonary function tests of the subject such as forced vital capacity (FVC), forced expiratory volume at 1st second (FEV1), FEV1/FVC, peak expiratory flow rate (PEFR), forced expiratory flow 25–75% (FEF25–75%) 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 American Thoracic Society (ATS) guidelines. Subjects having 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. rs17576 genotyping was done using 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 (SDS) version 1.4. Serum matrix metalloproteinase-9 levels were estimated by RayBio Human MMP-9 ELISA kit.

Statistical Analysis of Data

The results were analyzed using IBM PASW Statistics Version – 19.0 (SPSS version 19.0). The normality of the parameters was tested using Kolmogrov 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). Gender-wise difference in serum MMP-9 levels and serum MDA levels among different genotype groups in the study population was determined using Kruskal Wallis test. Correlation of serum MMP-9 levels and PFT parameters were done by Spearman's test.

RESULTS

Genotype and allele frequencies of Gln279Arg gene polymorphism in study population are shown in Figure 1 and 2. Gender-wise distribution of genotype frequencies are mentioned in Figure 3. No gender difference was found in allele frequencies. Descriptive statistics of pulmonary function test parameters and serum MMP-9 are shown in Table 1. On Comparison of serum MMMP-9 between different genotype groups, MMP-9 levels were found to be elevated in AA genotype group, as shown in Table 2. On gender wise comparison of serum MMP-9 levels between genotype groups, the difference in AA genotype group was observed in females, as shown in Table 3.

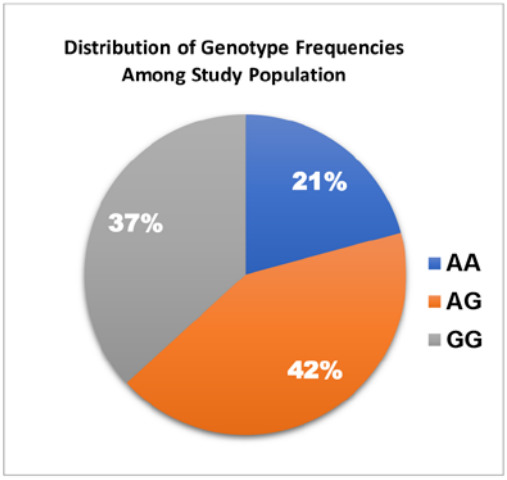

Figure 1: Distribution of genotype frequencies among study population.

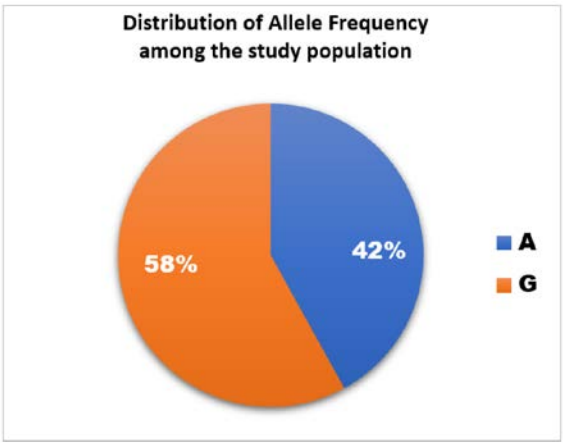

Figure 2: Distribution of allele frequencies among study population.

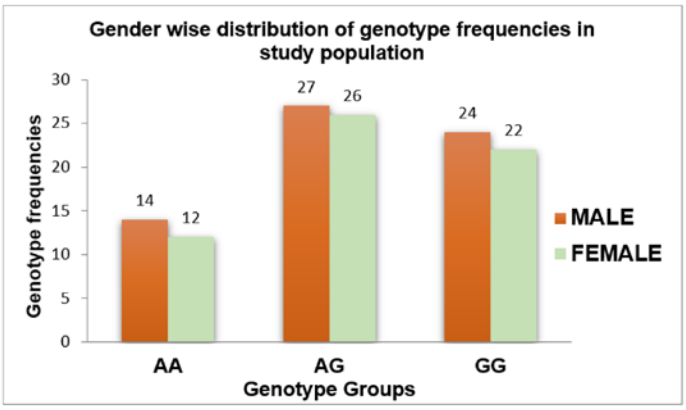

Figure 3: Gender wise distribution of genotype frequencies in study population.

DISCUSSION

Three major polymorphisms have been identified in MMP-9 gene. One among them is MMP-9 Gln279Arg.[8] The polymorphism studied was located at exon-6, codon 279, of MMP-9 gene and it involved substitution of allele 'G' for 'A'. This polymorphism leads to overexpression of MMP-9 gene causing over-activity of the enzyme. Overactivity of MMP-9 predisposes to extracellular matrix degradation.

The genotype frequency and allele frequency of the study population are represented in Figure 1 and 2 respectively. Gender-wise distribution of genotype frequencies are represented in Figure 3. There was no gender difference in allele frequencies in our study population. The genotype and allele frequencies obtained are in concordance with the results obtained in the other ethnic groups.[9]

The descriptive statistics of pulmonary function parameters and serum MMP-9 levels are mentioned in Table 1. The MMP-9 levels are more in females when compared to males. However, the difference in MMP-9 levels between male and female does not show statistical significance and this can be due to less sample size. On comparison of serum MMP-9 levels between the three genotype groups, it is found to be elevated in AA genotype group when compared to the other groups (Table 2). On further analysis of gender-wise comparison of serum MMP-9 levels between the genotype groups, significant difference in AA genotype is found in females (Table 3).

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.[4,10] Lung parenchymal destruction is attributed to the interplay of inflammation, overactivity of elastase enzymes and oxidative stress.[11] So, in the current study individuals of AA genotype group with increased serum MMP-9 levels may be predisposed to pulmonary matrix degradation. This is in favor of the reports from previous studies which showed increased severity and mortality in female COPD patients when compared to male.[7,12] The women exposed to cigarette smoke and environmental factors are found to have increased incidence of COPD, when compared to men with similar exposure status.[7,12,13] This is attributed to the pathophysiological role of sex hormones and hyper-responsiveness of immune system.[12]

Table 1: Descriptive statistics of pulmonary function parameters and serum MMP-9.

Sl. No	Parameters	Male	Female
1	FVC % predicted#	87±13	88 ± 9
2	FEV1 % predicted#	95 ±11	94 ± 11
3	FEV1/FVC % predicted#	111± 8	112 ± 6
4	PEF% predicted*	87 (71)	83 (56)
5	FEF 25-75% predicted*	94 (97)	80 (65)
6	MMP-9 (pg/ml)*	754 (5323)	885 (8292)

*Values are expressed as Median (Range). # Values are expressed as mean ± SD. FVC: Forced vital capacity; FEV_1: Forced expiratory volume at first second; PEF: Peak expiratory flow; MMP–9: Matrix metalloproteinase-9; MDA: Malondialdehyde.

Table 2: Comparison of serum concentration of MMP-9 between different genotype groups.

Genotype	*n*=71	MMP-9 levels (pg/ml)	P value
AA	23	2188 (6304)	0.002
AG	26	876.5 (8248)	(GG and AA)
GG	22	382 (3812)	(AG and AA)

MMP-9 levels are expressed as median (Range). The data was analyzed by Kruskal Wallis test. P value < 0.05 is considered statistically significant.
MMP-9: Matrix metalloproteinase-9.

Table 3: Gender – wise comparison of MMP-9 levels between different genotype groups.

Gender	AA genotype	AG genotype	GG genotype	Significance
Male	1299 (5167)	754 (4602)	496 (3792)	0.394
Female	5262 (3092)	998 (8027)	297 (2288)	0.009 (GG and AA)

MMP-9 levels are expressed as Median (Range). P values less than 0.05 is considered statistically significant. MMP-9: Matrix metalloproteinase – 9.

Limitations of the Study

Genotyping was done for 125 individuals but due to financial constraints MMP-9 levels could be estimated in 71 subjects (39 male and 31 female) only. Because of fewer samples no statistically significant correlation could be obtained between MMP-9 levels and pulmonary function parameters. We haven't estimated serum anti- elastase levels, estimation of which could have increased the specificity of the study.

CONCLUSION

From this current study we propose that the females with AA genotype have increased MMP-9 levels and so they may be more prone for COPD if they are exposed to the environmental risk factors.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MMP: Matrix Metalloproteinase; **COPD:** Chronic Obstructive Pulmonary Disease; **BMI:** Body Mass Index; **FVC:** Forced Vital Capacity; **FEV1:** Forced Expiratory Volume At 1st Second; **PEFR:** Peak Expiratory Flow Rate; **SDS:** Sequence Detection Software.

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