Original article:

Frequency distribution of Chronic Obstructive Pulmonary Disease related Matrix Metalloproteinase-9 gene polymorphism in healthy South Indian population

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ABSTRACT:

Background: Chronic Obstructive Pulmonary Disease (COPD) is an irreversible disorder of airway obstruction with multi factorial origin. The pathogenesis of COPD is multi factorial which includes genetic and environmental factors. Interaction between genetic and environmental factors attribute to the development of COPD. In India, as environmental air pollution is one of the major public health concern, establishing the role of genetic factors in the development of COPD will serve as a preventive tool. Hence the present study was aimed at assessing the normative frequency of COPD related Matrix Metalloproteinase -9 (MMP-9) gene polymorphism (*Gln279Arg – rs17576*) in a healthy South Indian population.

Methods: A total of 125 healthy South Indian adults were recruited for the study. Pulmonary Functions of the subjects were tested to ensure that they do not have pre-existing pulmonary diseases. Deoxyribonucleic Acid (DNA) extraction was done from anti-coagulated whole blood by kit method and genotyping was done by Real-Time PCR (RT PCR).

Results: The genotype frequency of AA was found to be 26 (21%), AG was 53 (42%) and GG was 46 (37%). The frequency of 'A' allele was 0.42 and 'G' allele was 0.58. The spirometry parameters were compared between the three genotype groups and there was no statistically significant difference.

Conclusion: The normative frequency of Gln279Arg polymorphism was assessed in the South Indian population. The obtained spirometry parameters were compared between the three genotype groups and there was no statistically significant difference between as the study population includes only healthy adults.

Key words: *Gln279Arg* , Frequency distribution of *rs17576* , COPD gene , Matrix metalloproteinase – 9.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disorder of airway obstruction that is not completely reversible. The etiology of COPD is multi factorial, which includes cigarette smoking, genetic and environmental factors. The interaction between genetic and environmental factors plays a vital role in the development of the disease.[1] In India, air pollution is a major public health concern.[2] According to World Health Organization (WHO), "*COPD is the third leading cause of death worldwide accounting for around 10 million deaths in United States and it is also suggested to be the third most common cause of death worldwide by the year 2020*".[1] According to WHO Global InfoBase updated on 20th January 2011, India was estimated to have the highest COPD mortality in the world with 64.7 estimated deaths per 1,00,000 population.[2]

The interaction of genetic and environmental factors is stated to be critical to the development of the disease, which activates variety of enzymes that can lead to extracellular matrix destruction.[1] Matrixins or Matrix Metalloproteinases (MMPs) 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.[3] Earlier studies had shown association of Single Nucleotide polymorphisms (SNP) of many of these identified MMPs like MMP-9, MMP-12, MMP-1 with COPD.[4] Polymorphisms result in over expression of MMPs leading to excessive extracellular matrix degradation, which cannot be overcome by the anti-proteinases. A study in Mexican population, showed significant association of *Gln279Arg* polymorphism at exon 6 of MMP-9 with COPD.[5] Till date, there is paucity in the literature to establish the frequency distribution of *Gln279Arg (rs17576)* polymorphism in South Indian population. Hence, in the present study we aimed at determining the normative frequency of COPD related *Gln279Arg* polymorphism at exon 6 of MMP-9 gene in south Indian population.

MATERIALS AND METHODS

This cross sectional study was conducted to estimate the normative frequency of MMP-9 *Gln279Arg (rs17576)* polymorphism among healthy south Indian adults aged 18 to 45 years. The study was approved by Institutional Ethics Committee and Postgraduate Research Monitoring Committee of the institute. The sample size was calculated to be 125.

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: Smoking, hypertension, diabetes mellitus, alcoholics, endocrinological disorders, acute illness, valvular heart diseases, chronic respiratory illness.

Subjects were asked to report to pulmonary function testing laboratory, at around 9 am, at least 1 hour after a light breakfast, as the maximum forceful expiratiory 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 were recorded. Pulmonary function tests of the subject were assessed by computerized Spirometry model (SPIROLAB III). The results are interpreted by comparing with the values predicted for height, weight and ethnicity of each individual. 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 mini kit from anti coagulated whole blood. *rs 17576* 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.

Statistical Methods

The results were analyzed using IBM PASW Statistics Version – 19.0 (SPSS version 19.0). The normality of the parameters were tested using Kolmogrov Smirnov test. Normally distributed parameters were expressed in Mean ± SD. Non-normally distributed parameters were expressed as median with Inter Quartile range. The genotype distribution in 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 allele frequency in the study population was compared with the allele frequencies in different other population group by Fischer's exact test.

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RESULTS

Gender and region–wise distribution of study population

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%).

Age, body mass index and FEV_1/FVC

Body mass index, FEV_1/FVC are expressed as Mean ± SD, age is expressed as median with inter quartile range in Table 1.

Table 1 (original): Mean ± SD / Median (IQR) of the subject characteristics (N=125)

S.No.	Parameters	Mean ± SD /Median (IQR)
1	Age (years)*	28 (20 - 35)
2	Body Mass Index (Kg/m2)#	23.6 ± 3.8
3	FEV1/FVC (%)*	97.9 ± 9.3

*Values are expressed as Median (IQR). # Values are expressed as mean ± SD.FEV_1 – Forced Expiratory Volume at first second; FVC – Forced Vital Capacity; MMP–9 – Matrix Metalloproteinase-9; MDA – Malondialdehyde; IQR - Inter Quartile Range, SD – Standard Deviation.

Genotype and allele frequencies

The distribution of genotype frequencies in the study population were AA (21%), AG (42%) and GG (37%). The frequency of A allele was 0.42 and G allele was 0.58. The observed genotype frequencies were found to be consistent with Hardy – Weinberg equilibrium (Chi – Square test P value was 0.146, with 1 degree of freedom).

Gender wise distribution of genotype and allele frequencies

Gender wise distribution of genotype and allele frequencies in the study population are shown in Table 2.

Table 2 (original): Gender wise distribution of genotype and allele frequencies

SNP	Genotype Frequency			Allele Frequency	
(rs17576)	AA (%)	AG (%)	GG (%)	A	G
Male	21.5	41.5	37	0.42	0.58
Female	20	43.3	36.7	0.42	0.58

Spirometry Parameters in different genotype groups:

The mean ± SD of FEV_1/FVC , PEFR, FEF_{25-75} are given in Table 3. On comparison by one way ANOVA, there was no significant difference in the spirometry parameters between the different genotype groups.

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Table 3 (original): Spirometry Parameters in different genotype groups

Genotype group	FEV1/FVC (%) (M ±SD)	PEFR (L/S) (M ± SD)	FEF25-75 (L/S) (M ± SD)
AA	91.67 ±6.2	7.3 ± 1.73	9 ± 1.2
AG	93.9 ±5.3	6.4 ± 1.63	6 ± 1.0
GG	93.82 ± 5.1	6.4 ± 1.93	6 ± 1.06

FEV_1 – Forced Expiratory Volume in first second; FVC – Forced Vital Capacity; PEFR – Peak expiratory Flow Rate; FEF_{25-75} – Forced Expiratory Flow 25 – 75 %;M ± SD - Mean ± Standard Deviation

Comparison of observed allele frequencies (A=0.42, G=0.58) in the study population with different population groups

The genotype and allele frequencies obtained in the study population were compared with the data available in the databases and results are tabulated in Table 4.

Table 4 (original): Comparison of observed allele frequencies (A=0.42, G=0.58) in the study population with different population groups

Study Population	'A' allele	'G' allele	P value
Indian Telugu in UK	0.42	0.58	1.00
Srilankan Tamil from UK	0.41	0.59	1.00
Punjabi from Lahore	0.50	0.50	0.32
Gujarat Indians in Texas	0.48	0.52	0.47
Bangladesh Bengali	0.45	0.55	0.70
Japanese	0.32	0.68	0.56
Han Chinese	0.25	0.75	0.01*
Southern Chinese	0.25	0.75	0.01*
British England	0.68	0.32	<0.000*
African American	0.68	0.32	<0.000*

Comparison was done by Fishers Exact test. *P value < 0.05 is considered statistically significant.

DISCUSSION

Studies in the past have proved that COPD runs in families and the interaction between genetic and environmental factors play a vital role in the development of COPD. The role of genetic factors in the etiology of COPD in South Indian population is less studied. Initially in 1970s, gene coding for alpha-1 anti-trypsin was the only gene identified. Alpha-1 anti-trypsin is a plasma protein that counteracts the action of serine proteinases, which are proteolytic in their action. Therefore, Alpha-1 anti-trypsin is protective against extracellular matrix degradation. Hence, genetic modification in the gene coding for alpha-1 anti trypsin can lead to defective extracellular matrix degradation.[6,7] In later years, polymorphisms in various genes had been reported to be associated with the development of COPD. Three major polymorphisms have been identified in MMP-9 gene. One among them is MMP-9 *Gln279Arg*.[8] The present study was conducted to assess the

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normative frequency of single nucleotide polymorphism of COPD related MMP-9 gene among healthy South Indian adults. The polymorphism studied was located at exon-6, codon 279, of MMP-9 gene and it involves substitution of allele 'G' for 'A'. In the study conducted in Mexican population, at exon 6 of MMP-9, 'G' is the rare allele and 'A' is the common allele. The presence of rare allele 'G' leads to degradation of extracellular matrix. 'G' allele codes for the amino acid Arginine (MMP-9R) and 'A' allele codes for Glutamine (MMP-9Q). MMP-9 R has reduced affinity for Tissue Inhibitors of MMPs (TIMP) thus contributing to extracellular matrix degradation leading to emphysematous changes.[5] Apart from COPD, Gln279Arg is also found to be associated with diabetes, transplant rejection, glaucoma and lung cancer metastasis.[9-12] The normative frequency of genotype/allele frequency of this Gln279Arg polymorphism in our study population is not available so far in the existing databases. So in the current study, as the foremost initiative, we have aimed at establishing the normative frequency of Gln279Arg polymorphism among healthy South Indian adults. So that this will serve as a control data to compare the same genotype/ allele frequency with the diseased group in our study population.

Age, body mass index and FEV_1/FVC

The median age of our study population was 28 years with inter quartile range of 20 – 35 years. Since pulmonary functions have been reported to decline with age (13), in the present study we have included individuals this age group. The mean Body mass index (BMI) of our study population was 23.6 Kg/m^2. Pulmonary functions were also found to be associated with Body mass index (BMI). Studies in the past have reported decline in pulmonary function in obese individuals when compared to non- obese (14) and the studies have also shown negative correlation between BMI and spirometry parameters (15). The mean FEV_1/FVC % ratio was 97.9%, and it was within normal limits

Comparison of observed allele frequencies (A=0.42, G=0.58) in the study population with different population groups

The comparison of allele frequencies in the study population with the other population groups available in databases are shown in Table 4.[15] The allele frequencies obtained in the study population were similar to those obtained in Indian Telugu population in UK, Srilankan Tamil population in UK, Punjabi Population, Gujarati Indians, Bangladesh population and Japanese population.[14]

In Mexican population, MMP-9 *Gln279Arg* was found to be associated with COPD. Those participants with homozygous GG genotype were found to have 3 fold increased risk of COPD.[5] In a study conducted in Tunisian population, MMP-9 *Gln279Arg* polymorphism was found to be associated with increasing severity of COPD.[13]

CONCLUSION

The study was conducted among healthy South Indians to assess the normative frequency of COPD related gene polymorphism. Future perspective of our study is that the frequency of the same Single Nucleotide polymorphism (Gln279Arg) is to be studied in COPD patients to determine the genotype group that is most commonly associated with COPD. Therefore in the current study, though the study subjects were healthy volunteers we have compared the three groups with respect to spirometry to determine any preexisting difference in pulmonary functions among the three genotype groups. There was no statistically significant difference in pulmonary functions among the three genotype groups studied. Further studies can be focused at comparing these genotype and allele frequencies between healthy adults and COPD patients.

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Future Perspectives:

The genotype and allele frequencies obtained in the study population can be used as the control data to compare the same with different disease conditions like COPD, diabetes, ischemic stroke and glaucoma that are found to be associated with the same genetic polymorphism.

REFERNCES:

1. Kasper DL, Fauci AS, Hauser SL, Lango DL, Jamson JL, Loccalzo J, editors. Harrison's principles of internal medicine. 19th ed. New York. Mc-Graw Hill; 2015

2. Bhome AB. COPD in India: Iceberg or volcano?, COPD in India: Iceberg or volcano. J Thorac Dis J Thorac Dis. 2012 Jun 1;4, 4(3, 3):298, 298–309.

3. Loffek S, Schilling O, Franzke C-W. Biological role of matrix metalloproteinases: a critical balance. Eur Respir J. 2011 Jul 1;38(1):191–208.

4. Jiang S, Yang ZH, Chen YY, He Z, Zhou Y, Gao Y, et al. MMP-9 genetic polymorphism may confer susceptibility to COPD. Genet Mol Res. 2016;15(2):1-11.

5. Tesfaigzi Y, Myers OB, Stidley CA, Schwalm K, Picchi M, Crowell RE, et al. Genotypes in matrix metalloproteinase 9 are a risk factor for COPD. Int J Chron Obstruct Pulmon Dis. 2006;1(3):267.

6. Cohen BH, Ball WC, Bias WB, Brashears S, Chase GA, Diamond EL, et al. A genetic-epidemiologic study of chronic obstructive pulmonary disease. I. Study design and preliminary observations. Johns Hopkins Med J. 1975 Sep;137(3):95–104.

7. Carrel RW, Jeppson J, Laurell C, Brennan SO, Owen MC, Vaughan L et al.Strucutre and variation of α_{1}- anti trypsin. Nature. 1982;298:329-333.

8. Vlaykova T, Dimov D. Polymorphisms of Matrix Metalloproteinases (*MMP*) in COPD. Biotechnol Biotechnol Equip. 2012 Jan;26(sup1):111–9.

9. Hu Z, Huo X, Lu D, Qian J, Zhou J, Chen Y, et al. Functional polymorphisms of Matrix Metalloproteinase-9 are associated with risk of occurrence and metastasis of lung cancer. Clin Cancer Res. 2005;11:5433–9.

10. Singh R, Srivastava P, Srivastava A, Mittal RD. Matrix metalloproteinase (MMP-9 and MMP-2) gene polymorphisms influence allograft survival in renal transplant recipients. Nephrol Dial Transplant. 2010;25:3393–401.

11. Saravani S, Yari D, Saravani R, Azadi Ahmadabadi C. Association of COL4A3 (rs55703767), MMP-9 (rs17576) and TIMP-1 (rs6609533) gene polymorphisms with susceptibility to type 2 diabetes. Biomed Rep. 2017;6:329-334.

12. Ahram DF, Alward WL, Kuehn MH. The genetic mechanisms of primary angle closure glaucoma. Eye Lond Engl. 2015;29:1251–1259.

13. Pruthi N, Multani NK. Influence of Age on Lung Function Tests. J Exerc Sci Physiother. 2012 Jun 1;8(1):1.

14. Wang S, Sun X, Hsia T-C, Lin X, Li M. The effects of body mass index on spirometry tests among adults in Xi'an, China. Medicine (Baltimore) [Internet]. 2017 Apr 14 [cited 2019 Mar 31];96(15).

15. Banerjee J, Roy A, Singhamahapatra A, Dey PK, Ghosal A, Das A. Association of Body Mass Index (BMI) with Lung Function Parameters in Non-asthmatics Identified by Spirometric Protocols. J Clin Diagn Res JCDR. 2014 Feb;8(2):12–4.

16. rs17576 - SNPedia [Internet]. [cited 2017 Apr 14]. Available from: https://www.snpedia.com/index.php/Rs17576

17. Bchir S, Nasr H ben, Hakim IR, Anes A ben, Yacoub S, Garrouch A, et al. Matrix Metalloproteinase-9 (279R/Q) Polymorphism is Associated with Clinical Severity and Airflow Limitation in Tunisian Patients with Chronic Obstructive Pulmonary Disease. Mol Diagn Ther. 2015 Dec;19(6):375–87.