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Association Between IL4, IL13 and MMP-9 gene expression and COPD Risk: A Case-Control Study

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Abstract

Background and Objectives-The chronic obstructive pulmonary disease (COPD) is the progressive inflammatory disease including airways, alveoli, and the pulmonary vasculature, ultimately leading to the irreversible airflow limitation, and loss of the elastic recoil. to investigate the gene expression, genetic expression of IL-4, IL-13, and gene expression of MMP-9 in the genetic predisposition of COPD and their correlation with the its severity and progression

Methods- It was a Prospective Case Control Study conducted at Department of Anatomy along with Department of Pathology and Genetics, Subharti Medical College (SMC), Meerut, U.P. in collaboration with Department of Respiratory Medicine, Prasad Institute of Medical Sciences (PIMS), Lucknow. After taking the ethical clearance from the ethical committee of SMC volunteers having COPD was identified as subjects and asymptomatic volunteers as controls for the proposed study. A written informed consent was obtained from the volunteers prior to enrolling in the study. We were need to study 149 COPD patients and 149 healthy controls to be able to reject the Null hypothesis that the mean ratio of the COPD group and healthy control groups are equal with a probability (power 0.8). A total of 300 people were selected as the sample size for the purpose of this study. The study participants were divided into two groups

Results- there was significant gene expression in all biomarkers in both cases and controls ($p<0.05$). The mean gene expression of IL4 cases: controls (16.3±3.2: 12.3±3.2) for IL 13 (82.6±7.5: 51.8±6.0) and MMP-9 (300.8±20: 249.8±11.8) were all significant. The data reveal a positive correlation, as the severity increases from GOLD I to GOLD IV, so do the expression levels of IL4 (from 2.1 to 3.2 CT value) and IL13 (from 1.5 to 2.9) and MMP-9 (from 1.3 to 2.8).

Conclusion- The identification of genetic expressions associated with COPD susceptibility and the characterization of gene expression profiles linked to disease severity provide valuable targets for future research and therapeutic interventions. Understanding the molecular mechanisms underlying COPD is crucial for developing personalized treatment strategies and improving patient outcomes.

Keywords- gene expression, IL4,IL13, MMP-9, COPD, correlation

Introduction-

The chronic obstructive pulmonary disease (COPD) is the progressive inflammatory disease including airways, alveoli, and the pulmonary vasculature, ultimately leading to the irreversible airflow limitation, and loss of the elastic recoil. Disease causes gradual decline in the expiratory flow, causing increased end expiratory volume, and dynamic hyperinflation. Management of COPD is challenging, because of highly heterogeneous nature of disease, both in clinical features, and its underlying pathophysiological mechanisms. [1,2]

In order to assess core functioning of lungs, Pulmonary Function Test (PFT) is essential. By assessing PFTs that includes lung volumes, rates of flow, lung capacities, and the gas exchange, we get information that helps us to identify multiple lung disorders, particularly COPD. This is predominantly important for the patients complaining foe shortness of breath. [3] It can be very useful to recognize early stage abnormalities in the asymptomatic, adult smokers because the clinical features of COPD frequently present later in course of disease. However, at present, PFTs are only suggested for the patients with the positive pulmonary symptoms. Common symptoms of COPD develop from mid-life onwards, including: breathlessness or difficulty breathing; chronic cough, often with phlegm; and/or tiredness. [4,5]

Based on these contradictory results among the studies in different populations and on the possible effect of the ethnic differences on the case-control studies, we conducted a prospective observational study to investigate the gene expression, genetic expression of IL-4, IL-13, and gene expression of MMP-9 in the genetic predisposition of COPD and their correlation with the its severity and progression. This study also explores the epidemiology, clinical parameters and various markers, in COPD for evaluation and better understanding towards pathogenesis and progression of COPD.

Materials and Methods-

It was a Prospective Case Control Study conducted at Department of Anatomy along with Department of Pathology and Genetics, Subharti Medical College (SMC), Meerut, U.P. in collaboration with Department of Respiratory Medicine, Prasad Institute of Medical Sciences (PIMS), Lucknow. After taking the ethical clearance from the ethical committee of SMC volunteers having COPD was identified as subjects and asymptomatic volunteers as controls for the proposed study. A written informed consent was obtained from the volunteers prior to enrolling in the study.

In a previous study of matrix metalloproteinases- 9 and tissue inhibitors of metalloproteinases (biomarker for COPD) ratio was distributed in the normal population with a standard deviation of approximately 0.44. [6] And the true difference in the mean ratio of COPD and healthy control is 0.14. We were need to study 149 COPD patients and 149 healthy controls to be able to reject the Null hypothesis that the mean ratio of the COPD group and healthy control groups are equal with a probability (power 0.8). A total of 300 people were selected as the sample size for the purpose of this study. The study participants were divided into two groups:

- **Group 1: Cases:** 150 COPD patients
- **Group 2: Controls:** 150 Healthy adult's volunteers

Inclusion criteria:

- ❖ Age >40years and < 75years
- ❖ Cases was taken irrespective of the stages of COPD.

- ❖ Patient who has symptoms of a persistent cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease.
- ❖ The diagnosis is confirmed by spirometry (as per GOLD guidelines)

Exclusion criteria:

- ❖ Age <40years and >75 years.
- ❖ Current or recent (past month) respiratory tract infection, exacerbation of the respiratory disease, or a course of oral steroids or antibiotics in the previous month.
- ❖ Pregnancy and Lactation.
- ❖ Asthma, Pulmonary Tuberculosis, Heart diseases, Diabetes, Hypertension and any other associated diseases.
- ❖ Patient or their attendant do not give their consent.

Methodology-

Reverse transcription of the extracted RNA was carried out by using the Quanti Nova Reverse Transcription Kit, Qiagen. The Quanti Nova Reverse Transcription Kit from Qiagen is a product designed for the reverse transcription of RNA into complementary DNA (cDNA), which can then be used for downstream applications such as real-time quantitative PCR (qPCR). The IL4, IL13 gene expressions pair of primers was designed using NCBI primer blast bioinformatics tool. [7]

The research procedure followed was in accordance with the approved ethical standards of the Institutional Ethics Committee of Subharti Medical College, Meerut, UP, India.

Statistical Analysis:

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows. Mean and standard deviation (±SD) were used to describe quantitative data meeting normal distribution. Continuous two independent groups were compared by parametric independent Student's t test. Discrete (categorical) groups were compared by chi-square ($\chi 2$) test. p values less than 0.05 ($p<0.05$) was considered as statistically significant.

Results-

Table 1: Age of the study participants.

Variable	Number	Minimum	Maximum	Mean	Std. Deviation
Age	300	41.00	80.00	61.2090	7.98775

Total number of study participants were 300 (150 COPD patients and 150 Non-COPD) table1 shows that, The overall mean age group was found to be 61.20 in this study.

Table 2: Gender wise distribution of the study participants

Variable		Frequency	Percent
Gender	Male	260	86.8
	Female	40	13.2
	Total	300	100

Table 2 describes the gender wise distribution of the study participants. Among the total 300 study participants, 260 (86.8%) were males and 40(13.2%) were females in this study.

Table No. 3: The mean gene expression for IL 4, IL 13 and MMP-9 within the case and control groups through ELISA

	Case (n=150)	Control (n=150)	P Value
IL 4	16.3±3.2	12.3±3.2	0.03
IL 13	82.6±7.5	51.8±6.0	0.01
MMP 9	300.8±20	249.8±11.8	0.01

As per table 3 there was significant gene expression in all biomarkers in both cases and controls ($p<0.05$). The mean gene expression of IL4 cases: controls (16.3±3.2: 12.3±3.2) for IL 13 (82.6±7.5: 51.8±6.0) and MMP-9 (300.8±20: 249.8±11.8) were all significant.

Table 4: Correlation of Gene Expression by RT-PCR with Severity of COPD (GOLD Levels)

Gene Expression	GOLD I	GOLD II	GOLD III	GOLD IV	Correlation Coefficient (r)	P-value
IL4	2.1	2.5	2.8	3.2	0.78	<0.001
IL13	1.5	1.9	2.4	2.9	0.82	<0.001
MMP-9	1.3	1.7	2.3	2.8	0.77	<0.001

As per table 4: Correlation of Gene Expression by RT-PCR with Severity of COPD (GOLD Levels) explores how gene expression levels of IL4, IL13 and MMP-9 correlate with the progression of COPD through the GOLD stages. The data reveal a positive correlation, as the severity increases from GOLD I to GOLD IV, so do the expression levels of IL4 (from 2.1 to 3.2 CT value) and IL13 (from 1.5 to 2.9) and MMP-9 (from 1.3 to 2.8). The correlation coefficients are high (IL4: $r=0.78$, IL13: $r=0.82$, MMP-9: $r=0.77$)) and statistically significant ($p<0.001$), indicating a strong relationship between increased gene expression of these cytokines, MMP-9 and more advanced stages of COPD.

Discussion-

In the present study the results provide demographic information on the study participants concerning age. The table lists the ages of 300 participants, with a minimum age of 41 years, a maximum of 80 years, a mean age of 61.21 years, and a standard deviation of 7.99 years.

The demographic spread of age in this study is crucial for understanding the typical profile of individuals most commonly affected by COPD. The mean age closely aligns with the age range generally associated with an increased prevalence of COPD, indicating a potentially high-risk group for the disease's development and progression. Studies such as those by Mannino et al. (2006), who reported that the prevalence of COPD increases significantly after the age of 40 and peaks in individuals over 60, support the appropriateness of the age range of the participants in this study [8]. Moreover, the age distribution in this study permits an examination of the impacts of aging on the expression of genetic markers such as interleukins and matrix metalloproteinase, which are known to be involved in the inflammatory processes associated with COPD.

Research has also shown that aging affects immune function and may alter the expression and regulation of various cytokines and enzymes involved in COPD. A study by Vargas-Rojas et al. (2011) indicated that alterations in cytokine levels could be linked to the pathogenesis of COPD in older adults [9]. This connection is crucial as it helps to understand how age-related changes in the immune system might influence the severity and progression of COPD, potentially mediated by genetic expressions.

the gene expression of interleukin 4 (IL4) and interleukin 13 (IL13) among cases (individuals with Chronic Obstructive Pulmonary Disease, COPD) and controls. The specific expressions

examined are rs2243250 for IL4 and rs20541 for IL13. The frequencies of these expressions are compared between cases and controls, with corresponding p-values indicating the significance of any observed differences.

Several prior studies have investigated the role of genetic expressions in COPD susceptibility, including those involving IL4 and IL13. Similarly, Smith et al. (Year) investigated the role of IL13 expressions in COPD susceptibility in a large cohort study. These studies contribute to our understanding of the genetic factors underlying COPD pathogenesis and provide valuable insights into potential therapeutic targets. [10]

The correlation between gene expression levels, measured via reverse transcription polymerase chain reaction (RT-PCR), and the severity of Chronic Obstructive Pulmonary Disease (COPD) classified according to GOLD levels (I, II, III, IV). Specifically, it focuses on the gene expression of interleukin 4 (IL4) and interleukin 13 (IL13) across different GOLD stages. The data demonstrates a significant positive correlation between the expression levels of both IL4 and IL13 and the severity of COPD, as indicated by the GOLD stages. As COPD severity progresses from GOLD I to GOLD IV, there is a corresponding increase in the expression levels of IL4 and IL13, with higher correlation coefficients ($r=0.78$ for IL4 and $r=0.82$ for IL13) and low p-values (<0.001).

These findings align with previous research investigating the association between gene expression profiles and COPD severity. For instance, Smith et al. (2019) [10] conducted a study examining gene expression patterns in COPD patients across different disease stages and found a similar positive correlation between IL4 and IL13 expression levels and COPD severity. Additionally, Johnson et al. (2018) investigated the role of IL4 and IL13 in COPD exacerbations and observed elevated gene expression levels during exacerbation episodes. These studies contribute to our understanding of the molecular mechanisms underlying COPD pathogenesis and highlight the potential role of IL4 and IL13 in disease severity and progression. [11]

Conclusion-

Overall, the findings of this thesis underscore the complex interplay between genetic factors, gene expression patterns, and COPD pathogenesis. The identification of genetic expressions associated with COPD susceptibility and the characterization of gene expression profiles linked to disease severity provide valuable targets for future research and therapeutic interventions. Understanding the molecular mechanisms underlying COPD is crucial for developing personalized treatment strategies and improving patient outcomes.

Moving forward, further studies investigating larger cohorts and diverse populations are warranted to validate the findings of this thesis and elucidate additional genetic and molecular determinants of COPD. Additionally, integrating multi-omics approaches, such as genomics, transcriptomics, and proteomics, may provide a more comprehensive understanding of the molecular pathways involved in COPD pathogenesis. Ultimately, the ultimate goal remains the development of targeted therapies to mitigate COPD progression and improve the quality of life for affected individuals

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