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Research Article

Association Of Leptin, Adiponectin And IL-8 Gene Polymorphism And Serum Biomarkers In COPD And COPD With T2DM In North Indian Population

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ABSTRACT

Background: Inflammation plays an important role in chronic obstructive pulmonary disease (COPD). Increasing evidence points to the role of inflammation in the pathogenesis of type 2 diabetes (T2D).

Aim & objectives: We intend to investigate the biomarkers in *Adiponectin*; *Leptin* and *IL-8* gene for COPD induced T2DM patients. Along with this we analyzed the effect of polymorphism on the levels of these cytokines.

Material & methods: A total of 500 subjects including 250 controls, 191 COPD and 59 COPD with T2DM each, aged 30 to 70 years were enrolled for the study after meeting the inclusion criteria. Written informed consent was obtained from each subject before inclusion in the study. The study was approved by the institutional ethics committee (ethical no 90TH ECM II B-FS/P2.).

Results: We found statistically significant results of serum biomarkers in all studied groups. Leptin, Adiponectin and IL-8 in serum levels were significantly higher with a p-value (<0.001) in COPD with diabetes when compared with COPD and healthy controls Genotyping of *Leptin* (C/T), *Adiponectin* (C/G) and *IL-8* (C/T) polymorphisms were performed. Genotype frequency of 'CT' of Leptin gene variant was showing significant association with risk of developing COPD by 3.7 folds. But it was not associated witGeh development of T2DM in COPD cases. Whereas genotype 'TT' was highly significant association with risk of developing T2DM in COPD patients by 2.4 folds. This means individuals with genotype 'TT' are at higher risk of developing of COPD and moreover they are at risk of T2DM

Conclusion: We also conclude that genotype 'TT' of *Leptin*, 'GG' of *Adiponectin* and 'TT' of *IL-8* may be exploited as biomarker for prognosis and diagnosis of T2DM in COPD and COPD respectively. While 'CT' of *IL-8* was protective biomarker against COPD.

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INTRODUCTION

Inflammation plays an important role in chronic obstructive pulmonary disease (COPD). Increasing evidence points to the role of inflammation in the pathogenesis of type 2 diabetes (T2D)[1].Irritation in COPD causes a risk factor for the development of type 2 diabetes mellitus(T2DM) as comorbid condition. While COPD is never-ending burning lung problem, perhaps the most significant idea is irritation occurring in the disease which is caused mainly by neutrophils. The prevalence of T2Dis increasing significantly worldwide while COPD is the third leading cause of death worldwide. The complications of T2D and COPD impact the quality of life and shorten life expectancy as well as increase the financial burden. Apart from being a major cause of sickness within oneself, COPD can be a potential risk factor for the development of pro-inflammatory conditions such as T2D. In many studies have found that DM causes an accelerated decline in lung functions as compared to non-diabetics [6]. Mannino *et al*, found that there is high risk of diabetes in patients with moderate or higher COPD. A study investigating about the factors which are associated with new-onset diabetes in the UK concluded that patients with frequent exacerbations of COPD were more likely to developT2DM. Higher exposure of inhaled corticosteroid (ICS), a commonly prescribed treatment in COPD was also found to be associated with increased incidence of T2DM.Diabetes especially uncontrolled is linked to worsening the outcomes such as (longer hospital stay and risk of death) in people that suffer from an exacerbation of COPD. The need to use of corticosteroid therapy during exacerbations can complicate the status of diabetes mellitus in those patients [7].

Adipose tissues acts as an endocrine organ, secreting various adipokines that are involved in metabolic processes.[2].Dysregulation in its endocrine capacity and aggravating fat tissues (AT) actuate second rate foundational irritation and insulin obstruction in fat patients, leading to T2DM. Adiponectin Secreted Frizzled-Related Protein 5 (SFRP5) calm adipokines created by fat tissues. It is the most popular and most copious adipokine found in human serum, with fixations ordinarily in the µg/mL [3].Leptin is a 16 kDa protein copied by the 'obese' (*ob*) gene (Zang et al, 1994). It is a member of cytokine family and perform variety of biological functions. Genius fiery cytokines are associated with COPD[4].Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) trigger a cytokine cascade that initially is composed of the pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-12, IFN-γ, IL-18, and TNF itself)[5]. These cytokines serve to contain and resolve the inflammatory foci through activation of local and systemic inflammatory responses. We intend to investigate the biomarkers in *Adiponectin*;*Leptin* and *IL-8* gene for COPD induced T2DM patients. Along with this we analyzed the effect of polymorphism on the levels of these cytokines.

MATERIAL AND METHODS

Study design

This was a case-control study of patients diagnosed with COPD as per Global Initiative for chronic obstructive lung disease (GOLD) criteria 2018carried out at the Inpatient and Outpatient Department of Respiratory medicine, at King George's Medical University (KGMU), Lucknow, India, from July 2018 to April 2019. A total of 500 subjects including 250 controls, 191 COPD and 59 COPD with T2DM each, aged 30 to 70 years were enrolled for the study after meeting the inclusion criteria. Written informed consent was obtained from each subject before inclusion in the study. The study was approved by the institutional ethics committee (ethical no 90^{TH} ECM II B-FS/P2.).

Diagnostic criteria for COPD

The criteria for diagnosis & severity of COPD were taken as per GOLD guidelines 2018.

According to it, COPD is diagnosed if, post-bronchodilator FEV1/FVC ratio<0.70. Based on the level of obstruction COPD severity was determined (GOLD, 2018).The patients were excluded from the study if they had pulmonary conditions other than COPD (e.g. TB, HIV infection), connective tissue disorders, chronic renal failure, chronic liver disease, and malignancies on long term steroid or cytotoxic drug therapy, chronic alcoholics. The information of the same was obtained from the detailed history, clinical examination, chest radiography and medications used by the patient currently or previously.

Diagnostic criteria for Diabetes

The screening and diagnosis of T2DM wasdone according to national guidelines, which stipulated that a fasting blood glucose (FBG) is used with cutoff thresholds in line with those recommended by WHO. In brief, FBG >126 mg/dL indicates DM; FBG from 110 mg/dL to <126 mg/dL indicates impaired fasting glucose; FBG <110 mg/dL is normal. The patients already known to have T2DM were directly enrolled in the study as "Known DM" cases with COPD. Patients whose diabetes status was not clear had gone for random blood sugar (RBS) testing, and if was more than 126 mg/dL, the subjects were further evaluated with fasting blood sugar (FBS) and post-prandial blood sugar (PPBS). If FBS was more than 126 mg/dL or PPBS more than 200 mg/dL the subjects were confirmed as having DM after which they were classified as new T2DM cases with COPD. All the participants will undergo a glycosylated hemoglobin (HbA1c) evaluation.

Healthy Control

Age and sex-matched controls were selected. In the age range of30-70 years was enrolled with no history of respiratory or any other disease.

Blood sampling and analysis

5.0 ml of the whole blood sample was collected from each participant in plain vials for serum separation and after that it was stored at –80˚C for further estimation of adiponectin, leptin andinterleukin-8 (IL-8) level by ELISA method. All the variables which were to be estimated in study group comprising of three types of subjects *i.e.*healthy controls, COPD and COPD with T2DM.

Genotyping

Frozen EDTA blood samples were used to extract DNA using salting out method (**Miller *et al.*, 1988**) with slight modifications.After isolation quantification and quality assessment was done. The quality of DNA was determined by gel elecrophoreis and amount was quantitated by using UV-VIS spectrophotometer (Shimadzu, Japan).Genotyping of *Adiponectin*; *Leptin* and *IL-8* was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)analysis. The primers were designed by Primer 3.0 (*ver* online) software and restriction enzymes were identified using NEB cutter (*ver* online). The reaction mixture for PCR contained DNA template- 100ng, Taq Buffer- 1X, Forward and Reverse primers (10 pmol each), dNTPs- 200 µM, Taq Polymerase-0.5U, and MQ water for final volume makeup. The amplified PCR products were digested according to the enzyme specifications. The digested fragments representing genotypes were electrophoresed on 12% polyacrylamide gel (PAG)/ 2.5% agarose gels. The details of primers, annealing temperature, Restriction Enzymes (RE) and product sizes were given in table 1.

Summary of SNPs, primer sequences, annealing temperatures, restriction enzyme and product sizes

Gene variant (rs ID)	Primer sequences	RE	Annealing temp (^{0}C)/ Product size (bp)	Product size after Digestion
*Adiponectin*A/G (rs710445)	F:5'GAATTGTCTGAACCTGGGAG'3 R:5'CAGCTCAAAACAACCAGGAT'3	Nla111 (CATG)	**56/300**	300, 159, 141
Leptin C/T (rs2060713)	F:5'GGGGCTCCACATTCTACTCT'3 R:5'TTTCCTCTGAGTGCTACTGAAG'3	BslHKAI (GWGCWC)	**59/271**	271, 192, 91
IL-8 C/T (rs2227306)	F-5'CTCTAACTCTTTATATAGGAATT-3'R:5'GATTGATTTTATCAACAGGCA-3'	EcoRI (CCCTTT)	**58/278**	278, 168, 98

Statistical analysis

Analysis was done using SPSS 20.0 version (Chicago, Inc., USA).The results are presented in average ± SD and percentages. Kolmogorov test was used to test patterns for normal distribution.Reconstruction showed a non-normal pattern of these parameters. Firstly for two group comparisons, Mann Whitney u test was used and for multiple comparisons between the groups, Kruskal Wallis test followed by Tukey's post hoc comparisonswere used to compare the levels of leptin, adiponectin, IL-4, IL-6 and IL-8 among different severity COPD. For determining associations of COPD with BMI and smoke pack years data, Spearman's rank correlation tests were performed. The p-value<0.05 was considered significant.

RESULTS:

In the present study, a total of 250 healthy controls, 250 COPD subjects were enrolled. Out of 250 COPD patients 59 were COPD with T2DM. Demographic profile, incidence, age wise distribution, HbA1C andGlycemic levels of COPD patients is given in table 1. Maximum no. of patients (32.0%) were in the age range of 60-70. The HbA1C and Glycemic levels were increasing with increasing severity and is highest for the GOLD IV stage in COPD with T2DM patients (Table 1).

Table 1: Demographic profile, incidence, age wise distribution, HbA1C and Glycemic levelsin patients of COPD and COPD+T2DM.

S.No	Variables	N (%)
1.	Total COPD patients	191
2.	COPD patients with T2DM	59 (24)
3.	Age distribution of COPD patients	
	40-50	46 (18.4)
	51-60	63 (25.2)
	61-70	80 (32.0)
	71-80	61 (24.4)
4.	Gender Distribution of COPD patients	
	Male	139 (55.6)
	Female	111 (44.4)
5.	Smoking Status of COPD patients	
	Smokers	204 (81.6)
	Non-Smokers	46 (18.4)
6.	HbA1C levels of COPD with T2DM patients (Mean±SD)	
	GOLD I	3.2±0.96

	GOLD II	4.2±0.9
	GOLD III	5.6±0.9
	GOLD IV	6.25±1.8
7.	Glycemia (mmol/l) levels of COPD with T2DM patients (Mean±SD)	
	GOLD I	2.63±0.65
	GOLD II	3.89±0.8
	GOLD III	5.62±0.9
	GOLD IV	7.67±3.7

All the Pulmonary function test (spirometry) parameters are given in table 2. It was observed that the post vital capacities were significantly different in all the three categories. The values of category third that is COPD with T2DM were most severely affected.

Table 2: Spirometry parameters of controls, COPD and COPD+T2DM.

S.No	Spirometry Parameters	Healthy Controls (N=250)	COPD (N=250)	COPD with diabetes (N=59)	P-Value
1.	Post fvc (ltrs)	4.03±0.74	2.09±0.68	2.98±0.68	**<0.001***
2.	Post fev1/fvc (%)	101.35±7.09	55.06±9.53	62.06±9.53	**<0.001***
3.	Post fev1 (ltrs)	2.48±0.60	1.13±0.47	1.98±0.47	**<0.001***
4.	Post fev1 % predicted	89.95±7.73	44.73±18.82	52.73±18.82	**<0.001***
5.	% change in fev1	7.86±7.05	10.26±13.22	11.26±13.22	**0.015***

Values are presented as (Mean±SD)

Estimation of Cytokine levels in serum

Distribution of Adiponectin levels (ng/mL) in serum of Healthy controls, COPD and COPD with Diabetes patients is shown in figure 1. It shows that Adiponectin serum levels (ng/mL) were significantly (P=< 0.001)highest in COPD with T2DM patients when compared with COPD and healthy control subjects

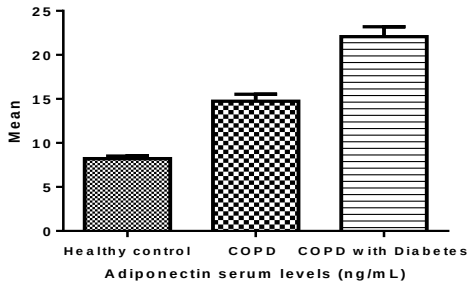

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Figure 1: Adiponectin serum levels (ng/mL) in Healthy control, COPD and COPD+T2DM patients.

Distribution of Leptin levels (ng/mL) in serum of Healthy controls, COPD and COPD with T2DM patients is shown in figure 2.It depicts that Leptin serum levels (ng/mL) were significantly (P=< 0.001)higher in COPD with T2DM patients when compared with COPD and healthy control subjects.

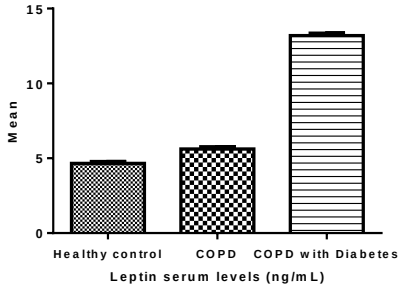

Figure 2: Leptin serum levels (ng/mL) in Healthy control, COPD and COPD+T2DM patients.

Distribution of IL-8 levels (pg/mL) in serum of Healthy controls, COPD and COPD with T2DM patients is shown in figure 3. It depicts that IL-8 levels (pg/mL) were significantly (P=< 0.001) higher in COPD with T2DM patients when compared with COPD and healthy control subjects.

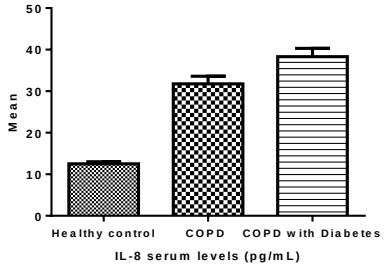

Figure 3:IL-8 serum levels (pg/mL) in Healthy control, COPD and COPD+T2DM patients.

Genotyping

Genotyping of *Leptin* (C/T), *Adiponectin* (C/G) and *IL-8* (C/T) polymorphisms were performed in all 250 healthy controls, 191 COPD and 59 COPD with T2DMsubjects. It was observed that in *Leptin* genotype 'CT' and 'TT' were significantly associated with COPD with high odds ratio (3.728, 4.579 respectively). Interestingly genotype 'TT' was also associated with COPD+T2DM and increases the risk ofdeveloping T2DMin COPD patients by 2.4 folds (Table 3).*Adiponectin* (C/G) polymorphism was not associated with COPD but it was showing significant association with COPD+ T2DM and increases the risk of developing T2DM in COPD patients by 2.6 folds(Table 3). *IL-8* (C/T) polymorphism was significantly associated with COPD, genotype 'TT' was seen to increase the risk by 5.1 folds. *IL-8* (C/T) was not found to be associated with the COPD+T2DM.

Table 3: Genotype frequencyof Leptin(C/T), Adiponectin (C/G) and IL-8(C/T) polymorphismsin controls, COPD and COPD+T2DM.

LeptinC/T (rs710445)							
Genotype	**Control (n=250)**	**COPD (n=191)**	**P- Value**	**OR (95% CI)**	**COPD+ T2DM (n=59)**	**P- Value**	**OR (95% CI)**
CC	208 (83.2)	106 (55.5)	Ref.	Ref.	28 (47.5)	Ref.	Ref.
CT	30 (12.0)	57 (29.8)	**0.001*** **	**3.728 (2.261–6.148)**	13 (22.0)	0.694	0.863 (0.415 – 1.796)
TT	12 (4.8)	28 (14.7)	**0.001*** **	**4.579 (2.239–9.365)**	18 (30.5)	**0.016***	**2.434 (1.180 – 5.019)**

AdiponectinC/G (rs2060713)							
CC	140 (56.0)	114 (59.7)	Ref.	Ref.	36 (61.1)	Ref.	Ref.
CG	87 (34.8)	66 (34.5)	0.731	0.932 (0.622–1.396)	14 (23.7)	0.257	0.672 (0.338–1.336)
GG	23 (9.2)	11 (5.8)	0.170	0.587 (0.275–1.256)	09 (15.2)	**0.051**	**2.591 (0.995–6.749)**
IL-8C/T(rs2227306)							
CC	90 (36.0)	34 (17.8)	Ref.	Ref.	13 (22.0)	Ref.	Ref.
CT	120 (48.0)	80 (41.9)	**0.022***	**1.765 (1.086–2.868)**	28 (47.5)	0.82	0.915 (0.424–1.978)
TT	40 (16.0)	77 (40.3)	**0.001*****	**5.096 (2.942–8.825)**	18 (30.5)	0.24	0.611 (0.269–1.388)

*** = *P* < 0.05, ** = *P* < 0.01, *** =*P* < 0.001**

The allele 'T' of Leptin C/T (rs710445) was significantly associated with risk of developing COPD by 3.5 folds and it was also associated with risk of developing T2DM along with COPD by 1.7 folds (Table 4).Adiponectin C/G (rs2060713) allele 'G' was significantly associated with COPD with T2DM(Table 4). IL-8 C/T (rs2227306) allele 'T' was significantly associated with risk of developing COPD by 2.4 folds but it was not showing association with COPD with T2DM(Table 4).

Table 4: Allele frequency of Leptin(C/T), Adiponectin (C/G) and IL-8(C/T) polymorphisms in controls, COPD and COPD+T2DM.

Leptin C/T (rs710445)							
Allele	**Control (n=500)**	**COPD (n=382)**	**P- Value**	**OR (95% CI)**	**COPD+ T2DM (n=118)**	**P- Value**	**OR (95% CI)**
C	446(89.2)	269(70.4)	**0.001*****	**3.470 (2.426-4.962)**	69 (58.5)	**0.016***	**1.691 (1.103-2.591)**
T	54 (10.8)	113(29.6)			49 (41.5)		
Adiponectin C/G (rs2060713)							
C	367 (73.4)	294 (76.9)	0.227	0.826 (0.606-1.126)	86 (72.9)	**0.034***	0.505 (0.269-0.948)
G	133 (26.6)	88 (23.1)			73 (27.1)		
IL-8 C/T (rs2227306)							
C	300 (60.0)	148 (38.7)	**0.001*****	**2.372 (1.806-3.115)**	54 (45.8)	0.18	0.750 (0.494-1.137)
T	200 (40.0)	234 (61.3)			64 (54.2)		

*** = *P* < 0.05, ** = *P* < 0.01, *** =*P* < 0.001**

DISCUSSION:

In the present study, 24% of patients with COPD were affected by T2DM, with significant gender differences and smoking status. In COPD patients males =139, females =111, smoker =204, non-smoker=46 were present. Our findings are in line with other studiesdone previously. Mahishale *et al.* observed that the prevalence of DM inCOPD patients was 25.63%. Few other studies observed that deranged blood glucose was seen in more than 50% of the patients admitted with acute exacerbation of COPD [7-9].

Our study showed that a significant number of diabetics 59 (24%) were newly detected. They were not aware of theirdiabetic status. It is

proved beyond doubt that COPD is acondition which predisposes to develop T2DM.Rana *et al*. observed that COPD patients had a multivariaterelative risk of 1.38 folds for new-onset T2DM.Feary*et al*. showed an odds ratioof 2.04 for the development of new-onsetdiabetes in COPD patients.

Furthermore, the present study showed that COPD patients with T2DM had statistically significant decline inthe FEV1, and duration of COPD was longer. Our findings are in line withother studies. The third National Health and NutritionExamination Survey observed declined lung functionsin people with diabetes compared to nondiabetics.Another important aspect worth mentioning here is that this impaired pulmonary function is likely to deterioraterapidly in uncontrolled diabetes. This is in agreement withthe Fremantle Diabetes Study which showed thatcomorbid DM was associated with lower values of FEV1,FVC, peak expiratory flow (PEF), and VC. El-Habashy*et al*(2013) showed that there was asignificant decrease in pulmonary function amongdiabetic patients (FEV1, FEV1/FVC%, forced expiratory flow –25%–75%, maximal voluntary ventilation, and PEF)compared with healthy controls and further proved thatdecline was exaggerated in poorly controlled DM.

India is a major contributor to the burden of COPD, whichis considered to beoneof the highest in the world. Mortalityfrom COPD is four times higher in India than in the US and Europe.This number is expected to increase dramatically due to increased exposure to smoking and biomass fuel. COPD is viewed as a novel risk factor for emerging T2DM due to chronic inflammation, oxidative stress, insulin resistance, weight gain, and fat dysfunction. On the other hand, the spreadof DM is increasing rapidly worldwide. India is considered the diabetes capital of theworld, with 41 million people sufferingfrom DM, and one in fivediabetics in the world is Indian.A growingDM epidemic has emerged in both rural and rural areas, given the significant change in lifestyle, eating habits, decrease in physical activity and obesity in the Indian population urban population of India.

As far as we are aware, this is the first study that specifically investigated the association between COPD and T2DM in North Indian population. This study also signifies that adipokine and pro-inflammatory cytokine were higher in COPD with T2DM, when compared to COPD and healthy control group. Our data showed that the association of T2DM with COPD was present; there was also a significant association with pack years and BMI with COPD and COPD with diabetes in all biomarkers which we had studied. To best of our knowledge, this is the first cross sectional studies to quantitatively clarify the association between COPD, lung function and T2DM. A previous meta-analysis by Malte Rasmussenet al. found a higher risk of T2DM in the COPD group (OR 1.17, 95% CI 1.01–1.35); however, this meta analysis combined four cohort studies and three casecontrol studies, and subsequent subgroup analysesseparating cohort studies and case-control studies did not find any relationship between COPD and T2DM, which was inconsistent with our findings.

In our study, we found statistically significant results of serum biomarkers in studied groups. Leptin serum levels were significantly higher with a p-value (<0.001) in COPD with diabetes when compared with COPD and healthy controls. Likewise, adiponectin serum levels were also increased in both the groups COPD with diabetes when compared with COPD and healthy controls. Proinflammatory cytokines were also increased in COPD with diabetes group and were statistically significant with a p value (<0.001) when they were compared with COPD and healthy controls. So we cansay that, adipokine and proinflammatory cytokines plays an important role in all the studied groups. The association of diabetes and decline in lung function are linked at multiple levels namely systemic inflammation, glucotoxicity and insulin resistance. A large body of evidence has demonstrated systemic inflammation to be the major culprit underlying the impairment of lung function by diabetes.

Adipokine and pro-inflammatory cytokine biomarkers are increased in patients with COPD and have been observed to be up-regulated in patients with T2D also when compared to that in healthy control group, suggesting that inflammation may be the common link[8]. Elevated levels of leptin, adiponectin, IL-8, IL-6 and IL-4 have been shown to predict the development of the insulin resistance syndrome and T2D, supporting a role for inflammation in the pathogenesis of diabetes. We also found that the incidence of diabetes mellitus was statistically significant in a diagnosed patient of COPD than in those without COPD [9].

When COPD patients were contrasted with sound subjects, patients with COPD have higher insulin levels, which are identified with incendiary markers, for example, IL-8, IL-6, IL-4 and solvent receptors for TNF-α and adipokine leptin and adiponectin. The methodology of our examination didn't permit us to verify or refute this speculation. In any case, it demonstrated that cigarette smoking isn't a connection between diabetes mellitus and COPD. This is critical data since tobacco smokes introduction is key to the advancement of COPD and a free and modifiable determinant of diabetes mellitus. Smoking produces oxidative pressure that can enact neighborhood and foundational irritation. In the event that cigarette smoking isn't a connection, it is conceivable that different instruments past fundamental irritation may clarify the connection between diabetes mellitus and COPD.

A noteworthy correlation between COPD and the risk of T2DM has enormously raised interest in studying the underlying biological mechanisms. It is well-known that genetic susceptibility is one of notable characteristics of T2DM, with a 40% life-time risk of T2DM for descendants when a parent has T2DM and the risk increases to 70% when both parents have T2DM This feature might indicate that COPD and diabetes tend to develop simultaneously and indirectly support the hypothesis that COPD and diabetes mellitus could be two different expressions of the systemic inflammatory syndrome[10].

Our results also supports this hypothesis that when correlation was done with pack years versus leptin (healthy control, COPD, COPD with diabetes), adiponectin (healthy control, COPD, COPD with diabetes), IL-8 (healthy control, COPD, COPD with diabetes), IL-6 (healthy control, COPD, COPD with diabetes), IL-4 (healthy control, COPD, COPD with diabetes). There was a significant positive correlation was found in all the groups with p-value (<0.001**), or we can say that as the pack years and BMI increases the serum markers also increases accordingly.

In terms of prognostic implications, there is the need to better understand the link between COPD and T2DM, and in particular, whether the centre of therapy should be shifted to the systemic inflammatory state[11]. We should also understand whether treatment of COPD influences the course of diabetes mellitus or is altered by the presence of the concomitant comorbid disease. It is also important to know whether the treatment of T2DM can alter the natural history of concomitant COPD [5].

Leptin is a primarily pro-inflammatory adipokine that affects both innate and adaptive immune responses[12]. Leptin is increased in COPD, COPD with diabetes when compared with healthy control resulting a significant increase in both the diseases with a significant p-value (<0.001). Leptin differentially increases the production of TH1 cytokines (Interleukin-2, interferon-γ and Tumor Necrosis Factor) and suppresses the production of TH2 cytokines (IL-4, IL-5, and IL-10).When BMI (kg/m2) and pack years was correlated with leptin serum levels of healthy control, COPD, COPD with Diabetes, we found a significant positive correlation with a statistically significant p-value (<0.001), so we can say that as the BMI and pack years of patient increases leptin serum levels also increases in all three groups i.e.: Healthy control, COPD and COPD with Diabetes, so they are directionally proportional to each other.

Some human studies have detected higher circulating adiponectin levels in COPD and COPD with diabetes patients when compared with healthy controls. Some researchers showed that plasma adiponectin levels were elevated in both normal- and underweight patients with COPD and COPD with diabetes. Furthermore, previous smoking has been found to decrease serum adiponectin levels in a dose-dependent manner[11]. Recently, it was claimed that higher plasma adiponectin levels were associated with decreased body mass index, female sex, older age and lower bronchial reversibility in patients with COPD. These findings suggest that adiponectin is associated with BMI and smoke pack years with COPD, COPD with diabetes but virtually nothing is known about the associations of adiponectin with important clinical parameters like lung function, symptoms or treatment responsiveness[13]. When BMI (kg/m2) and pack years was correlated with adiponectin serum levels of healthy control, COPD, COPD with Diabetes, we found a significant positive correlation with a statistically significant p-value (< 0.001), so we can say that as the BMI and pack years of patient increases adiponectin serum levels also increases in all three groups i.e.: Healthy control, COPD and COPD with Diabetes, so they are directionally proportional to each other.

IL-8 is a pro-neutrophilic chemokine that is secreted by various cell types. For more than 10 years, IL-8 has been suggested to play an important role in COPD, and especially in the severity of the disease. In our study, analyzing IL-8 serum levels in regard to clinical parameters of COPD, demonstrated several significant results. IL-8 is a selective chemo-attractant of neutrophils[14]. In this study, the concentration of serum IL-8 in asthmatic and COPD patients was significantly higher than that from controls [15]. One study found a significant increase in levels of IL-8 in plasma and sputum of asthmatic and COPD patients compared with normal subjects. The increased levels of IL-8 in the airway secretions from patients with asthma, asthma with diabetesand COPD, COPD with diabetes may be a marker of an ongoing inflammatory process which is pronounced in patients with asthma and COPD. IL-8 may play a primary role in the activation of neutrophils in asthma and COPD and they may serve as a marker in evaluating the severity of airway inflammation [16]. When BMI (kg/m2) and pack years was correlated with IL-8 serum levels in healthy control, COPD, COPD with Diabetes, we found a significant positive correlation with a statistically significant p-value (< 0.001), so we can say that as the BMI and pack years of patient increases IL-8 serum levels also increases in all three groups i.e.: Healthy control, COPD and COPD with Diabetes, so they are directionally proportional to each other.

It is obscure why individuals with COPD are influenced with T2DM more frequently than controls. There are a few systems, for example, expanded corpulence, decreased physical movement, expanded tobacco smoke and corticosteroid presentation and sickness related irritation, oxidative pressure and hypoxia, which may add to the expanded commonness of diabetes in COPD patients [21].

Not long ago, we have archived a huge impact size relationship between pack years and BMI with COPD and COPD with diabetes. Despite the fact that this is a cross-sectional investigation, which in its inclination can't recognize the genuine reason for the connection between factors, fundamental irritation which may clarify why patients with COPD have an expanded danger of creating diabetes mellitus.

The characteristic pathological change of COPD is a chronic inflammatory response in the respiratory tract and lung parenchyma, particularlyduring acute exacerbations. Moreover, available evidence has found that insulin resistance in COPD patients is associated with inflammation mediators such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor–α. Based on the above studies, some articles have suggested that enhanced levels of inflammation and oxidative stress were plausible links with insulin resistance in COPD patients .This is an

interesting theory thinking about that cigarette smoking can expand the danger of insulin obstruction and diabetes by activating fundamental irritation and oxidative pressure, the two of which are normal in COPD [22].

In spite of the fact that the components clarifying the connection between respiratory debilitation and diabetes stay indistinct, Lin et al. proposed that fundamental irritation could be a conceivable clarification. In patients with COPD, the degrees of fiery go betweens, (for example, tumor necrosis factor alpha, interleukin-6 or C-reactive protein) were demonstrated to be expanded. As per a similar report, these mediators may add to the development of T2DM[23].

As featured right now, COPD patients with persistent aggravation are bound to create T2DM. So also, high introduction to breathed in corticosteroids (ICS), which is the most generally utilized treatment for these patients, demonstrated a critical relationship with the beginning of diabetes. Right now, would be fascinating to rethink the hazard advantage profile of this treatment in COPD clinical consideration and cutoff the utilization of high-portion ICS to patients where the advantage is clear.

The processes by which COPD canpose a T2DMhazard are not fullyunderstood. The most conceivable clarification is that these diseasescangiveT2DM a typical provocative premise. COPD are burning lung problems thatinvolve variousroutes ofprovocation [24]. The atomic factor-kB (NF-kB) signaling pathway may play a centralrole in the pathogenesis of obstructive aspiration obstruction infection (COPD). NF-kB is a translation factor that triggers the declaration of a variety of cool burn qualities, including IL-6, TNF-α, and binding atoms. Elevated circulating levels of these provocative cytokines have been linkedto the progression of prediabetic insulin obstruction and movement toward clear T2DM. The etiology of T2DM is enigmatic and multifactorial, including various hereditary and ecological variables. COPD with T2DMall had strong effects, indicating that there may be a typical route of exacerbation and that the expert'skey provocative components largely reach mirror levels forbothdiseases [25].

Genotyping of *Leptin* (C/T), *Adiponectin* (C/G) and *IL-8* (C/T) polymorphisms were performed. Genotype frequency of 'CT' of Leptin gene variant was showing significant association with risk of developing COPD by 3.7 folds. But it was not associated witGeh development of T2DM in COPD cases. Whereas genotype 'TT' was highly significant association with risk of developing T2DM in COPD patients by 2.4 folds. This means individuals with genotype 'TT' are at higher risk of developing of COPD and moreover they are at risk of T2DM (Table 3).*Adiponectin* (C/G) polymorphism did not show association with COPD but it was showing significant association with COPD+T2DM i.e. COPD cases with genotype 'GG' are at increased risk of encountering by 2.6 folds (Table 3). Genotype frequency of 'TT' of *IL-8* (C/T) polymorphismwas significantly associated with risk of COPD by 5.1 folds. Whereas in heterozygous condition it was seen to be protective against risk of COPD by 1.8 folds.*IL-8* (C/T) was not found to be associated with the COPD+T2DM.To best of our knowledge we are the first one to analyze the association of leptin, adiponectin and IL-8 polymorphism with T2DM in COPD.

CONCLUSION

The biomarkers leptin, adiponectin and IL-8 are implicatedin the pathogenesis of COPD, so we can also say that these cytokines may have an interest in making fiery changes in COPD patients. IL-8 also havethe task of reducingthe work of aspiration andincreasing the severity of the wind current blockage in COPD patients. COPD wasat a higher risk of developing type 2 diabetes regardless of common risk factors for diabetes, including smoking. Ourresultsconfirm the theory that the inexorable deterioration of the airways can increase the risk of type 2 diabetes dueto hiddenpro-inflammatory components. Thelink between COPD andyour comorbid diabetes

mellitus shows the importance of expanding mindfulness and reducing therisk of diabetes for patients with pneumonialoss. Based on future knowledge, prevention methods should be developed and updated to improve the general health problem in potential COPD patients who are also at high risk for other persistent comorbidities.We also conclude that genotype 'TT' of *Leptin*, 'GG' of *Adiponectin* and 'TT' of *IL-8* may be exploited as biomarker for prognosis and diagnosis of T2DM in COPD and COPD respectively. While 'CT' of *IL-8* was protective biomarker against COPD.

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