Original Article

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Plasma Levels of Fetuin B and Fibrinogen in Chronic Obstructive Pulmonary Disease: A Preliminary Cross-sectional Study

Biochemistry Section

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ABSTRACT

Introduction: Recent exploratory proteomics study reported increased blood levels of Fetuin B (FETUB) in Chronic Obstructive Pulmonary Disease (COPD). Growing body of evidence demonstrates fibrinogen not only as acute phase reactant but also possesses features advocating as biomarker of COPD.

Aim: To determine the blood levels of FETUB and fibrinogen independently as well as in combination COPD and among severity of COPD.

Materials and Methods: A single-centred cross-sectional study of eight months duration was conducted during May to December 2017. A total of 76 blood samples (38 COPD patients and 38 controls) were recruited. Based on distribution, parametric and non parametric statistical tools were used. Analysis of co-variance and multiple multivariate linear regression models were utilised to assess the influence of clinical data on FETUB. Steel-Dwass-Critchlow-Fligner method was employed for multiple pairwise comparisons. Receiver Operating Characteristic (ROC) curve were generated to compute cut-off of FETUB, fibrinogen and their combination.

Results: The median age of subjects were 57 years with an IQR of 50-66 years for controls whereas 58 years (IQR 50-65 years) for COPD subjects. In comparison to controls, a significant increase in blood levels of both FETUB and fibrinogen in COPD and Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages of COPD was prominent. Among COPD grades, differences of FETUB levels were significant in GOLD I vs II and GOLD IV vs I, II and III whereas fibrinogen levels in GOLD III from GOLD IV. FETUB showed independent correlation with Forced Expiratory Volume (FEV) 1% pred and severity of COPD. A moderate improvement in combined (FETUB+fibrinogen) analysis of control vs COPD was noticed.

Conclusion: The present study demonstrates significant increase in blood levels of FETUB in COPD and among the severity of COPD in comparison to fibrinogen. Hence, FETUB can be a more promising probable inflammatory biomarker in COPD.

Keywords: Forced expiratory volume, Global initiative for chronic obstructive lung disease, Smoking and biomass exposure

INTRODUCTION

The COPD is a devastating condition posing major health challenge and recognised as fourth leading cause of death in the world [1]. In India, it is the second biggest cause of mortality [2,3]. The pathogenesis is attributed to aberrant inflammatory response to cigarette smoke, aerotoxins and biomass fumes [4]. Most of the recent studies were targeted to understand the patterns of various inflammatory markers in COPD and their relation with respect to clinical outcome, response to therapy and prognosis. Plasma fibrinogen has been demonstrated to exhibit the features of relatively stable prognostic indicator relative to Body Mass Index (BMI), Airflow Obstruction, Dyspnea and Exercise Capacity (BODE) index; the exacerbation rate; and the mortality [5]. On the other side, in one of the recent exploratory studies, on gel free isobaric Tags for Relative and Absolute Quantification (iTRAQ)-based proteomic technique, elevated plasma levels of Fetuin-B (FETUB) along with fibrinogen in stable COPD and Acute Exacerbation COPD (AECOPD) was demonstrated only in cigarette smoking male subjects [6,7].

Globally tobacco smoke is projected as the major prevalent cause of COPD. Especially in Low- and Middle-Income Countries (LMICs) like India, beedi and cigarette are the most common sources of tobacco smoke with varying degree of harmful impact [8]. Whereas biomass smoke exposure group encompassing household utilisation of wood, coal, dung cakes, agricultural residues or Liquefied Petroleum Gas (LPG) for domestic energy purposes attributing to indoor air pollution is well-recognised as an independent major risk factor of COPD. More than 70% of population utilises biomass fuels for domestic purpose attributing indoor air pollution [4,9]. In view of that even non smokers are also prone to develop COPD. Hence, smoking in males and biomass smoke exposure in females are recognised as more predominant hazard and cause of COPD in India. Though, various causal factors lead to different types of inflammatory response yet the pattern of inflammation seen in indoor air pollution induced COPD was reported as similar to that of smoking induced COPD [10].

Hence, the aberrant inflammatory response of respiratory tract is similar in both cigarette smoking and indoor air pollution induced COPD. However, external validation in an independent population of various races under different conditions is warranted before contemplating to routine use in clinical setup. In view of this, the present pilot study was undertaken to determine the variations in the plasma levels of both FETUB and fibrinogen in newly diagnosed stable COPD subjects comprising either genders with cigarette smoking in males and biomass fumes in females as the causal agents.

MATERIALS AND METHODS

The present cross-sectional study was carried out for eight months during May to December 2017 in Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. Institutional Human Ethics Committee (IHEC) Ref No: IHEC/91/Biochemistry/04.2017 dated: 26-04-2017 has approved the present cross-sectional study. The study was conducted in accordance with the Declaration of Helsinki. Informed consent was procured from each subject.

Sample size calculation: As evident from literature and in accordance to 'flat rule of thumb' [11,12], a minimum composite sample size of

30 or 12 per arm (i.e., controls and COPD) is warranted for a pilot study. As well as, in accordance to 'stepped rule of thumb' [13], for a study design with standardised effect size of 0.8 and power >90%; the recommended sample size for a pilot study is 32 per arm. Hence, a total of 76 participants were enrolled in the present study. It comprises 38 newly diagnosed and clinically approved COPD patient samples procured from the Department of Tuberculosis (TB) and Chest Disease, Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu. The remaining 38 participants of control group comprised healthy volunteers belonging to either relatives/attendees of patient (or) relatives/attendees of other patients visiting the hospital on Outpatient Department (OPD) basis. A medical questionnaire for gathering subject's characteristics (smoking details, exposure period to biomass fumes, medical, surgical and gynaecological-obstetrics history, current medications if any and dyspnea) was used.

Inclusion criteria: Only newly diagnosed and clinically approved COPD patients were involved in the present study. Age, gender, BMI, smoking status, smoking index, Biomass Exposure Index (BEI) and menopausal status matched healthy volunteers were recruited as controls.

Exclusion criteria: The patients with hypertension, hepatitis, coronary artery disease, hyperlipidemia, diabetes mellitus, nonalcoholic fatty liver disease, abnormal Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) levels were excluded. Even the patients under long-term treatment with anticoagulant, statin or systemic glucocorticoids were not included.

Anthropometric, Smoking and Biomass Fumes Exposure Indices Measurements

A trained person was reserved for the extraction of blood pressure and anthropometric indices comprising weight, height and BMI. These measurements were carried out on all participants with light clothes and no shoes. Stadiometer with an accuracy of ±0.1 cm and seca scale with 0.1 Kg accuracy were used as standard approaches for extracting height and weight information, respectively. BMI was computed as weight (Kg) per height squared (m^2). The smoking status, smoking index and BEI were stratified according to the earlier studies on Indian population [14,15]. BEI was computed as product of average hours spend on cooking per day and number of years of cooking.

Spirometric Measurements

As per guidelines of American Thoracic Society [16], spirometry indices were computed from the best out of the three technically satisfactory performances for the assessment of pulmonary function under the supervision of specially trained and dedicated personnel. Forced Expiratory Volume in 1 second (FEV1), Forced Vital Capacity (FVC), and FEV1/FVC ratio were measured. Individuals with FEV1% predicted ≥80% were selected as controls. The severity of clinically diagnosed COPD subjects was graded as per the guidelines of GOLD [17]. Reversibility assessment was conducted after the inhalation of short acting β_2 agonist i.e., 400 µg salbutamol.

Laboratory Measurements

The White Blood Cells (WBC) in whole blood was analysed in the cell counter Sysmex XS-800i (Sysmex India Pvt., Ltd., New Delhi). Quantia-CRP-US turbidometric immunoassay kit (Coral Clinical System) was employed for detecting serum high-sensitivity C-Reactive Protein (hs-CRP) levels. Concentration of ALT and AST were measured on clinical chemistry automatic analyser Erba Manheim EM360 (Transasia Bio-medicals Ltd., Mumbai, 400 072) using system pack kits. Only the plasma samples for FETUB analysis were stored at -20°C until their analysis. A 96-well plated Enzyme-Linked Immunoassay (ELISA) kit was utilised according to the manufacturer's protocol (BOSTER Biological Technology, 3492 B Valley, Pleasanton, CA 94566) for assaying FETUB with intra-assay and inter-assay coefficient of variations <9% and <11.8%. The sensitivity of the assay was <12 pg/mL. Clauss method was used for quantification of plasma fibrinogen [18]. The entire process of sample collection, processing and analysis were strictly performed under aseptic conditions in accordance to standard laboratory protocols.

STATISTICAL ANALYSIS

Statistical analysis was conducted with SPSS version 24.0 software (Statistical Package for the Social Sciences, Chicago, IL., USA), Trial version of GraphPad Prism 7.0 (GraphPad, San Deigo, CA, USA) and Trial version of NCSS 12 (NCSS Statistical, LLC, Utah 84037, USA). All data were examined with Shapiro-Wilk (S-W) and Levene's test for normal distribution and homogeneity of variance, respectively. Continuous variables were presented as means±standard deviation (SD), or median {Interquartile Range, (IQR)} in accordance with their distribution state. Chi-square test analysed categorical variables were presented as observed number and percentage. The correlation coefficients between FETUB levels and other variables were analysed using the Pearson's or Spearman's test. Normally distributed data of the patients with COPD and healthy controls were compared using Student's t-tests, and non-normally distributed data were analysed using Mann-Whitney U and Kruskal-Wallis H test. Analysis of Covariance (ANCOVA) and multiple Multivariate Linear Regression (MVLR) with step method were used to evaluate the relationship between dependent and independent variables. Steel-Dwass-Critchlow-Fligner method was employed for pairwise multiple comparisons. Plasma levels of FETUB and fibrinogen were subjected to ROC curve to compute the Area Under Curve (AUC) with 95% confidence interval (95% CI) and cut-off value with corresponding sensitivity and specificity. All assumptions of each statistical approach employed were respected. A two-sided $p<0.05$ was considered significant for all analyses.

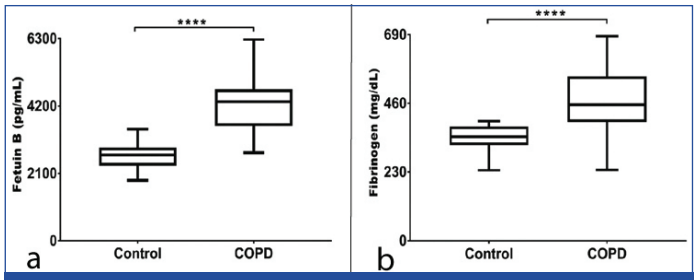
RESULTS

A total of 76 subjects (38 Control+38 COPD) were enrolled. Only newly diagnosed COPD subjects without any co-morbid conditions and in accordance to inclusion and exclusion criteria were recruited. General characteristics of the participants in the study are provided in [Table/Fig-1]. The median age of subjects were 57 years with an IQR of 50-66 years for controls whereas 58 years (IQR 50-65 years) for COPD subjects. The computed gender based distribution in each group i.e., control and COPD was 57.89% and 42.11% for males and females, respectively. The mean of BMI was 25.5±3.6 for healthy participants and 24.8±3.9 for COPD diagnosed patients. Among the males 17 (77.3%) comprised current smokers. The remaining 5 (22.7%) were ex-smokers. Current smokers with similar proportions in either groups (control and COPD) were further stratified into heavy (36.3%), moderate (59.09%) and mild smokers (4.5%). The BEI of females was 83.3±6.8 and 83.5±9.3 in controls and COPD subjects, respectively. All the female subjects were in postmenopausal phase and without a history of tobacco consumption. In the present study, the gender based differences in the blood levels of FETUB within controls and COPD group were not significant.

Plasma levels of both FETUB and fibrinogen significantly increased in COPD, as apparent in [Table/Fig-2]. Mean distribution of FETUB was 2672±417 pg/mL for controls and 4234±844 pg/mL for COPD group [Table/Fig-2a]. Similarly, fibrinogen indicated median of 348 mg/dL (IQR: 320-382 mg/dL) and 450 mg/dL (IQR: 392-567 mg/dL) for controls and COPD, respectively [Table/Fig-2b]. Among the COPD group, a gradual rise in the blood levels of both FETUB and fibrinogen with increasing severity of disease was apparent. Pairwise multiple

	Stages of COPD					
Variable	**Control (n=38)**	**COPD (n=38)**	**GOLD I (n=12)**	**GOLD II (n=11)**	**GOLD III (n=8)**	**GOLD IV (n=7)**
Age (Years)	57 (50-66)	58 (50-65)	52±9	56±10	61±8	66±4
Gender						
Male	22 (57.89%)	22 (57.89%)	8 (66.66%)	6 (54.54%)	4 (50%)	4 (57.14%)
Female	16 (42.11%)	16 (42.11%)	4 (33.33%)	5 (45.45%)	4 (50%)	3 (42.85%)
Smoking index (SI) in males						
Mild	1 (4.5%)	1 (4.5%)	0 (0%)	0 (0%)	1 (25.0%)	0 (0%)
Moderate	13 (59.09%)	13 (59.09%)	6 (75%)	4 (66.7%)	2 (50.0%)	1 (25.0%)
Heavy	8 (36.3%)	8 (36.3%)	2 (25%)	2 (33.3%)	1 (25.0%)	3 (75.0%)
Smoking status in males						
Current	17 (77.7%)	17 (77.7%)	7 (87.5%)	4 (66.7%)	4 (100%)	2 (50.0%)
Ex-smoker	5 (27.7%)	5 (27.7%)	1 (12.5%)	2 (33.3%)	0 (0%)	2 (50.0%)
Biomass Exposure Index (BEI) in females						
BEI	83.3±6.8	83.5±9.3	84.0±12.8	83.8±12.7	82.2±5.0	84.3±5.6
BMI (Kg/m^2)	25.5±3.6	24.8±3.9	25.5±5.4	25.1±4.1	24.3±2.7	23.5±1.2
FEV1 (%Pred)	88 (86-91)	66 (46-83)	84±2	70 (63-74)	49±5	29±3
Fibrinogen (mg/dL)	348 (320-382)	450 (392-567)	481±146	426 (395-575)	447 (355-461)	560±147
Fetuin B (pg/mL)	2672±417	4234±844	3488±705	4399 (3611-4707)	4466±438	5349±642
WBC (cells/mm^3)	6855 (6280-8569)	6490±2579	5303±2640	6553±2713	7874±2200	6673±2134
hs-CRP (mg/dL)	0.161 (0.106-0.200)	0.150 (0.078-0.237)	0.112 (0.048-0.234)	0.122±0.068	0.245±0.104	0.203±0.050

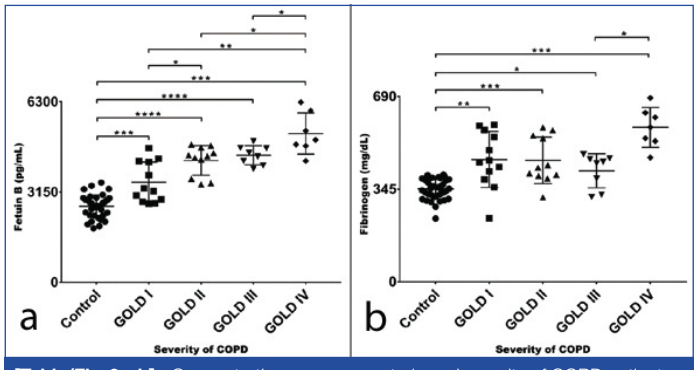
[Table/Fig-1]: General characteristics of study group.
Normally distributed data are presented as Mean±standard deviation, not normally distributed data are presented as Median (Interquartile range) and categorical variables are presented as number (%); BMI: Body mass index; FEV1: Forced expiratory volume frist; WBC: White blood cells; hs-CRP: High-sensitivity C-reactive protein



[Table/Fig-2a-b]: Concentration among controls and COPD patients. a) Fetuin B in pg/mL, and b) Fibrinogenin mg/dL.
(****: p<0.0001)

comparisons elucidated significant differences among control, GOLD I, GOLD II, GOLD III and IV as evident from the scattered plots of FETUB [Table/Fig-3a] and fibrinogen [Table/Fig-3b]. In both scattered plots the pairwise differences of GOLD I, II, III and IV against control were significant. Among the COPD subjects, significant variations in distribution of FETUB in GOLD I vs GOLD II and GOLD IV against remaining grades (GOLD I, II and III) in a pairwise comparisons were noticed. Whereas the fibrinogen exhibited limited differences among the COPD group i.e., only between GOLD III and GOLD IV.

Among the various combinations [Table/Fig-4], FETUB has exhibited significant linear correlation only with FEV1%pred, severity of lung disease and age. Although, FETUB showed significant negative



[Table/Fig-3a-b]: Concentrations among controls and severity of COPD patients. a) Fetuin B in pg/mL; and b) Fibrinogen in mg/dL.
(*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001)

Variable	**Pearson correlation**		**Spearman correlation**	
	r-value	p-value	ρ	p-value
Age	-	-	0.267	0.020
Gender			0.140	0.229
BMI	-0.146	0.209	-	-
Smoking status				
Heavy smokers	-	-	0.084	0.469
Moderate smokers	-	-	-0.221	0.055
Mild smokers	-	-	0.034	0.772
Ex-smokers	-	-	0.084	0.469
Biomass exposure index	-	-	0.140	0.229
FEV1	-	-	-0.771	0.001
Grades of lung function	-	-	-0.843	0.001
WBC	-0.072	0.538	-	-
hs-CRP	0.148	0.202	-	-

[Table/Fig-4]: Linear correlation analysis between FETUB and other clinical data.

correlation with both FEV1%pred (-0.771) and severity of lung disease (-0.843) yet FETUB had relatively very strong correlation with severity of lung disease. Despite of significance in positive correlation of FETUB with age but the strength of association (0.267) was negligible. Moreover, ANCOVA [Table/Fig-5a,b] and multiple MVLR [Table/Fig-6] corrected for age, BMI, smoking status, smoking index, BEI, WBC, hs-CRP demonstrated independent correlation of FETUB only with FEV1%pred and severity of lung disease.

The AUC with 95% CI computed from ROC curve analysis of FETUB, fibrinogen and their combination were apparent from [Table/Fig-7]. In the present study, ROC plots of control vs COPD were significant with respect to FETUB (cut-off value: 3031.2 pg/mL; sensitivity: 89.4%; specificity: 86.8%; positive predictive value: 0.8718; and accuracy: 0.8816), fibrinogen (cut-off value: 390 mg/dL; sensitivity: 78.9%; specificity: 86.8%; positive predictive value: 0.8718; and accuracy: 0.8816). A combination of both exhibited a moderate improvement in discrimination of COPD group from control in terms of AUC with 95% CI.

Variable	p-value	Partial eta squared
Group (Control vs COPD)	0.001	-
Age	0.073	0.041
Gender	0.072	0.044
BMI	0.257	0.018
Heavy smokers	0.838	0.001
Moderate smokers	0.334	0.011
Mild smokers	0.973	0.001
Ex-smokers	0.892	0.001
Biomass exposure index	0.072	0.044
WBC	0.316	0.014
hs-CRP	0.153	0.028

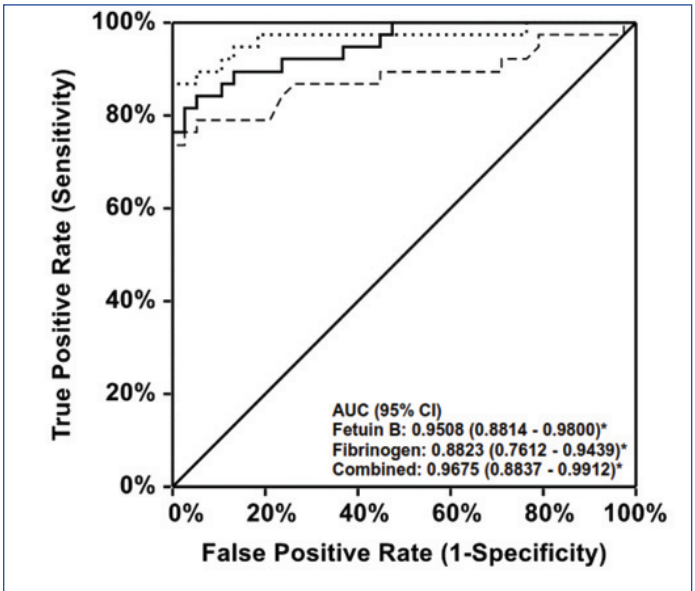
[Table/Fig-5a]: Analysis of covariance (ANCOVA) on fetuin B between groups. a) Impact of covariates on fetuin B in Control vs COPD group.

Variable	p-value	Partial eta squared
Group (Control vs GOLD I, II, III IV)	0.001	-
Age	0.053	0.012
Gender	0.077	0.044
WBC	0.712	0.001

[Table/Fig-5b]: Impact of covariates on fetuin B in control vs severity of COPD group.

	FEV1% pred		Grades of lung function	
Variable	Beta (β)	p-value	Beta (β)	p-value
FEV1% pred	-0.808	0.001	-	-
Grades of lung function	-	-	0.846	0.001
Gender	0.065	0.380	0.100	0.109
Age	0.036	0.649	0.117	0.065
BMI	-0.040	0.593	-0.034	0.591
Smoking status				
Heavy smoker	-0.052	0.524	-0.087	0.196
Moderate smoker	-0.061	0.465	-0.120	0.083
Mild smoker	-0.023	0.761	-0.029	0.652
Ex-smoker	-0.394	0.694	0.008	0.902
Biomass exposure index	0.065	0.380	0.100	0.109
WBC	-0.140	0.057	-0.061	0.330
hs-CRP	-0.072	0.344	-0.037	0.564

[Table/Fig-6]: Multivariate linear regression model with Fetuin B as dependent variable.



[Table/Fig-7]: Receiver Operating Characteristic (ROC) curve of Fetuin B (thickline), Fibrinogen (broken line) and combined (Fetuin B + Fibrinogen as dotted line).
AUC: Area under the curve; CI: Confidence interval; *:p<0.05

DISCUSSION

As is well known, involvement of both innate and adaptive immune responses linked through activation of dendritic cells is a classical feature of COPD inflammatory response. Though, systemic inflammation is considered as hallmark of COPD yet not all patients are consistent with elevated concentrations of popular inflammatory biomarkers [19-23]. However, accumulating data of evidence demonstrated the association of poor clinical outcomes (including exacerbations and mortality) in the subjects persistent with elevated level of systemic inflammation biomarkers [20-22]. Hence, subjects with persistent systemic inflammation may be considered as a distinct COPD subgroup or phenotype. A constantly growing body of evidence unraveled the pivotal attributes of fibrinogen in describing the degree of local or systemic inflammation [23] and also the latest proteomics based study [7] explored as well as demonstrated the probable role of FETUB in the same direction. In lines with previous studies [7,8,19,24], in the present study also blood levels of both fibrinogen and FETUB were elevated in COPD.

Fibrinogen, a soluble heterohexameric glycoprotein of hepatic origin with a molecular weight of ~340,000 and a half-life of approximately three to five days [25]. It is not only popular as major circulating coagulation protein but it is also renowned as an acute-phase reactant with IL-6 being an important cytokine mediating its release [26]. Despite of unclear underlying mechanism in various chronic conditions [27,28]; especially in COPD subset of population with adverse outcomes in terms of exacerbations and all cause mortality, persistence of increased plasma levels of fibrinogen emerged as a distinct feature [18,19]. In one of the largest meta-analysis [28], every 1-g/L raise of plasma fibrinogen is associated with an increased risk of 3.7 fold COPD specific mortality. In another multicenter intervention and characterisation study based on COPD-related hospitalisations also demonstrated the association of 1-g/L increase in plasma fibrinogen with a risk of 77% raise in severe COPD exacerbations [24]. In view of the above attributes, in the present study, the fibrinogen was included as reference blood inflammatory biomarker of COPD. In the present study, blood fibrinogen levels were significantly elevated in COPD. The pairwise comparisons of COPD GOLD stages, the statistically significant differences in distribution was noticed only between GOLD III and GOLD IV. These observations are in lines with earlier studies [18,19,24].

The FETUB, a novel adipokine/hepatokine, member of fetuin family belongs to cystatin superfamily of cysteine protease inhibitors [29-33]. The underlying molecular mechanism of FETUB is not yet clearly elucidated in various clinical conditions. However, FETUB is an unambiguous paralogue of fetuin A. The role of fetuin A in regulation of inflammatory signaling mediated via Toll-like receptors and as an endogenous inhibitor of human metalloproteases is well-addressed [34]. Hence, it is apparently plausible that even FETUB might possess similar property in the inflammatory progression of COPD also. The farnesoid X-receptors were shown to induce FETUB expression [35]. In the present study also, the blood levels of FETUB were elevated in COPD. A distinct and significant gradual rise of FETUB levels with increasing severity of COPD was also apparent. Among the GOLD stages of COPD pairwise comparisons, significant difference in FETUB levels was apparent only in GOLD I vs GOLD II, GOLD I vs IV, GOLD II vs IV and GOLD III vs IV. The remaining pairwise comparisons were insignificant. The ROC curve based computations also substantiated significant AUC with 95% CI and cutoff values with reasonable sensitivity, specificity, positive predictive value and accuracy in control vs COPD analysis. Whereas in the earlier study comprising only tobacco smoke induced COPD male subjects [7], significant differences were apparent in: control vs COPD, control vs GOLD II, III and IV, GOLD I vs GOLD II and GOLD IV. As apparent in the present study, FETUB and fibrinogen exhibited disparity in their blood levels only among GOLD stages of COPD. A significant difference was noticed in FETUB levels of

GOLD I from GOLD II and GOLD IV from GOLD I, II and III in pairwise comparisons whereas fibrinogen levels were different only between GOLD III and GOLD IV.

Till date, ineptitude of most of the explored biomarkers of COPD in to aid diagnosis, define clinical phenotypes or monitoring response to therapeutic strategies enforced the attention towards the feasibility of composite biomarkers. In the same perspective, especially fibrinogen as a part of multiple biomarker is evident in literature [19,22,26]. Epidemiological study reported the prognostic value of fibrinogen alongwith CRP and WBC in COPD with respect to incident co-morbidities [19]. In another combination study fibrinogen alongwith CRP and IL-8 is correlated with disease severity as measured by GOLD stage of COPD [36]. In view of that, in the present study also, the feasibility of combination of fibrinogen with FETUB in distinguishing the COPD group from controls was explored. The ROC computations derived from combination of fetuin B and fibrinogen expressed slightly superior AUC with 95% CI in Control vs COPD.

The earlier study encompassed COPD group with varying degree of co-morbid conditions [7]. The ROC curve computations (AUC; 95% CI, Cut-Off value; sensitivity and specificity) of fetuin B (0.747; 0.642-0.834; 1375 ng/mL; 73.6% and 67.6%), fibrinogen (0.715; 0.608-0.806; 2.9 g/L; 66.0% and 73.5%) and combination (0.804; 0.705-0.881) were shown to be significant in their control vs COPD analysis. Even in the present study observation were in corroboration with the earlier study. However, in comparison to present study, reasonably decreased ROC attributes in control vs COPD analysis of earlier study reveals the declination in predictive ability of COPD on association with comorbid conditions [7]. Owing to the poor sample size, ROC curve analysis was not extended among the GOLD stages of COPD in the present study.

The strengths of the present study is involvement of both genders; inclusion of smoking status, smoking index, and BEI (as per Indian references); and recruitment of age, gender, menopausal status, BMI, smoking status, smoking index and BEI matched healthy volunteers as controls. Another lucrative aspect of the present pilot study is involvement of newly diagnosed COPD subjects of either gender without any co-morbid conditions to understand the difference in primary pattern of these biomarkers before commencement of clinical management of COPD. Whereas in the previous analogous study only the active cigarette smoking males with varying degree of co-morbid conditions were involved [7].

Growing body of evidence demonstrates the increased blood levels of FETUB in type 2 diabetes mellitus [31], Acute myocardial infarction [32] and COPD [6,7]. Literature survey further evidences COPD as an independent risk factor for a range of cardiovascular disease but a reciprocal relationship with type 2 diabetes mellitus. Most of these conditions commonly shares oxidative stress and inflammation as the underlying components. Hence, the complex and multifaceted mechanistic that underpin this association have yet to be elucidated. And such understandings in the future studies will lead to the discovery of sorely needed novel targets for therapy.

Limitation(s)

The limitations of the present study comprise: sample size especially in severity of COPD, non adoption of new classification system and a bias in the participant selection based on the exclusion criteria. Though, the present pilot study suffers with weak sample size in severity of COPD related analysis yet the most of the interpretations are in corroboration with previous studies. However, robust studies with increased sample size is warranted for an unbiased understanding of FETUB levels among the severity of COPD.

CONCLUSION(S)

Plasma levels of both FETUB and fibrinogen exhibited significant variations in their plasma levels between control and COPD. It was also apparent between controls and severity of COPD as per GOLD guidelines. Pairwise multiple comparisons revealed limited significance in differences among severity of COPD. FETUB levels in GOLD I showed significant differences from GOLD II and GOLD IV from GOLD III, II and I whereas fibrinogen levels only between GOLD IV and GOLD III. The multiple MVLR model indicated independent correlation of severity of COPD and FEV1% pred with FETUB. ROC curve analysis of FETUB, fibrinogen and combination of both (FETUB+fibrinogen) suggested significant AUC with 95% CI between COPD vs control. In view of the above observations, the present pilot study further demonstrates the feasibility of relative plasma levels of FETUB discretely and also in coalition with fibrinogen as a probable inflammatory index of COPD group and may assist in assessing risk of CVD and type 2 diabetes mellitus at an early stage.

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REFERENCES

[1] Soriano JB, Abajobir AA, Abate KH, Abera SF, Agarwal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and year lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: A systemic analysis for the Global Burden of Disease Study. Lancet Respir Med. 2017;5(9):691-706.
[2] Dandona L, Dandona R, Kumar GA, Shukla DK, Paul VK, Balakrishnan K, et al. Nations within a nation: Variations in epidemiological transition across the states of India, 1990-2016 in the Global Burden of Disease Study. Lancet. 2017;390:2437-60.
[3] Salvi S, Kumar GA, Dhaliwal RS, Paulson K, Agarwal A, Koul PA, et al. The burden of chronic respiratory diseases and their heterogeneity across the states of India: The Global Burden of Disease Study 1990-2016. Lancet Glob Health. 2018;6:e1363-74.
[4] Augusti AG. COPD, a multicomponent disease: Implications for management. Respir Med. 2005;99:670-82.
[5] Duvoix A, Dickens J, Haq I, Mannino D, Miller B, Tal-Singer R, et al. Blood fibrinogen as a biomarker of chronic obstructive pulmonary disease. Thorax. 2013;68:670-76.
[6] Franciosi L, Postma DS, Van den Berge M, Govorukhina N, Horvatovich PL, Fusetti F, et al. Susceptibility to COPD: Differential proteomic profiling after acute smoking. PLoS Pm. 2014;9:e102037.
[7] Diao WQ, Shen N, Du YP, Liu BB, Sun XY, Xu M, et al. Fetuin-B (FETUB): A plasma biomarker candidate related severity of lung function in COPD. Sci Rep. 2016;6:30045.
[8] Sheetu S, Soumya M, Anirudh S, Varun M, Udai VS, Singh V. Breath carbon monoxide levels in different forms of smoking. Indian J Chest Dis Allied Sci. 2011;53:25-28.
[9] Gupta D, Agarwal R, Agarwal AN, Maturu VN, Dhooria S, Prasad KT, et. al. Guidelines for diagnosis and management of chronic obstructive pulmonary disease: Joint ICS/NCCP [I] recommendations. Lung India. 2013;30:228-67.
[10] Iyer N, Brashier B, Madas S, Londhe J, Salvi S, Blames PJ. Physiological and inflammatory phenotypic comparisons between non-smoking and smoking COPD. Eur Resp J. 2013;42(Suppl 52):3042.
[11] Browne RH. On the use of a pilot sample for sample size determination. Stat Med. 1995;14:1933-40.
[12] Julious SA, Owen RJ. Sample size calculations for clinical studies allowing for uncertainty about the variance. Pharmaceut Stat. 2006;5:29-37.
[13] Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomized trial to minimize the overall trial sample size for the external pilot and main trial for continuous outcome variable. Stat Methods Med Res. 2016;25(3):1057-73.
[14] Jindal SK, Malik SK. Smoking index- A measure to quantify cumulative smoking exposure. Lung India. 1988;4:195-96.
[15] Mahesh PA, Jayaraj BS, Prabhakar AK, Chaya SK, Vijayasimha R. Identification of a threshold for biomass exposure index for chronic bronchitis in rural women of Mysore district, Karnataka, India. Ind J Med Res. 2013;137:87-94.
[16] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Resp J. 2005;26:319-38.
[17] Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for diagnosis, management and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Resp Crit Care Med. 2013;187:347-65.
[18] Thyagarajan B, Jacobs DR, Apostol GG, Smith LJ, Lewis CE, Williams OD. Plasma fibrinogen and the lung function: the CARDIA study. Int J Epidemiol. 2006;35:1001-08.

[19] Thomsen M, Dahl M, Lange P, Vestibo J, Nordestgaard BG. Inflammatory biomarkers and comorbidities in chronic obstructive pulmonary disease. Am J Respir Crt Care Med. 2012;186:982-88.
[20] Mannino DM, Tal-Singer R, Lomas DA, Vestbo J, Graham Barr R, Tetzlaff K, et al. Plasma fibrinogen as a biomarker for mortality and hospitalized exacerbations in people with COPD. Chronic Obstr Pulm Dis. 2015;5:23-34.
[21] Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory biomarkers improve clinical predictions of mortality in chronic obstructive pulmonary disease. Am J Respir Crt Care Med. 2012;185:1065-72.
[22] Agusti A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent inflammation is associated with poor clinical outcomes in COPD: A novel phenotype. PLoS One. 2012;7:e37483.
[23] Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol. 2012;34:43-62.
[24] Alvar A, Sin DD. Biomarkers in COPD. Clin Chest Med. 2014;35:131-41.
[25] Doolittle RF. Chapter 17- Structural basis of signaling events involving fibrinogen and fibrin. In: Bradshah RA, Dennis DA, editors. Handbook of Cell Signaling, 2nd ed. Tokyo: Academic Publishers; 2010. Pp.111-14.
[26] Anderson L, Stone V, Donaldson K, Guy K. Fibrinogen synthesis in the lung in response to particulate air pollution. Ann Occup Hyg. 2002;46:440-43.
[27] Reinhar WH. Fibrinogen-marker or mediator of vascular disease? Vasc Med. 2003;8:211-16.
[28] Danesh J, Lewington S, Thompson SG, Lewington S, Thompson SG, Lowe GD, et al. Plasma fibrinogen level and the risk of major cardiovascular disease and nonvascular mortality: An individual participant meta-analysis. JAMA. 2005;294:1799-809.
[29] Meex RC, Hoy AJ, Morris A, Brown RD, Lo JC, Burke M, et al. Fetuin B Is a Secreted Hepatocyte Factor Linking Steatosis to Impaired Glucose Metabolism. Cell Metab. 2015;22:1078-89.
[30] Zhu J, Wan X, Wang Y, Zhu K, Wan X, Li C, et al. Serum fetuin B level increased in subjects of nonalcoholic fatty liver disease: A case-control study. Endocrine. 2017;56:208-11.
[31] Li Z, Lin M, Liu C, Wang D, Shi X, Chen Z, et al. Fetuin-b links nonalcoholic fatty liver disease to type 2 diabetes via inducing insulin-resistance: Association and path analyses. Cytokine. 2018;108:145-50.
[32] Jung SH, Won KJ, Lee KP, Kim HJ, Seo EH, Lee HM, et al. The serum protein fetuin-B is involved in the development of acute myocardial infarction. Clin Sci (Lond). 2015;129:27-38.
[33] Floehr J, Dietzel E, Neulen J, Rosing B, Weissenborn U, Jahnen-Dechent W. Association of high fetuin-B concentrations in serum with fertilization rate in IVF: A cross-sectional pilot study. Hum Reprod. 2016;31:630-37.
[34] Dabrowska AM, Tarach JS, Wojtysiak-Duma B, Duma D. Fetuin-A (AHSG) and its usefulness in clinical practice. Review of the literature. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015;159:352-59.
[35] Murakami T, Walczak R, Caron S, Duhem C, Vidal V, Darteil R, et al. The farnesoid X-receptors induces fetuin-b gene expression in human hepatocytes. Biochem J. 2007;407:461-69.
[36] Walter RE, Wilk JB, Larson MG, Vasan RS, Keaney JF Jr, Lipinska I, et al. Systemic inflammation and COPD: The Framingham Heart Study. Chest. 2008;133:19-25.

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