Microalbuminuria and Hypoxemia in Patients with COPD

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Abstract

Background: Microalbuminuria (MAB), a marker of endovascular dysfunction, is a predictor of cardiovascular events and all-cause mortality in the general population. There is evidence of vascular dysfunction in patients with chronic obstructive pulmonary disease (COPD).

Objectives: (i) To evaluate the practical role of microalbuminuria in patients with COPD. (ii) To establish relationship between degree of Microalbuminuria and Hypoxemia in COPD. (iii) To study the frequency of microalbuminuria in stable COPD patients.

Study design: The study was prospective, conducted jointly in the Department of Internal medicine and Department of Immunology and Molecular Medicine at Sher-e-kashmir institute of medical sciences, srinagar(India), over a period of two years. Stable COPD patients without any comorbidity, with wide range of airflow obstruction were taken in study. Age matched smokers of more than 10 pack-years without airflow obstruction served as controls.

Methods: We measured spot urinary albumin, smoking history, arterial blood pressure, body mass index, lung function, kidney function tests and BODE index in 97 patients with stable COPD and 94 age matched smokers with normal spirometry without known cardiovascular disease. MAB levels were compared between groups. A multivariate analysis was performed to determine the best determinants of MAB levels.

Results: Microalbuminuria was more frequent in COPD patients compared to smokers without obstruction (20.6% versus 7.4% respectively); p=0.007. There was an inverse association of the PO2 with MAB (r=-0.35, p<0.0001). Multivariate analysis with MAB as the dependent variable showed that PO2 (odds ratio: 1.003; 95%CI, 0.767-0.974; p=0.021) was only independent and significant predictor of MAB.

Conclusions: MAB is frequent in patients with COPD and is associated with hypoxemia independent of other cardiovascular risk factors.

Keywords: Microalbuminuria; Hypoxemia; COPD

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a disease state characterized by airflow limitation that is not fully reversible. It is a preventable and treatable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patient. The airflow limitation is usually progressive and associated with abnormal inflammatory response of lung to noxious particles or gases [1].

COPD is the fourth leading, but under recognized cause of morbidity and mortality worldwide [2], and affects >10 million persons in the United States. Worldwide COPD was the sixth leading cause of death in 1990, and presently is the fifth [3]. The global burden of disease study projects that, by 2020, COPD will become the third leading cause of death worldwide [4].

COPD is associated with an abnormal inflammatory response in the lungs, with important extra pulmonary manifestations and with the presence of multiple co-morbidities. Several cytokines and inflammatory mediators have been implicated, but the link between COPD and its systemic expressions are not well understood [5,6]. Cardiovascular disease is a major cause of mortality in COPD, particularly in patients with mild to moderate severity [7-9]. The likelihood of identifying cardiovascular subclinical abnormalities in patients with COPD during daily clinical practice strongly depends on the diagnostic techniques used. The widespread use of sensitive diagnostic tests, such as ultrasound scan and a new generation of computed tomography (CT) scans are good options [10,11].

Recent studies have shown an association between lower FEV1 and emphysema severity with arterial stiffness [12,13] and endothelial dysfunction [14]. Also, vascular alterations, measured from the cross-sectional area of small pulmonary vessels by CT scan, correlate with the magnitude of pulmonary hypertension [15]. However, because of the high prevalence of COPD, these tools may not always be practical in the general population for both logistic and economical reasons.

The discovery of novel biomarkers to help identify cardiovascular risk in patients with COPD could help individualize therapy for that particular phenotype. Ideally, the biomarker should be inexpensive, noninvasive, and easily assessable. C-reactive protein (CRP) has been proposed as one such biomarker and increased serum levels of CRP have been related to increased cardiovascular mortality in mild to moderate COPD [16]. However, this finding was described only in epidemiological cohorts and was not replicated in patients with more severe disease [17]. In addition, CRP appears not to provide additional prognostic information beyond traditional risk factors in the general population [18].

Microalbuminuria (MAB) is a sensitive marker of cardiovascular risk [19,20]. The presence of MAB is consistently associated with arterial stiffness assessed by pulse wave velocity and worse cardiovascular outcomes in patients with diabetes and hypertension, but most

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Microalbuminuria is defined as urinary excretion of albumin that is persistently increased above normal although below the sensitivity of conventional semi-quantitative test strips. Microalbuminuria is a sign of glomerular dysfunction in general and a sign of tubulointerstitial to a lesser extent. Microalbuminuria can be diagnosed from a 24-hour urine collection (between 30 to <300 mg/24 hours) more commonly, from elevated concentrations in a spot sample (20 to <200 mg/L). For timed overnight urine collections an albumin excretion rate greater than 20 microgram/min is considered to be diagnostic of microalbuminuria [24]. An at excretion rate of about 200 microgram/min, conventional semi-quantitative strips give a positive result for albuminuria; this defines the upper limit for microalbuminuria. An early morning urine sample with an albumin: creatinine ratio greater than 3.0 reliably predicts an overnight excretion rate greater than 30 microgram/min, making it a useful screening method [28,29]. This negates the need for precisely timed and conducted urine collections, which are necessary only to confirm positive screening values.

The prevalence of microalbuminuria in the general population is 2.2% in white adults aged 20-65 [30] and 13-20% in those aged 60-74 [31] and in Mexican Americans aged 25-64 [32]. It also predicts death from cardiovascular disease in elderly patients [33] and perhaps also coronary and peripheral vascular disease in the general population [34]. Two broad explanations have been proposed to account for this: microalbuminuria is associated with an excess of known and potential cardiovascular risk factors and is a marker of established cardiovascular disease.

Hypoxia causes both systemic and pulmonary arteries to constrict [35] but the mechanism involved has not been elucidated as yet. However, based on their location, endothelial cells of the vascular pulmonary bed are thought to play a key role. In keeping with this hypothesis, changes in blood oxygen tension induce endothelial cells to release a number of different vasoactive agents including endothelin-1 [36], platelet derived growth factor [37] nitric oxide [38] and other unknown substances [39] which can modify the contractile and proliferative state of the underlying smooth muscle cells.

MAB is frequent in patients with COPD and was associated with hypoxemia independent of other cardiovascular risk factors [40]. There are few studies that have reported a relationship between hypoxia and microalbuminuria [40,41]. Studies at high altitude suggest that systemic hypoxia may cause an elevation in the urinary albumin excretion with increasing the renal capillary permeability despite unchanged tubular functions [42] and albuminuria is strongly related with the degree of hypoxia [43].

In vitro studies have also shown that cultured endothelial cells exposed to low oxygen concentrations become larger, and small intercellular gaps appear. These phenomena are also reversible [44,45]. Temporary proteinuria has been reported in patients with sleep apnea syndrome which regressed with oxygen therapy and the protein leakage was attributed to the increase in glomerular filtration rate resulting from tissue hypoxia [46].

A limited number of studies have evaluated the presence of MAB in patients with COPD, mostly during exacerbations [47]. In those studies, the influence of other cardiovascular risk factors was not considered. Patients with co-morbidities (hypertension, diabetes) were not excluded [48]. We hypothesized that MAB is elevated in patients with COPD independently of other cardiovascular risk factors. To test this hypothesis, we determined the prevalence of MAB in a group of patients with stable COPD without any co-morbidities and evaluated the relationship of MAB levels with clinical and physiological descriptors of COPD severity and cardiovascular risk factors. A group of smoking subjects without COPD served as control subjects.

**Patients and Methods**

**Study group**

Outpatients with COPD and wide range of airflow obstruction were taken in study. COPD was defined by post bronchodiurator FEV1/FVC<0.7. Patients included in study were stable for 6 weeks and had received optimal therapy according to guidelines.

**Controls**

Persons included in controls were above 40 yrs of age, smokers of more than 10 pack-years without airflow obstruction (FEV1/FVC>0.7).

**Exclusion criteria**

I. History of renal disease or presence of macroalbuminuria (spot urinary albumin >200 mg/L).

II. Cardiovascular disease (IHD, CCF).

III. Diabetes

IV. Uncontrolled co-morbidities such as malignancy, asthma or confounding diseases.

**Study design and conduct**

Cases were taken from COPD clinic and controls included age matched smokers accompanying patients in outpatient departments and inpatient departments. An informed consent was taken from the participants found fit for study.

After taking proper history regarding hypertension, diabetes, fever, dysuria, any other co morbid illness, smoking history (pack years), drug history and time since last exacerbation (weeks), all the baseline parameters (like pulse, B.P, height, weight, BMI) were measured.

Predesigned questionnaire regarding smoking in locally understandable language was tested. The questionnaire was administered through a personal interview with specific enquiry about history of smoking, current smoking status, pack years of smoking, amount of smoking, specific history about the pattern (hookah, cigarettes, mixed), average amount of tobacco smoked, water change habit, etc. Pack years of smoking in participants who were hukkah smokers was calculated by using the formula; 1 chattakh of tobacco (ie 60 gram tobacco)=30 cigarettes (each cigarette of 1 gram) [49].

Baseline laboratory tests (like CBC, kidney function tests, liver function tests, blood glucose(fasting), lipid profile, urine microscopy, 24 hr urinary proteins) were checked before subjecting the participants for six minute walk test and spirometry. Participants having hypertension, diabetes, renal disease, cardiovascular diseases, and any other confounding comorbidity (fever, malignancy, urinary tract infections) were excluded from study.

Also the participants having leucocytosis, deranged kidney function tests and abnormal urine microscopy were excluded from study.

Glomerular filtration rate (GFR) was estimated using the validated...
Modification of Diet in Renal Disease (MDRD) equation [50].

The 6MWD was measured in COPD participants. Pre test oxygen saturation (by pulse oximeter), total distance walked in six minutes and post test oxygen saturation was taken [51].

Arterial blood gases by arterial puncture, sitting at rest for fifteen minutes were taken.

**Spirometry**

The spirometer used during the study was ndd Easyone spirometer made by ndd Medezink Switzerland. The spirometer fulfilled ATS and ERS criteria for accuracy and precision. The spirometry testing was performed according to guidelines. Before doing spirometry testing, spirometry Questionnaire was administered and spirometry was not performed on participants who had any of the contraindications for performing spirometry [52]. COPD was classified as per GOLD guidelines [1].

**Urinary albumin**

Urinary albumin was measured using commercially available quantitative microalbumin kits from AXIS – SHIELD Poc AS, Norway. The kits are available as Nycocard – U = albumin and are measured on Nycocard reader. The patients who passed our inclusion criteria were asked to give spot urine samples. 10 ml of urine sample was collected in clean polypropylene screw capped vial from each participant and stored at -20°C until samples were processed. The sensitivity of this test is between 5-200 mg/L.

MAB was defined from elevated concentrations of albumin in a spot sample (20 to <200 mg/L) [29]. The study was approved by the Institute Postgraduate and Ethics committee and informed consent was obtained for all participants in the study. Results have been expressed as mean ± SD, and a p-value of <0.05 was considered significant.

**Statistical Analysis**

Statistical Package for Social Sciences (SPSS) ver. 19 from IBM-SPSS was used for data analysis. The results are expressed as proportions or mean ± SD, as specified. Pearson’s Chi-square method was used for comparing proportions and percentages whereas Student’s t-test was used for comparison of continuous variables. Where the data was not uniformly distributed, a non-parametric test like Mann-Whitney U test was used. ANOVA was be used wherever needed. Multivariate logistic regression was performed using MAB as the dependent variable. A two-tailed P value was used for calculating statistical significance; a value of <0.05 was be taken as significant.

**Results and Observations**

The total number of individuals in the study was 191 among which 97 were cases and 94 were controls. Out of 97 cases i.e. COPD patients 28(27.1%) were females and in 94 controls i.e. smokers without obstruction 13(12.2%) were females and this difference was statistically significant; P=0.009.

Patients with COPD were slightly older (60.90 ± 6.99 versus 59.70 ± 7.43) than control subjects but was statistically insignificant; p=0.117, and had longer pack years of cigarette exposure than controls (31.86 ± 14.3 versus 17.99 ± 4.94) and was statistically significant; p=<0.0001.

GFR values were normal (≥60 ml/min/1.73 m²) in all study participants and had no significant relation to degree of microalbuminuria.

Patients with COPD had significantly higher levels of microalbuminuria than control subjects (median 20.55 ± 30.63 versus 11.37 ± 17.46 mg/l); p=0.012 (Table 1).

COPD patients having microalbuminuria (MAB) were significantly higher in mean age (61.4 ± 6.56 versus 60.06 ± 6.9) compared to COPD patients without microalbuminuria (MAB), P=0.021. Out of 20 COPD patients having microalbuminuria 7 (35%) were females and 13(65%) were males and the difference was not statistically significant; p=0.033.

COPD patients with MAB were more hypoxic (mean pO₂=63.45 ± 7.14) and more hypercapnic (mean pCO₂=46.1 ± 6.11) compared to COPD patients without MAB (mean pO₂=72.4 ± 11.21 and mean pCO₂=42.72 ± 6.53) and was statistically significant. Patients with COPD having MAB had low FEV1%, FEV1/FVC and higher Bode index compared to COPD patients without MAB and was statistically significant (Table 2).

To evaluate the impact of age on MAB, we performed an analysis by grouping the cases and controls into two age groups (45-65 years and ≥65 years). There were 62 COPD patients between age group of 45-65 years and 35 COPD patients were ≥65 years, while 59 controls were in age group of 45-65 years and 35 controls were in age group of ≥65 years.

Most of the COPD patients having MAB were in age group of ≥65 years (13 out of 20) and was statistically significant=0.003, and also most of the control subjects having MAB were in age group of ≥65 years (5 out of 7): p=0.052 (insignificant) (Table 3).

Microalbuminuria was significantly more in COPD patients having P02 below 70 mm Hg as compared to COPD patients having P02 above 70 mm Hg,(90% versus 10.0%):p<0.0001 (Table 4).

Patients of COPD having MAB were more hypercapnic compared to COPD patients without MAB. Out of 20 COPD patients with microalbuminuria, 11 (55%) had pCO₂ >45 mm Hg and 9 (45%) had pCO₂ below 45 mm Hg and this difference remained statistically insignificant; p=0.086 (Table 5).

Majority of COPD patients with microalbuminuria (MAB) had GOLD stage of III and stage IV(33.3%), while COPD patients without microalbuminuria had GOLD stage of I and II (95.3%) and this difference was statistically significant; p=0.001 (Table 6).

COPD patients with microalbuminuria had significantly lower levels of FEV1. Out of 20 COPD patients with MAB 17 (85%) had FEV1 below 50 and 3 (15%) had FEV1 above 50 and was statistically significant; p=0.003 (Table 7).

Out of 20 COPD patients with MAB, 95% had BODE index of >3 and was statistically significant; p=0.016 (Table 8). Majority of COPD patients with microalbuminuria(MAB) had MMRC dyspnea grade III-IV (32.5%), while most of COPD patients without MAB had MMRC dyspnea grade I (100.0%) f/by grade II (86.3%), indicating that COPD patients with MAB were more dyspneic/hypoxic compared to controls and was statistically significant; p=0.039 (Table 9).

COPD patients with MAB had significant drop in oxygen saturation after performing 6 minute walk test compared to COPD patients without MAB and this difference remained statistically significant; p=0.008 (Table 10). Multivariate analysis with MAB as the dependent variable Showed that P02 (odds ratio: 1.003; 95%CI, 0.767-0.974; p=0.021) was only independent and significant predictor of MAB (Table 11).

There was an inverse association of the PO2 and MAB in patients
**MAB.** To evaluate the impact of age on MAB, we performed an analysis by grouping the cases and controls into two age groups (45-65 years and ≥65 years). Most of the MAB positive cases and controls were in the age group of ≥65 years. MAB was significantly associated with higher pack years of smoking exposure (p<0.0001) possibly because of endothelial cells could be directly affected by the cigarette smoke products and this

### Table 4: Relationship of PO2 and Microalbuminuria (MAB) In COPD Patients.

| PO2 (mm Hg) | Total | P value |
|-------------|-------|---------|
| <70         | 52    | 45      | 97       |
| >70         |       |         | <0.0001  |

### Table 5: Relationship of PCO2 and Microalbuminuria (MAB) in COPD Patients.

| PCO2 (mm Hg) | Total | P value |
|--------------|-------|---------|
| <45          | 59    | 38      | 97       |
| >45          |       |         | 0.086    |

### Table 6: Urinary albumin (MAB) and Stage of COPD.

| FEV1 (%) | Total | P value |
|----------|-------|---------|
| <50      | 55    | 42      | 97       |
| >50      |       |         | 0.003    |

### Table 7: Relationship between BODE index and Microalbuminuria (MAB) in COPD patients.

| BODE Index | Total | P value |
|------------|-------|---------|
| <3         | 24    | 73      | 97       |
| ≥3         |       |         | 0.016    |

### Table 8: Relationship Between Microalbuminuria (MAB) and Dyspnea Grades (MMRC) (0-4) in COPD patients.

| Dyspnea grades | Total | P value |
|----------------|-------|---------|
| I              | 6     | 0       | 6        |
| II             | 44    | 7       | 51       |
| III-IV         | 27    | 13      | 40       |
| Total          | 77    | 20      | 97       |

### Table 9: Relationship Between Microalbuminuria (MAB) and Dyspnea Grades (MMRC) (0-4) in COPD patients.

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with COPD ($r=-0.35, p<0.0001$) (Figure 1).

**Discussion**

To our knowledge there are very few studies, reporting a higher prevalence of MAB in stable COPD patients compared with age matched control subjects who smoked >10 pack years and both groups had no comorbidities like cardiovascular, diabetes, hypertension, malignancy or renal disease. The study was carried out with the purpose to find the prevalence and relationship of MAB with clinical and physiological parameters in stable patients with COPD.

Our study included total 191 subjects, out of which 97 subjects were stable COPD patients without any comorbidity and 94 healthy subjects (age matched) with smoking exposure >10 pack years served as controls. Out of 97 COPD patients (cases) 28 (27.1%) were females and 69.9% were males and in 94 controls 13 (12.2%) were females and 81 (76.1%) were males.

Of the studied subjects, mean age in cases was 60.9 ± 6.9 yrs and mean age in controls was 59.7 ± 7.4 yrs. COPD patients with MAB had significantly higher mean age compared to COPD patients without MAB +ve cases (n=20) 94.6 ± 3.16 82.1 ± 19.9 0.008

MAB -ve cases (n=77) 95.1 ± 3.17 93.1 ± 3.73 0.083

**Table 10:** Six minute walk test in COPD Patients with and without microalbuminuria.

| Odds ratio | 95% C.I | P value |
|------------|--------|---------|
| $PO_2$ 1.033 | 0.767-0.974 | 0.021 (significant) |

**Table 11:** Relative risk of presence of microalbuminuria in patients with COPD using multivariate logistic regression including Age, $PO_2$, $PCO_2$, BODE Index, Pack years Stage of COPD and Dyspnea Grade.

GFR values were normal (≥60 ml/min/1.73 m²) in all study participants and had no significant relation to degree of microalbuminuria. In patients with COPD, the GFR had no relation to the degree of MAB. No subject in our study (cases and controls) was on ACE inhibitors/ARBs/statins.

COPD patients with microalbuminuria had significantly lower levels of FEV1. Out of 20 COPD patients with MAB 17 (85%) had FEV1 below 50% and 3 (15%) had FEV1 above 50% and was statistically significant; $p=0.0003$. Majority of COPD patients with microalbuminuria had GOLD stage of III and IV (33.3%), while COPD patients without microalbuminuria had GOLD stage of I and II (95.3%) and this difference was statistically significant; $p<0.0001$. The possible explanation for this is that impaired lung function, including COPD, has been associated with increased systemic arterial stiffness. An increase in arterial stiffness should result in increased kinetic energy transmission to the distal microcirculation, and hence may result in microvascular damage. Given the association between abnormal lung function and arterial stiffness [53].

Majority of COPD patients with microalbuminuria (MAB) had MMRC dyspnea grade III and IV (32.5%), while most of COPD patients without MAB had MMRC dyspnea grade I(100.0%) f/by grade II (86.3%) and was statistically significant; $p=0.006$, likely because of more endothelial damage due to hypoxia in patients having dyspnea grade of III and IV leading to microalbuminuria.

COPD patients with MAB were more hypoxic (mean $pO_2$=63.45 ± 7.14); $p<0.0001$ and more hypercapnic (mean $pCO_2$=46.1 ± 6.11); $p=0.040$, compared to COPD patients without MAB (mean $pO_2$=72.4 ± 11.21 and mean $pCO_2$=42.72 ± 6.53) and was statistically significant. Multivariate analysis with MAB as the dependent variable to study the effect of various factors like age, $PO_2$, $PCO_2$, BODE index, pack-years and COPD Stage showed that $PO_2$ was the only independent and significant predictor of MAB (Odds ratio,1.003; 95%CI, 0.767-0.974;p=0.021). $PO_2$ was the most important predictive factor and showed a negative association with MAB ($r=-0.35, p<0.0001$).

Most of the patients with MAB had hypoxemia and was observed in patients with $PO_2$ levels less than 70 mm Hg. Nevertheless, some patients with hypoxemia did not show MAB, suggesting that other factors, such as genetic susceptibility to oxidative stress, may play an important role [54]. The development of anorexia in patients with COPD and in normal subjects exposed to high altitude support the importance of hypoxia on metabolic regulatory mechanisms through hypoxia inducible factor 1 [55]. An equally likely explanation is that endothelial dysfunction may be directly implicated in the pathogenesis of COPD. Endothelial cells could be directly affected by the cigarette smoke products and this might be associated with reactivity to hypoxic stimulus, thereby altering ventilation perfusion matching (VA/Q) and resulting in hypoxemia [56].

In fact, as suggested by the work of Rodriguez-Roisin et al. [57], this vascular dysfunction could explain the substantial increase of A-aPO2 due to VA/Q mismatch in GOLD stage I and the modest increase of VA/Q in more severe stages of the disease. In addition, hypoxemia could result in up-regulation of vascular endothelial growth factor, reduced expression of endothelial nitric oxide synthase, and increase in oxidative stress, which in turn might amplify and perpetuate the dysfunction. The cross-sectional nature of this study does not allow us to establish a causal relationship between hypoxemia and MAB.

Results of numerous studies demonstrate the value of MAB as a...
clinically relevant tool for the identification of generalized endothelial dysfunction and detection of patients at risk for the development of end-organ damage and cardiovascular disease [16-18,23]. Therefore, MAB might help in the identification of a subgroup of patients with COPD at increased cardiovascular risk and potential adverse prognosis. In fact, previous studies have shown the predictive value of MAB in the development of acute respiratory failure and multiple organ failure in ICU patients [58].

Interestingly we observed that COPD patients with MAB had significant drop in oxygen saturation after performing six minute walk test compared to COPD patients without MAB and this difference remained statistically significant; p=0.008, possibly due to more hypoxia in these patients compared to COPD patients without MAB.

Conclusion

Patients with COPD had longer pack year of cigarette exposure than controls and had significantly higher levels of MAB than smokers without obstruction (controls). There was no significant difference of microalbuminuria among COPD males and females.

COPD patients with MAB were more hypoxic and more hypercapnic compared to COPD patients without MAB. PO2 was most important predictor and showed a negative association with MAB. Microalbuminuria was significantly more in COPD patients having PO2 below 70 mm Hg as compared to COPD patients having PO2 above 70 mm Hg. COPD patients with microalbuminuria had significantly lower levels of FEV1. Majority of the COPD patients with microalbuminuria were of GOLD stage III and IV and had MMRC dyspnea grade III and IV. COPD patients with MAB had significant drop in oxygen saturation after performing 6 minute walk test compared to COPD patients without MAB.

To conclude, the determination of MAB is simple, inexpensive, and noninvasive. As such, it could be a promising biomarker to identify COPD patients at increased cardiovascular risk. Longitudinal studies in different settings with larger populations are needed to evaluate the practical role of MAB in patients with COPD.

Limitations of Study

1. The less number of women included in study made impossible any evaluation of possible sex differences in MAB. The limited number of women was not by design, because we offered the opportunity to join the study independent of sex and also possibly due to less prevalence of female smokers in this region.
2. We had no follow up MAB levels in COPD patients to look for stability of MAB levels over a period of time.
3. In this study we did not compare the MAB with other biomarkers, such as CRP. However, CRP is not a biomarker that has proven useful in COPD and therefore is not a comparator gold standard.

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