Original Article

Spirometry and Diffusion Studies in Patients with Type-2 Diabetes Mellitus and Their Association with Microvascular Complications

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ABSTRACT

Background. Diabetes is a systemic disease with-well known complications involving eyes, kidneys and nerves. The presence of an extensive pulmonary microvascular circulation and abundant connective tissue raises the possibility that lung may also be a target organ in diabetes.

Methods. A total of 45 subjects were included in the study. All patients were evaluated for diabetic microangiopathies: nephropathy (by 24-hour protein excretion), retinopathy (by direct ophthalmoscopy) and neuropathy (by clinical examination). The patients were divided into following three groups: *Group A:* patients with type-2 diabetes mellitus (DM) with evidence of microangiopathy (n=15); *Group B:* patients with type-2 DM without any evidence of microangiopathy (n=15); *Group C:* non-diabetic subjects (n=15) as controls. Glycosylated haemoglobin (HbA1C) was measured as an indicator of glycemic control. Spirometry and single-breath diffusion capacity for carbon-monoxide (DLCO) were performed on all patients using Elite Series Body Plethysmograph machine.

Results. A significant reduction of diffusion capacity corrected for alveolar volume (%DL/VA) was observed in group A ($p<0.001$), as compared to the other groups. There were no differences among the three groups for other pulmonary functions. There was a significant correlation between DL/VA percent predicted and albuminuria ($r= -0.975$, $p<0.001$), and DL/VA percent predicted and the retinopathy ($r = -0.550$, $p< 0.05$).

Conclusion. This study shows a mild reduction in diffusing capacity in patients with type-2 DM with microangiopathy.
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Key words: Spirometry, Diffusion, Diabetes, Microangiopathy.

INTRODUCTION

Diabetes mellitus (DM) is a significant public health problem worldwide.[1] It is associated with widespread hormonal, metabolic, and microvascular abnormalities, as well as with disturbances of the function of many organic systems. The macro-angiopathic and microangiopathic complications affect eyes, kidneys, nerves, cardiovascular system and respiratory system. The development of these complications may be related to biochemical alterations in connective tissue constituents, particularly collagen and elastin, as well as microangiopathy due to a non-enzymatic glycosylation of proteins induced by chronic hyperglycemia.[2]

Due to an alarming increase in the incidence and prevalence of DM particularly in Asian Indians, it would be important to study pulmonary functions in patients having DM and examine their correlation with microangiopathic complications. Several clinical studies[2-6] have suggested a possible association between pulmonary function abnormalities and diabetic renal microangiopathy and retinopathy. Changes in pulmonary diffusing capacity for carbon monoxide (DLCO) as a manifestation of pulmonary microangiopathy have also been reported.[7]

The aim of the present study was to investigate pulmonary function in patients with type-2 DM and examine its association with microangiopathy.

MATERIAL AND METHODS

Consecutive patients with type-2 DM between ages 31 to 60 years were included from the out-patient clinics of the Department of Medicine, Government Medical College, Nagpur. After investigating for the presence of microvascular complications, the patients were divided into three groups: *Group A:* patients

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with evidence of microangiopathy (n=15); *Group B:* patients without any evidence of microangiopathy (n=15); and *Group C:* control group of non-diabetic subjects (n=15). Diabetes mellitus was defined as per American Diabetes Association criteria.[8]

Patients with macrovascular diabetic complications, such as ischemic heart disease with previous myocardial infarction or typical anginal chest pain along with any significant electrocardiographic changes were excluded from the study. Other exclusion criteria included any history of stroke or transient ischemic attacks, intermittent claudication or non-healing foot ulcers and/or absence of ≥ 2 foot pulses (taken as indicative of peripheral vascular disease). Patients with chronic obstructive pulmonary disease, bronchial asthma, history of pulmonary tuberculosis or evidence of any other lung disease as determined by chest radiographs and, smokers with regular smoking of one year or more were also excluded.

The presence of diabetic glomerulopathy was determined by measuring the 24-hour protein excretion rate by a semi-automated analyser. An albumin excretion rate between 30-300mg per day was considered as indicative of microalbuminuria. The presence of retinopathy was determined by using direct ophthalmoscopy, carried out by the same ophthalmologist in all the patients. The disease was graded according to the diabetic retinopathy disease severity scale as recommended by the American Academy of Ophthalmology.

Biochemical investigations included blood sugar (fasting and post meal), lipid profile and glycosylated haemoglobin(HbA1C). Spirometry was performed and adequate spirograms as per the American Thoracic Society (ATS) guidelines[9] were obtained. The following parameters were measured: forced expiratory volume in one second, forced vital capacity, peak expiratory flow rate, maximum voluntary ventilation and forced expiratory flow at 25%-75% of vital capacity. The DLCO was performed on Elite Series Body Plethysmograph (Make: Medical Graphics Corporation, USA; Software- Breeze Suite Version 6.3). The DLCO was measured using single breath technique based on the joint statement of ATS and the European Respiratory Society.[10] Acceptable test criteria included: (1) inspiratory vital capacity of >85% of largest vital capacity in <4s, (2) a stable calculated breath hold for 10±2s with no evidence of leaks, or Valsalva or Mueller manoeuvres, (3) expiration in <4s (and sample collection time <3s), with appropriate clearance of dead space gas and proper sampling/analysis of the alveolar gas. There were at least two acceptable tests that met the repeatability requirements of either being within 3mL CO mL/min/mmHg (or 1 mmoL/min/kPa) of each other or within 10% of the highest value. The average of at least two acceptable tests that met this repeatability requirement was reported. Maximum five tests were attempted. The DLCO corrected for alveolar volume (DLCO/VA) was obtained.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare mean values in the three groups. In case of overall statistical significance in ANOVA, Scheffe's post-hoc test was used to compare pair-wise means. Pearson's correlation coefficient was used to quantify the extent of relationship between % DLCO/VA and age, duration of diabetes, HbA1c and micro-albuminuria. The correlation between DLCO and grade of retinopathy was made with Spearman's rank sum test. All the statistical tests used for analysis were two-tailed (SPSS software version 8.0). A p value of <0.05 was considered as statistically significant.

RESULTS

Of the total of 45 patients, 28 (62.2%) were males and 17 (37.8%) were females. The mean age was 45.6±6.7, 43.6±8.3 and 45.4±2.6 years in three groups, respectively. There were no significant differences in age, sex and anthropometric measurements across the three groups (Table 1). The duration of diabetes was 8.4±4.8 years in group A and 2.5±1.9 years in group B. The duration was calculated from the time of first diagnosis. The difference was significant ($p<0.001$). The level of microalbuminuria was 136.9±72.6 mg/dL in group A and 15±10.1 mg/dL in group B. The degree of retinopathy varied across group A: one patient had grade 4, two had grade 3, two had grade 2, five had grade 1 while five had no evidence of retinopathy.

Table 1. Demographic and anthropometric profile of study group

Parameters	Group A (n=15)	Group B (n=15)	Group C (n=15)
Age (years)	45.6±6.7	43.6±8.3	45.4±12.6
Gender: Male/ Female	9/6	8/7	11/4
Weight (Kg)	47.86±12	52.03±9.20	46.63±8.91
Height (cm)	148.95±11.51	150.54±11	145.11±11.32
BMI (Kg/m^2)	21.23±2.39	22.82±2.26	21.93±1.96

* Data are presented as mean±SD; BMI=Body mass index

On comparing the three groups using ANOVA, a significant reduction of DL/VA percent predicted was observed in group A ($p<0.001$), as compared to the other groups (Table 2). There were no differences among the three groups for other pulmonary functions. When the groups were compared in pairs

for DL/VA percent predicted, a significant difference was observed between groups A and B (p=0.001); and groups A and C (p<0.00001); but not between groups B and C (p=0.949) (Table 2).

Table 2. Pulmonary function and DLCO values in the three groups

Variables		Group A	Group B	Group C
FEV_1 (L)	Absolute^{ns}	1.98±0.43	2.11±0.47	2.09±0.41
	% predicted^{ns}	80.2±13.26	83.0 ± 8.0	85.67±8.15
FVC (L)	Absolute^{ns}	2. 3±0.56	2.41±0.42	2.33±0.43
	% predicted^{ns}	80.0±9.34	83.13±7.36	86.6±10.57
$FEF_{25\%-75\%}$ (L/s)	Absolute^{ns}	2.57±0.89	2.87±0.69	2.92±0.53
	% predicted^{ns}	79.4±18.06	87.67±9.37	89.07±8.76
PEFR (L/s)	Absolute^{ns}	5.27±1.80	5.68±1.02	5.96±1.07
	% predicted^{ns}	81.4±18.0	86.6±13.09	89.67±10.16
MVV (L/min)	Absolute^{ns}	81.0±31.12	89.67±13.37	94.6±12.02
	% predicted^{ns}	84.73±153.0	87.13±12.15	90.67±12.86
DLCO unc (mL/min/ mmHg)	Absolute^{ns}	17.19±5.94	19.93±4.81	21.08±3.61
	% predicted^{ns}	72.33±16.0	82.67±14.88	84.53±13.9
DL/VA (mL/min/ mmHg)	Absolute**	5.48±0.88	5.91±0.84	6.05±0.84
	% predicted^{ns}	76.4±10.22	88.22±5.21	90.93±10.51

FVC=Forced vital capacity, FEV_1=Forced expiratory volume in one second, $FEF_{25\%-75\%}$=Forced expiratory flow at 25%-75% of vital capacity, PEFR=Peak expiratory flow rate, MVV=Maximum voluntary ventilation, DLCOunc=Diffusing capacity for carbon monoxide uncorrected for alveolar volume, DL/VA=Diffusing capacity for carbon monoxide corrected for alveolar volume, NS=Not significant, S=Significant

Superscripts on pulmonary function parameters indicate ANOVA results (ns, p>0.05; **=p<0.001); For DLCO/VA group A *vs* B (p<0.001); group A *vs* C (p<0.00001), group B *vs* C (p>0.015).

The mean levels of fasting blood glucose, post-prandial blood glucose, blood urea nitrogen and HbAlc were significantly higher (p<0.01) in group A as compared to the other two groups. Mean serum levels of triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein were statistically comparable among the three groups (Table 3).

Table 3. Biochemical parameters in the three groups

Parameters	Group A	Group B	Group C
FBS* (mg/dL)	172±57.01	157.67±22.64	90.13±5.55
PLBS* (mg/dL)	232.2±85.8	240.47±52.68	111.4±7.24
HbA1c* (%)	8.89±2.5	8.54±1.29	5.99±0.62
TC^{ns} (mg/dL)	192.47±32.93	174.4±13.75	183.93±23.3
TG^{ns} (mg/dL)	138.2±41.69	120.13±19.52	128.33±15.68
HDL-C^{ns} (mg/dL)	42.4±6.30	40.53±5.29	39.87±3.83
LDL-C^{ns} (mg/dL)	128.83±19.3	118.79±14.27	113.73±22.47
BUN* (mg/dL)	24.79±12.22	31.57±7.73	20.97±2.23

Superscripts against parameters indicate ANOVA results (ns=not significant, p>0.05, *=p<0.01), FBS=Fasting blood glucose, PLBS=Post-prandial blood glucose, HbA1c=Glycosylated haemoglobin, TC=Total cholesterol, TG=Triglycerides, BUN=Blood urea nitrogen, HDL-C=High density lipoprotein, LDL-C=Low density lipoprotein

There was a significant correlation between DL/VA percent predicted and the albuminuria (r= -0.975, p<0.001), and DL/VA percent predicted and the retinopathy (Spearman's r = -0.550, p< 0.05) (Table 3). There was no significant correlation between DL/VA percent predicted with age, duration of diabetes or level of HbA1c (Table 4).

Table 4. Correlation of DL/VA percent predicted in group A patients

	r Value	p Value
DLCO/VA and age^{+}	-0.116^{ns}	
DLCO/VA and diabetes duration^{+}	-0.205^{ns}	
DLCO/VA and HbAlc^{+}	-0.287^{ns}	
DLCO/VA and microalbuminuria^{+}	-0.975**	< 0.001 (S)
DLCO/VA and retinopathy*	-0.550***	< 0.05 (S)

+=Pearson's correlation coefficient, *=Spearman's rank sum test, ns=Not significant, p>0.05, S=Significant, **=p<0.001, ***=p<0.05

DISCUSSION

The major finding of this study was a significant reduction in diffusing capacity in type-2 DM patients with microangiopathy as compared to those without microangiopathy and healthy controls. There was a significant correlation of DL/VA percent predicted with microalbuminuria, and with retinopathy.

The findings of this study are in agreement with previous studies.[2-6] An Indian study[4] reported that the mean value of absolute uncorrected DLCO was significantly lower in patients with type-2 DM with microangiopathy as compared to those without microangiopathy and healthy controls. Ljubic *et al*[2] has demonstrated the relationship between pulmonary functions and chronic complications in diabetes with proteinuria as the significant independent predictor of DL/VA, while in another study,[11] only retinopathy was found to be related. Other studies[2-6] have also observed a correlation between diffusing capacity and microalbuminuria. It was also observed that spirometric values did not differ in the diseased and healthy controls. In our study too no significant difference in spirometric values was noted among the three groups. Asanuma *et al*,[11] however, reported a significantly lower forced vital capacity in diabetics along with a decreased diffusing capacity.

In contrast to the above findings, Bulbou *et al*[12] did not find any correlation between reduced diffusion capacity in diabetics with diabetic complications and others[13] have reported no significant difference in diffusing capacity between healthy subjects and diabetics.

Larger population-based studies have been more consistent, demonstrating reduced pulmonary

functions in patients with an elevated plasma glucose level and a diagnosis of DM.[7,14-17]

Complications in the lung, kidneys, and eyes are comparable in frequency and severity due to an identical aetiopathogenic mechanism. Changes in connective tissue, particularly collagen and elastin, as well as microangiopathy due to a non-enzymatic glycosylation of proteins induced by chronic hyperglycemia, are proposed as the main reasons for the development of complications.[18,19]

Other mechanisms postulated to explain a decreased diffusing capacity include modification of surfactant and its actions and an altered affinity of glycosylated haemoglobin to carbon-monoxide.[19,20] Further, there may be an increase in the activity of lysyl oxidase, an enzyme that plays a major role in connective tissue formation and whose activity has been shown to be increased in rats with experimentally-induced diabetes.[21]

It has been suggested that the increased systemic inflammation associated with diabetes[22] may result in pulmonary inflammation,[16] and hence, airway damage.[23] Alternatively, a reduction in antioxidant defenses resulting from increased oxidative activity associated with diabetes[24] may lead to a secondary reduction in the antioxidant defenses of the lung, and hence, increased susceptibility to environmental oxidative insults, resulting in subsequent loss of lung function. Postmortem studies have shown histopathologic changes in the lungs of patients with diabetes, specifically in the connective tissue and small vessels.[25]

A small sample size and non-measurement of TLC are the limitations of the present study. Further, histological studies on pulmonary microvasculature and compliance measurements of the lung would be useful to investigate reasons for reduced DLCO values.

In conclusion, our study has shown that the microalbuminuria and retinopathy in type-2 DM patients was correlated with reduced diffusing capacity.

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