Original Article

Role of Nitric Oxide in the Diuresis and Natriuresis Occurring in Patients with Obstructive Sleep Apnoea Syndrome

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ABSTRACT

Objective. To determine whether nitric oxide (NO) has any role in the diuresis and natriuresis observed in patients with obstructive sleep apnoea syndrome (OSAS).

Methods. We measured 12-hour urine volume in the day and in the night in patients with OSAS (n=20) and determined the concentrations of urinary sodium and nitrate. The frequency of urination in the night was also noted. The measurements were done again after two nights of continuous positive airway pressure (CPAP) therapy and after putting the patients on oral anti-oxidant treatment (vitamin C–100mg BD and vitamin E–400IU BD) for 45 days. Ten healthy normal subjects underwent the same protocol except the CPAP therapy.

Results. In patients with OSAS, the night urine volume and sodium concentration were similar and the nitrate levels were higher compared to those in the day. After CPAP therapy, while the urine volume and sodium concentration decreased, the nitrate level became similar to that in the day. Such effects were not observed after anti-oxidant treatment. The frequency of urination was decreased in both the instances. The effects observed after CPAP therapy were similar to those observed in control subjects with or without anti-oxidant treatment.

Conclusion. Renal NO promotes diuresis and natriuresis in patients with OSAS. **[Indian J Chest Dis Allied Sci 2011;53:11-20]**

Key words: Diuresis, Natriuresis, Nitric oxide, Obstructive sleep apnoea syndrome, Oxidative stress.

INTRODUCTION

Even though obstructive sleep apnoea syndrome (OSAS) has been associated with various cardiovascular disorders, such as arterial hypertension,[1,2] coronary artery disease[3] and cerebrovascular disease,[4] the mechanisms that link it with the latter diseases is under intense investigation for the last several years. Among the various mechanisms that have been postulated, one that has gained substantial attention of late is increased oxidative stress in patients with OSAS.[5-7] This postulation is understandable as there is evidence that there is an increased production of reactive oxygen species (ROS) in these patients possibly because of repetitive nocturnal hypoxia-reoxygenation.[6,7]

Along with ROS, the reactive nitrogen species (RNS) also contribute to oxidative/nitrosative stress and cause tissue damage under certain pathological situations.[8] Together, they may cause inflammation, endothelial dysfunction,[9] hypertension,[1,2] coronary atherosclerosis and heart failure.[10] Peroxynitrite and other RNS are generated by the reaction between superoxide and nitric oxide (NO). Thus, it is reasonable to expect that when there is an increase in ROS, there will be an increase in RNS also. In such a scenario, there will be a decrease in NO, and hence, a compromise in the NO mediated effects in situations in which NO functions as a signalling molecule. Indeed, there are reports that there is a decrease in plasma NO levels in patients with OSAS[11] and it has been suggested as a mechanism for the development of cardiovascular disorders in them.[11]

Nitric oxide has an important role to play in regulating renal function. It affects renal haemodynamics,[12] renin secretion[13] and tubuloglomerular feedback.[14] *In vivo* experiments have demonstrated that NO promotes natriuresis and diuresis due to decreased sodium chloride and fluid reabsorption.[15]

Nocturnal polyuria and natriuresis have been reported in patients with OSAS.[16] The urge to urinate is high among these patients and they complain that it leads to frequent arousals from sleep.[16] The mechanisms for these phenomena are largely

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unknown. For the present study, we hypothesised that the diuresis and natriuresis occurring in patients with OSAS were due to NO generation in the kidney and reduction of oxidative/nitrosative stress by oral intake of anti-oxidants would mitigate these responses. Urinary nitrate/nitrite levels were determined as an index of renal NO production.[17] The results were compared with two nights of CPAP therapy.

MATERIAL AND METHODS

The present study was conducted strictly in accordance with the ethical guidelines for biomedical research on human subjects by Central Ethics Committee on Human Research, Indian Council of Medical Research-2000 and those contained in "Declaration of Helsinky" and was approved by the Ethics Committee of the Institute.

The patients with OSAS included in the present study were the same in whom a sleep study and measurements of oxidative stress parameters, before and after oral intake of anti-oxidants vitamins E and C were performed by us in a previous study.[7] It is indeed a contemporaneous study. The criteria used for the selection of the patients with OSAS, the study plan, venous sample collections for estimation of oxidative stress parameters (lipid peroxidation and reduced glutathione levels), the methodology adopted for their determination, etc had been described in detail by us in our previous publication.[7]

Briefly, the study was completed in 20 patients. Polysomnography (PSG) was carried out in each patient for two consecutive nights after they got accustomed to sleep in the sleep laboratory. Analysis and interpretation of the sleep study was done as per the standard criteria and described previously.[7] As was the case earlier, the same 10 healthy males served as control subjects in this study too.[7] In OSAS patients as well as in controls, urine was collected during the day and night before PSG, after two consecutive nights of CPAP therapy and after treatment with anti-oxidants as described below. Both the groups were then put on anti-oxidants (Vitamin E – 400IU BD and Vitamin C – 100mg BD) for 45 days.

Urine Sample Collection

For Estimation of Urine Volume, Urine Sodium and Creatinine

The patients were given instructions to collect whole of the urine in a measuring jar each time they went to pass urine and note its volume before transferring it into a bottle for delivery to our laboratory. Thus, the cumulative volume collected between 8 AM and 8 PM (over a period of 12 hours) was designated as the day sample and that collected between 8 PM and 8 AM (over a period of 12 hours) was designated as the night sample. Urine samples were collected six times (twice before the sleep study, once, on the 2nd night of CPAP application and once, the next day and twice, after treatment with anti-oxidants for 45 days).

For Estimation of Urinary Nitrate

Urine samples were collected one day prior to the performance of the sleep study (a sample collected at 11.30 AM was designated as the day sample and another sample collected at 6 AM [before the patient got out of bed] was designated as the night sample). The nitrate values determined were taken as the baseline values. Urine samples were collected in a similar way after two nights of CPAP therapy and after anti-oxidant treatment for 45 days.

Blood Sampling

Venous blood samples were collected early in the morning before the patient got out of the bed in ethylene diamine tetra acetic acid (EDTA) vials and plain vials for various biochemical estimations. Plasma was separated for estimation of lipid peroxidation. Whole blood was lysed for estimation of total reduced glutathione. From the blood in plain vials, serum was separated for determining the lipid profile. The collections were done thrice, first before sleep study, second, after two nights of CPAP therapy and third, after anti-oxidant treatment. All the samples collected were stored at -80 °C before subjecting them to various analyses.

Biochemical Estimations

Urine Nitrate

Estimation was done by the method of Grisham *et al*.[18] 100µL of sample was incubated for 30 minutes at 37 °C in the presence of 0.2U/mL of *Aspergillus* nitrate reductase, 50mM 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) buffer, 5µM flavin adenine dinucleotide (FAD) and 0.1mM nicotinamide adenine dinucleotide phosphate (NADPH) in a total volume of 500µL. Following incubation, 55µL of potassium ferricyanide was added to oxidise the unreacted NADPH; 1mL of Greiss reagent was then added and after 10 minutes incubation at room temperature, the absorption was determined at 543nm.

Total Glutathione Assay

It was estimated in venous blood samples collected early in the morning. Total glutathione assay was carried out by the method of Griffith[19] using Ellmans reagent. Whole blood was lysed by the addition of 6% acetic acid and total glutathione was immediately

precipitated by the addition of 10% 5-sulfosalicylic acid. After centrifugation at 4 ºC, the supernatant was kept at -80 ºC. The standard assay mixture contained 700µL of 0.3mM/L NADPH, 100µL of 6mM/L 5, 5′-dithio-bis (2-nitrobenzoic acid), 5µL sample and 95µL sodium EDTA buffer (100mM/L, pH-7.5). All the reagents were made in sodium phosphate (125mm/L)-EDTA(6.3 mM/L) buffer (pH-7.5). To start the reaction, 100µL of glutathione reductase (15U/L) was added and the absorption at 412nm was observed for three minutes.

Lipid Peroxide Assay

It was done in plasma by the method of Yagi[20] in venous blood samples collected early in the morning. Free radicals trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side chain methylene carbon of cell membrane, resulting in transformation of polyunsaturated fatty acids into lipid hydroperoxides. The level of thiobarbituric acid reactive substances (p-TBARS) is an indicator of lipid peroxidation and oxidative stress. This assay was carried out in plasma by precipitation of lipid peroxides in phosphotungstic acid-sulfuric acid system and malondialdehyde levels were determined by reaction with thiobarbituric acid (TBA). The assay mixture contained 200µL of distilled water, 200µL of plasma, 50µL of butylated hydroxyl toluene (11mg/10mL ethanol) and 400µL of orthophosphoric acid (OPA) (1.115 OPA upto 50mL distilled water). To the assay mixture, 50µL of TBA (160mg/10mL of 0.1M sodium hydroxide) was added and incubated in boiling water bath for 45 minutes. The eppendorfs were ice-cooled and color was extracted with 100µL of butanol. After centrifugation at 10000 rpm for 5 minutes, absorption of the supernatant was read at 535nm.

Serum creatinine was estimated using standardised Bayer diagnostic kit.

Serum sodium and potassium were estimated on arterial blood gas analyser.

Creatinine clearance was calculated using the Cockcroft-Gault equation:

Creatinine clearance (mL/min)= (140-age)×body weight (kg)/(72×serum creatinine)

Urine sodium was estimated on flame photometer (Systronics flame photometer 129).

Statistical Analysis

Data were expressed as mean±standard error of mean. Paired t-test with two-tail significance was used to compare the changes in study parameters in the same patient before and after the treatment. Unpaired t-test was used to compare the baseline data in the control subjects and the patients. The tests were considered significant if they yielded p value <0.05.

RESULTS

Of the 8918 patients who attended the out-patient department of Viswanathan Chest Hospital of our Institute, 70 had excessive day-time sleepiness, loud snoring and had a body mass index (BMI) >25kg/m^2. Among them, only 30 agreed to undergo over-night polysomnography (PSG). Since OSAS was not evident in four and one patient was using anti-depressants (which he concealed previously), they were excluded from the study. Follow-up was not possible in four other patients. Thus, there were finally 21 patients suffering from OSAS who stayed for the full length of the study. As there was some doubt about the compliance *vis-à-vis* the consumption of anti-oxidants in the case of one patient, he was not included in the study.

The results presented here are from the data collected from 20 patients with OSAS. Their anthropometric parameters, blood pressure, pulse rate, fasting blood sugar (FBS) and haemoglobin have been reported in our previous study.[7] Their mean age, mean body weight, mean BMI, mean FBS were 44±2.4 years, 93.2±4.2kg, 32.9±1.3kg/m^2 and 98.4±2.1mg/dL, respectively. Any abnormality in the kidney function was ruled out from the normal blood urea (25.6±0.6mg/dL), serum creatinine (0.8±0.1mg/dL) and creatinine clearance (141.0±12.9mL/min) values obtained. Among them, three were hypertensive and were on anti-hypertensive medication. Their blood pressure was under control on medication. Two patients were diabetic and were on oral hypoglycaemic drugs and their FBS was well controlled. The rest of the patients had only OSAS. Six patients had deviated nasal septum and mild to moderate nasal obstruction on computed tomography (CT) of the paranasal sinuses. Any chest disease in all the 20 patients was ruled out by auscultation of the chest, chest radiography, pulmonary function tests, arterial blood gases, total and differential leukocyte counts and if needed CT. Cardiac disease was ruled out by history, auscultation, electrocardiogram and chest radiography. Ten healthy normal subjects not suffering from OSAS were included in the study as controls (C). Their mean age, FBS, creatinine clearance and haemoglobin were 30.7±1.2 years, 90±2.5g/dL, 129±1.6mL/min and 14.8±1.1g/dL, respectively. Anti-oxidant treatment did not change any of these parameters in the controls as well as the OSAS patients.

Sleep Parameters

The details of the changes in PSG and sleep parameters recorded in patients with OSAS were reported in our previous publication.[7] Briefly, there were significant increases in the Epworth sleepiness score, apnoeic episodes and apnoea-hypopnoea index (per hour) in these patients. Anti-oxidant treatment for 45 days produced significant reductions in all of them and the patients reported that they had more refreshing sleep. Their frequency of falling asleep during day time became less and there was an overall improvement in their quality of life.[7]

Oxidative Stress Parameters

The details of the oxidative stress parameters determined in patients with OSAS were reported in our previous publication.[7] Briefly, there was a significant increase in lipid peroxidation products and a significant decrease in reduced glutathione levels in them. The former got decreased and the latter got increased both by CPAP therapy and anti-oxidant treatment.[7]

Urine Parameters

Urination Frequency

In patients with OSAS, the baseline frequency of urination was 2.6±0.3 episodes per night. It became 0.7±0.3 episodes per night after two nights of CPAP therapy and 1.2±0.2 episodes per night after anti-oxidant treatment. The decreases observed were significant (Figure 1). In controls, the baseline frequency of urination was 0.4±0.2 episodes per night and it remained as 0.5±0.2 episodes per night after anti-oxidant treatment (p>0.05).

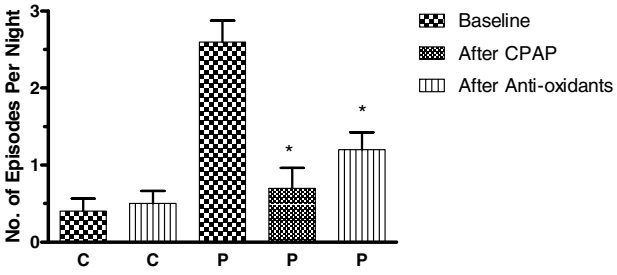

Figure 1. Urination frequency in patients with OSAS (P) and controls (C).

*** p<0.01 Compared to baseline values of P**

Urine Volume

In patients with OSAS, the 12 hour urine volume in the night was similar to that in the day (p>0.05, Figure 2, Table 1). Two nights of CPAP therapy decreased the night urine volume significantly (p<0.05) but had no effect on the day urine volume (Figure 2 Table 1). Anti-oxidant treatment produced a different response. It increased the night urine volume significantly (p<0.001) but produced no change in day urine volume (Figure 2, Table 1).

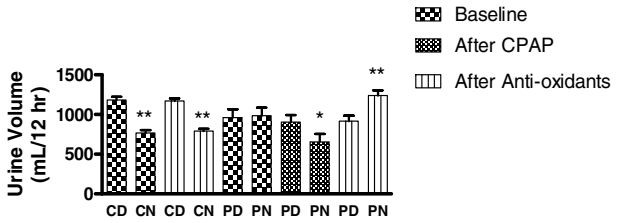

Figure 2. Urine volume in patients with OSAS during day (PD) and night (PN) and in controls during day (CD) and night (CN).

*** p<0.05 PN *versus* PD after CPAP; ** p<0.001 PN *versus* PD and CN *verses* CD after anti-oxidants and, CN *versus* CD baseline**

Table 1. Urine parameters in patients with OSAS

Parameters	OSAS Patients					
	Baseline		After CPAP		After Anti-oxidants	
	Day	Night	Day	Night	Day	Night
Urine volume (mL/12hr)	960.7±104.2	983.3±102.1	904.8±89.8	657.1±96.1*	916.7±70.3	1236±65.3**
Urine nitrate (µmol/L)	1009±44.6	1273±26.8**	998.9±43.2	1020±38.6	1006±41.1	1198±37.3**
Urine creatinine (mg/dL)	40.7±2.4	40.2±1.9	39.9±1.9	39.5±1.7	39.9±2.1	39.2±1.8
Urine sodium (mmol/L/12hr)	168.3±9.3	147.9±5.8	148±3.9	130.5±4.8**	150±4.1	146.7±6.1

* p<0.05, After CPAP (night) *versus* after CPAP (day); ** p<0.001, Baseline (night) *versus* baseline (day); after CPAP (night) *versus* after CPAP (day); after anti-oxidant (night) *versus* after anti-oxidant (day)

In controls, the urine volume in the night was significantly lower compared to that in the day (p<0.001, Figure 2, Table 2). It remained so after anti-oxidant treatment (p<0.001, Figure 2, Table 2).

Table 2. Urine parameters in controls

Parameters	Controls			
	Baseline		After Anti-oxidants	
	Day	Night	Day	Night
Urine volume (mL/12hr)	1180±40.9	765±39.5*	1170±30.9	790±32.3*
Urine nitrate (µmol/L)	788.5±44.4	785.5±43.1	787±43.5	780±42.5
Urine creatinine (mg/dL)	37.6±3.1	37.4±3.1	37.6±3.1	37.6±3.1
Urine sodium (mmol/L/12hr)	158.1±2.80	139.8±2.3*	155.5±1.9	139.5±2.1*

* p<0.001 Baseline (night) *versus* baseline (day); after anti-oxidants (night) *versus* after anti-oxidants (day)

Urine Nitrate

In patients with OSAS, the urine nitrate levels were significantly higher in the night sample as compared to that in the day sample (p<0.001, Figure 3, Table 1). These became similar to that in the day sample after two nights of CPAP therapy (p>0.05, Figure 3, Table 1). Such a reversal was not evident after anti-oxidant treatment. The urine nitrate levels remained significantly higher in the night sample as compared to that in the day sample (p<0.001, Figure 3, Table 1).

In controls, the urine nitrate levels were similar in the day and the night samples (p>0.05, Figure 3, Table 2) and anti-oxidant treatment did not produce any change in them (p>0.05, Figure 3, Table 2).

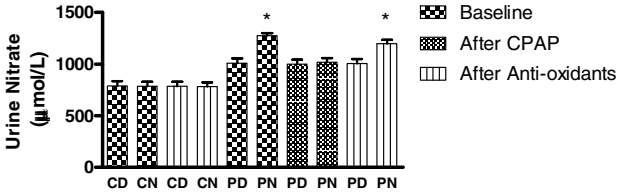

Figure 3. Urine nitrate levels in patients with OSAS during day (PD) and night (PN) and in controls during day (CD) and night (CN).

*** p<0.001 PN *versus* PD at baseline and PN *versus* PD after anti-oxidants**

Urine Sodium

In patients with OSAS, the urine sodium concentrations were similar in the day and the night samples (p>0.05, Figure 4, Table 1). Two nights of CPAP therapy decreased the urine sodium concentration in the night sample significantly (p<0.001, Figure 4, Table 1). Such a reversal was not evident after anti-oxidant treatment. The urine sodium concentrations were once again similar in the day and the night samples (p>0.05, Figure 4, Table 1).

In controls, the urine sodium concentration was significantly lower in the night sample as compared to that in the day sample (p<0.001, Figure 4, Table 2). It remained so after anti-oxidant treatment (p<0.001, Figure 4, Table 2).

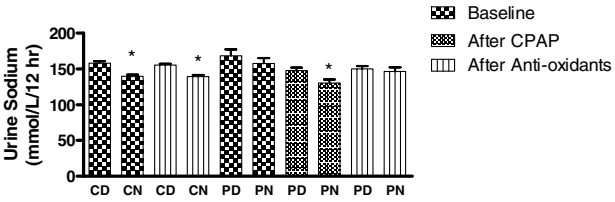

Figure 4. Urinary sodium concentrations in patients with OSAS during day (PD) and night (PN) and in controls during day (CD) and night (CN).

*** p<0.001 CN *versus* CD at baseline, CN *versus* CD after anti-oxidants and PN *versus* PD after CPAP**

Urine Creatinine

In patients with OSAS, the urine creatinine levels were similar in the day and the night samples (p>0.05, Table 1). It remained so after two nights of CPAP therapy (p>0.05, Table 1). After anti-oxidant treatment also, the urine creatinine levels were similar in the day and the night samples (p>0.05, Table 1). A similar observation was noted in the controls (p>0.05, Table 2).

Serum Sodium and Potassium

In patients with OSAS, the serum sodium was 141±1meq/L and the serum potassium was 4±0.1meq/L. These did not change significantly after CPAP therapy or by anti-oxidant treatment.

DISCUSSION

The main findings of the present study are that in patients with OSAS, there occurs diuresis and natriuresis. These responses get corrected by CPAP therapy but persist after oral intake of anti-oxidants. Nitric oxide appears to be the signalling molecule for these responses as diuresis and natriuresis do not manifest when there is attenuation of urine nitrate level.

Nocturia is a term used often when the frequency to urinate is higher than normal. Several investigators have suggested that one event per night is within normal limits and the ratio between day time (8 AM to 8 PM) and night time (8 PM to 8 AM) urination is 2:1 in young adults.[21] In patients with sleep disordered breathing (SDB), two or more awakenings from sleep to urinate per night is considered abnormal.[21] In the elderly, it is known that the frequency of urination is higher in the night due to several causes such as bladder dysfunction, diabetes mellitus, diabetes insipidus, chronic kidney diseases, etc. Even in them, Dahlstrand *et al*[22] reported that their voiding disturbance got corrected with CPAP therapy. This observation prompted them to state that a medical history including snoring habits and potential obstructive apnoeic events should be obtained from patients coming to OPD with complaints of nocturnal voiding.[22]

From their studies on patients with OSAS, Pressman *et al*[23] reported that most of them awoke from sleep to urinate as they felt that their bladders were full. Thus, it appeared that the nocturia was independent of the SDB. However, it needs to be noted that in their study, the urge to urinate was immediately preceded by an obstructive apnoea or a snoring episode. Thus, there is the possibility that the former could have led to the latter.

There was nocturia (two or more awakenings per night for urination) in our patients. Some of them reported that they woke up at night because of difficulty in breathing and then went to pass urine. Nocturia in our patients got corrected with two nights of CPAP therapy. This finding is in agreement with the results from other laboratories.[24, 25] Along with this effect, CPAP therapy corrected the SDB. Hence, it is proposed that SDB may be the cause of nocturia in these patients.

This conclusion is supported by the findings with the anti-oxidant therapy. As observed with CPAP therapy, the number of awakenings to urinate decreased in our patients after treatment with anti-oxidants too. Simultaneously, there was a decrease in the number of apnoeic episodes and an increase in the time spent in stage 3 and stage 4 of sleep.[7] Thus, it appears that it is the SDB that promotes nocturia in these patients. Extending this observation, it is recommended that sleep studies should be performed in young children who snore and wet their bed and the elderly, who exhibit frequent voiding in the night.

Previous studies[24] and our present results have shown that in normal subjects, there is a circadian rhythm of renal function, with significant decreases in urine volume and sodium excretion during the night. Such a rhythm was not evident in our patients with OSAS. In fact, both urine volume and urine sodium concentration of the night sample were similar to those observed during the day time suggesting that in the night, there was either less absorption or more excretion of water and sodium by the kidneys. In our patients, renal function was normal as evidenced by normal blood urea and serum creatinine levels. Three of our patients had hypertension and two others had type 2 diabetes mellitus. The remaining 15 patients did not have any other underlying disease. In all of them, we observed that the diurnal rhythm in urine volume and urine sodium concentration returned after CPAP therapy. This finding is in agreement with previous published reports.[24] However, till date no satisfactory explanation has been provided either for the increase in urine volume/sodium concentration that is observed initially or its correction after CPAP therapy. As discussed below, the present study provides a lead that these may be due to NO produced in the kidney.

There are reports which state that there is a rise in plasma catecholamine concentration and an increase in sympathetic nerve activity in patients with OSAS accounting for the rise in blood pressure observed in them.[26] The CPAP therapy has been reported to reduce cardiovascular disorders by decreasing both these effects.[27] There is a reversal in sympathetic nerve activity even when there is a temporary withdrawal of CPAP therapy,[28] suggesting that it is a fast responding system.

An increase in renal sympathetic nerve activity would promote water and sodium re-absorption through several mechanisms. It causes constriction of the afferent and efferent arteriole, decreases the glomerular filtration rate and thus reduces the filtered load of sodium and water. Through the renin-angiotensin-aldosterone mechanism, it promotes sodium and water re-absorption. Finally, it has a direct stimulatory effect upon the nephron facilitating sodium and water re-asborption.[29] All these actions suggest that in patients with OSAS, there should be retention of water and sodium. These responses would be compounded by the oxidative stress in renal medulla.[30] But, the converse namely, diuresis and natriuresis, was noted in them. It is possible that during sympathetic stimulation, along with catecholamines, there is the liberation of some other signalling molecule which promotes diuresis and natriuresis and we propose that it is NO.

Of late, there has been an increasing interest on the role of NO as a modulator of renal function. NO synthase (NOS) catalyses the generation of NO. All the three isoforms of NOS, namely eNOS, iNOS and nNOS have been identified in the kidney to varying degrees in the cortex as well as the medulla.[8] Nitrergic neurons have been reported to be present in the kidney and are often co-localised with the sympathetic innervations of the kidney.[31]

Ravi *et al*[32] showed that a reflex diuresis occurred following an increase in pulmonary extravascular fluid volume. This response was abolished following sectioning of the renal sympathetic nerves. Later on, it was demonstrated that the diuresis was due to generation of renal NO.[33] There are reports which show that the renal NO generation is mediated by activation of alpha-2 receptors.[34] These studies establish that sympathetic excitation can lead to NO generation by activation of nNOS through alpha-2 receptors.[34] Further evidences in support of these propositions are provided by the observations of Mount and Power[35] who demonstrated that in the kidney, NO was involved in promoting natriuresis and diuresis and Majid and Navar[36] who reported that NO was involved in pressure natriuresis. While evaluating the role of NO, it is emphasised that the NO generated may produce antinatriuresis and antidiuresis also depending upon its site of action and the existing sympathetic drive.[15]

There are just a few studies which have determined NO levels in patients with OSAS. In them, the blood sample was taken for the measurement of NO. Schulz *et al*[37] have shown that plasma nitrate levels are low in patients with OSAS and they get corrected with CPAP therapy. Mary *et al*[11] have also shown that circulating NO is suppressed in patients with OSAS and with one night of CPAP therapy, it becomes equal to that in controls. The low NO levels have been attributed to the diminished eNOS activity in the systemic vasculature.[17] The night-time hypoxia produces oxygen desaturation which may suppress transcription of eNOS gene and the stability of its mRNA.[17] Additionally, NOS inhibitors have been reported to be elevated in patients with OSAS. Finally, NO may be scavenged by the increased ROS production in patients with OSAS.[8]

Unlike the previous investigations, for the present study, instead of blood, we measured urinary nitrate levels for the following reasons: (1) measurement of blood NO does not always reflect the NO status of a particular organ/system. For instance in pregnant women with pre-eclampsia, there is no significant change in blood NO while there is a significant decrease in urinary NO level,[38] (2) exhaled NO but not blood NO has been reported to be a better marker for interstitial lung disease in systemic sclerosis;[39] and (3) NO produced locally in the kidney regulates the blood flow and the tubular functions of the nephron.[15]

Contrary to the fall in blood nitrate levels reported by other investigators,[11] there was an increase in urinary nitrate levels in the night urine sample in patients with OSAS suggesting an increased generation of NO in the kidney. As explained previously, we suggest that it is due to the increased sympathetic nerve activity reported in these patients. It follows then that when the sympathetic nerve activity is reduced by CPAP therapy, there should be a decrease in renal NO generation, and hence, a decrease in urinary nitrate level. Indeed, that was the case in our patients — the nitrate level in the night urine sample was similar to that measured in the day urine sample. It is possible that the NO mediated effects would be hampered by several mechanisms including its scavenging by the free radicals that are produced in patients with OSAS.[8]

We had reported previously[7] that in these patients oral intake of the anti-oxidants, vitamins C and E, reduced the oxidative stress. Anti-oxidant intake improved the quality of sleep by reducing the apnoea/hypopnoea index and by increasing the time spent in sleep stages 3 and 4.[7] But, this treatment did not reduce the urinary nitrate level. It is proposed that in this situation, the anti-oxidants will scavenge the renal superoxide and reduce the formation of peroxynitrite and the subsequent hydroxyl radical and nitrogen dioxide formation.[8] Thus, there will be an increase in the bio-availability of NO. Indeed, after anti-oxidant treatment, there was a significant increase in the night urine volume compared to that during the day. Such an exaggeration was not evident in the urinary sodium concentration and it remained similar to that in the day sample.

The bio-availability of NO is influenced by several factors including dietary NO. However, it is unlikely that dietary nitrate would have influenced the results of the present study. This is because in both the controls and the patients with OSAS, for estimation of nitrate in the night sample, urine was collected at 6 AM (before the controls/patients got out of bed). This was clearly a fasting sample as our subjects took their dinner at 7 PM, the previous night, during the entire period of the present study. For estimation of nitrate in the day sample, urine was collected at 11.30 AM after their breakfast. In the control subjects, there was no change in nitrate concentration between the night and the day samples both before and after anti-oxidant treatment suggesting that diet did not affect nitrate measurements in the present study. In patients with OSAS, the nitrate concentration in the night (fasting) sample was higher than that during day. These became similar after CPAP therapy suggesting once again that diet did not influence our results. Thus, the increase in nitrate concentration in the night urine sample which persists after anti-oxidant treatment in patients with OSAS is most probably due to NO produced locally in the kidneys.

Decreased bio-availability of NO is considered as a mechanism for coronary artery disease and the effect of anti-oxidant intake is a strategy that is being tried for its treatment.[40] Ascorbic acid, given either as an infusion or oral supplementation has been reported to reverse the NO-dependent endothelial dysfunction in coronary and peripheral arteries of patients with atherosclerosis.[41] Ascorbic acid not only protects NO from inactivation by scavenging superoxide anion but also potentiates NO formation by stabilising tetrahydrobiopterin.[42] Like ascorbic acid, alpha-tocopherol also enhances endothelial NO synthesis. An increase in the production of NO from human platelets by alpha-tocopherol has been reported.[43] Alpha-tocopherol increases eNOS activity which is amplified by ascorbic acid.[42] These studies support our contention that anti-oxidant intake is a useful method for increasing NO bio-availability.

The nitrate concentrations were determined in controls twice before anti-oxidant intake — once in the day sample and once in the night sample. Nitrate concentrations were determined similarly after anti-oxidant intake. The mean nitrate concentrations in the four samples were 788, 786, 787 and 780 μmol/L, respectively. Thus, there was minimal variation in the measurements. These values are in agreement with those reported by other investigators[44]. The mean urinary nitrate in healthy controls has been reported to be 895 μmol/L (range; 533-1354 μmol/L).[44]

Other possible mechanisms for diuresis and natriuresis could be altered levels of hormones such as aldosterone, atrial natriuretic peptide (ANP), and anti-diuretic hormone (ADH). Krieger *et al*[25] reported that urinary flow, urinary sodium (Na^+), chloride (Cl^-) and cyclic guanyl mono phosphate (cGMP) excretion were higher without CPAP therapy than with CPAP therapy in patients with OSAS. The authors concluded that increased water and salt excretion in OSAS during sleep were due to increased ANP levels. However, their findings were not supported by the observations of Warley and Stradling[45] who studied six patients with moderate to severe OSAS with and without CPAP therapy. They measured plasma ANP levels at three different times during the night and reported that the ANP levels were in the normal range in all the three samples, demonstrating that time or CPAP therapy does not affect ANP levels.

There is no consistent report on the role of aldosterone. Rodenstein *et al*[24] found no change in the aldosterone levels in patients with OSAS before and after CPAP therapy. Charloux *et al*[46] reported that acute total sleep deprivation dampened the night-time elevation of aldosterone levels and increased natriuresis. Krieger *et al*[25] examined the effects of CPAP therapy on ADH release during sleep in patients with OSAS. They found no change in ADH levels after CPAP therapy. Based upon these observations, we propose that these hormones may have a negligible role to play in the observations of the present study.

The diuresis and natriuresis observed in our patients may be an independent phenomena or it is possible that increased water excretion may be secondary to sodium excretion. One way of resolving this issue is determination of free water clearance. We did not determine that in our study. However, the increase in urine volume with no change in urinary sodium concentration in the night urine sample seen after anti-oxidant treatment suggests that both may be independent. Finally, we propose that just as frequent awakenings from sleep save the lives of patients with OSAS (even though they are disturbing to the patients and affect their quality of life), the diuresis and natriuresis may be another protective mechanism in them. Besides waking them up, these may minimise the blood pressure rise in them. Like their effects on sleep behaviour, anti-oxidant treatment is beneficial in this situation also since it reduces the urination frequency without affecting the water and sodium excretion. With this treatment patients with OSAS can sleep better without frequent arousals.

The controls chosen for the present study were not age matched with the OSAS patients. They had a BMI which was significantly lower also. We used them to document the diurnal variation in urination frequency and, water and sodium excretion in normal healthy individuals and for validation of the techniques used for various determinations. In the present study, each patient was his own control.

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REFERENCES

1. Neito FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline SD, *et al*. Association of sleep disordered breathing, sleep apnea, and hypertension in a large community based study. Sleep Heart Health Study. *JAMA* 2000;283:1829-36.
2. Peppard PE, Young T, Palta M, Straturd J. Prospective study of the association between sleep disordered breathing and hypertension. *N Engl J Med* 2000;342:1378-84.
3. Peber Y, Hedner J, Norem J, Kraicizi H, Carlson J. Increased incidence of cardiovascular disease in middle aged men with, obstructive sleep apnea: a 7-year follow up. *Am J Respir Crit Care Med* 2002;166:159-65.
4. Yaggi HK, Concato J, Kernan WN, Lichtman JH. Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* 2005;353:2034-41.

5. Barcello A, Miralles C, Barbe F, Vila M. Abnormal lipid peroxidation in patients with sleep apnea. *Eur Respir J* 2000;16:644-7.
6. Deegan PC, McNicholas WT. Pathophysiology of obstructive sleep apnea. *Eur Respir J* 1995;8:1161-78.
7. Singh TD, Patial K, Vijayan VK, Ravi K. Oxidative stress and obstructive sleep apnoea syndrome. *Indian J Chest Dis Allied Sci* 2009;51:217-24.
8. Pallone TL, Mattson DL. Role of nitric oxide in regulation of the renal medulla in normal and hypertensive kidneys. *Curr Opin Nephrol Hypertens* 2002;11:93-8.
9. Grebe M, Eisele HJ, Weissman N, Schafer C, Tillmans H, Seeger W, *et al*. Anti-oxidant vitamin C improves endothelial function in obstructive sleep apnea. *Am J Respir Crit Care Med* 2006;173:897-901.
10. Sin DD, Fitzgerald F, Parker JD. Risk factors for central and obstructive sleep apnea in 450 men and women with congestive heart failure. *Am J Respir Crit Care Med* 1999; 160:1101-6.
11. Mary SMIP, Lam B, Chan LY, Zheng L, Tsang KWT, Fung PCW, *et al*. Circulating nitric oxide is suppressed in OSAS and is reversed by nasal CPAP. *Am J Respir Crit Care Med* 2000;162:2166-71.
12. Kurtz A, Gotz KH, Hamann M, Sandner P. Mode of nitric oxide action on the renal vasculature. *Acta Physiol Scand* 2000;168:41-5.
13. Kurtz A, Wagner C. Role of nitric oxide in control of renin secretion. *Am J Physiol Renal Physiol* 1998;275:F849-F862.
14. Ren YL, Garvin JL, Carretero OA. Role of macula densa nitric oxide and cGMP in the regulation of tubuloglomerular feedback. *Kidney Int* 2000;58:2053-60.
15. Ortiz PA, Garvin JL. Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physio* 2002;282: F777-F784.
16. Coyne KS, Zhou Z, Bhattacharyya SK, Thompson CL, Dhawan R. The prevalence of nocturia and its effect on health related quality of life and sleep in a community sample in the USA. *BJU Int* 2003;92:948-54.
17. Schulz R, Schmidt D, Ribeiro XL, Lucke L, Mayer K, Olschewski H, *et al*. Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnea: response to CPAP therapy. *Thorax* 2000;55:1046-51.
18. Grisham MB, Johnson GG, Gautreaux MD, Berg RD. Measurement of nitrate and nitrite in extracellular fluids: a window to systemic nitric oxide metabolism. *Methods Enzymol* 1995;7:84-90.
19. Griffith OW. Determination of glutathione and glutathione reductase and 2-Vinyl Pyridine. *Annal Biochem* 1980;106:207-12.
20. Yagi K. Assay for blood plasma or serum. *Methods Enzymol* 1984;105:328-31.
21. Asplund R, Aberg HE. Micturition habits of older people: voiding frequency and urine volumes. *Scand J Urol Nephrol* 1992;26:345-9.
22. Dahlstrand C, Hedner J, Wang YH, Pettersson S. Snoring: a common cause of voiding disturbance in elderly men. *Lancet* 1996;347:270-1.
23. Pressman MR, Figueroa WG, Kendrick-Mohamed J, Greenspon LW. Nocturia: a rarely recognized symptom of sleep apnea and other occult sleep disorders. *Arch Intern Med* 1996;156:545-550.
24. Rodenstein DO, Odemont JP, Pieters T, Aubert TG. Diurnal and nocturnal diuresis and natriuresis in obstructive sleep apnoea: effects of nCPAP. *Am Rev Respir Dis* 1992;145:1367-71.
25. Krieger J, Imbs JL, Schmidt M, Kurtz D. Renal function in patients with OSAS: effects of nasal continuous positive airway pressure. *Arch Intern Med* 1988;148:1337-40.
26. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* 1993;103: 1763-8.
27. Somers VK, White DP, Amin R. Sleep apnea and cardiovascular disease: An American Heart Association/ American College of Cardiology Foundation. Scientific statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council of Cardiovascular Nursing in collaboration with the National Heart, Lung and Blood Institute National Center on Sleep Disorders Research. *Circulation* 2008;118:1080-111.
28. Teramoto S, Yamaguchi Y, Yamamoto H, Hanaoka Y, Ishii M, Hibi S, ***et al.*** Cardiovascular and metabolic effects of CPAP in obese obstructive sleep apnoea patients. *Eur Respir J* 2008;31:223-5.
29. Wu XC, Johns EJ. Interactions between nitric oxide and superoxide on the neural regulation of proximal fluid reabsorption in hypertensive rats. *Exp Physiol* 2003;89: 255-61.
30. Juncos R, Garvin JL. Superoxide enhances Na-K-2Cl co transporter activity in the thick ascending limb. *Am J Physiol Renal Physiol* 2005;288:F982-F987.
31. Wu XC, Johns EJ. Nitric oxide modulation of neurally induced proximal tubular fluid reabsorption in the rat. *Hypertension* 2002;39:790-3.
32. Ravi K, Bravo M, Kappagoda CT. Effect of pulmonary lymphatic obstruction on rabbit urine flow. *J Physiol* 1997;505:833-40.
33. McCormick KM, Gunawardena S, Ravi K, Bravo EM, Kappagoda CT. Role of nitric oxide in the reflex diuresis in rabbits during pulmonary lymphatic obstruction. *Exp Physiol* 2004;89:487-96.
34. McCormick KM, Bravo EM, Kappagoda CT. Role of adrenergic receptors in the reflex diuresis in rabbits during pulmonary lymphatic obstruction. *Exp Physiol* 2005;90: 341-7.
35. Mount PF, Power DA. Nitric oxide in the kidney: functions and regulation of synthesis. *Acta Physiol* 2006; 187:433-46.
36. Majid DS, Navar LG. Nitric oxide in the mediation of pressure natriuresis. *Clin Exp Pharmcol Physiol* 1997;24: 595-9.
37. Schulz R, Schmidt D, Blum A, Lopes-Ribeiro X, Lucke C, Mayer K, ***et al.*** Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnoea: response to CPAP therapy. *Thorax* 2000;55:1046-51.
38. Davidge ST, Stranko CP, Roberts, JM. Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. *Am J Obstet Gynecol* 1996;174:1008-13.
39. Tiev KP, Dong NNL, Quy SD, Huy TH, Cabane J. Exhaled nitric oxide, but not serum nitrite and nitrate, is a marker of interstitial lung disease in systemic sclerosis. *Nitric Oxide* 2009;20:200-6.
40. Tousoulis D, Antoniades C, Stefanadis, C. Nitric oxide in coronary artery disease: effects of anti-oxidants. *Eur J Clin Pharmacol* 2006;62:101-7.
41. Heller, R, Werner, ER. Ascorbic acid and endothelial NO synthesis. In: Packer L, Traber MG, Kraemer K, Frei B, editors *The Anti-Oxidant Vitamins C and E*. Illinois: AOCS Press; 2002:pp66-88.
42. Heller R, Werner-Felmayer, G, Werner, ER. Anti-oxidants and endothelial nitric oxide synthesis. *Eur J Clin Pharmacol* 2006;62:21-8.
43. Li D, Saldeen T, Romeo F, Mehta JL. Different isoforms of tocopherols enhance nitric oxide synthase

mphosphorylation and inhibit human platelet aggregation and lipid peroxidation: implications in therapy with vitamin E. *J Cardiovasc Pharmacol Therapeut* 2001;6:155-61.
44. Moshage H, Stegeman CA, Jansen PLM. Determination of nitrite and nitrate in stored urine. *Clin Chem* 1998; 44:1780-1.
45. Warley AR, Stradling JR. Abnormal diurnal variation in salt and water excretion in patients with obstructive sleep apnoea. *Clin Sci* 1988;74:183-5.
46. Charloux A, Gronfier C, Chapotot F. Sleep deprivation blunts the night increase in aldosterone release in humans. *J Sleep Res* 2001;10:27-33.

thoracoscopy. Medical thoracoscopy can be used for therapeutic procedures, such as adhesiolysis and evacuation of pleural fluid in patients with empyema, pleurodesis in patients with malignant pleural effusion and spontaneous pneumothorax.[2]

In the present study, we describe our experience with the technique of medical thoracoscopy in patients who underwent thoracoscopy for diagnostic purposes.

MATERIAL AND METHODS

This was a retrospective study conducted in the Department of Pulmonary Medicine, PGI, Chandigarh, between January 2007 and December 2008. We performed thoracoscopy for diagnosis of undiagnosed pleural effusions. Undiagnosed pleural effusion was defined as failure to achieve a diagnosis by initial pleural fluid analysis including pleural fluid adenosine deaminase (ADA) levels and at least three pleural fluid analyses negative for malignant cells. All patients underwent detailed clinical evaluation with history and clinical examination. Computed tomography (CT) of the chest was performed to assess feasibility of thoracoscopy. Patients with excess rib crowding with narrow intercostal space and loculated pleural effusion could not undergo thoracoscopy. All patients undergoing thoracoscopy were investigated with complete blood count including prothrombin time (PT), activated plasma thrombin time (aPTT) and platelet count to rule out bleeding diathesis. Patients with platelet count less than 75,000/mm^3 and those with PT or aPTT prolonged by more than four seconds above control were not subjected to thoracoscopy. Other contraindications for thoracoscopy included haemodynamic instability, arrhythmias and intractable cough.

Patients were kept fasting for six hours prior to the procedure. Vascular access was achieved with intravenous cannula inserted in the upper limb opposite to the side of thoracoscopy. In patients with small pleural effusion, an artificial pneumothorax was created by injecting approximately one liter of air into pleural cavity just prior to the procedure. This allowed lung to collapse and reduces the chances of lung being injured while introduction of trocar. Patients were positioned in lateral decubitus with diseased side up. Arm on the side of thoracoscopy was positioned above the patient's head. This allowed better access and widens the intercostal spaces. Thoracoscopy was conducted under conscious sedation. Chest wall was draped with sterile cloth after cleaning the skin with 7.5% povidone iodine. Patients were sedated with intravenous midazolam (0.5mg/kg body weight) and intravenous tramadol 5mg was given for analgesia prior to the start of procedure. The skin, subcutaneous tissue, intercostal muscle and parietal pleura were anesthetised with 10mL 2% lignocaine to achieve local anaesthesia. During the procedure intravenous midazolam and tramadol boluses were repeated as required to achieve adequate sedation and analgesia. Intravenous pethidine 25mg was given as bolus to control pain if analgesia could not be achieved with tramadol.

We used single port for visualising and taking pleural biopsy. A 1.5cm to 2cm long skin incision along the line of intercostal space was given in 4^{th} or 5^{th} intercostal space in mid-axillary line using sterile surgical blade. After blunt dissection of subcutaneous tissue and the intercostal muscles with curved artery forceps, a cannula of 10mm diameter with blunt trocar is inserted into the pleural cavity. The trocar was then replaced with rigid video thoracoscope (Richard Wolf GmbH, Knettligen, Germany). Pleural fluid was suctioned to enable clear visualisation of entire pleural surface. Thoracoscope was manoeuvered to see visceral, costal, diaphragmatic surface as well as the costophrenic recess. Adhesions were gently lysed using thoracoscope or biopsy forceps to allow visualisation of pleura.

After selecting suitable site on parietal pleura for biopsy, biopsy forcep was introduced through working channel of the thoracoscope. Pleura was grasped under vision and biopsy is taken with a shearing movement of the thoracoscope. After the procedure is completed, thoracoscope and the cannula were removed and a 28 to 32 Fr chest tube was inserted. Chest drain was connected to water-seal drainage bag. Once the lung had expanded and drain output had decreased to less than 50mL per 24 hours, chest drain was removed.

Demographic characteristics of the patient including the age, gender, clinical diagnosis, pleural fluid analysis, including total and differential count, protein and glucose values, ADA levels, stain for acid-fast bacilli (AFB) and cytology findings and findings of the CT of the chest were recorded. Data are presented in a descriptive fashion.

RESULTS

During the study period, 35 patients (71.4% men and 28.6% women; mean [SD] age 48.68 [14] years) with undiagnosed pleural effusion underwent thoracoscopy for diagnostic purposes (Table 1). The representative images of pleural abnormalities visualised during thoracoscopy are shown in the figure.

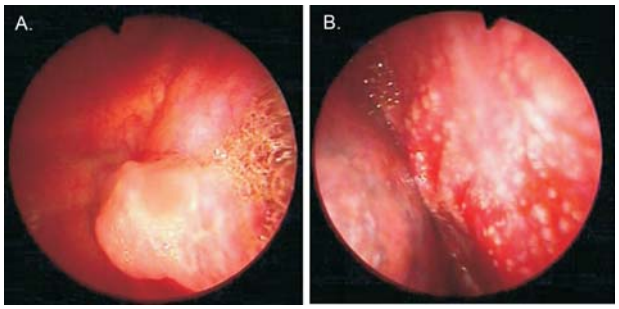

Figure. Thoracoscopic pictures of pleural abnormalities: (A) large pleural nodule in malignant pleural effusion and (B) sago nodules appearance in tubercular pleural effusion.

Of the 35 patients with undiagnosed pleural effusion, the initial clinical diagnosis was malignant pleural effusion in 26 (74.3%) cases. Three patients with clinical suspicion of TB and no diagnosis after initial pleural fluid analysis underwent thoracoscopy. One patient was thought to have Churg-Strauss syndrome with eosinophilic pleural effusion, but no histological diagnosis. In five patients, despite pleural fluid and radiological investigations, no clinical diagnosis could be made. Demographic details and radiological features of these patients are shown in table 1. Pleural fluid ADA was not elevated (>70 IU/L) in any of the patients with suspected malignant pleural effusion or tuberculosis effusion.

Table 1. Demographic characteristics and details of investigations of 35 patients undergoing thoracoscopy for undiagnosed pleural effusion

Demographic Characteristic	Result
Age (years)	48.68 (14.0)
Male:Female	25:10
Initial clinical diagnosis	
Malignant pleural effusion	26
Tuberculosis	3
No diagnosis	5
Churg-Strauss syndrome	1
Pleural nodules in CT chest	25.7%
Pleural fluid	
TLC (/mm^3)	1,525 (1,795)
Differential count	50% lymphocytic effusions 50% neutrophilic effusions
Protein (g/dL)	4.89 (1.21)
Sugar (mg/dL)	72.22 (38.3)
AFB	0%
ADA (I/L)	39.1 (19.5)
Malignant cells	0

The results are depicted as mean±SD or No. (%) unless otherwise stated
CT=Computed tomography; TLC=Total leukocyte count; AFB=Acid-fast bacilli; ADA=Adenosine deaminase activity

Thoracoscopic pleural biopsy could achieve diagnosis in 26 of the 35 patients (74.3%). Final diagnosis of pleural malignancy was made in 17 patients and a diagnosis of TB was made in eight patients. Pleural biopsy confirmed eosinophilic inflammation in the patient with Churg-Strauss syndrome. Of 17 patients with proven pleural malignancy, only one had mesothelioma and the remaining had metastatic pleural cancer. Seven patients had metastatic adenocarcinoma, five patients had poorly differentiated metastatic pleural malignancy, two patients had squamous cell lung cancer and one patient each had small cell lung cancer and lymphoma. In eight patients, thoracoscopic pleural biopsy showed granulomatous inflammation consistent with TB. Biopsy revealed AFB in one case. In nine out of 35 (25%) patients with pleural effusion, thoracoscopic pleural biopsy did not reveal any specific diagnosis, and these cases were classified as idiopathic pleural effusions.

We analysed the yield of biopsy according to the initial clinical diagnosis and found that 19 of the 26 (73%) patients with initial diagnosis of malignant pleural effusion had a definitive diagnosis on thoracoscopic pleural biopsy. Sixteen of them had pleural malignancy and incidentally three of them had chronic granulomatous inflammation consistent with TB (Table 2). We could confirm diagnosis of TB in two out of three patients with initial diagnosis of TB. Among five patients with no initial clinical diagnosis, three had TB diagnosed on pleural biopsy and one had lymphoma (Table 2).

Table 2. Results of diagnostic thoracoscopy

Initial Clinical Diagnosis	Yield of Thoracoscopic Pleural Biopsy	Final Diagnosis on Thoracoscopic Pleural Biopsy
Malignant pleural effusion (n=26)	19/26 (73%)	Pleural malignancy - 16 (mesothelioma - 1 and metastatic pleural malignancy - 15) Tuberculosis - 3 Idiopathic - 7
Tuberculosis (n=3)	2/3 (66.6%)	Tuberculosis - 2 Idiopathic - 1
No clinical diagnosis (n=5)	4/5 (80%)	Tuberculosis - 3 Lymphoma - 1 Idiopathic - 1
Churg-Strauss syndrome (n=1)	1/1(100%)	Churg-Strauss syndrome - 1
Total	**26/35 (74.3%)**	

Of the 35 thoracoscopic procedures, two cases developed empyema (5.2%). There were no instances of haemorrhage, shock or subcutaneous emphysema.

DISCUSSION

In this study, we have presented the data of 35 consecutive patients who underwent thoracoscopy for the diagnosis of undiagnosed pleural effusions. We included patients with undiagnosed pleural

effusions for thoracoscopy in whom initial diagnostic work-up with pleural fluid analysis including pleural fluid ADA and three pleural fluid cytologies were inconclusive. The yield of thoracoscopic pleural biopsy was 74.3% (26/35) patients in this group. Similar experience with medical thoracoscopy has been described from other centers. Kendall *et al*[5] reported yield of thoracoscopic pleural biopsy to be 83% in their study which included 48 patients. Tscheikuna *et al*[6] described their experience from Thailand (n=86) and thoracoscopy was diagnostic in 95% of 34 patients. Ng *et al*[7] could achieve diagnosis with thoracoscopic pleural biopsy in 45.5% (10/22) patients with undiagnosed pleural effusions. In a majority of patients in our study, thoracoscopic pleural biopsy yielded diagnosis of pleural malignancy. A significant proportion of patients, 45.7% (16/35) with un-diagnosed pleural effusion had pleural malignancy. Similar observations were made by Tscheikuna *et al*[6] who found pleural malignancy in 45% of patients with undiagnosed pleural effusions undergoing thoraco-scopy. Ng *et al*[7] found that 45.5% of patients with undiagnosed pleural effusions had pleural malignancy.

Pleural metastasis is the more common cause of malignant pleural effusions than mesothelioma. We could diagnose only one case of mesothelioma whereas 16 of the 17 cases were due to pleural metastasis. Among the patients with metastatic pleural malignancy diagnosed with thoracoscopic pleural biopsy, the most common site of primary malignancy was the lung. In fact, in 50% (8/16) of patients the primary cancer was bronchogenic and in 12.5% (2/16) patients, the primary was breast and in 31.2% (5/16) of cases, the primary site remained unidentified. Among the patients with metastatic pleural effusion from lung cancer, adenocarcinoma was the most common diagnosis. Among those with malignant pleural effusion from primary lung cancer, five of the eight patients had adenocarcinoma. Small cell lung cancer and squamous cell lung cancer were less common diagnosis. These findings are in concordance with the findings of others.[8,9] Eight out of 35 (22.9%) patients in whom we performed thoracoscopy had pleural TB on pleural biopsy. Only one of the eight patients had AFB in their biopsy specimens. This is in stark contrast to the findings of Kendall *et al*[5] who did not find any case of TB in their study of 48 patients undergoing thoracoscopy for undiagnosed pleural effusions. This is probably due to low prevalence of TB in the West.

Thoracoscopic pleural biopsy is considered gold standard in diagnosis of malignant pleural effusion and TB pleural effusion. Diagnostic yield of thoracoscopic pleural biopsy can be as high as 95% in malignant pleural effusions and 99% in TB pleural effusions which is far superior to that of pleural fluid analysis and closed pleural biopsy.[4] These findings along with results of our study and similar studies mentioned above suggest that thoracoscopic pleural biopsy should be considered in all patients with pleural effusions who remain undiagnosed after initial pleural fluid analysis.

A variety of complications associated with thoracoscopy have been described in the literature,[2,6,10-13] such as subcutaneous emphysema (0.6%-4.9%), air leak (0.5%-8.1%), empyema (0.5%-2.7%), haemorrhage (0.3%-0.4 %), shock (0.2%), chest wall seeding by malignancy (0.5%-4.0%). We had only 2 (5%) cases of empyema and noted no other complications.

CONCLUSIONS

The results of this study suggest that medical thoracoscopy should be considered in patients with undiagnosed pleural effusions, particularly those lymphocytic exudative effusions where TB and malignant pleural effusion are clinical possibilities and initial pleural fluid analysis is inconclusive.

REFERENCES

1. Jacobeus HC. The cauterization of adhesions in artificial pneumothorax treatment of pulmonary tuberculosis under thorascopic control. *Proc R Soc Med* 1923;16:45-62.
2 Casal RF, Eapen GA, Morice RC, Jimenez CA. Medical thoracoscopy. *Curr Opin Pulm Med* 2009;15:313-20.
3 Loddenkemper R. Thoracoscopy: state of the art. *Eur Respir J* 1998;11:213-21.
4 Loddenkemper R, Grosser H, Gabler A, Mai J, Presseuler H, Brandt HJ. Prospective evaluation of biopsy methods in diagnosis of malignant pleural effusions: intra patient comparision between pleural fluid cytology, blind needle biopsy and thoracoscopy. *Am Rev Respir Dis* 1983;127:114.
5 Kendall SW, Bryan AJ, Large SR, Wells FC. Pleural effusions: is thoracoscopy a reliable investigation? A retrospective review. *Respir Med* 1992;86:437-40.
6 Tscheikuna J, Silairatana S, Sangkeaw S, Nana A. Outcome of medical thoracoscopy. *J Med Assoc Thai* 2009; 92 (Suppl. 2):S19-S23.
7 Ng TH, How SH, Kuan YC, Hasmah H, Norra H, Fauzi AR. Medical thoracoscopy: Pahang experience. *Med J Malaysia* 2008;63:298-301.
8 Johnston WW. The malignant pleural effusion: a review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. *Cancer* 1985;56:905-9.
9 Chernow B, Sahn SA. Carcinomatous involvement of the pleura: an analysis of 96 patients. *Am J Med* 1977;63: 695-702.
10 Boutin C, Viallat JR, Cargnino P, Farisse P. Thoracoscopy in malignant pleural effusions. *Am Rev Respir Dis* 1981; 124:588-92.
11 Viskum K, Enk B. Complications of thoracoscopy. *Poumon Coeur* 1981;37:25-8.
12 Menzies R, Charbonneau M. Thoracoscopy for the diagnosis of pleural disease. *Ann Intern Med* 1991;114:271-6.
13 de Campos JR, Vargas FS, de Campos Werebe E, Cardoso P, Teixeira LR, Jatene FB, *et al*. Thoracoscopy talc poudrage: a 15-year experience. *Chest* 2001;119:801-6.

Original Article

Breath Carbon Monoxide Levels in Different Forms of Smoking

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ABSTRACT

Background and Objectives. *Bidi*, cigarette, *hookah* and *chillum* are common modes of tobacco smoking in India. Many people consider *hookah* and *chillum* smoking less toxic because smoke is filtered through water or wet cloth. We evaluated the toxicity of tobacco smoking by measuring end-tidal carbon monoxide (eCO) levels after various modes of smoking.

Methods. Eighteen healthy smokers who smoked *bidi*, cigarette, *hookah* and *chillum* on six days were studied. They smoked one *bidi*, one cigarette, five minutes *hookah*, one serve (15 minutes) *hookah*, five minutes *chillum* and one serve (15 minutes) *chillum* on six days randomly. The eCO values were measured before initiation of smoking and for a period of one hour after the smoking session. Increase in eCO values in comparison to baseline after different modes of smoking was compared.

Results. In comparison to baseline, mean eCO levels were raised by 4.94 (0.96) parts per million (ppm) immediately and 4.17 (1.07) ppm 60 minutes after cigarette smoking. *Bidi* smoking caused slightly less increase in mean eCO levels (3.17 [0.82]). One serve of *hookah* and *chillum* smoking caused elevation of mean eCO values by almost eight-folds higher than that of cigarette smoking. Five minutes of smoking with *hookah* (22.18 [5.29]) and one serve of *hookah* (33.0 [8.76]) and *chillum* (40.14 [12.73]) caused significantly higher values of mean increase in eCO in comparison to cigarette smoking ($p<0.001$).

Conclusion. With regard to eCO levels, *hookah* and *chillum* smoking are much more toxic than cigarette smoking.
[Indian J Chest Dis Allied Sci 2011;53:25-28]

Key words: *Hookah*, Tobacco smoking, *Bidi* smoking, *Chillum* smoking, End-tidal carbon monoxide.

INTRODUCTION

Bidi, cigarette, *chillum* and *hookah* are commonly used methods of smoking in India.[1] Amongst them, *bidi* is the most common form of tobacco smoking (50%).[1] Less than 20% smoke cigarettes, whereas, *hookah* smokers are even lesser. A *bidi* is made of a piece of *tendu* (*Diospyros melanoxylon*) leaf in which dried tobacco is rolled. Primarily it is popular in the adults but now flavoured *bidis* (chocolate, mango, cherry) are also in vogue.[2] They are freely available on the net and are a tempting attraction for children.[2] *Chillum* consists of a clay pipe 10cm-15cm long that is held vertically. They are locally made and are inexpensive. The smoke passes through a fold of wet cloth before getting inhaled. *Chillum* has also been used to smoke opium and other narcotic substances. *Hookah* is also called as *water-pipe, narghille and sheesha* in different parts of the world. The tobacco smoke in a *hookah* passes through water before inhalation. *Chillum* and *hookah* are mainly smoked in rural areas. However, now these are becoming popular amongst youngsters in metropolitan cities because of availability of *hookah* joints. *Hookah* is becoming a popular way of smoking tobacco not only in India, but also in many countries in the world. In a recently carried out study in United States of America (USA), it was found that 15% of college students have used *hookah* at least once in life time which was much more than any other substance of abuse.[3]

As the smoke in *hookah* is filtered through water and in *chillum* it passes through a wet cloth, many smokers consider these modes of smoking to be less harmful. Though data are available to show harmful effects of cigarette smoking, more convincing comparative data are needed, especially for less conventional modes of tobacco smoking, such as *bidi*, *hookah* and *chillum*. We planned to study levels of carbon monoxide in exhaled breath after use of *hookah*, *bidi* and *chillum* in comparison to cigarette smoking.

MATERIAL AND METHODS

Eighteen healthy smokers were included in the study after obtaining the informed consent. The study was approved by Institutional Ethics Committee. All subjects were familiar and have been using all the

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four modes of smoking; however, routinely they were using one particular mode of smoking. The subjects reported to the respiratory laboratory after abstaining from any smoking for 24 hours. The eCO levels were measured using a breath analyser (Bedfont UK) at baseline and for one hour after using different modes of smoking on six days. Subjects smoked one *bidi*, one regular cigarette, *hookah* for five minutes, one serve *hookah* (15 minutes), *chillum* for five minutes and one serve of *chillum* (15 minutes) on six days randomly. Usually, a smoker takes five minutes in smoking a cigarette. Therefore, to have comparable data for same duration we also studied *hookah* and *chillum* smoked for five minutes. *Chillum* and *hookah* were used on two days with two types of serving. After obtaining baseline values, eCO levels were determined immediately, 10 minutes, 20 minutes, 30 minutes and 60 minutes after completion of smoking. The same volunteers were evaluated on subsequent days with random allocation of smoking modes.

Increase in end-tidal carbon monoxide values from baseline (ΔeCO) were calculated after different modes of smoking. The mean ΔeCO values among various modalities of smoking were compared by application of analysis of variance.

RESULTS

The mean age of volunteers was 48.0±11.0 years. At baseline eCO levels in different groups, such as *bidi*, cigarette, *hookah* (5 minutes), *hookah* (one serve), *chillum* (5 minutes) and *chillum* (one serve) were 10.2±3.2, 10.4±4.2, 12.1±5.6, 11.7±5.6, 10.7±4.2 and 10.7±4.2 ppm, respectively (Table). The baseline values on different days were comparable (F=0.496, p=0.778). The eCO values were increased from baseline values with different modes of smoking (Figure). It was observed that one serve of *chillum* raised the eCO levels maximally. One serve and five minutes of *hookah* smoking also raised the eCO levels significantly higher in comparison to cigarette smoking (p<0.001). *Bidi* smoking also caused increased levels of eCO and the magnitude of increase was slightly less than that of cigarette but the difference was not significant statistically.

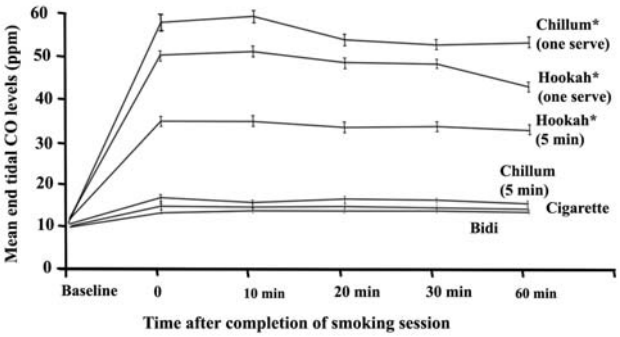

Figure. Mean end-tidal carbon monoxide after various modes of smoking
***= P<0.001**
CO=Carbon monoxide; ppm=Parts per million

DISCUSSION

Cigarette smoke consists of around 2% to 4% carbon monoxide.[4] Our study showed that *bidi* smoke also raised eCO level comparable to cigarette smoking. Both *hookah* and *chillum* raised eCO much higher levels than cigarette smoking. The level of eCO

Table. Pre-smoking mean baseline and post-smoking increase in eCO (Δ) values after various modes of smoking

	Pre-smoking Baseline eCO Values (ppm)+	Post-smoking Increase in eCO from Baseline (ppm)++				
		At the end of smoking	10 min after smoking	20 min after smoking	30 min after smoking	60 min after smoking
Bidi	10.2±3.2	3.00±0.72	3.61±0.84	3.67±0.86	3.61±0.83	3.17±0.82
Cigarette	10.4±4.2	4.94±1.07	4.56±0.91	4.89±1.07	4.67±0.99	4.17±0.96
***Hookah* (5 min)**	12.1±5.6	25.27±6.30*	25.09±6.32*	23.45±5.61*	23.55±5.43*	22.18±5.29*
***Hookah* (One serve)**	11.7±5.6	41.57±11.23*	42.57±10.22*	39.14±9.69*	39.00±10.20*	33.00±8.76*
***Chillum* (5 min)**	10.7±4.2	5.55±0.79	4.64±0.65	5.18±0.66	5.00±0.61	4.27±0.48
***Chillum* (One serve)**	10.7±4.2	45.00±17.04*	46.14±14.41*	41.29±11.72*	39.86±11.82*	40.14±12.73*

One serve is equivalent to 15 minutes of smoking
+=expressed as mean±SD; ++=expressed as mean±(SEM); * P<0.001;
ppm=Parts per million; SD=Standard deviation; SEM=Standard error of mean

remained high for one hour. Clinical significance of such levels of eCO is yet not known but in animal experiments even low levels of CO have been shown harmful.[5]

It has been a popular myth that the water in a *hookah* detoxifies the smoke. Old historical accounts state that the *hookah* was invented by a physician named Hakim Abul Fath during the reign of Emperor Akbar in India.[6] The physician suggested that this form would be less toxic. So this popular belief of *hookah* being safe is as old as the origin of *hookah*. It had been shown in older studies that *hookah* smoking was less toxic as compared to cigarette smoking because in it smoke was passed through water.[7] However, a study done in Pakistan[9] showed that the eCO hazard is similar in *hookah* and cigarette smokers. It was also substantiated in another study carried out by Shafagoj and Mohammed.[10] They documented an increase in end-expiratory increase in eCO, heart rate, systolic, diastolic and mean arterial blood pressure after *hookah* smoking. In this study,[10] it has been suggested that eCO levels were comparable to cigarette smoking. Since they did not measure comparative eCO values after *hookah* and cigarette smoking, objective data were lacking.

Our study showed that eCO levels after *hookah* smoking was much higher than cigarette smoking, therefore, suggesting substantial higher toxicity than cigarette smoking. A newer study[8] has also suggested that *hookah* smoke is more toxic in terms of CO and smoke exposure.[8]

Hookah joints claim that the nicotine content is 0.5% while tar content is 0%.[11] *Hookah* smokers continue smoking till they have enough nicotine to satisfy them. *Hookah* smoke is made less irritating by moisturising it and adding fruity flavours. Thus, *hookah* smokers inhale more smoke and are exposed to higher levels of CO, carcinogens and heavy metals present in *hookah* smoke.[9] This exposes water-pipe smokers to the risk of same kind of diseases as caused by cigarette smoking which includes cancer, heart and respiratory diseases.[12] The concentration of toxins inhaled during *hookah* smoking depends on the frequency, depth of inhalation and total duration of the smoking session.[12]

Bidi is a slim, unfiltered and a more dangerous form of tobacco. Some studies indicate that the amount of nicotine and other toxic substances delivered by a *bidi* is as great as that by an ordinary cigarette.[13,14] In a recent study,[15] the average breath CO levels were equal to or higher for *bidi* smokers than cigarette smokers. However in our study, eCO levels were slightly lower during *bidi* than cigarette smoking. Use of *bidi* is not limited to rural India but even in USA as many as 5% adolescents use *bidi* believing that they are healthy alternatives to traditional cigarettes.[16] The irony of *bidi* smoking is that it is smoked with more intensity and frequency leading to higher nicotine intake[14] and 2-3 times greater tar inhalation than cigarettes.[17]

One serve of *chillum* smoking was found most toxic in increasing eCO levels in the breath. Though *chillum* smoking is not widely practiced in urban areas but many people in rural India still smoke *chillum*. Since smoke through *chillum* passes through wet cloth many people believe it less toxic. Unfortunately, none of the studies in the past evaluated the toxic effects of *chillum*. In view of the results of the present study showing highest eCO levels after *chillum* smoking, there is need to study the effect of this mode of smoking more extensively. Curiously five minutes of *chillum* smoking could increase eCO level much less than five minutes *hookah* and one serve of *chillum* smoking. Probably it may due to the fact that *chillum* takes longer time in ignition and smoke generation.

CONCLUSIONS

Bidi, the most commonly used mode of smoking, is as toxic as a regular cigarette in terms of end-tidal CO levels. *Hookah* and *chillum* use commonly considered harmless were associated with almost eight-fold higher rise in breath CO levels in comparison to cigarette smoking, indicating substantially higher toxicity. One serve of *chillum* smoking lead to maximal increase in eCO levels indicating the possibility of *chillum* being the most dangerous mode of smoking.

REFERENCES

1. Food and Agriculture Organization of the United Nations. Issues in the global tobacco economy: selected case studies. Rome: Food and Agricultural Organization; 2003:p.54.
2. World Health Organization. Tobacco: Deadly in any form or disguise – No Tobacco Day 2006. Geneva: World Health Organization;2006:p.21.
3. Grekin ER, Ayna D. Argileh use among college students in the United States: an emerging trend. *J Studies Alcohol Drugs* 2008;69:472-5.
4. Coburn RF, Forster RE, Kane PB. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. *J Clin Invest* 1965;44:1899-1910.
5. Mirza A, Eder V, Rochefort GY, Hyvelin JM, Machet MC, Fauchier L, *et al*. CO inhalation at dose corresponding to tobacco smoke worsens cardiac remodeling after experimental myocardial infarction in rats. *Toxicol Sci* 2005;85:976-82.
6. Chattopadhyay A. Emperor Akbar as a healer and his eminent physicians. *Bull Indian Institute Hist Med* 2000; 30:151-8.
7. Weiss W. The toxicity of tobacco smoke solutions for paramecium: the influence of various forms of filtration. *Arch Environ Health* 1965;10:904-9.

8. Eissenberg T, Shihadeh A. Waterpipe tobacco and cigarette smoking: direct comparison of toxicant exposure. *Am J Prevent Med* 2009;37:518-23.
9. Sajid KM, Akhter M, Malik GQ. Carbon monoxide fractions in cigarette and hookah smoke. *J Pak Med Assoc* 1993;43:179-82.
10. Shafagoj YA, Mohammed FI. Levels of maximum end-expiratory carbon monoxide and certain cardiovascular parameters following hubble-bubble smoking. *Saudi Med J* 2002;23:953-8.
11. World Health Organization. WHO study group on tobacco regulation (Tob Reg). (2005). *Advisory Note:* Waterpipe tobacco smoking: health effects, research needs and recommended action by regulators. Geneva: World Health Organization;2005:p.1.
12. Knishkowy B, Amitai Y. Water-pipe (narghile) smoking: an emerging health risk behavior. *Pediatrics* 2005;116:e113-19.
13. United States Centre for Disease Control. Bidi use among urban youth: Massachusetts, March-April 1999. *MMWR Morb Mortal Wkly Rep* 1999;48:796-9.
14. Malson JL, Sims K, Murty R, Pickworth WB. Comparison of the nicotine content of tobacco used in beedis and conventional cigarettes. *Tob Control* 2001;10:181-3.
15. Kumar R, Prakash S, Kushwah AS, Vijayan VK. Breath carbon monoxide concentration in cigarette and *bidi* smokers in India. *Indian J Chest Dis Allied Sci* 2010;52:19-24.
16. Healton C, Messeri P, Reynolds T, Wolfe C, Stokes C, Ross J. Tobacco use among middle and high school students – US, 1999. *MMWR Morb Mortal Wkly Rep* 2000;49:49-53.
17. Rahman M, Fukui T. Bidi smoking and health. *Public Health* 2000;114:123-7.

Original Article

Adiposity: Determinant of Peak Expiratory Flow Rate in Young Indian Adults Male

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ABSTRACT

Background. Although several factors such as respiratory muscle strength, lung compliance, resistance to airflow, and even obesity affect the lung functions, the nature of relationship with markers of adiposity is not clear. We hypothesised that central pattern of fat distribution is a significant predictor of decreased peak expiratory flow rate (PEFR). The present study was designed with the aim to examine the effects of adiposity on PEFR in males.

Methods. One hundred young healthy male volunteers were analysed in the study. They were classified into non-obese, and obese groups based on body mass index (BMI) (obese ≥30Kg/m^2 and non-obese <30Kg/m^2). The PEFR was measured by using Wright's peak flow meter. Data was analysed using unpaired 't' test for statistical significance of differences between the non-obese and the obese, stratified into age groups of 20 to 30 years and 30 to 40 years. A partial correlation adjusted to age, height and BMI followed by regression analysis was conducted using adiposity markers as a predictor of PEFR.

Results. The model adjusted to age, height, weight and BMI revealed waist hip ratio (WHR) as the only parameter which shows significant variance in PEFR with a Pearson's r=-0.59, F (1, 100)=12.23, p=0.04. The resulting linear regression equation is y=-388.72xWHR+850.68.

Conclusions. Our findings suggest that obesity itself and especially the pattern of body fat distribution have independent effects on PEFR. These results suggest that abdominal adiposity, measured as WHR, is a better predictor of expiratory flow than weight or BMI. **[Indian J Chest Dis Allied Sci 2011;53:29-33]**

Key words: Pulmonary function, PEFR, Obesity, Adiposity marker.

INTRODUCTION

Pulmonary functions are generally determined by respiratory muscle strength, compliance of the thoracic cavity, airway resistance and elastic recoil of the lungs.[1] It is well known that pulmonary functions may vary according to the physical characteristics including age, height, body weight[2], and altitude (hypoxia or low ambient pressure).[3] Significant regional differences in lung functions in healthy Indians have been reported.[4,5]

For demonstrating the narrowing of airways, different expiratory flow rates are employed. Peak expiratory flow rate (PEFR) is one such parameter that can be easily measured by a peak flow meter and is a convenient tool to measure lung functions in a field study.[6] It is a fairly good indicator of bronchial hyperresponsiveness,[7] and does not require body temperature pressure saturated (BTPS) correction. The PEFR values are affected by various factors, such as sex, body surface area, obesity, physical activity, posture, environment and racial differences.[8-10] Obesity has been linked with impaired pulmonary function and airway hyperresponsiveness,[11,12] but not in all studies[13] and with asthma in adults.[14-16]

Excess body weight as in an obese or overweight person is normally due to accumulation of extra body fat.[17] However, it could also be due to other causes and can show variations in regional distribution.[18] Weight and body mass index (BMI) as measures of overall adiposity are used as predictors of pulmonary function in many epidemiological studies.[19-21] While these measures are widely accepted as determinants of pulmonary function, waist hip ratio (WHR)[22] and waist circumference (WC),[23,24] often used as a surrogate measure for abdominal or upper body obesity may influence pulmonary function

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mechanically[25] by changes in compliance, work of breathing and the elastic recoil.[26,27] Therefore, markers of obesity, such as BMI,[28] WC[29] and WHR[30] may be correlated to PEFR.

Most of the studies regarding the effect of obesity on pulmonary function tests have been conducted in males, in the age group of 5 to 16 years or in the elderly age groups.[31,32] Further, these studies have not considered the pattern of fat distribution that may affect the pulmonary function.

The PEFR in the obese individuals should be lower, as the extra fat would exert a mechanical effect on the movement of chest or abdomen but the predictability of different adiposity markers for deranged PEFR may vary across populations. Therefore, the present study was undertaken to establish the relationship between lung functions and adiposity measures in young male adults of Garhwal.

MATERIAL AND METHODS

The study was conducted on healthy young male volunteers from university population and nearby area of Bhaniyawala, Dehradun, Uttarakhand. One hundred volunteers were selected between the age group of 20 to 40 years. The nature of the study was explained and informed consent was obtained from each subject prior to participation in the study. The protocol of the study was approved by the Institutional Ethics Committee.

To rule out any obvious cardio-pulmonary compromise, a detailed history was taken and clinical examination of the subjects was done. Subjects with history of smoking, severe chest trauma, obvious chest and spinal deformity, personal/family history of asthma, chronic obstructive pulmonary diseases and other cardio-respiratory diseases were excluded from the study. The volunteers were asked to avoid beverages, like tea and coffee and other stimulants with light breakfast before reporting to the Department of Physiology, HIMS, in the forenoon to avoid diurnal variation in respiratory parameters. Volunteers were subjected to anthropometry at the point of entry using the standard procedures and instruments as per the study protocol.

Age was recorded from date of birth to the nearest completed/approaching year (<6 months and >6 months). Standing height was recorded without shoes and with light clothes on a wall mounted measuring tape to the nearest centimetre (<5 mm and >5 mm). Weight was recorded without shoes and with light clothes on a Krups weighing machine with a least count of 100 grams. Body mass index was calculated by the formula of weight (in Kg) and height (in meters).[2]

$$BMI = Weight\ (Kg)/Height\ (meter)^2$$

Waist circumference (WC) measurement was done with minimal, adequate clothing (light clothes) with feet 25 to 30 cm apart and weight equally balanced with a tailor's measuring tape in a plane perpendicular to the long body axis at the level of umbilicus without compression of the skin to the nearest 0.1cm (WC≥90cm in males and ≥80cm in females were defined as abdominal obesity using World Health Organization Asia Pacific prospective guidelines).[33] Hip circumference (HC) measurement was done with minimal, adequate clothing (light clothes) across the greater trochanter with legs and feet together by a measuring tape without compressing the skin fold. The ratio of WC, WHR and HC, was calculated. It is a measure of central pattern of fat distribution (>0.9 for males and >0.8 for females).[33]

The PEFR was recorded with Wright's portable peak flow meter according to the standard procedure. At least three readings were obtained under supervision and the best of the three was recorded.[11,12] A close watch was kept to ensure that a tight seal was maintained between lips and the mouthpiece of the peak flow meter. The procedure was performed in a spacious room with regulated temperature during the morning hours between 9 AM to 11 AM in the months of March and April. The pooled data were subjected to statistical analysis.

Statistical Analysis

Patients were stratified according to the age groups of 20 to 30 years and 30 to 40 years into obese and non-obese volunteers according to the WHO criteria with BMI ≥30 Kg/m^2 as obese and BMI <30 as non-obese. Analysis was carried out to identify which of the adiposity markers showed difference in the PEFR among the groups within 95% of confidence limit. Partial correlation among the PEFR and adiposity markers was determined to see the association of the parameter with adiposity. Most significant predictable markers were analysed by step-wise regression analysis to make the model acceptable for the epidemiological studies.

RESULTS

No significant differences in age or height were found among the two groups studied. However, as expected, weight, BMI, WC and WHR were significantly higher in obese as compared to non-obese groups (Table 1). Mean values of PEFR showed significantly lower values (393.6±51.1) only in obese males of the higher age group. The absolute values of PEFR were higher in the lower age group as compared to the higher age group.

Table 1. Mean anthropometric data, body mass index (BMI), waist circumference (WC), and waist hip ratio (WHR) in obese and in non-obese males

	20-30 years		30-40 years	
	Non-Obese (n=25)	Obese (n=25)	Non-Obese (n=25)	Obese (n=25)
Age (years)	25.4±3.0	24±3.5	36±3.1	34.8±3.0
Height (m)	1.6±0.1	1.7±0.1	1.69±0.8	1.7±0.1
Weight (Kg)	64.2±5.7	96.5±4.6*	71.9±11.3	96.2±8.4*
BMI (Kg/m^2)	24.7±1.1	34.5±2.1*	24.9±2.4	32.1±3.8*
WC (cm)	88.8±2.8	107.5±7.5*	105.1±15.5	124.4±7.5*
WHR	0.8±0.1	0.9±0.1*	0.9 0.1	1.1±0.1*
PEFR (L/min)	489±56.2	518.2±24.5	507.6±41.6	393.6±51.1*

Variables are expressed as mean±SD; *=p<0.05
PEFR=Peak expiratory flow rate

Partial correlation analysis of the adiposity markers after adjusting for age and height showed an inverse correlation of PEFR with all the adiposity markers but significant association was seen only with WHR (Table 2). Linear regression analysis was conducted including independent parameters like age, height, BMI, WC and WHR as predictors of PEFR.

Table 2. Partial correlation coefficient of adiposity markers with PEFR adjusted for age and height in males (n=100)

	Weight (Kg)	BMI (Kg/m^2)	WC (cm)	WHR	PEFR (L/min)
Weight (Kg)	1.00	0.997*	0.845*	0.462**	-0.2078
BMI (Kg/m^2)	-	1.00	0.8257*	0.4363**	-0.1811
WC (cm)	-	-	1.00	0.4916***	-0.3118***
WHR	-	-	-	1.00	-0.5951+
PEFR (L/min)	-	-	-	-	1.00

*=p<0.0001, **=p<0.01, ***=p<0.05, +=p<0.001
PEFR=Peak expiratory flow rate; BMI=Body mass index; WC=Waist circumference; WHR=Waist hip ratio

The model revealed that WHR was the only significant predictor of the variance in PEFR with a Pearson's r=-0.59, F (1, 100)=12.23, p=0.04 (Table 3). Scatter plot between PEFR and WHR among the males show a negative correlation. The resulting linear regression equation is y=-388.72xWHR+ 850.68 (Figure).

Table 3. Regression coefficients Beta for adiposity markers entered into model with PEFR (n=100)

Variable	Beta (p value)
Age (years)	-0.056 (0.08)
Height (m)	0.313 (0.12)
WC (cm)	-0.15 (0.7)
BMI (kg/m^2)	0.11 (0.68)
WHR	-0.56 (0.04)

PEFR=Peak expiratory flow rate; WC=Waist circumference; BMI=Body mass index; WHR=Waist hip ratio

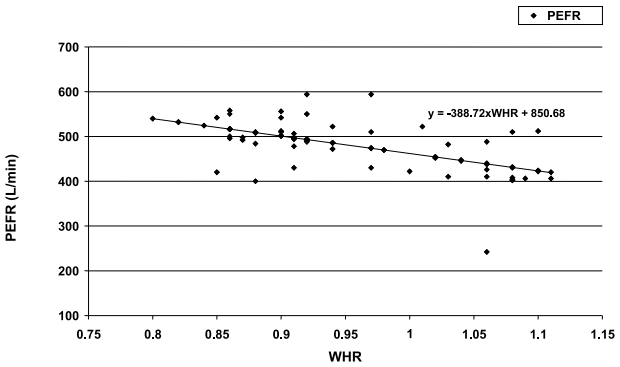

Figure. Scatter plot showing relationship of PEFR and WHR.

DISCUSSION

The primary factors that affect PEFR are the strength of the expiratory muscles generating the force of contraction, the elastic recoil pressure of the lungs and the airway size.[32]

Abdominal adiposity may influence pulmonary functions by restricting the descent of the diaphragm and limiting lung expansion as compared to overall adiposity which may compress the chest wall. We found that PEFR is negatively associated with adiposity markers, as measured by BMI, WC and WHR, after the effects of variation in age and height were removed.

On multivariate analysis, WHR was found to be the only most significant parameter that showed significant negative association with PEFR while age, height, BMI and WC were not. Similar findings were observed by Collins *et al*[30] who reported a lower FEV_1 in subjects with higher WHR even without adjustment for age, stature and relative obesity. However, regression analysis reported no significant effect of WHR on flow rates.[30] Chen *et al*[28] in a six-year follow-up study on patients with the extreme obesity (W/H >0.9) have reported that forced expiratory flow during mid expiratory phase was significantly reduced. In another study, Chen *et al*[23] showed a positive correlation between maximum mean expiratory flow (MMEF) and increasing BMI, that was significant in the middle age group of 40 to 69 years. The MMEF is generally regarded as "effort independent" and it may be that higher levels of BMI are associated with increased chest wall elastic recoil, and thus, with a change in the balance of elastic recoil.[27] Shaheen *et al*[14] found that obese men, but not women, had reduced maximum expiratory flow rates at 50% and 75% of exhaled vital capacity. In contrast to our study, Lazarus *et al*[21] observed no effect of the central pattern of fat distribution (WHR) in the mean age 35.2±1.3 years. Rather, upper body subcutaneous fat was significantly associated with the flow rates.

In the present study, PEFR values for obese individuals were found to be lower than the non-obese individuals only in the higher age group. However, the results were not significant when BMI was taken as a parameter of obesity. Correlation study has shown a negative relationship between BMI and PEFR. The study by Chinn *et al*[7] on young adults found evidence of linearity in relation of slope to BMI. The "Slope" declined with increasing BMI in males, that is, bronchial hyperresponsiveness increased. The statistical significance of the results was similar to our study. In the study conducted by Carey *et al*[22] on obese healthy subjects suggests that both total respiratory resistance and airway resistance increased significantly with the level of obesity, disclosing a significant linear relationship between airway conduction and functional residual capacity. However, the study by Ghabashi and Iqbal[34] on asthmatic patients suggested that, although obesity was prevalent in asthmatic patients, BMI did not correlate with any of the spirometric variables.

The lower values of PEFR could be linked to obesity through several mechanisms, such as mechanical effects on the diaphragm (impeding descent into the abdominal cavity) and also because of the fat deposition between the muscles and the ribs that can lead to increase in the metabolic demands and work-load of breathing.

Although the magnitude of the effect is relatively small from a public health perspective, our findings in the present study indicate the consequence of increased abdominal obesity on lung function. A larger sample size and a longitudinal study will definitely be of a great value in predicting the relationship between pulmonary function tests and abdominal obesity. Further, the association needs to be studied in female subjects.

CONCLUSIONS

The study concludes that adiposity, measured as WHR, affects the PEFR in young males in the age group of 20 to 40 years, especially in higher age group. After adjusting for BMI, a central pattern of fat distribution, as measured by WHR was associated with lower values for PEFR in young male adults. The findings of the present study suggest that the pattern of body fat distribution have independent effect on PEFR. These results suggest that WHR is a better predictor of pulmonary function than weight or BMI, and should be considered when investigating the determinants of pulmonary function.

REFERENCES

1. Cotes JE. *Lung Function Assessment and Application in Medicine*; 3rd edn. Oxford: Blackwell Scientific Publications;1975:pp281-7.
2. Polgar G, Weng TR. The functional development of the respiratory system. *Am Rev Respir Dis* 1979;170:625-95.
3. Kashyap S, Puri DS, Bansal SK. Peak expiratory flow rates of healthy tribal children living at high altitudes in the Himalayas. *Indian Pediatr* 1992;29:283-6.
4. Jain SK, Ramaiah TJ. Normal study of pulmonary function tests for healthy Indian men 15-40 years and comparison of different regression equations (predicted formula). *Indian J Med Res* 1969;57:1453-66.
5. Kamath SR, Sarma BS, Raju VRK, Venkataraman C, Balkrishna M, Bhavsar RC, *et al*. Indian norms for pulmonary function. *J Assoc Physicians India* 1977;25:531-40.
6. Wright BM, McKerrow CB. Maximum forced expiratory flow as a measure of ventilatory capacity. *Br Med J* 1959; 2:1041-7.
7. Chinn S, Jarvis D, Burney P. Relation of bronchial responsiveness to body mass index in the ECRHS. *Thorax* 2002;57:1028-33.
8. Benjaponpitak S, Direkwattanachai C, Kraisarin C, Sasisakulporn C. Peak expiratory flow rate values of students in Bangkok. *J Med Assoc Thai* 1999;82 (Suppl.): 137-43.
9. Srinivas P, Chia YC, Poi PJ, Ebrahim S. Peak expiratory flow rate in elderly Malaysians. *Med J Malaysia* 1999;54: 11-21.
10. Raju PS, Prasad KV, Ramana YV, Murthy KJ. Pulmonary function tests in Indian girls prediction equations. *Indian J Pediatr* 2004;71:893-7.
11. Gibson GJ. Obesity, respiratory function and breathlessness. *Thorax* 2000;55 (Suppl. 1): S41-S44.
12. Rubinstein I, Zamel N, DuBarry L, Hoffstein V. Airflow limitation in morbidly obese, nonsmoking men. *Ann Intern Med* 1990;112:828-32.
13. Schachter LM, Salome CM, Peat JK, Woolcock AJ. Obesity is risk for asthma and wheeze but not airway hyperresponsivness. *Thorax* 2001;56:4-8.
14. Shaheen SO, Sterne JA, Montgomery SM, Azima H. Birth weight, body mass index and asthma in young adults. *Thorax* 1999;54:396-402.
15. Young SY, Gunzenhauser JD, Malone KE, McTiernan A. Body mass index and asthma in the military population of the northwestern United States. *Arch Intern Med* 2001;161:1605-11.
16. Ferretti A, Giampiccolo P, Cavalli A, Milic-Emili J, Tantucci C. Expiratory flow limitation and orthopnea in massively obese subjects. *Chest* 2001;119:1401-8.
17. World Health Organization Tech Rep Series, 854. Overweight adults. In: Physical status: The use and interpretation of anthropometry;1995:pp 312-4.
18. Bjorntorp P. The regulation of adipose tissue distribution in humans. *Int J Obese* 1996;20:291-302.
19. Raison J, Cassuto D, Orvoen-Frija E, *et al*. Disturbances in respiratory function in obese subjects. In: Ailhaud G, editor *Obesity in Europe '91 Proceedings of the 3rd Congress on Obesity*. London: John Libbey and Company Ltd; 2001: pp227-30.
20. Zerah F, Harf A, Perlemuter L, Lorino H, Lorino A, Atlan G. Effects of obesity on respiratory resistance. *Chest* 1993; 103:1470-6.
21. Lazarus R, Sparrow D, Weiss ST. Effect of obesity and fat distribution on ventilatory function: the normative aging study. *Chest* 1997;111:891-8.
22. Carey IM, Cook DG, Strachan DP. The effects of adiposity and weight change on forced expiratory volume decline in a longitudinal study of adults. *Int J Obes Relat Metab Disord* 1999;23:979-85.
23. Chen R, Tunstall-Pedoe H, Bolton-Smith C, Hannah MK,

Morrison C. Association of dietary antioxidants and waist circumference with pulmonary function and airway obstruction. *Am J Epidemiol* 2001;153:157-63.
24. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, *et al.* Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 1994;73:460-8.
25. King GG, Brown NJ, Diba C, Thorpe CW, Muñoz P, Marks GB, *et al*. The effects of body weight on airway calibre. *Eur Respir J* 2005;25:896-901.
26. Biring MS, Lewis MI, Liu JT, Mohsenifar Z. Pulmonary physiologic changes of morbid obesity. *Am J Med Sci* 1999;318:293-7.
27. Luce JM. Respiratory complications of obesity. *Chest* 1980;78:626-31.
28. Chen Y, Horne S, Dosman J. Body weight and weight gain related to pulmonary function decline in adults: a six year follow up study. *Thorax* 1993;48:375-80.
29. World Health Organization. Obesity: preventing and managing the global epidemic. *WHO Obesity Technical Report Series 894*. Geneva:WHO 2000.
30. Collins L, Hoberty P, Walker J, Fletcher E, Peiris A. The effect of body fat distribution on pulmonary function tests. *Chest* 1995;107:1298-1302.
31. Ray CS, Sue DY, Bray G, Hansen JE, Wasserman K. Effects of obesity on respiratory function. *Am Rev Respir Dis* 1983;128:501-6.
32. Sahebjami H. Dyspnea in obese healthy men. *Chest* 1998;114:1373-7.
33. World Health Organization (WHO), International Association for the Study of Obesity (IASO), and International Obesity Task Force (IOTF). The Asia-Pacific Perspective: Redefining Obesity and Its Treatment. 2000;Australia:Health Communication Pvt Ltd pp 17-19.
34. Ghabashi AE, Iqbal M. Obesity and its correlation with spirometric variables in patients with asthma. *Med Gen Posted* 2006;8:58.

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Original Article

Prevalence of Depression in Stable Chronic Obstructive Pulmonary Disease

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ABSTRACT

Background. Psychological impairment is a significant co-morbid condition of chronic obstructive pulmonary disease (COPD). No studies from India have been conducted to assess the prevalence of depression in COPD.

Methods. We investigated the prevalence of depression in 100 consecutive stable COPD patients during their routine out-patient department visits. Patients diagnosed to have depression or chronic systemic diseases were excluded. Severity of the COPD was classified according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. Hindi translations of patient health questionnaire-9 (PHQ-9) were administered and severity of depression was assessed at each stage of the COPD.

Results. All subjects were males with a mean age of 61.7±9.6 years. Six patients in stage I, 32 patients in stage II, 40 patients in stage III and 22 patients in stage IV of the COPD were enrolled. The cumulative prevalence of depression in the study population was 72 percent.

Conclusions. Symptoms of depression were observed at all stages of COPD and its severity increased with an increase in severity of the COPD. High prevalence of depressive symptoms in Indian patients with COPD may be due to various confounding factors. Screening for symptoms of depression in patients with COPD by simple and quick validated questionnaires during their out-patient visits will be helpful in early diagnosis and appropriate treatment or referral.
[Indian J Chest Dis Allied Sci 2011;53:35-39]

Key words: Depression, PHQ-9, Chronic obstructive pulmonary disease.

INTRODUCTION

Depression is a significant co-morbid condition of chronic obstructive pulmonary disease (COPD). The prevalence of depression in stable COPD ranges between 10 percent and 42 percent.[1-4] The estimates are variable either due to the use of different tools of measurement or variations in the degree of severity of illness across the studies.

Depression in COPD is associated with poor compliance to treatment, frequent hospitalisation or doctor visit, prolonged hospital stay, poor quality of life and high cost of treatment.[5] The impact of depression in the management of COPD is receiving interest and the American College of Chest Physicians organised a multi-disciplinary workshop to shed the light on the current understanding and to identify the areas of future research needs.[4]

Detection of depression in patients with COPD by a simple questionnaire will help respiratory physicians to diagnose it and appropriate treatment or referral. Patient health questionnaire-9 (PHQ-9) is a part of the primary care evaluation of mental disorders (PRIME-MD) and utilises a semi-structured psychiatric interview using the *Diagnostic and Statistical Manual of Mental Disorders* (fourth edition) (DSM-IV) criteria to assess the severity and functional impairment due to depression.[6] The PHQ-9 is a standardised, brief and easy screening instrument designed to diagnose depression in primary care settings for the busy clinician. In PHQ-9, patients indicate for each of the nine depressive symptoms during the previous two weeks. Hindi (local Indian language) translation of PHQ-9 is well validated by Kochhar *et al*[7] for the diagnosis of depression as per DSM-IV.

The present study was conducted to assess the prevalence and severity of depression using the Hindi translation of PHQ-9 in different stages of chronic stable COPD.

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MATERIAL AND METHODS

This cross-sectional study was carried out on consecutive patients with COPD during their routine out-patient visits in Bhopal Momorial Hospital and Research Centre, Bhopal from July 2008 to April 2009. The subjects were recruited on the basis of a written informed consent. The study was approved by the Institutional Ethics Committee.

Those patients who meet the following criteria were included in this study: (1) age more than 40 years, (2) ex-smoker or current smoker with a smoking history of more than 10 pack years (Ex-smoker was defined as a person who stopped smoking for more than one year); and (3) ratio of forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) less than 0.70 and FEV_1 or FVC fails to increase absolute volume greater than and equal to 200mL and 12% after 200µg of salbutamol inhalation.

Patients with prior diagnosis of depression or subjects with other chronic systemic illness, like malignancy, diabetes mellitus, coronary artery disease, renal or hepatic disease were excluded from the study.

The spirometric measurements (FVC, FEV_1 and FEV_1/FVC) and bronchodilator responses were performed in sitting position as per the American Thoracic Society guidelines.[8] Depending on the post-bronchodilator FEV_1 (%) values, the patients were classified in four stages of COPD as per Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations[9]: Stage I (>80), Stage II (50-79), Stage III (30-49) and Stage IV (<30).

Hindi translation of PHQ-9 was self administered to literate patients. For illiterate patients, help was sought from either relative or paramedical workers to read out the questionnaire and to record the responses. Each of the nine items of PHQ-9 was scored from 0 (not at all) to 3 (nearly every day). Total score can range from 0 to 27. Depending upon total score, the severity of depression was classified as follows: none (0-4), mild (5-9), moderate (10-14), moderately severe (15-19) and severe (20-27). Major depression was diagnosed if five or more of the depressive nine symptom criteria were present for at least "more than half the days" in the past two weeks, and one of the symptoms was depressed mood or anhedonia. Other depression was diagnosed if 2, 3, or 4 depressive symptoms were present for at least "more than half the days" in the past two weeks, and one of the symptoms was depressed mood or anhedonia.

Statistical Analysis

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS)-version 9.0, (USA) and a p-value of <0.05 was considered significant. Data are presented as mean± standard deviation (SD). For comparison of mean, we have used one-way analysis of variance (ANOVA) and categorical data was compared by Chi-square test.

RESULTS

One hundred stable subjects with COPD were studied. All subjects were men and their mean age was 61.7±9.6 years. The demographic characteristics of the patients and spirometric values are summarised in the table. The average FEV_1 of the study population was 1.22±0.52 liters.

Table. Demographics, spirometry data and PHQ-9 score of the study population

Characteristics	Stage I	Stage II	Stage III	Stage IV
No. of subjects	6	32	40	22
Age (years)	63.0±10.4	64.8±8.9	61.4±10.6	57.5±7.2
FVC (L)	3.69±0.88	2.98±0.63	2.39±0.51	2.01±0.43
FEV_1 (L)	2.22±0.37	1.59±0.40	1.06±0.25	0.68±0.17
Mean PHQ-9 Score	12.7±3.6	13.0±5.4	15.5±3.6	16.8±4.6*

*p<0.009; FVC=Forced vital capacity; FEV_1=Forced expiratory volume in one second; PHQ-9=Patient health questionnaire-9

The mean PHQ-9 score for the entire study population was 14.8±4.7. Seventeen out of the 100 patients (17%) had PHQ-9 scores of 20 or more, suggestive of severe depression. Figure 1 shows the PHQ-9 scoring severity in different stages of COPD. The mean PHQ-9 scores increased significantly with increasing severity of COPD (p=0.009). The prevalence of major depression and other depression in stages I, II, III and IV were 83.3%, 56.3%, 72.5% and 86.4%, respectively (Figure 2).

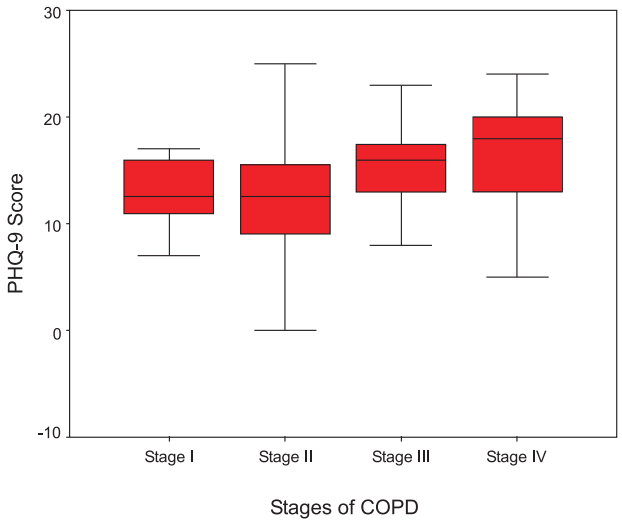

Figure. 1. PHQ-9 scoring severity in different stages of COPD.

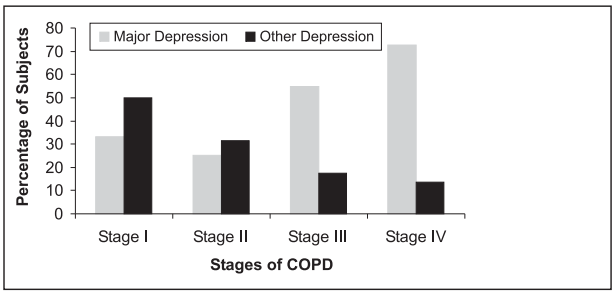

Figure 2. Prevalence of depressions in different COPD stages.

The cumulative prevalence of depression (both major depression and other depression) in the present study was 72% and prevalence of depression increases with the severity of COPD (p=0.024).

DISCUSSION

In the present study, we investigated the prevalence of undiagnosed depression in different stages of COPD patients. *To the best of our knowledge, this is the first study to evaluate the prevalence of depression in patients with COPD from India.* The prevalence of depression in the present study is 72 percent. The raised mean PHQ-9 (14.66±4.5) scoring in all stages of COPD indicates that most of the subjects are suffering from subclinical depression irrespective of COPD severity. In a review of three studies, Solano *et al*[10] had observed the prevalence of depression ranged from 37% to 71% of COPD patients and the cumulative prevalence rate of depression in our study is comparable with their results.

Chronic obstructive pulmonary disease is a leading cause of morbidity and the prevalence of COPD is rising. Co-morbid psychiatric and physical illness presents a unique health-care challenge for the respiratory physician. The risk of developing depression in COPD is high compared to healthy individuals. The presence of unrecognised sub-clinical depression in patients with COPD is a major concern, as they are at the risk of developing major depression and may increase the burden of physical disability.[1] Several factors are attributed for developing depression in patients with COPD. Severe dyspnoea, progressive irreversible condition and associated hypoxia may responsible for organic causes of depression in severe COPD. In addition, advanced age, low socio-economic condition and the chronic nature of the disease may result in social isolation and leads to more depressive feelings.[2] Even after adjusting the severity of COPD, depression is responsible for fatigue, shortness of breath and disability.[4]

Several screening tests are available to diagnose depression in primary care settings. Overall sensitivity and specificity of these tests to detect depression are 84% (95% confidence interval [CI], 79% to 89%) and 72% (95% CI, 67% to 77%), respectively and, there are no significant differences between the screening tests.[11] The PRIME-MD is highly sensitive and has a reasonably good positive predictive value for screening for anxiety and depression, and this test is useful and an easily administered tool for primary care physicians.[12] The PHQ-9 diagnostic validity and symptom severity with clinician-detected severity have a good correlation (0.84).[6]

The prevalence of depression varies widely in different populations, which could be attributed to different ethnicity, different cultural backgrounds and heterogeneous demography of the study populations and different screening tools. Kunik *et al*[12] observed that the prevalence of depression is 70% using the PRIME-MD in 1,334 persons with chronic breathing disorders. However, all types of chronic breathing disorders (COPD, asthma and bronchiectasis) were included in their study. We have used specific well-defined inclusion criteria in the present study to eliminate other chronic respiratory illness and co-morbidities.

The prevalence rates of depression in general population of India varies from 21% to 83% and one large study[13] from urban area of south India had reported the prevalence of depression is 25.7% among population of more than 60 years of age. The prevalence of depression in Indian COPD is lacking. In a group of 13 in-patients with chest diseases, Singh *et al*[14] had observed the prevalence of depression as 53.8 percent. Prevalence of depression in a community has a strong relationship with low level of education, poor socio-economic conditions and advanced age. All the patients in the present study were from either low or middle socio-economic families and average annual income is less than US $ 3000. The relatively high prevalence of depression in our study population is possibly due to poverty, poor education and high prevalence of common mental disorders in general Indian population.[15]

Screening questionnaires for psychological impairment in COPD may be less precise since they include many somatic symptoms which occur as part of the disease or ageing process.[3] Questionnaires increase the likelihood of diagnosis of depression in high risk population. However, questionnaires to determine depressive disorders can result in over estimation and may not be the same as a clinically verified event. Wagena *et al*[16] failed to show any significant association between the severity of COPD and the level of depression. Whereas, Manen *et al*[17] observed that the patients with mild to moderate COPD severity are not at increased risk for depression but patient with severe COPD had 2.5 times (95% CI, 1.2 to 5.4) higher risk of depression. Present study showed that the prevalence of depression increases

with the severity of COPD. Cognitive behavioural therapy, pharmacotherapy and pulmonary rehabilitation all are useful for treating depression in patients with COPD. However, the evidence for the role of anti-depressant for depression in COPD is limited. Randomised controlled trials have shown pulmonary rehabilitation improve symptoms of anxiety and depression as a consequence of training-related gains in functional capacity.[18] None of the patients in the present study were treated with any anti-depressant or were participating in any pulmonary rehabilitation programme.

Smoking associated depression is highest among people who try to quit, followed by those who consider quitting and lowest among those who left smoking for more than one year.[19] The association of smoking and depression is due to nicotine dependency rather than smoking index.[20] The population in the present study did not participate in any smoking cessation programme and 78% of them stopped smoking due to their respiratory symptoms.

The limitations of the present study need to be mentioned. The samples were selected from a single centre and all subjects were males. The clinic based study may not represent population from any geographical area. Other than tobacco smoking, indoor and outdoor pollution, exposure to dust and fume and low socio-economic status plays an important role in pathogenesis of COPD especially in female from developing countries.[21] In the present study population, female patients with fixed airway obstruction had the history of exposure to other risk factors, but none were current or ex-smoker. Hence, female subjects were not enrolled in the study. Female patients with COPD are likely to have higher psychological impairment.[22]

Six patients with mild COPD were enrolled in the present study, as mild COPD patients usually do not seek regular medical advice. The prevalence of depression may be high even in mild COPD and that might increase their level of dyspnoea.[22] This may be the possible reason for the presence of relatively high depression in small number of patients with mild COPD in the present study.

CONCLUSIONS

Depressive symptoms are common in all stages of COPD and the prevalence of depression in Indian patients with COPD is high. Patients with COPD should be screened for depression and those with higher depression score should undergo further evaluation. Further studies involving larger number of subjects from several centres are required to study the prevalence of depression in Indian patients with COPD.

REFERENCES

1. Yohannes AM, Baldwin RC, Connolly MJ. Prevalence of sub-threshold depression in elderly patients with chronic obstructive pulmonary disease. *J Geriatric Psychiatry* 2003;18:412-6.
2. Bemt LVD, Schermer T, Bor H, Smink R, Baumgarten EW, Lucassen P, *et al*. The risk for depression co morbidity in patients with COPD. *Chest* 2009;135:108-14.
3. Hill K, Geist R, Goldstein RS, Lacasse Y. Anxiety and depression in end-stage COPD. *Eur Respir J* 2008;31:667-77.
4. Maurer J, Rebbapragada V, Borson S, Goldstein R, Kunik ME, Yohannes AM, *et al* for the ACCP Workshop Panel on Anxiety and Depression in COPD. Anxiety and depression in COPD: current understanding, unanswered questions, and research needs. *Chest* 2008;134:43-56.
5. Gudmundsson G, Gislason T, Janson C, Lindberg E, Ulrik CS, Brøndum E, *et al*. Depression, anxiety and health status after hospitalisation for COPD: a multicentre study in the Nordic countries. *Respir Med* 2006;100:87-93.
6. Spitzer RL, Kroenke K, Williams JBW and the Patient Health Questionnaire Primary Care Study Group. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. *JAMA* 1999;282:1737-44.
7. Kochhar PH, Rajadhyaska SS, Suvarna VR for PRIME-MD Study Group. Translation and validation of brief patient health questionnaire against DSM IV as a tool to diagnose major depressive disorder in Indian patients. *J Psotgrad Med* 2007;53:102-7.
8. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al*. Standardization of spirometry. *Eur Respir J* 2005;26:319-38.
9. Global Strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. Updated 2008. *Available at URL*: http:\\www.goldcopd.com (Accessed on April 4, 2009).
10. Solano JP, Gomes B, Higginson IJ. A comparison of symptom prevalence in far advanced cancer, AIDS, heart disease, chronic obstructive pulmonary disease and renal disease. *J Pain Symptom Manage* 2006;31:58-69.
11. Mulrow CD, Williams JW, Gerety MB, Ramirez G, Montiel OM, Kerber C. Case-finding instruments for depression in primary care settings. *Ann Intern Med* 1995;122:913-21.
12. Kunik ME, Roundy K, Veazey C, Souchek J, Richardson P, Wray NP, *et al*. Surprisingly high prevalence of anxiety and depression in chronic breathing disorders. *Chest* 2005; 127:1205-11.
13. Poongothai S, Pradeepa R, Ganesan A, Mohan V. Prevalence of depression in a large urban south Indian population: the Chennai Urban Rural Epidemiology Study (Cures-70). *PLoS ONE* 4(9): e7185. doi:10.1371/journal.pone.0007185.
14. Singh G, Sachdev JS, Kaur H. Prevalence of depression among medical in-patients. *Indian J Psychiatry* 1979;21: 274-8.
15. Avasthi A, Varma SC, Kulhare P, Nehra R, Grover S, Sharma S. Diagnosis of common mental disorders by using PRIME-MD patient health questionnaire. *Indian J Med Res* 2008;127:159-64.
16. Wagena EJ, Arrindell WA, Wouters EFM, Schayck CPV. Are patients with COPD psychologically distressed? *Eur Respir J* 2005;26:242-8.
17. Manen JGV, Bindels PJE, Dekker FW, Jzermans CJI, Zee JSV, Schadé E. Risk of depression in patients with chronic obstructive pulmonary disease and its determinants. *Thorax* 2002;57:412-6.

18. Güell R, Resqueti V, Sangenis M, Morante F, Martorell B, Casan P, *et al*. Impact of pulmonary rehabilitation on psychosocial morbidity in patients with severe COPD. *Chest* 2006;129:899-904.
19. Khaled SM, Bulloch A, Exner DV, Patten SB. Cigarette smoking, stages of change, and major depression in the Canadian population. *Can J Psychiatry* 2009;54:204-8.
20. Breslau N, Kilbey MM, Andreski P. Nicotine dependence, major depression and anxiety in young adults. *Arch Gen Psychiatry* 1991;48:1609-74.
21. Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. *Lancet* 2009;374:733-43.
22. Marco FD, Verga M, Reggente M, Morante F, Martorell B, Casan P, *et al*. Anxiety and depression in COPD: how much do they contribute to physical symptoms and functional status? *Respir Med* 2006;100:1767-74.

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Editor-in-Chief

Review Article

Immune Responses to Mycobacterial Antigens in Sarcoidosis: A Systematic Review

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ABSTRACT

From the time sarcoidosis has been described, there has always been a viewpoint that the disease is in some way related to tuberculosis (TB). Sarcoidosis is a granulomatous disease, which is likely a result of continued presentation of a poorly degradable antigen. *Mycobacterium tuberculosis* has been a very strong contender for this antigen. Besides the molecular studies demonstrating mycobacterial deoxyribonucleic acid (DNA) in the sarcoid tissue, assessment of immune responses against mycobacterial antigens provides a useful tool to study the role of mycobacteria in the pathogenesis of sarcoidosis. We reviewed the studies focussing on T-cell and B-cell responses to tubercular antigens in patients with sarcoidosis. Pooled data from various studies does provide a suggestive, though not unequivocal evidence in favour of mycobacteria as a cause of sarcoidosis. These findings not only reinforce the possible pathogenic role of mycobacterial antigens in sarcoidosis, but at the same time also limit the clinical utility of molecular and serological studies based on mycobacterial antigens in the differential diagnosis of TB from sarcoidosis, particularly in a country with high endemicity for TB.
[Indian J Chest Dis Allied Sci 2011;53:41-49]

Key words: Sarcoidosis, *Mycobacterium tuberculosis*, IGRA, T-cell responses, B-cell responses.

INTRODUCTION

Sarcoidosis is a granulomatous disease, which like all other granulomatous diseases is most likely a result of continued presentation of a poorly degradable antigen.[1] In a quest to identify this 'poorly degradable antigen', numerous aetiologic agents have been incriminated, both infective and non-infective.[2] Non-infective agents have been implicated because of their epidemiologic association,[3] but have not stood the test of time.[4] Examination of the sarcoid granuloma with an electron microscope and immunohistochemical techniques has identified structures similar to organisms, such as *Leptospira* species, *Mycoplasma* species, and *Propionibacterium* species.[2] Other micro-biological agents that have been implicated from time to time include herpes virus, retrovirus, *Chlamydia pneumoniae*, *Borrelia burgdorferi*, *Rickettsia helvetica*, and finally *Pneumocystis jirovecii*.[2,5] However, one of the strongest contender remains the *Mycobacterium*.[6-8]

The probability of a causative link between TB and sarcoidosis has intrigued physicions.[9] However, the inability to identify mycobacteria by histologic staining or culture from pathologic tissues of sarcoidosis continues to be one of the strongest arguments against a potential role for mycobacteria. A recent meta-analysis[10] showed that mycobacterial deoxyribonucleic acid (DNA) was present in 30% of sarcoid samples, although individual studies had reported detection rates from zero percent to 50 percent. It needs to be emphasised that most of these studies were published from countries with a low prevalence for TB. If indeed mycobacteria are aetiologically linked to sarcoidosis, then the detection rates for mycobacterial DNA in sarcoid samples should be higher in countries with a high prevalence of TB. In a recent prospective, case-control study from India[11] aimed at detection of mycobacterial DNA in patients with sarcoidosis, we have demonstrated mycobacterial DNA by polymerase chain reaction (PCR) for 65 kDa protein gene in 48% of samples (bronchoalveolar lavage [BAL] or biopsy) from freshly diagnosed patients of sarcoidosis. This reinforces the hypothesis of mycobacteria as a causative agent for sarcoidosis.

The factors that favour mycobacteria as a trigger for sarcoidosis include histopathological appearances of the granulomas,[1] reports of mycobacterial disease either existing before, during or after sarcoidosis,[12,13] and the finding of mycobacteria in occasional granulomas of sarcoidosis.[14-16] Passage experiments have also suggested that mycobacteria with

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characteristics of *Mycobacterium tuberculosis* may be the incriminating agent.[17-20] It has also been suggested that the organism might exist in a cell wall deficient L-form and may be difficult to isolate.[21] Recent studies on humoral immunity to mycobacterial antigens from sarcoidosis patients have renewed interest in a potential role of mycobacteria in sarcoidosis.[22] Assessment of immune responses against microbial proteins is a useful tool to study the role of mycobacteria in the pathogenesis of sarcoidosis.

In this review, we look into the immunopathogenesis of sarcoidosis in brief and review the studies focussing on T-cell and B-cell responses to tubercular antigens in patients with sarcoidosis. For the purpose of this review, we have performed a systematic search of the electronic databases — MEDLINE and EMBASE — for relevant studies published from 1965 till current date using the free text term: sarcoidosis. In addition, we reviewed our personal files. A total of 100 articles were reviewed in detail for the purpose of this review.

IMMUNOPATHOGENSIS OF SARCOIDOSIS

A non-caseating granuloma is the hallmark of sarcoidosis. The granulomatous reaction is a protective response to the inciting agent. It limits inflammation and protects the tissue. Granuloma formation briefly involves four stages. In the first stage, the inciting antigen comes in contact with antigen processing cells (APCs), which are usually the alveolar macrophages. These APCs then interact with CD4+ T-lymphocytes in the second stage to initiate the formation of a simple granuloma. In the third stage, there is a further recruitment of CD4+ T-lymphocytes and type 1 and type 2 helper responses are elicited, that lead to the final or fourth stage of formation and maintenance of complex granulomas. Macrophages differentiate to form epithelioid cells under the influence of cytokines. These epithelioid cells fuse to form multi-nucleated giant cells and gain secretory properties. Granulomas secrete many humoral substances including calcitriol and angiotensin converting enzyme. These events, however, are dependent on a susceptible genetic background described by a variety of functional polymorphisms.[23,24]

Extensive research over the past two decades has helped to delineate the sequence of events in the immunopathogenesis of sarcoidosis. The earliest event is the accumulation of CD4+ helper T cells and release of interleukin (IL)-2 in the alveoli and interstitium.[25-27] This is followed by a progressive and selective oligoclonal expansion of αβ T cells.[28] There is an increased *in situ* production of Th1 cell-derived cytokines (IL-2 and interferon [IFN]-γ) during granuloma formation.[29,30] The alveolar macrophages in sarcoidosis have immense secretory properties and there is an increased release of macrophage-derived cytokines (IL-1, IL-6, IL-8, IL-15, tumour necrosis factor [TNF]-α, IFN-γ, granulocyte macrophage-cerebrospinal fluid [GM-CSF]) and chemokines (RANTES, MIP-1α, IL-16). Most of these cytokines favour granuloma formation and lung damage.[31-34] The progression and maintenance of the granulomatous reaction is also favoured by the accumulation of these monocyte-macrophages with antigen-presenting cell capacity and expressing increased levels of activation markers (human leukocyte antigen [HLA]-DR, HLA-DQ, CD71) and adhesion molecules (CD49a, CD54, CD102). The chemo attractant cytokines, such as IL-8, IL-15, IL-16 and RANTES, recruit the CD4+ T cells from the peripheral blood to the site of inflammation, while IL-2 induces an *in situ* proliferation of these cells.[35,36] The mechanism(s) that result in spontaneous resolution or progression to chronic disease and fibrosis are unclear but may be linked to host susceptibility and genetic factors. The sarcoid granulomatous inflammation is characterised by an altered balance of Th1/Th2 responses with a dominant expression of Th1 cytokines (IFN-γ and IL-2) with low levels of expression of Th2 cytokines including IL-4 and IL-5.[27,37] The IL-12 contributes to proliferation of activated T cells in early disease. Elevated levels of IL-6 and IL-8 are reported in the BAL fluid of active sarcoidosis, and these may modify the disease process. The IL-15 may aid in the proliferation of T- and B- cells.[38] The IL-12 and IL-18 are also increased in the lungs of sarcoidosis, and they stimulate IFN-γ production.[27,39] Transforming growth factor (TGF)-β is an inhibitor of IL-12 and IFN-γ production. Its production is increased in patients undergoing remission of sarcoidosis, suggesting a key role for TGF-β in the down regulation of the granulomatous inflammation of sarcoidosis.[40]

IMMUNE RESPONSES AGAINST MYCOBACTERIAL ANTIGENS

Along with the detection of mycobacterial DNA in the sarcoid granulomas as described above, mycobacterial peptide fragments have also been demonstrated within sarcoidosis granulomas and both T-cell-mediated immune responses and B-cell-mediated humoral immune responses against these antigens have been reported. Identification of genes in the *M. tuberculosis* genome has enabled the detection of proteins such as 6-kDa early secreted antigenic target (ESAT-6) and the 10-kDa culture filtrate protein (CFP-10) encoded by genes located on the region of difference 1 (RD1).[41] These genes are not shared by Bacille-Calmette Guerin (BCG) strains and most non-tuberculous mycobacteria (except *M. kansasii*, *M. szulgai*, and *M. marinum*).[42] These antigens are highly specific indicators of *M. tuberculosis* complex infection, and

have been shown to elicit strong IFN-γ responses from the T cells of persons infected with *M. tuberculosis*.[43] Immune responses to these and several other antigens have been studied in sarcoidosis.

T-cell Responses

Sarcoidosis is characterised by polarised CD4+ T cells with a Th1 immunophenotype.[44] Dubaniewicz, *et al* studied *M. tuberculosis*-heat shock protein stimulated T-cell subsets and Th1/Th2 cytokine patterns in the peripheral blood mononuclear cell cultures from 22 sarcoidosis patients, 20 TB patients and 20 healthy controls.[44] Their results showed that mtb-hsp stimulation lead to increased levels of pro-inflammatory cytokines, TNF-α, and IL-6 in sera from sarcoidosis and TB patients in comparison with healthy controls. Moreover, sarcoidosis patients demonstrated the lowest levels of IL-4 and the highest levels of IL-10. Two different studies[45,46], identified Th1 immune responses in peripheral blood mononuclear cells (PBMCs) to ESAT-6, katG and superoxide dismutase (SodA) in 20/56 (*versus* 2/50 purified protein derivative [PPD] negative controls), 22/56 (*versus* 0/50 PPD negative controls) and 12/30 (*versus* 0/26 PPD negative controls), respectively in patients with sarcoidosis. These studies[45,46] suggested that the sarcoidosis immune response may be against mycobacterial virulence factors. This was further studied by cellular recognition patterns against virulence factors, such as antigen 85A (Ag85A), that can differentiate mycobacterial species.[47] In another study, T-cell responses to Ag85A (mycolyl transferase) were found in 15 of 25 patients with sarcoidosis as against 2 of 22 PPD negative controls.[48] Immune responses against Ag85A in sarcoidosis have confirmed that similar to patients with mycobacterial infections such as TB or leprosy, sarcoidosis patients recognised multiple distinct epitopes of Ag85A. However, Ag85A peptides recognised by sarcoidosis patients were distinct from the Ag85A peptides recognised by patients infected with *M. tuberculosis* or *M. leprae*. Another mycobacterial virulence factor, sodA, has been isolated from sarcoidosis specimens and its characterisation by molecular techniques have demonstrated nucleic acid sequences closest to *M. tuberculosis*, yet distinct. Further, peptides translated from these sequences has been shown to evoke Th-1 immunophenotype in the sarcoidosis PBMCs.[49]

T-cell immune responses against *M. tuberculosis* antigens have also been studied in BAL in sarcoidosis. Although the Th1 immune responses were present systemically, it was shown that katG-reactive CD4+ Th1 cells preferentially accumulated in the lung, indicating a compartmentalised response.[50] This study also demonstrated that circulating katG-reactive T cells were found in chronic active sarcoidosis but not in patients with inactive disease. Further, the responses were similar in patients with or without Lofgren's syndrome, and were not influenced by phenotypic, genetic, or prognostic characteristics. Similar loss of immune responses to mycobacterial virulence factors after resolution of TB has also been observed.[51] More recently, another study on diagnostic BAL in sarcoidosis[52] demonstrated similar compartmentalised immune responses, and that induction of innate immunity by toll-like receptor 2 contributes to the polarised Th1 immune response. Recognition was significantly absent from BAL fluid cells from patients with other lung diseases, including infectious granulomatous diseases.

The detection of T-cell responses against ESAT-6, katG, and SodA provides a mechanism for more in-depth analysis of pathogenesis of sarcoidosis. It suggests exposure of sarcoidosis patients to pathogenic mycobacterial species. These proteins are typically secreted during the stage of active mycobacterial replication, compared with expression of other proteins that are expressed when mycobacteria are in the latent state.[53] However, a recent study from Germany[54] failed to replicate these findings. It was observed that IFN-γ production in response to *ex vivo* contact with PPD, ESAT-6 or CFP-10 by mononuclear cells in the BAL or peripheral blood was comparable among patients with sarcoidosis and controls, but was less frequently observed in both groups compared to patients with TB. It has also been shown that mycobacterial ESAT-6 and katG are recognised by sarcoidosis CD4+ T cells when presented by known sarcoidosis susceptibility allele, DRB1*1101.[55] It is possible that the presence of mycobacterial infection or BCG vaccination in genetically predisposed host may be involved in the development of autoimmunity.[56]

Tuberculin Anergy and Interferon-Gamma Release Assays

Another clinically important phenomenon that occurs in sarcoidosis is the depression of delayed type hypersensitivity. Commonly utilised as a diagnostic tool, this is often impaired in active sarcoidosis and not seen when sarcoidosis resolves. Various mechanisms have been postulated to explain this phenomenon. In one of the earlier studies,[57] it was shown that the T-lymphocytes from blood and BAL of sarcoidosis patients had reduced proliferative responses to various antigens, such as PPD, *Candida* and tetanus. Similar weaker responses to PPD were demonstrated in patients with ocular sarcoidosis from Japan.[58] A subgroup of CD4+ T cells may account for this anergy by abolishing the IL-2 production and inhibiting the T-cell proliferation.[59,60] It is also suggested that the anergy state may be

related to diminished dendritic cell function.[61] Anergy to tuberculin skin test (TST) in sarcoidosis has also been well described from India. In our earlier study,[62] despite the high prevalence of latent TB infection (LTBI) in the population, most of the patients (95%) with sarcoidosis were 'TST negative' with 1 tuberculin units (TUs) PPD using a cut-off >10mm. A negative TST with a cut-off of 10mm reaction to 5 TU PPD had virtually 100% sensitivity for a diagnosis of sarcoidosis, making TST an important test in the diagnostic work-up of sarcoidosis. Tuberculin testing is widely applied in sarcoidosis, primarily to rule out TB as a differential diagnosis in countries with high prevalence of TB and to rule out LTBI prior to initiating these patients on therapies known to cause increased risk of reactivation of TB, such as TNF-α inhibitors.

Interferon-gamma release assays (IGRAs) have several advantages over the TST and are superior to TST in several groups of healthy individuals.[63-65] As the test is done *in vitro* and does not involve measurements such as skin induration, the results are less subjective, and a single visit by the patient is sufficient. Newer, RD1-based IGRAs are also thought to be more sensitive and specific than the PPD-based TST. The test is based on the principle that T cells from a whole blood sample, when exposed and incubated with a specific *M. tuberculosis* antigen (ESAT-6, CFP-10) will produce IFN- γ. These proteins are absent from all BCG strains and from most non-tuberculosis mycobacteria, making these tests very specific for *M. tuberculosis*. Carlisle and colleagues,[46] found a significant difference among the sarcoidosis and tuberculin negative controls to ESAT-6. Similarly, Drake and colleagues[45] detected Th1 immune responses to *M. tuberculosis* ESAT-6 and KatG peptides from peripheral blood mononuclear cells in sarcoidosis but peripheral anergy to PPD. In contrast, the responses of Japanese patients with sarcoidosis to QuantiFERON-TB Gold (QFT) using ESAT-6 and CFP-10 showed positivity rate of QuantiFERON-TB Gold in only 3.3 percent.[66] The differing responses to ESAT-6 and CFP-10 as measured by the two IGRAs used in Japanese study and previous study by Drake *et al*[45] may reflect the differing methodology used in the two tests.[67] In a recent study,[68] we have shown that there is high positivity of QFT in patients with sarcoidosis, which is similar to the positivity in healthy volunteers, reflecting the high population prevalence of LTBI in our country. In clinical practice, we have often observed that a diagnosis of TB in patients with sarcoidosis is made based on a positive QFT. Our studies have shown that there is an acquired tuberculin anergy in patients of sarcoidosis; however results of QFT are not similarly affected. The QFT being more sensitive test than the TST continues to remain positive in many patients with sarcoidosis, and thus, may be more accurate to detect LTBI in these patients. Also, in high TB prevalence countries, a negative TST had a better value in the diagnosis of sarcoidosis and a positive QFT should not be considered to rule out sarcoidosis.

B-cell Responses

Clear elucidation of the appropriate antigen and humoral responses to them in cohorts of TB and sarcoidosis patients promises a novel approach to study the relationship between these two granulomatous pathologies. Few investigators have reported on the detection and humoral response of sarcoidosis patients to *M. tuberculosis* antigens. In one of the earliest reports, Levy *et al*[68] had reported that sarcoidosis patients showed lower values of mean optical density of serum immunoglobulin (Ig)-G using adsorbed mycobacterial sonicates as antigens when compared to active TB. Song *et al*[22] found anti-mycobacterial katG IgG in the sera of 12 of 25 (48%) patients with sarcoidosis subjects compared with 0 of 11 PPD-negative healthy controls.[22] Using matrix-assisted laser desorption ionisation time-off flight (MALDI-TOF) mass spectrometry, they identified peptide sequences that corresponded to *M. tuberculosis* katG and one potential match for *M. tuberculosis* topoisomerase. In a separate sarcoidosis specimen, they found four of 26 peptides that corresponded to the katG of *M. smegmatis*.[22] Moller[69] also reported that myco-bacterial KatG was detectable in Kviem's reagent and that IgG responses to recombinant mKatG was detectable in more than 50% of patients with sarcoidosis but rarely in PPD-negative controls. In another study of immune responses to mycobacterial proteins among patients with sarcoidosis, TB and BCG-vaccinated controls; Dubaniewicz and colleagues[70] reported that 12 of 37 patients with sarcoidosis demonstrated a humoral response to *M. Tuberculosis*-heat shock protein 70 (hsp70), compared with none of the 18 controls, and six of 29 TB patients.

The humoral response in sarcoidosis against RD1 antigens of *M. tuberculosis* has rarely been studied despite numerous studies of T-cell responses to RD1 antigens. There are several drawbacks of using IGRAs in developing countries to assess the relationship between TB and sarcoidosis, and distinguishing between them based on these responses. These include the high-cost, necessity of technical expertise, utilisation/separation of a living cell, and lack of widespread availability given that LTBI is not treated in the tropics. Besides, serologic responses to ESAT-6 as evidenced by antibody levels and detection of antigens may reflect a qualitatively different aspect of the immunopathogenesis in sarcoidosis as opposed to IGRAs. We evaluated the antibodies against RD1 (ESAT-6 and CFP-10) antigens in serum of patients

with sarcoidosis and demonstrated a high prevalence of antibodies against RD1 antigens in patients with sarcoidosis. The overall reactivity for any antigen was seen in 44.4% sarcoidosis samples and if only PPD-patients were used as controls, the positivity rose to 61 percent. None of the PPD positive cases were positive either for ESAT-6 or CFP-10 antibodies by the PPD negative cut-off.[71]

IMPLICATIONS AND FUTURE DIRECTIONS

Pooled data from various studies discussed above does provide a suggestive, though not unequivocal evidence in favour of mycobacteria as a cause of sarcoidosis (Table and Figures 1 and 2). Studies on the recognition of mycobacterial antigens and host T-cell and B-cell responses to them have generated many interesting hypotheses in understanding the complex immunopathogenesis of sarcoidosis and the role of mycobacteria in them. So much so, in a recent computer simulation study,[71] it was suggested that Lofgren's syndrome represents the hyper-reactive end of a spectrum of granulomatous responses to specific mycobacteria, whereas pulmonary TB and atypical mycobacterial infections might represent the opposite end. Similarly, du Bois *et al*[4] hypothesised that analogous to leprosy wherein tuberculoid (pauci-bacillary) and lepromatous (exuberant bacilli) forms represent different levels of host immune responses to the pathogen; sarcoidosis might represent the tuberculoid form of the pathological responses to mycobacteria.

There have been counter arguments to the role of mycobacteria in pathogenesis of sarcoidosis and the major points among them are: (1) *Mycobacteria* spp have never been cultured from sarcoidosis lesions; (2) the absence of nucleic acid in 50% of patients; and (3) the absence of a response to anti-tuberculosis treatment together with tuberculin anergy. However, studies do suggest that mycobacteria are a trigger, if

Table. Summary of major studies on mycobacterial antigens in sarcoidosis

Author (year)	Experimental Details	Positivity in Sarcoidosis % (n/N)	Positivity in Controls		Significance
			PPD+/Tuberculosis % (n/N)	PPD- % (n/N)	
T-cell responses					
Nishino *et al* (2000)[58]	IFN-γ by PBMCs in response to PPD	14	10	-	Weaker but similar responses to controls
Carlisle *et al* (2007)[46]	Th1 immune responses in PBMCs to: (a) ESAT-6; (b) katG; (c) SodA	(a) 40 (12/30) (b) 30 (9/30) (c) 40 (12/30)	(a) 60 (6/10)** (b) 50 (5/10)** (c) 60 (6/10)**	(a) 3.8 (1/26)* (b) 0 (0/26)* (c) 0 (0/26)*	*(a) P=0.0014; *(b) and (c) P= 0.002; ** NS
Drake *et al* (2008)[45]	Th1 immune responses in PBMCs to: ESAT-6, mkatG	57.7 (15/26)	87.5 (7/8)**	4.2 (1/24)*	* P<0.001; **P=0.21
Inui *et al* (2007)[66]	IGRA in response to ESAT-6 and CFP-10 (QFT) in blood	3.33 (3/90)	–	–	Low prevalence of T-cell response
Dubaniewicz *et al* (2007)[44]	T-cell subsets and Th1/Th2 cytokine patterns in response to Mtb-hsp	22	20	20	Increased IL-10 and decreased IL-4 in sarcoidosis
Chen *et al* (2008)[50]	T-cell responses to mKatG in PBMCs and BALMCs	Higher median spot forming cells			Higher as compared to PPD-controls and higher in BAL as compared to PBMCs
Horster *et al* (2009)[54]	IGRA in response to ESAT-6 and CFP-10 by: (a) PBMCs; (b) BALMCs	(a) 29.4 (5/17) (b) 46.7 (7/15)	(a) 93.9 (31/33)** (b) 84.4 (27/32)**	(a) 48.6 (17/35)* (b) 24 (7/29)*	*(a) P=0.23; *(b) P=0.17; ** (a) P< 0.05; **(b) P<0.001
Oswald-Richter *et al* (2009)[52]	T-cell responses in BAL to: ESAT-6, mkatG	72.7 (32/44)	–	3.7 (1/27)*	* P<0.001
Gupta *et al* (2010)[68]	IGRA in response to ESAT-6 and CFP-10 (QFT) in blood	30 (9/30)	80.9 (9/11)**	30.1 (5/16)	* P=0.03; ** P=0.009
B-cell responses					
Levy *et al* (1988)[69]	Anti TB IgG by ELISA to adsorbed mycobacterial sonicates	11	7	7	TB patients had significantly higher mean OD for IgG compared to sarcoidosis or controls
Song *et al* (2005)[22]	IgG antibodies to recombinant mKatG	48 (12/45)	40 (4/10)**	0 (0/11)*	*P=0.005; **P=0.72
Dubaniewicz *et al* (2006)[71]	Serum anti-Mtb-hsp70 antibodies	32.4 (12/37)	20.6 (2/29)**	0 (0/18)*	*P=.000; **P=0.43
Hajizadeh *et al* (2007)[48]	Recognition of mycobacterial antigen 85A by the PBMCs	60 (15/25)	87.5 (14/16)**	9.1 (2/22)*	*P=0.0006; **P=0.08
Agarwal *et al* (2009)[72]	Antibodies against RD1 (ESAT-6 and CFP-10) antigens in serum	61.1 (11/18)	90 (9/10)**	0 (0/20)*	*P=0.0002; **P=0.24

PPD=Purified protein derivative, IFN-γ=Interferon-gamma, PBMCs=Peripheral blood monocytes, ESAT-6=Early secretory antigen-6, SodA=Superoxide dismutase A, mkatG=Mycobacterial catalase-peroxidase, IGRAs=Interferon-gamma release assays, CFP-10=Culture filtrate protein-10, QFT=Quanti FERON-TB, IL=Interleukin, BALMCs=Bronchoalveolar lavage monocytes, BAL=Bronchoalveolar lavage, TB=Tuberculosis, IgG=Immunoglobulin G, ELISA=Enzyme linked immunosorbent assay, OD=Optial density, Mtb-hsp70=*Mycobacterium tuberculosis*-heat shock protein 70, RD1=Region of differentiation 1

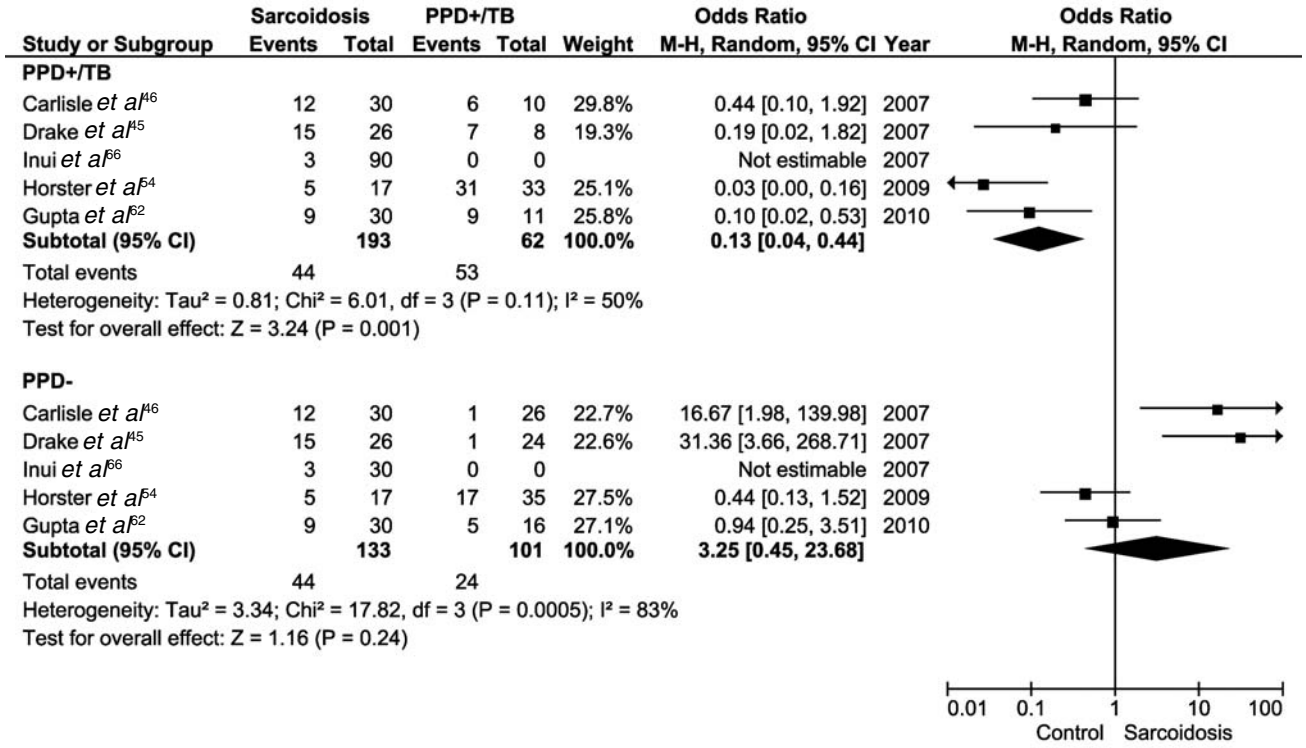

Figure 1. Forest plot (odds ratio [OR], 95% confidence interval [CI]) displaying the studies that have assessed T-cell responses against RD1 antigens (ESAT-6, CFP-10) in patients with sarcoidosis compared to PPD+/TB patients and PPD- controls. The plot shows that T-cell responses are significantly demonstrable in PPD+/TB compared to sarcoidosis and there is a trend towards higher responses in sarcoidosis as compared to PPD- controls (random effects model).

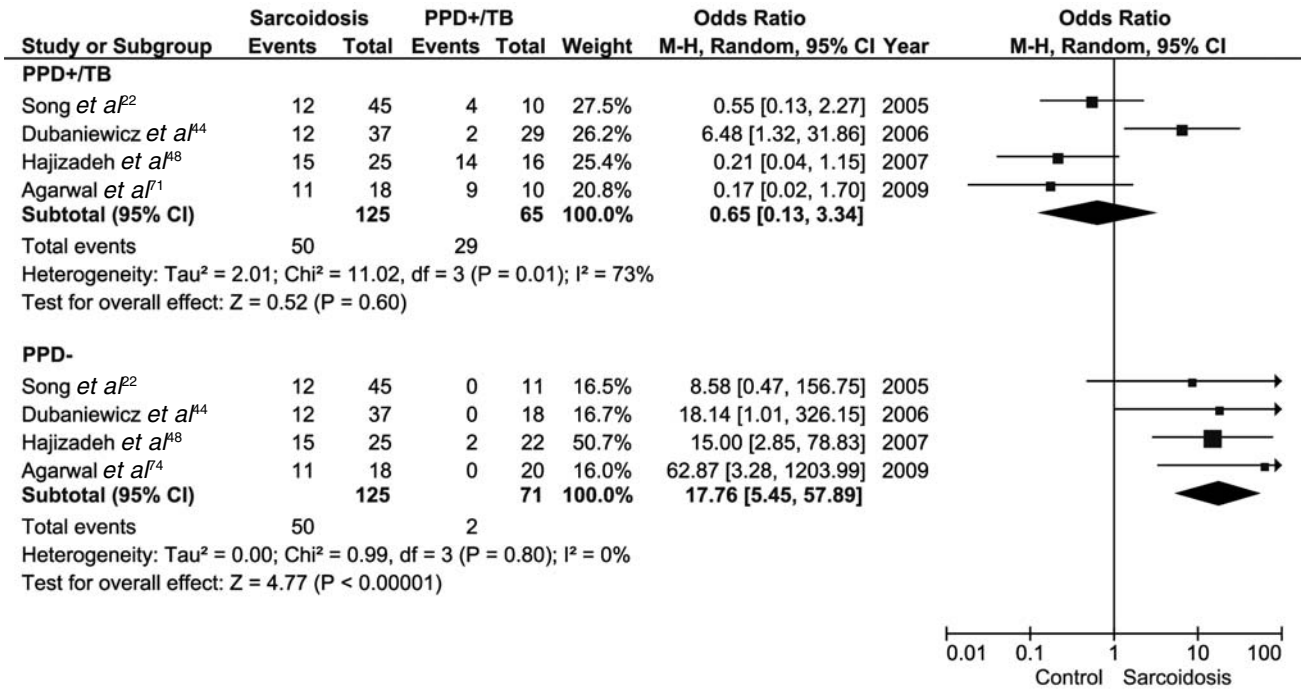

Figure 2. Forest plot (odds ratio [OR], 95% confidence interval [CI]) displaying the studies that have assessed B-cell responses against specific mycobacterial proteins (ESAT-6, CFP-10, katG, sodA) in patients with sarcoidosis compared to PPD+/TB patients and PPD- controls. The plot shows that humoral responses are significantly demonstrable in sarcoidosis compared to PPD- controls and are similar in sarcoidosis compared to PPD+/TB subjects (random effects model).

not an infection, and therefore there may not necessarily be a response to anti-tubercular therapy.

Further research, in addition to identifying microbial species that may have a role in the pathogenesis of sarcoidosis, should also focus on identification of microbial proteins that contribute to sarcoidosis resolution or disease progression. Future molecular efforts should delineate whether the nucleic acids or proteins detected reflect actively replicating organisms or persistent proteins. Focus on other microbial virulence factors would also provide greater insight into pathogenic mechanisms of sarcoidosis and also identify likely therapeutic targets.

To conclude, even if there is no clear evidence to support the contention that tubercle bacillus causes sarcoidosis, the various studies discussed above reinforce the possible pathogenic role of mycobacterial antigens in sarcoidosis. At the same time, these also limits the clinical value of the molecular and serological studies based on mycobacterial antigens in the differential diagnosis of TB from sarcoidosis, particularly in a country like India with high endemicity for TB.

REFERENCES

1. Perez RL, Rivera-Marrero CA, Roman J. Pulmonary granulomatous inflammation: from sarcoidosis to tuberculosis. *Semin Respir Infect* 2003;18:23-32.
2. Newman LS. Aetiologies of sarcoidosis. *Eur Respir Mon* 2005;32:23-48.
3. Thomeer M, Demedts M, Wuyts W. Epidemiology of sarcoidosis. *Eur Respir Mon* 2005;32:13-22.
4. du Bois RM, Goh N, McGrath D, Cullinan P. Is there a role for microorganisms in the pathogenesis of sarcoidosis? *J Intern Med* 2003;253:4-17.
5. Vidal S, de la Horra C, Martin J, Montes-Cano MA, Rodriguez E, Respaldiza N, *et al*. *Pneumocystis jirovecii* colonisation in patients with interstitial lung disease. *Clin Microbiol Infect* 2006;12:231-5.
6. Drake WP, Newman LS. Mycobacterial antigens may be important in sarcoidosis pathogenesis. *Curr Opin Pulm Med* 2006;12:359-63.
7. Ishige I, Eishi Y, Takemura T, Kobayashi I, Nakata K, Tanaka I, *et al*. Propionibacterium acnes is the most common bacterium commensal in peripheral lung tissue and mediastinal lymph nodes from subjects without sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;22:33-42.
8. Oswald-Richter KA, Drake WP. The etiologic role of infectious antigens in sarcoidosis pathogenesis. *Semin Respir Crit Care Med* 2010;31:375-9.
9. Sharma OP, Murray Kornfeld, American College of Chest Physicians, and sarcoidosis: a historical footnote: 2004 Murray Kornfeld Memorial Founders Lecture. *Chest* 2005; 128:1830-5.
10. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. *Eur Respir J* 2007;30:508-16.
11. Mootha VK, Agarwal R, Aggarwal AN, Gupta D, Ahmed J, Verma I, *et al*. The sarcoid-tuberculosis link: evidence from a high TB prevalence country. *J Infect* 2010; 60:501-3.
12. Kent DC, Houk VN, Elliott RC, Sokolowski JW Jr, Baker JH, Sorensen K. The definitive evaluation of sarcoidosis. *Am Rev Respir Dis* 1970;101:721-7.
13. Hatzakis K, Siafakas NM, Bouros D. Miliary sarcoidosis following miliary tuberculosis. *Respiration* 2000;67:219-22.
14. Vanek J, Schwarz J. Demonstration of acid-fast rods in sarcoidosis. *Am Rev Respir Dis* 1970;101:395-400.
15. Cantwell AR Jr. Variably acid-fast bacteria in a case of systemic sarcoidosis and hypodermitis sclerodermiformis. *Dermatologica* 1981;163:239-48.
16. Cantwell AR Jr. Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis: a report of 3 cases. *Growth* 1982;46:113-25.
17. Mitchell DN, Rees RJ. A transmissible agent from sarcoid tissue. *Lancet* 1969;2:81-4.
18. Mitchell DN, Rees RJ. An attempt to demonstrate a transmissible agent from sarcoid material. *Postgrad Med J* 1970;46:510-4.
19. Mitchell DN, Rees RJ, Goswami KK. Transmissible agents from human sarcoid and Crohn's disease tissues. *Lancet* 1976;2:761-5.
20. Mitchell DN. The nature and physical characteristics of a transmissible agent from human sarcoid tissue. *Ann N Y Acad Sci* 1976;278:233-48.
21. Almenoff PL, Johnson A, Lesser M, Mattman LH. Growth of acid fast L forms from the blood of patients with sarcoidosis. *Thorax* 1996;51:530-3.
22. Song Z, Marzilli L, Greenlee BM, Chen ES, Silver RF, Askin FB, *et al*. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. *J Expir Med* 2005;201:755-67.
23. Rybicki BA, Hirst K, Iyengar SK, Barnard JG, Judson MA, Rose CS, *et al*. A sarcoidosis genetic linkage consortium: the sarcoidosis genetic analysis (SAGA) study. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;22:115-22.
24. Schurmann M. Genetics of sarcoidosis. *Semin Respir Crit Care Med* 2003;24:213-22.
25. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med* 1981;305:429-34.
26. Semenzato G, Pezzutto A, Chilosi M, Pizzolo G. Redistribution of T lymphocytes in the lymph nodes of patients with sarcoidosis. *N Engl J Med* 1982;306:48-9.
27. Grunewald J, Eklund A. Role of CD4+ T cells in sarcoidosis. *Proc Am Thorac Soc* 2007;4:461-4.
28. Silver RF, Crystal RG, Moller DR. Limited heterogeneity of biased T-cell receptor V beta gene usage in lung but not blood T cells in active pulmonary sarcoidosis. *Immunology* 1996;88:516-23.
29. Konishi K, Moller DR, Saltini C, Kirby M, Crystal RG. Spontaneous expression of the interleukin 2 receptor gene and presence of functional interleukin 2 receptors on T lymphocytes in the blood of individuals with active pulmonary sarcoidosis. *J Clin Invest* 1988;82:775-81.
30. Robinson BW, McLemore TL, Crystal RG. Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. *J Clin Invest* 1985;75:1488-95.
31. Baughman RP, Strohofer SA, Buchsbaum J, Lower EE. Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. *J Lab Clin Med* 1990;115:36-42.
32. Moller DR, Forman JD, Liu MC, Noble PW, Greenlee BM, Vyas P, *et al*. Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. *J Immunol* 1996;156:4952-60.
33. Agostini C, Trentin L, Facco M, Sancetta R, Cerutti A, Tassinari C, *et al*. Role of IL-15, IL-2, and their receptors in the development of T cell alveolitis in pulmonary sarcoidosis. *J Immunol* 1996;157:910-8.
34. Kreipe H, Radzun HJ, Heidorn K, Barth J, Kiemle-Kallee J, Petermann W, *et al*. Proliferation, macrophage colony-

stimulating factor, and macrophage colony-stimulating factor-receptor expression of alveolar macrophages in active sarcoidosis. *Lab Invest* 1990;62:697-703.
35. Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N Engl J Med* 1983;308:793-800.
36. Hunninghake GW, Bedell GN, Zavala DC, Monick M, Brady M. Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am Rev Respir Dis* 1983;128:634-8.
37. Agostini C, Adami F, Semenzato G. New pathogenetic insights into the sarcoid granuloma. *Curr Opin Rheumatol* 2000;12:71-6.
38. Girgis RE, Basha MA, Maliarik M, Popovich J Jr, Iannuzzi MC. Cytokines in the bronchoalveolar lavage fluid of patients with active pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1995;152:71-5.
39. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, *et al*. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs. *J Immunol* 2001;166:642-9.
40. Bingisser R, Speich R, Zollinger A, Russi E, Frei K. Interleukin-10 secretion by alveolar macrophages and monocytes in sarcoidosis. *Respiration* 2000;67:280-6.
41. Berthet FX, Rasmussen PB, Rosenkrands I, Andersen P, Gicquel B. A Mycobacterium tuberculosis operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). *Microbiology* 1998;144:3195-203.
42. Lalvani A, Nagvenkar P, Udwadia Z, Pathan AA, Wilkinson KA, Shastri JS, *et al*. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. *J Infect Dis* 2001;183:469-77.
43. Kunst H. Diagnosis of latent tuberculosis infection: the potential role of new technologies. *Respir Med* 2006; 100:2098-106.
44. Dubaniewicz A, Trzonkowski P, Dubaniewicz-Wybieralska M, Singh M, Mysliwski A. Mycobacterial heat shock protein-induced blood T lymphocytes subsets and cytokine pattern: comparison of sarcoidosis with tuberculosis and healthy controls. *Respirology* 2007;12:346-54.
45. Drake WP, Dhason MS, Nadaf M, Shepherd BE, Vadivelu S, Hajizadeh R, *et al*. Cellular recognition of Mycobacterium tuberculosis ESAT-6 and KatG peptides in systemic sarcoidosis. *Infect Immun* 2007;75:527-30.
46. Carlisle J, Evans W, Hajizadeh R, Nadaf M, Shepherd B, Ott RD, *et al*. Multiple Mycobacterium antigens induce interferon-gamma production from sarcoidosis peripheral blood mononuclear cells. *Clin Exp Immunol* 2007;150:460-8.
47. Launois P, DeLeys R, Niang MN, Drowart A, Andrien M, Dierckx P, *et al*. T-cell-epitope mapping of the major secreted mycobacterial antigen Ag85A in tuberculosis and leprosy. *Infect Immun* 1994;62:3679-87.
48. Hajizadeh R, Sato H, Carlisle J, Nadaf MT, Evans W, Shepherd BE, *et al*. Mycobacterium tuberculosis antigen 85A induces Th-1 immune responses in systemic sarcoidosis. *J Clin Immunol* 2007;27:445-54.
49. Allen SS, Evans W, Carlisle J, Hajizadeh R, Nadaf M, Shepherd BE, *et al*. Superoxide dismutase A antigens derived from molecular analysis of sarcoidosis granulomas elicit systemic Th-1 immune responses. *Respir Res* 2008;9:36.
50. Chen ES, Wahlstrom J, Song Z, Willett MH, Wiken M, Yung RC, *et al*. T cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. *J Immunol* 2008;181:8784-96.
51. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, Pasvol G, *et al*. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment. *J Immunol* 2001;167:5217-25.
52. Oswald-Richter KA, Culver DA, Hawkins C, Hajizadeh R, Abraham S, Shepherd BE, *et al*. Cellular responses to mycobacterial antigens are present in bronchoalveolar lavage fluid used in the diagnosis of sarcoidosis. *Infect Immun* 2009;77:3740-8.
53. Andersen P. The T cell response to secreted antigens of Mycobacterium tuberculosis. *Immunobiology* 1994; 191: 537-47.
54. Horster R, Kirsten D, Gaede KI, Jafari C, Strassburg A, Greinert U, *et al*. Antimycobacterial immune responses in patients with pulmonary sarcoidosis. *Clin Respir J* 2009; 3:229-38.
55. Oswald-Richter K, Sato H, Hajizadeh R, Shepherd BE, Sidney J, Sette A, *et al*. Mycobacterial ESAT-6 and katG are recognized by sarcoidosis CD4+ T cells when presented by the American sarcoidosis susceptibility allele, DRB1*1101. *J Clin Immunol* 2010;30:157-66.
56. Dubaniewicz A. Mycobacteriumtuberculosis heat shock proteins and autoimmunity in sarcoidosis. *Autoimmun Rev* 2010;9:419-24.
57. Lecossier D, Valeyre D, Loiseau A, Cadranel J, Tazi A, Battesti JP, *et al*. Antigen-induced proliferative response of lavage and blood T lymphocytes: comparison of cells from normal subjects and patients with sarcoidosis. *Am Rev Respir Dis* 1991;144:861-8.
58. Nishino K, Yoshida H, Yoshida O, Watanabe M, Fukushima A, Ueno H. Analysis of responses of peripheral blood lymphocytes from sarcoidosis patients to purified protein derivative. *Jpn J Ophthalmol* 2000;44:165-70.
59. Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, *et al*. The immune paradox of sarcoidosis and regulatory T cells. *J Expir Med* 2006;203:359-70.
60. Hudspith BN, Flint KC, Geraint-James D, Brostoff J, Johnson NM. Lack of immune deficiency in sarcoidosis: compartmentalisation of the immune response. *Thorax* 1987;42:250-5.
61. Mathew S, Bauer KL, Fischoeder A, Bhardwaj N, Oliver SJ. The anergic state in sarcoidosis is associated with diminished dendritic cell function. *J Immunol* 2008;181:746-55.
62. Gupta D, Chetty M, Kumar N, Aggarwal AN, Jindal SK. Anergy to tuberculin in sarcoidosis is not influenced by high prevalence of tuberculin sensitivity in the population. *Sarcoidosis Vasc Diffuse Lung Dis* 2003;20:40-5.
63. Hardy AB, Varma R, Collyns T, Moffitt SJ, Mullarkey C, Watson JP. Cost-effectiveness of the NICE guidelines for screening for latent tuberculosis infection: the QuantiFERON-TB Gold IGRA alone is more cost-effective for immigrants from high burden countries. *Thorax* 2010; 65:178-80.
64. Winje BA, Oftung F, Korsvold GE, Mannsaker T, Ly IN, Harstad I, *et al*. School based screening for tuberculosis infection in Norway: comparison of positive tuberculin skin test with interferon-gamma release assay. *BMC Infect Dis* 2008;8:140.
65. Lee SS, Liu YC, Huang TS, Chen YS, Tsai HC, Wann SR, *et al*. Comparison of the interferon-gamma release assay and the tuberculin skin test for contact investigation of tuberculosis in BCG-vaccinated health care workers. *Scand J Infect Dis* 2008;40:373-80.
66. Inui N, Suda T, Chida K. Use of the QuantiFERON-TB Gold test in Japanese patients with sarcoidosis. *Respir Med* 2008;102:313-5.
67. Ferrara G, Losi M, D'Amico R. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet* 2006;367:1328-34.

68. Gupta D, Kumar S, Verma I, Agarwal R. Interferon gamma release assay (IGRA) in sarcoidosis patients from a high tuberculosis (TB) prevalence country. *Am J Respir Crit Care Med* 2010;181:A2366.
69. Levy H, Feldman C, Wadee AA, Rabson AR. Differentiation of sarcoidosis from tuberculosis using an enzyme-linked immunosorbent assay for the detection of antibodies against Mycobacterium tuberculosis. *Chest* 1988;94:1254-5.
70. Moller DR. Potential etiologic agents in sarcoidosis. *Proc Am Thorac Soc* 2007;4:465-8.
71. Dubaniewicz A, Kampfer S, Singh M. Serum anti-mycobacterial heat shock proteins antibodies in sarcoidosis and tuberculosis. *Tuberculosis (Edin)* 2006;86:60-7.
72. Agarwal R, Gupta D, Srinivas R, Verma I. Role of proteins encoded by RD1 of Mycobacterium tuberculosis in differentiating tuberculosis (TB) from sarcoidosis in a high TB prevalence country. *Chest* 2009;136:44S-f-
73. Saltini C, Pallante M, Puxeddu E, Contini S, Voorter CE, Drent M, *et al*. M. avium binding to HLA-DR expressed alleles in silico: a model of phenotypic susceptibility to sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2008;25:100-16.

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Case Report

Transthoracic Decompression of Emphysematous Bulla: A Novel Experience

P. Bhattacharyya[1], S. Bardhan[1], S. Nag[1], S. Mukherjee[2] and A. Verma[3]

Institute of Pulmocare and Research[1], and Departments of Cardiothoracic Surgery[2] and Anaesthesia[3], Calcutta Medical College, Kolkata, India

ABSTRACT

Emphysematous bullae are closed air containing spaces in lung parenchyma that may severely compromise lung function in patients of chronic obstructive pulmonary disease (COPD). We describe a simple and minimally invasive procedure to decompress a large emphysematous bullae in a patient with advanced COPD and high surgical risk. Transthoracic decompression of the bulla was accomplished under short-acting anaesthesia and muscle relaxation resulting in significant symptomatic, radiological and functional improvement. **[Indian J Chest Dis Allied Sci 2011;53:51-53]**

Key words: COPD, Emphysema, Chronic bronchitis, Transthoracic decompression.

INTRODUCTION

Bullae are closed air containing spaces in the lung parenchyma.[1,2] At times, bullae may be large enough to produce symptoms and functional disability. Simultaneous compression atelectasis of surrounding normal parenchyma further jeopardises the lung function.[3,4] Hence, management of emphysematous bulla often becomes very important. Surgery happens to be the most commonly practiced definitive therapy for bullae.[3] However, it has significant intra- and post-operative morbidity and mortality due to generally poor lung functions.[4] Video assisted thoracoscopy has been used successfully for the resection of emphysematous bullae. However, intra-operative air leakage has proven to be a frequent problem with this procedure.[5] Intra-cavitatory drainage has been attempted earlier. However, the procedure resulted in the development of prolonged air leaks, further necessitating fibreoptic intrabronchial intervention.[6] Options are limited for patients who refuse surgery or are unfit for it.

We report here a case of a patient having multiple emphysematous bullae with stage IV COPD who was treated with transthoracic decompression.

CASE REPORT

A 58-year-old male, a known case of very severe COPD (stage IV) with multiple emphysematous bullae, was treated with a novel technique of transbronchial decompression of the largest bulla, five years back. The patient had significant improvement in symptoms and lung function parameters following the procedure and was doing quite well. However in the last six months, he had recurrent episodes of acute exacerbations with a rapid down-hill course over the last four weeks. Clinical deterioration was to such an extent that the patient was totally confined within his premises and could barely manage to carry out his personal care. His lung function had deteriorated significantly (forced expiratory volume in one second [FEV_1] less than 0.44L and the ratio of FEV_1 and forced vital capacity [FEV_1/FVC] less than 0.33) with a resting oxygen saturation (SaO_2) of around 90% breathing room air. Chest radiograph (Figure 1) and high resolution computed

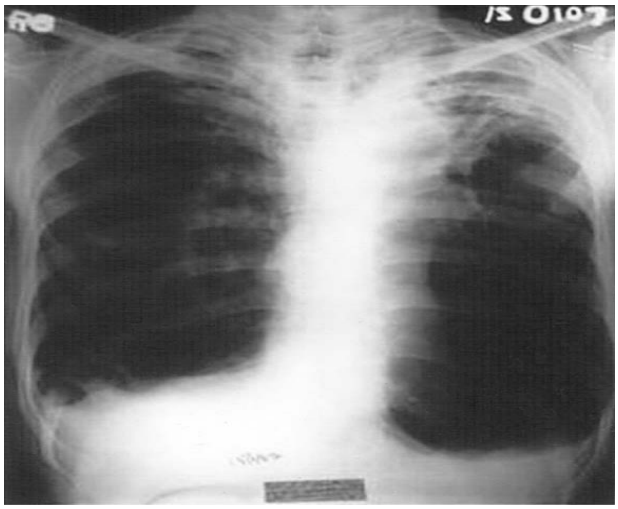

Figure 1. Chest radiograph showing a large bulla in the left lower zone with concavity of the left dome of diaphragm.

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tomography (HRCT) of thorax (Figure 2) showed a huge bulla in the left lower zone with inversion of the ipsilateral diaphragm. He was hospitalised on further deterioration of his clinical status. A repeat transbronchial decompression was attempted but failed. A transthoracic decompression was then planned since the surgical options were limited due to high risk.

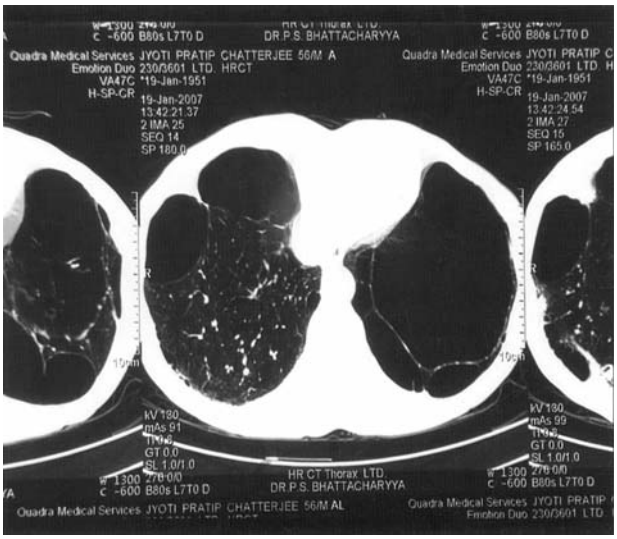

Figure 2. Pre-procedure HRCT showing a large bulla in the left lower lobe.

The proposed procedure was discussed with the patient and his relatives and the risks of thoracic surgery as an alternative mode of therapy were also explained. After an informed consent, we planned for a transthoracic decompression under short-acting anaesthesia with a cardio-thoracic surgery team on stand-by for emergency support.

In the operation theatre, the patient was oxygenated with low flow oxygen to maintain oxygen saturation levels around 98 percent to 100 percent. The prospective site for puncture was determined from the chest radiograph and HRCT views. Deep sedation was induced by propofol and a short-lasting muscle relaxation was attained with succinylcholine. Propofol being a sedative hypnotic with a rapid onset and termination of action, best suited our purpose. Thereafter, ventilation was maintained by an anaesthetic mask connected to Bain's Circuit with supplementation of 100% oxygen. Once deeply sedated and relaxed, a small (22 gauge) needle was used to puncture the bulla transthoracically at the selected site under aseptic measures. A guide wire was then passed through the needle and a paediatric subclavian catheter (Arrow International, Inc., 16Fr 0.9mm, 22G) was placed into the bulla following the dilatatation of the passage using the Seldinger's technique. Thereafter, the air inside the bulla was aspirated with the help of a 50cc syringe and the aspirated air was expelled to the atmosphere through an under-water drainage system with the help of a three-way stop cock fitted in between the catheter hub and the syringe (Figure 3).

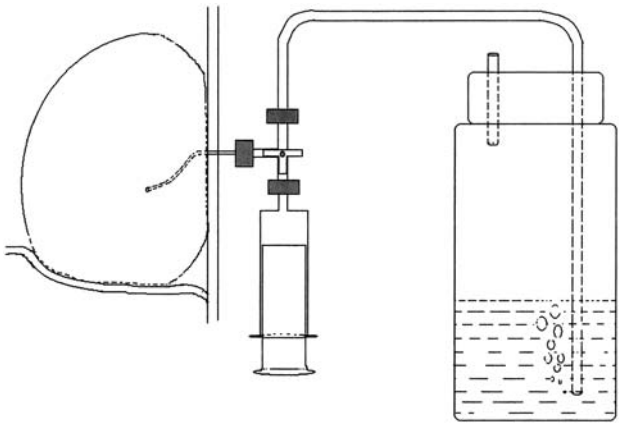

Figure 3. Diagrammatic representation of the procedure using a syringe, three-way cannula and water-seal drainage.

After aspirating about 800mL of air, the process was stopped and 10mL of freshly drawn (non-heparinised) blood from the patient's antecubital vein was quickly injected into the bulla before withdrawal of the catheter. The patient had a quick recovery from sedation and muscle relaxation. There was improvement of breath sound over the left infra-axillary and infra-mammary areas. Other than an occasional bout of cough on withdrawal of sedation, the entire procedure was uneventful. Immediately after the procedure, the patient had a pulse rate of 96 per minute, respiratory rate of 28 per minute, with an improved SaO_2 of 98% on room air. He was able to go to the toilet within 30 minutes of the procedure without much distress.

A chest radiograph obtained half an hour after the procedure showed elevation of the left dome of diaphragm and a significant reduction in the size of the bulla. The spirometry performed next morning showed a significant improvement, with FEV_1 increasing to 0.99L, and FEV_1/FVC to 0.43. The patient was discharged subsequently on the same day. A follow-up visit after 10 days revealed significant improvement in air entry, with a SaO_2 of 96% to 97% on room air, a resting pulse rate of 80 per minute and a comfortable respiratory pattern. A repeat chest radiograph revealed the persistently decompressed state of the bulla obvious from its reduced diameter and correction of the diaphragm contour and position (Figure 4).

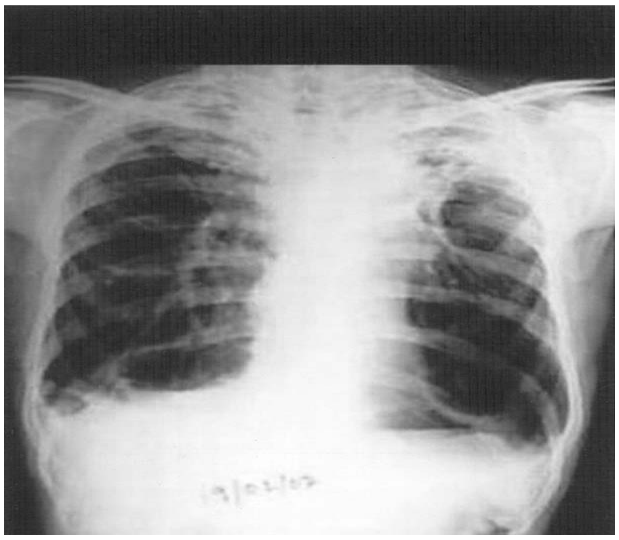

Figure 4. Cheat radiograph done on the 10th post-procedure day showing reduction in size of the treated bulla.

DISCUSSION

Our patient was an index case of a novel bronchoscopic decompression in the past.[7] However, the procedure failed this time due to inadvertent bleeding, probably from a vessel injury on attempting to penetrate the wall of the bulla with the transbronchial needle aspiration. This resulted in difficulty in visualisation along with worsening of hypoxaemia.

Transthoracic decompression of intra-pulmonary cavities had been attempted by Monaldi[8] for treatment of tuberculosis and the concept has already been applied to emphysematous bullae in the past.[9] We used short-acting anaesthesia (with propofol)[10] and muscle relaxation mainly to avoid the chance of pneumothorax resulting from any respiratory or unintentional voluntary movement during the process of insertion and withdrawal of the needle from the bulla. The pediatric subclavian catheter was chosen for its thin bore. Bullae have a tendency to enlarge progressively, trapping an increasingly larger volume of air over time.[11] Hence, autologous blood was instilled inside the bulla with an intention to induce an aseptic inflammation that might facilitate the closure of bronchial communications, if any. This may also result in thickening of the wall of the bulla so as to make it non-compliant for further expansion.[12] Significant decompression was achieved, reflected by the elevation and correction of contour and position of the diaphragm. The bulla had become smaller and well defined. The functional improvement of the patient was remarkable and maintained on the follow-up visits.

A patient with a single big bulla is perhaps a more suitable candidate to be treated with transthoracic decompression than a patient with multiple emphysematous bullae. The advantage of this procedure is that it is practically bloodless and targets only a selected bulla thought to be causing the maximum functional disability. Although the long-term effect of instilling blood into the bulla is yet to be appreciated, the procedure is much less invasive than surgery and even thoracoscopy. Moreover, it is economical. In our opinion, this procedure deserves a trial with larger number of patients in future and may prove to be an alternate but easy and cost-effective method for the treatment of emphysematous bullae.

REFERENCES

1. CIBA Foundation Guest Symposium. Terminology, definitions and classification of chronic pulmonary emphysema and related conditions. *Thorax* 1959;14: 286-99.
2. Uyama T, Monden Y, Harada K, Kimura S, Taniki T. Drainage of giant bulla with balloon catheter using chemical irritant and fibrin glue. *Chest* 1988;94:1289-90.
3. Kinnear WJ, Tattersfield AE. Emphysematous bulla. *Br Med J* 1990;300:208-9.
4. Shamii FM, Sachs HJ, Perkins DG. Cystic disease of lung. *Surg Clinic North Am* 1988;68:581-620.
5. Mc Kenna RJ Jr, Brenner M, Gelb AF, Mullin M, Singh N, Peters H, *et al.* A randomised prospective trial of stapled lung reduction *versus* laser bullectomy for diffuse emphysema. *J Thorac Cardiovasc Surg* 1996;111:317-22.
6. Takizawa H, Kondo K, Sakiyama S, Monden Y. Computed tomography guided drainage for large pulmonary bulla. *Int Cardiovasc Thorac Surg* 2004;3:283-5.
7. Bhattacharyya P, Sarkar D, Nag S, Ghosh S, Roychowdhury S. Transbronchial decompression of emphysematous bulla: a new therapeutic approach. *Eur Respir J* 2007;29:1003-6.
8. Head JR, Avory EE. Intracavity suction (Monaldi) in the treatment of emphysematous bulla and blebs. *J Thoracic Surg* 1949;18:761-76.
9. Macarthur AM, Fountain SW. Intracavity suction and drainage in the treatment of emphysematous bullae. *Thorax* 1977;32:668-72.
10. Schnittger T. Intravenous propofol anesthesia. *West J Med* 1990;153:312.
11. Snider GL. Reduction pneumoplasty for giant bullous emphysema. *Chest* 1996;109:540-8.
12. Stone DJ, Schwartz A. A long term study of natural history and effects of therapy. *Am Rev Respir Dis* 1960; 94:493-507.

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Case Report

Diagnostic Dilemma of Antineutrophil Cytoplasmic Antibody Seropositivity in Human Immunodeficiency Virus Infection

Prasanta R. Mohapatra, Sushant Khanduri, Naveen Dutt, Preeti Sharma and Ashok K. Janmeja

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ABSTRACT

We present a case of a 48-year-old male who was diagnosed and treated for Wegener's granulomatosis on the basis of history, clinical features, computed tomography (CT) and antineutrophil cytoplasmic antibodies (ANCA) positivity. The patient initially improved and later on during course of the disease he was found to be human immunodeficiency virus (HIV) seropositive. The potential pitfalls of cANCA in a HIV-infected patient are discussed.
[Indian J Chest Dis Allied Sci 2011;53:55-57]

Key words: Wegener's granulomatosis, HIV, ANCA, Radiology.

INTRODUCTION

Wegener's granulomatosis is a necrotising granulomatous vasculitis involving respiratory tract and kidneys. The available literature suggests that ANCA are highly specific for Wegener's granulomatosis. Clinicians use cytoplasmic pattern of ANCA (cANCA) for serologic confirmation of Wegener's granulomatosis.

CASE REPORT

A 48-year-old male was admitted to hospital with cough, low-grade fever, minimal haemoptysis and progressive dyspnoea for six weeks. He had taken some treatment for nearly three weeks from a private practitioner but no records were available. Repeated sputum smear examinations were negative for *Mycobacterium tuberculosis*. On admission, the haemoglobin was 12 gm/dL, total leukocyte counts (TLC) were 17,900/cmm with 64% neutrophils, platelets 2.5 lakh/cmm^3 and the erythrocyte sedimentation rate (ESR) was 47mm at first hour (Westergren). Serum urea and creatinine levels were 20mg/dL and 1.3mg/dL, respectively. Urinalyses (routine and microscopic) was normal. Chest radiograph and CT of thorax at the time of admission are shown in figures 1 and 2, respectively. Based on chest radiograph and CT of thorax, the patient was put on standard antituberculosis treatment (ATT) empirically and bronchoalveolar lavage (BAL) for cytology and acid-fast bacilli (AFB) was planned. Flexible bronchoscopic examination revealed no abnormality up to the level of sub-segmental bronchi. BAL cytology was non-contributory. Wegener's granulomatosis was considered as next possible differential diagnosis. Subsequently, he was found positive for cANCA by both indirect immunofluorescence and proteinase 3 (PR3) capture assays (quantitative test, kit used- Varelisa™, Phadia GmbH, Freiburg, Germany).

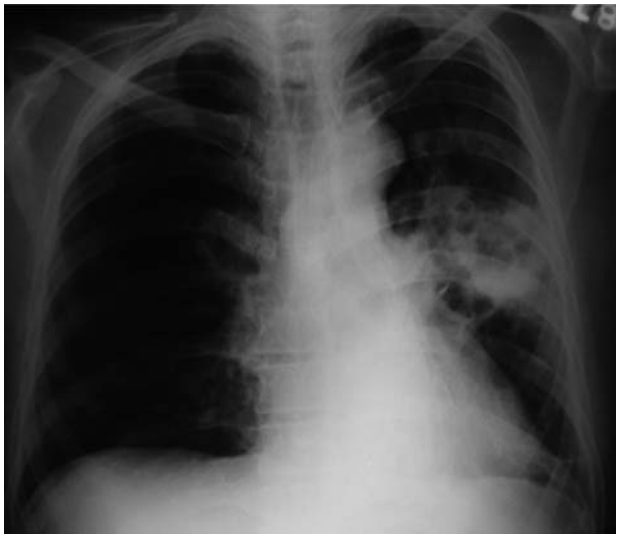

Figure 1. Chest radiograph (postero-anterior view) showing left mid-zone consolidation with multiple cavitations.

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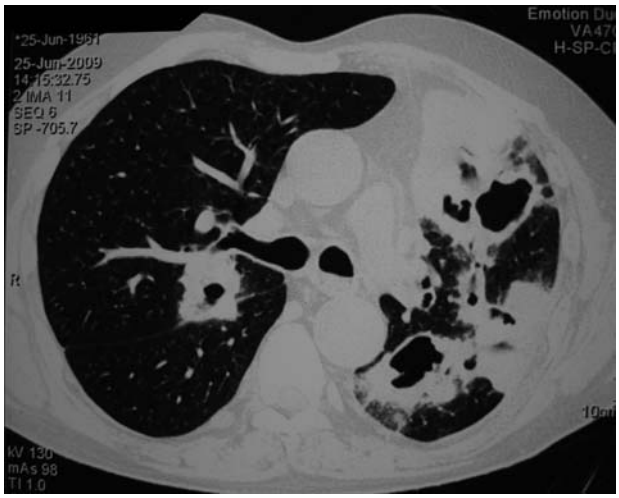

Figure 2. Computed tomography of thorax at level carina showing bilateral (left > right) consolidation with thick-walled cavitating lesions.

Based on abnormalities on CT thorax and laboratory reports of positive cANCA, we raised the diagnosis to Wegener's granulomatosis, and ATT was stopped. He was now treated with cyclophosphamide and prednisolone. The patient improved both clinically as well as radiologically during the hospital stay. The TLC was reduced to 11,300/cmm with 70% neutrophils. He was on regular follow-up and showed further improvement clinically and radiologically (Figure 3). After four-and-a-half months the patient was admitted again with clinical deterioration. At this time, haemoglobin was 11.6 gm/dL, TLC was 7,000/cmm with 88% neutrophils and platelets was 1.78 lakh/cmm^3. Serum urea and creatinine levels were 51mg/dL and 1.2mg/dL, respectively. Sputum for AFB was negative. He was found positive for HIV (confirmed by three different methods) with a CD4 cell count of 09/μL.

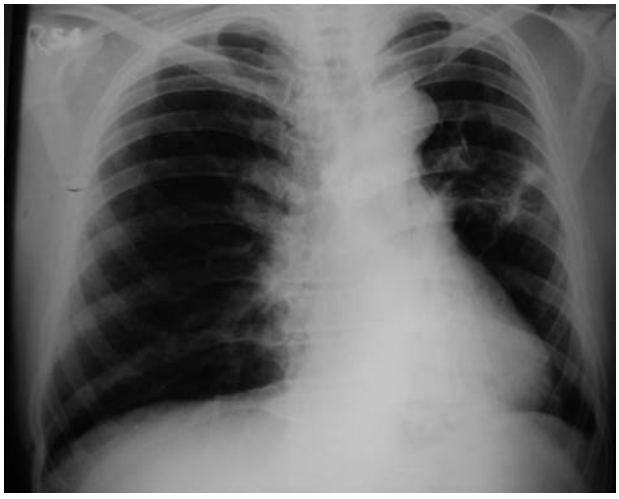

Figure 3. Follow-up chest radiograph (PA view) after three months reveals significant resolution of left mid-zone consolidation.

He was referred to nearest apex hospital for anti-retroviral treatment. After four days, the patient succumbed while on anti-retroviral treatment.

DISCUSSION

The patient either had concurrent HIV and Wegener's granulomatosis or there was a false positivity to ANCA. It is difficult to conclude that our clinical interpretations and subsequent treatment with cyclophosphamide and prednisolone was erroneous.

Antineutrophil cytoplasmic antibodies are immunoglobulin (Ig) G autoantibodies directed against constituents of primary granules of neutrophils and monocytes' lysosomes. Although, numerous antigenic targets have been recognised, the ANCA directed to proteinase 3(PR3) or myeloperoxidase (MPO) are clinically important, whereas the importance of other ANCA remains unknown. The PR3 is the usual target of cANCA. The cANCA have provided clinicians with a serological test that is useful to assist in the diagnosis of Wegener's granulomatosis and few other vasculitides. Further, when positive results from indirect immunofluorescence and enzyme linked immunosorbent assay are combined, specificity for ANCA-associated vasculitides is 99% and sensitivity for Wegener's granulomatosis is 73 percent.[1] When there is a high clinical suspicion, the determination of ANCA is most valuable tool to support the diagnosis of Wegener's granulomatosis (positive predictive value 95%) in the setting of high suspicion.[2]

Search of the literature on ANCA positivity secondary to HIV infection revealed several interesting studies. Savige *et al*[3] found 44 patients (42%) with ANCA on immunofluorescence testing out of 105 HIV-infected patients, including 26 with MPO specificity, whereas Cornely *et al*[4] found 40 ANCA-positive patients (20%) out of 199 HIV-infected patients, 67 of whom revealed an atypical pattern and 33% a pANCA pattern. Cornely *et al*[4] found MPO positivity in only one out of 199 HIV patients (0.5%), whereas Koderisch *et al*[5] found a faint cANCA positivity in 24 out of 29 HIV-infected patients (83%).

The cytopathic effect of HIV on CD4 T-cells and the active autoimmune mechanism play a vital role in the pathogenesis of the infection. The tumour necrosis factor alpha (TNF-α) is an important cytokine produced by the monocyte-macrophage series in HIV infection. This cytokine induces antigens such as PR3 or MPO. Antineutrophil cytoplasmic antibodies are directed against these antigens.[6] So ANCA positivity may be seen in HIV-infected patients. A wide range of vasculitic manifestations have been reported in HIV-infected individuals. Vasculitis has been described in both early in the disease with CD4 counts >500/μL and later in patients with CD4 counts <200/μL.[7]

Granulomatous necrotising vasculitis has also been observed in HIV infection.[8]

In case we consider this as a case of HIV infection and not Wegener's granulomatosis, we need to correlate the clinical features, radiographic and CT findings. The patient had daily treatment (possibly ATT) for about three weeks before being admitted in our hospital and ATT was also continued in our hospital for nearly 10 days for which microscopic diagnosis could not be established. The ANCA positivity made us to believe that the patient had Wegener's granulomatosis and further diagnostic work-up was not pursued.

Selective or limited knowledge as well as premature closure of the diagnostic process, and publication bias on the specificity of ANCA might sometimes lead to erroneous interpretation particularly with a confounding clinical presentation of pulmonary symptomatology, as in the present case. Such treatment may result in a potentially dangerous situation as cytotoxic therapy for Wegener's granulomatosis may well be fatal in acquired immunodeficiency syndrome. Clinicians should be aware of the possibility of a false positive ANCA, particularly in view of the current HIV epidemiology in this region. We conclude that in view of the increasing screening application of ANCA, one should be aware of false-positive results in all clinical presentations. There seems to be a real risk that clinicians may jump to conclusions in cases with a clinical suggestion of Wegener's granulomatosis with cANCA positivity. History taking in ANCA-positive patients should include the risk factors for HIV infection and all cANCA positive patients should under go HIV screening.

A careful work-up including biopsy is required in patients in whom Wegener's granulomatosis is suspected, even when cANCA is found positive. One must be cautious in interpreting a positive ANCA in HIV-infected patients.

REFERENCES

1. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, *et al*. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int* 1998;53:743-53.
2. Jennette JC, Wilkman AS, Falk RJ. Diagnostic predictive value of ANCA serology. *Kidney Int* 1998;53:796-8.
3. Savige JA, Chang L, Crowe SM. Anti-neutrophil cytoplasm antibodies in HIV infection. *Adv Exp Med Biol* 1993;336:349-52.
4. Cornely OA, Hauschild S, Weise C, Csernok E, Gross WL, Salzberger B, *et al*. Seroprevalence and disease association of antineutrophil cytoplasmic autoantibodies and antigens in HIV infection. *Infection* 1999;27:92-6.
5. Koderisch J, Andrassy K, Rasmussen N, Hartmann M, Tilgen W. "False-Positive" anti-neutrophil cytoplasmic antibodies in HIV infection. *Lancet* 1990;335:1227-8.
6. Habegger de Sorrentino A, Motta P, Iliovich E, Sorrentino AP. [Anti-neutrophil cytoplasmic antibodies (ANCA) in patients with symptomatic and asymptomatic HIV infection]. *Medicina (B Aires)* 1997;57:294-8.
7. Otedo AE, Oyoo GO, Obondi JO, Otieno CF. Vasculitis in HIV: report of eight cases. *East Afr Med J* 2005;82:656-9.
8. Garcia-Garcia JA, Macias J, Castellanos V, Fernández-Rivera J, Lozano-Gutiérrez F, Rivera JM, *et al*. Necrotizing granulomatous vasculitis in advanced HIV infection. *J Infect* 2003;47:333-5.

Case Report

Cholinergics, Airway Eosinophils and Asthma Exacerbation in the Elderly

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ABSTRACT

Cholinomimetic agents have a number of potential indications in an ageing population. This case series emphasises the need to exercise caution while prescribing cholinergic drugs in elderly patients with asthma, particularly in patients with a history of virus-induced exacerbations and airway eosinophilia. **[Indian J Chest Dis Allied Sci 2011;53:59-61]**

Key words: Asthma, Elderly, Cholinergics, Sputum eosinophils.

INTRODUCTION

There is a steady increase in the prevalence of asthma from adolescence to old age.[1] However, in the elderly patients, asthma is often under-recognised and under-treated.[2] Asthma control in the elderly is further complicated by numerous co-morbidities. Coexisting conditions may exacerbate asthma, hinder effective therapy and reduce asthma control. We highlight the risks of the use of drugs with cholinergic activity in asthmatic patients using the following examples.

CASE REPORTS

Case-1

A 65-year-old male with chronic airflow limitation due to asthma and smoker's bronchitis was admitted to the intensive care unit (ICU) for ventilatory support and intercostal drainage of pneumothorax and mediastinal emphysema. At the time of admission, he was on 10mg daily of prednisone and a combination of inhaled fluticasone (500µg) and salmeterol (50µg) twice daily. A paracardiac mass was detected and resected that turned out to be a lymphocyte-rich thymoma. At the time of discharge from the hospital, the values for forced expiratory volume in one second (FEV_1) and vital capacity (VC) were 1.4L (64%) and 2.2L (66%), respectively. He had further three episodes of bacterial bronchitis associated with sputum neutrophilia and normal eosinophils (total cell count greater than 25 million cells/gm, neutrophil >85%).[3] These did not result in asthma exacerbation. However, an episode of respiratory syncytial virus bronchitis that was associated with sputum neutrophils and eosinophils (total cell count 11 million/gm, neutrophil 88%, eosinophil 4%) resulted in another admission with an FEV_1 of 0.6L. He recovered within 14 days, with FEV_1 improving to 1.3L. The patient was discharged on 30mg daily prednisone. Subsequently, prednisone was tapered off without any further decline in the FEV_1 by regular monitoring of the sputum cell counts that did not show any eosinophils as the prednisone dose was stopped. Four months after discontinuing prednisone, he started complaining of increased fatigue and exertional breathlessness. The FEV_1 and VC had declined to 1.0L and 1.6L, respectively and the inspiratory mouth pressure was 30% of the predicted. Sputum cell counts were normal. A clinical diagnosis of *Myasthenia gravis* was confirmed by demonstrating anti-cholinesterase receptor antibodies. However, treatment with pyridostigmine resulted in increasing chest tightness and wheezing and the FEV_1 dropped to 0.7L that improved to 1.2L upon discontinuing the drug. He is now on 7.5mg daily of prednisone for his *Myasthenia gravis.*

Case-2

An 82-year-old female was evaluated for cough and exertional breathlessness. The FEV_1 and VC were 1.0L and 1.8L, respectively. Sputum showed 5%

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eosinophils. Symptoms and lung function improved significantly (FEV_1=1.4L) and remained stable for almost three years after she was treated with fluticasone (250µg) and salmeterol (25µg). In August 2008, her family doctor prescribed doneprezil (Aricept®) 10mg daily for slight cognitive decline and memory loss. Within two weeks of starting therapy, she had six episodes of central chest heaviness and shortness of breath, all occurring between 2 AM and 3 AM. She was seen at the emergency room during four of these episodes and was documented to have wheezing with mild hypoxaemia (SpO_2=91%), low peak expiratory flow (PEF=100 L/min), and normal electrocardiogram (ECG) and serial cardiac enzymes. On each occasion symptoms improved with inhaled salbutamol and oxygen. The emergency room physician also recommended ipratropium bromide (4 puffs four times a day). Post-bronchodilator FEV_1 in between these episodes had decreased to 0.9 L, sputum, for the first time, showed 3% eosinophils. Since discontinuing donepezil, she had no recurrence of these symptoms during 12 weeks of follow-up. Currently, FEV_1 is 1.3L and sputum does not show eosinophils.

Case-3

A 79-year-old female with chronic open angle glaucoma and frequent winter bronchitis presented with new onset wheezing shortly after she had been prescribed timolol maleate (Timoptic XE ®) 0.5% eye drops by her family physician. PC_{20} methacholine was 1.2mg/mL. Sputum cell counts were normal indicating that she did not have bronchitis. Timolol was discontinued. Four weeks later, PC_{20} methacholine was 5.6mg/mL and wheezing had improved without any further need for daily salbutamol inhaler. Treatment was substituted with pilocarpine 0.5% eye drops three times a day. After seven days, she presented again with daily and nocturnal wheezing. Sputum showed 2% eosinophils and the PC_{20} methacholine had dropped to 2.4 mg/mL and FEV_1 had decreased by 400mL. Pilocarpine was discontinued and substituted with latanoprost (prostaglandin F2 alpha analogue) 0.005% by the ophthalmologist. She has not since required salbutamol inhalation.

DISCUSSION

These cases highlight three clinically relevant aspects of asthma management in the elderly. First, caution needs to be exercised in prescribing cholinergic agents for beneficial effects in diseases that particularly affect an ageing population. These include Parkinson's disease, Alzheimer's disease and glaucoma. Second, this phenomenon may be particularly problematic in patients who have a history of virus-induced bronchoconstriction. Thirdly, cholinergic stimulation may increase airway responsiveness and airway eospinophilia resulting in asthma exacerbations.

Acetylcholine is the primary para-sympathetic neuro-transmitter in the airways and is thought to induce airway smooth muscle contraction by acting on the M3 receptors on the smooth muscle. Viral infections cause a marked increase in vagally-mediated bronchoconstriction. This is likely due to increased acetylcholine release as a result of loss of inhibitory M2 muscarinic receptors in the cholinergic nerve fibers resulting from the increased bronchial hyperreactivity associated with inflammation.[4] Possible mechanisms are illustrated in the figure below. As illustrated in the first case history, presence of airway eosinophilia, not neutrophilia, at the time of viral bronchitis, may exacerbate muscarinic receptor dysfunction.[5] It is also likely that cholinergic stimulation can increase eosinophil recruitment to the airway by indirect mechanisms. This needs further investigation. Examination of sputum cell counts enables the recognition of the type of bronchitis, and thus, initiate appropriate treatment.

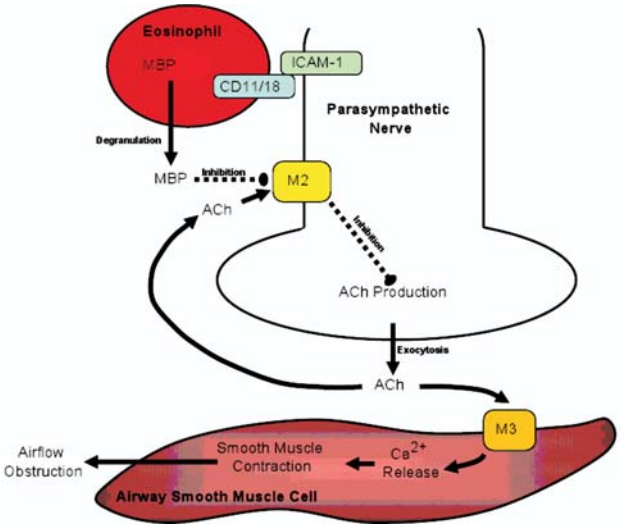

Figure. The interactions between airway smooth muscle, parasympathetic neurons, and eosinophils. Parasympathetic neurons innervate and contribute to the tone of airway smooth muscle by release of acetylcholine (ACh). ACh release from neurons mediates contraction of airway smooth muscle via M3 muscarinic receptor[s]. Neuronal release of ACh can also bind to the M2 muscarinic receptor on the parasympathetic neurons to inhibit further neuronal ACh production. Eosinophil degranulation results in major basic protein (MBP) release which can inhibit the M2 muscarinic receptor. Eosinophils are able to interact with parasympathetic neurons through CD11/18 and ICAM-1 interactions. Solid lines represent positive signalling. Dashed lines represent inhibitory signalling.

Cholinomimetic agents have a number of potential indications in an ageing population. They are used for diseases of the eye (glaucoma), the gastro-intestinal and urinary tract (post-operative atony and neurgoenic bladder), the neuromuscular junction (*Myasthenia gravis*), central nervous system (Alzheimer's), and the cardiovascular system (hypertension, myocardial infarction). This case series emphasises the need to exercise caution while prescribing cholinergic drugs in patients with asthma particularly in those with history of virus-induced exacerbations and airway eosinophilia.

REFERENCES

1. Evans R , Mullally DI, Wilson RW, Gergen PJ, Rosenberg HM, Grauman JS, Chevarley FM, *et al*. National trends in morbidity and mortality in asthma in the U.S. *Chest* 1987; 91:65s-74s.
2. Parameswaran K, Hildreth AJ, Chadha D, Keaney NP, Taylor IK, Bansal SK. Asthma in the elderly: underperceived, underdiagnosed and undertreated: a community survey. *Respir Med* 1998;92:573-7.
3. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000;161:475-8.
4. Jacoby DB, Fryer AD. Interaction of viral infections with muscarinic receptors. *Clin Exp Allergy* 1999;29 (Suppl. 2): 59-645.
5. Jacoby DB, Gleich GJ, Fryer AD. Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M2 receptor. *J Clin Invest* 1993; 91:1314-8.

Free the Mother Earth from Tobacco Sacrilege

Burnt out lips nail tips cheek palate ruminate
Inflamed mucosa cancer in wait knocking at gate
Stained mutilated teeth putrid gum in dire state
Tell tale signs of smoke and lurking death fate!

Coarse wrinkles and salt pepper define hair line
Signs of getting old and grey at young age shine
Ravages of tobacco indelible entire body line
Foolishly embracing the killer on dotted line!

Air passage filled with fumes tar coal most time
Tiny air sacs rendered useless bloated all time
Hacking cough and difficult breathing every night-time
Phthisis and cancer cutting lifeline giving no time!

Poisonous carbon monoxide well stealth in smoke
Nicotine arecoline clogging coronaries to broke
Numerous insults, attacks after attacks and stroke
Heart stops suddenly slaps of tobacco and smoke!

Why don't we wipe the tobacco *gutka* and gul?
Protect all our siblings' kids' kith kin and '*kul*'
Curse of deadly poisonous substances and bull
Going hell and gruesome ringing of tobacco death bell!

Let us take this pledge with all our grit and might
Not to cultivate sell share deal chew or alight
Say no more tobacco good bye *gutka, bidi* and smoke
Never to touch pipe spice or snuff even in joke!

Big no to surti spitting smoking around the ground
Our mother earth free from tobacco sacrilege abound
Dawn shining fragrant filled with breeze and sound
Making this planet a heaven to lounge around!

Dr Shridhar Dwivedi

[Indian J Chest Dis Allied Sci 2011;53:63-67]

Abstracts' Service

Evaluating the Risks of Clinical Research

Annette Rid, Ezekiel J. Emanuel and David Wendler

JAMA 2010;304:1472-1479

The ethical appropriateness of clinical research depends on protecting participants from excessive risks. Yet no systematic framewor has been developed to assess research risks, and as a result, investigators, funders, and review boards rely only on their intuitive judgments. Because intuitive judgments of risk are subject to well-documented cognitive biases, this approach raises concern that research participants are not being adequately protected. To address this situation, we delineate a method called the systematic evaluation of research risks (SERR), which evaluates the risks of research interventions by comparing these interventions with the risks of comparator activities that have been deemed acceptable. This method involves a 4-step process: (1) identify the potential harms posed by the proposed research intervention; (2) categorize the magnitude of the potential harms into 1 to 7 harm levels on a harm scale; (3) quantify or estimate the likelihood of each potential harm; and (4) compare the likelihood of each potential harm from the research intervention with the likelihood of harms of the same magnitude occurring as a result of an appropriate comparator activity. By explicitly delineating, quantifying and comparing the risks of research interventions with the risks posed by appropriate comparator activities, SERR offers a way to minimize the influence of cognitive biases on the evaluation of research risks and thereby better protect research participants from excessive risks.

Diagnosing and Managing Common Food Allergies: A Systematic Review

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JAMA 2010;303:1848-1856

Context. There is heightened interest in food allergies but no clear consensus exists regarding the prevalence or most effective diagnostic and management approaches to food allergies.

Objective. To perform a systematic review of the available evidence on the prevalence, diagnosis, management, and prevention of food allergies.

Data Sources. Electronic searches of PubMed, Cochrane Database of Systematic Reviews, Cochrane Database of Abstracts of Reviews of Effects, and Cochrane Central Register of Controlled Trials. Searches were limited to English-language articles indexed between January 1988 and September 2009.

Study Selection. Diagnostic tests were included if they had a prospective, defined study population, used food challenge as a criterion standard, and reported sufficient data to calculate sensitivity and specificity. Systematic reviews and randomized controlled trials (RCTs) for management and prevention outcomes were also used. For foods where anaphylaxis is common, cohort studies with a sample size of more than 100 participants were included.

Data Extraction. Two investigators independently reviewed all titles and abstracts to identify potentially relevant articles and resolved discrepancies by repeated review and discussion. Quality of systematic reviews and meta-analyses was assessed using the AMSTAR criteria, the quality of diagnostic studies using the QUADAS criteria most relevant to food allergy, and the quality of RCTs using the Jadad criteria.

Data Synthesis. A total of 12 378 citations were identified and 72 citations were included. Food allergy affects more than 1% to 2% but less than 10% of the population. It is unclear if the prevalence of food allergies is increasing. Summary receiver operating characteristic curves comparing skin prick tests (area under the curve [AUC], 0.87; 95% confidence interval [CI], 0.81-0.93) and serum food-specific IgE (AUC, 0.84; 95% CI, 0.78-0.91) to food challenge showed no statistical superiority for either test. Elimination diets are the mainstay of therapy but have been rarely studied. Immunotherapy is promising but data are insufficient to recommend use. In high-risk infants,

hydrolyzed formulas may prevent cow's milk allergy but standardized definitions of high risk and hydrolyzed formula do not exist.

Conclusion. The evidence for the prevalence and management of food allergy is greatly limited by a lack of uniformity for criteria for making a diagnosis.

Genetic Ancestry in Lung-Function Predictions

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The New England Journal of Medicine 2010;363:321-30

Background. Self-identified race or ethnic group is used to determine normal reference standards in the prediction of pulmonary function. We conducted a study to determine whether the genetically determined percentage of African ancestry is associated with lung function and whether its use could improve predictions of lung function among persons who identified themselves as African American.

Methods. We assessed the ancestry of 777 participants self-identified as African American in the Coronary Artery Risk Development in Young Adults (CARDIA) study and evaluated the relation between pulmonary function and ancestry by means of linear regression. We performed similar analyses of data for two independent cohorts of subjects identifying themselves as African American: 813 participants in the Health, Aging and Body Composition (HABC) study and 579 participants in the Cardiovascular Health Study (CHS). We compared the fit of two types of models to lung-function measurements: models based on the covariates used in standard prediction equations and models incorporating ancestry. We also evaluated the effect of the ancestry-based models on the classification of disease severity in two asthma-study populations.

Results. African ancestry was inversely related to forced expiratory volume in 1 second (FEV_1) and forced vital capacity in the CARDIA cohort. These relations were also seen in the HABC and CHS cohorts. In predicting lung function, the ancestry-based model fit the data better than standard models. Ancestry-based models resulted in the reclassification of asthma severity (based on the percentage of the predicted FEV_1) in 4 to 5% of participants.

Conclusions. Current predictive equations, which rely on self-identified race alone, may misestimate lung function among subjects who identify themselves as African American. Incorporating ancestry into normative equations may improve lung-function estimates and more accurately categorize disease severity. (Funded by the National Institutes of Health and others.)

Reduced Treatment Intensity in Patients with Early-Stage Hodgkin's Lymphoma

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The New England Journal of Medicine 2010;363:640-52

Background. Whether it is possible to reduce the intensity of treatment in early (stage I or II) Hodgkin's lymphoma with a favorable prognosis remains unclear. We therefore conducted a multicenter, randomized trial comparing four treatment groups consisting of a combination chemotherapy regimen of two different intensities followed by involved-field radiation therapy at two different dose levels.

Methods. We randomly assigned 1370 patients with newly diagnosed early-stage Hodgkin's lymphoma with a favorable prognosis to one of four treatment groups: four cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) followed by 30 Gy of radiation therapy (group 1), four cycles of ABVD followed by 20 Gy of radiation therapy (group 2), two cycles of ABVD followed by 30 Gy of radiation therapy (group 3), or two cycles of ABVD followed by 20 Gy of radiation therapy (group 4). The primary end point was freedom from treatment failure; secondary end points included efficacy and toxicity of treatment.

Results. The two chemotherapy regimens did not differ significantly with respect to freedom from treatment failure (P=0.39) or overall survival (P=0.61). At 5 years, the rates of freedom from treatment failure were 93.0% (95% confidence interval [CI], 90.5 to 94.8) with the four-cycle ABVD regimen and 91.1% (95% CI, 88.3 to 93.2) with the two-cycle regimen. When the effects of 20-Gy and 30-Gy doses of radiation therapy were compared, there were also no significant differences in freedom from treatment failure (P=1.00) or overall survival (P=0.61). Adverse events and acute toxic effects of treatment were most common in the patients who received four cycles of ABVD and 30 Gy of radiation therapy (group 1).

Conclusions. In patients with early-stage Hodgkin's lymphoma and a favorable prognosis, treatment with two cycles of ABVD followed by 20 Gy of involved-field radiation therapy is as effective as, and less toxic than, four cycles of ABVD followed by 30 Gy of involved-field radiation therapy. Long-term effects of these treatments have not yet been fully assessed. (Funded by the Deutsche Krebshilfe and the Swiss Federal Government; ClinicalTrials.gov number, NCT00265018.)

Susceptibility to Exacerbation in Chronic Obstructive Pulmonary Disease

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The New England Journal of Medicine 2010;363:1128-38

Background. Although we know that exacerbations are key events in chronic obstructive pulmonary disease (COPD), our understanding of their frequency, determinants, and effects is incomplete. In a large observational cohort, we tested the hypothesis that there is a frequent-exacerbation phenotype of COPD that is independent of disease severity.

Methods. We analysed the frequency and associations of exacerbation in 2138 patients enrolled in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) study. Exacerbations were defined as events that led a care provider to prescribe antibiotics or corticosteroids (or both) or that led to hospitalization (severe exacerbations). Exacerbation frequency was observed over a period of 3 years.

Results. Exacerbations became more frequent (and more severe) as the severity of COPD increased; exacerbation rates in the first year of follow-up were 0.85 per person for patients with stage 2 COPD (with stages defined in accordance with Global Initiative for Chronic Obstructive Lung Disease [GOLD] stages), 1.34 for patients with stage 3, and 2.00 for patients with stage 4. Overall, 22% of patients with stage 2 disease, 33% with stage 3, and 47% with stage 4 had frequent exacerbations (two or more in the first year of follow-up). The single best predictor of exacerbations, across all GOLD stages, was a history of exacerbations. The frequent-exacerbation phenotype appeared to be relatively stable over a period of 3 years and could be predicted on the basis of the patient's recall of previous treated events. In addition to its association with more severe disease and prior exacerbations, the phenotype was independently associated with a history of gastroesophageal reflux or heartburn, poorer quality of life, and elevated white-cell count.

Conclusions. Although exacerbations become more frequent and more severe as COPD progresses, the rate at which they occur appears to reflect an independent susceptibility phenotype. This has implications for the targeting of exacerbation-prevention strategies across the spectrum of disease severity. (Funded by GlaxoSmithKline; ClinicalTrials.gov number, NCT00292552.)

Four-arm Robotic Lobectomy for the Treatment of Early-Stage Lung Cancer

Giulia Veronesi, Domenico Galetta, Patrick Maisonneuve, DipEng, Franca Melfi, Ralph Alexander Schmid, Alessandro Borri, Fernando Vannucci and Lorenzo Spaggiari

The Journal of Thoracic and Cardiovascular Surgery 2010;140:19-25

Objectives. We investigated the feasibility and safety of four-arm robotic lung lobectomy in patients with lung cancer and described the robotic lobectomy technique with mediastinal lymph node dissection.

Methods. Over 21 months, 54 patients underwent robotic lobectomy for early-stage lung cancer at our institute. We used a da Vinci Robotic System (Intuitive Surgical, Inc, Mountain View, Calif) with three ports plus one utility incision to isolate hilum elements and perform vascular and bronchial resection using standard endoscopic staplers. Standard mediastinal lymph node dissection was performed subsequently. Surgical outcomes were compared with those in 54 patients who underwent open surgery over the same period and were matched to the robotic group using propensity scores for a series of preoperative variables.

Results. Conversion to open surgery was necessary in 7 (13%) cases. Postoperative complications (11/54, 20%, in each group) and median number of lymph nodes removed (17.5 robotic vs 17 open) were similar in the 2 groups. Median robotic operating time decreased by 43 minutes (P = .02) from first tertile (18 patients) to the second-plus-third tertile (36 patients). Median postoperative hospitalization was significantly shorter after robotic (excluding first tertile) than after open operations (4.5 days *vs* 6 days; P = .002).

Conclusions. Robotic lobectomy with lymph node dissection is practicable, safe, and associated with shorter postoperative hospitalization than open surgery. From the number of lymph nodes removed it also appears oncologically acceptable for early lung cancer. Benefits in terms of postoperative pain, respiratory function, and quality of life still require evaluation. We expect that technologic developments will further simplify the robotic procedure.

Neuromuscular Blockers in Early Acute Respiratory Distress Syndrome

Laurent Papazian, Jean-Marie Forel, Arnaud Gacouin, Christine Penot-Ragon, Gilles Perrin, Anderson Loundou, Samir Jaber, Jean-Michel Arnal, Didier Perez, Jean-Marie Seghboyan, Jean-Michel Constantin, Pierre Courant, Jean-Yves Lefrant, Claude Guerin, Gwenael Prat, Sophie Morange and Antoine Roch, for the ACURASYS Study Investigators

The New England Journal of Medicine 2010;363:1107-16

Background. In patients undergoing mechanical ventilation for the acute respiratory distress syndrome (ARDS), neuromuscular blocking agents may improve oxygenation and decrease ventilator-induced lung injury but may also cause muscle weakness. We evaluated clinical outcomes after 2 days of therapy with neuromuscular blocking agents in patients with early, severe ARDS.

Methods. In this multicenter, double-blind trial, 340 patients presenting to the intensive care unit (ICU) with an onset of severe ARDS within the previous 48 hours were randomly assigned to receive, for 48 hours, either cisatracurium besylate (178 patients) or placebo (162 patients). Severe ARDS was defined as a ratio of the partial pressure of arterial oxygen (PaO_2) to the fraction of inspired oxygen (FIO_2) of less than 150, with a positive end-expiratory pressure of 5 cm or more of water and a tidal volume of 6 to 8 ml per kilogram of predicted body weight. The primary outcome was the proportion of patients who died either before hospital discharge or within 90 days after study enrollment (i.e., the 90-day in-hospital mortality rate), adjusted for predefined covariates and baseline differences between groups with the use of a Cox model.

Results. The hazard ratio for death at 90 days in the cisatracurium group, as compared with the placebo group, was 0.68% (95% confidence interval [CI], 0.48 to 0.98; P=0.04), after adjustment for both the baseline PaO_2:FIO_2 and plateau pressure and the Simplified Acute Physiology II score. The crude 90-day mortality was 31.6% (95% CI, 25.2 to 38.8) in the cisatracurium

group and 40.7% (95% CI, 33.5 to 48.4) in the placebo group (P=0.08). Mortality at 28 days was 23.7% (95% CI, 18.1 to 30.5) with cisatracurium and 33.3% (95% CI, 26.5 to 40.9) with placebo (P=0.05). The rate of ICU-acquired paresis did not differ significantly between the two groups.

Conclusions. In patients with severe ARDS, early administration of a neuromuscular blocking agent improved the adjusted 90-day survival and increased the time off the ventilator without increasing muscle weakness. (Funded by Assistance Publique-Hopitaux de Marseille and the Programme Hospitalier de Recherche Clinique Regional 2004-26 of the French Ministry of Health; ClinicalTrails.gov number, NCT00299650.)

A Controlled Trial of Sildenafil in Advanced Idiopathic Pulmonary Fibrosis

The Idiopathic Pulmonary Fibrosis Clinical Research Network

The New England Journal of Medicine 2010;363:620-8

Background. Sildenafil, a phosphodiesterase-5 inhibitor, may preferentially improve blood flow to well-ventilated regions of the lung in patients with advanced idiopathic pulmonary fibrosis, which could result in improvements in gas exchange. We tested the hypothesis that treatment with sildenafil would improve walk distance, dyspnea, and quality of life in patients with advanced idiopathic pulmonary fibrosis, defined as a carbon monoxide diffusion capacity of less than 35% of the predicted value.

Methods. We conducted a double-blind, randomized, placebo-controlled trial of sildenafil in two periods. The first period consisted of 12 weeks of a double-blind comparison between sildenafil and a placebo control. The primary outcome was the proportion of patients with an increase in the 6-minute walk distance of 20% or more. Key secondary outcomes included changes in oxygenation, degree of dyspnea, and quality of life. The second period was a 12-week open-label evaluation involving all patients receiving sildenafil.

Results. A total of 180 patients were enrolled in the study. The difference in the primary outcome was not significant, with 9 of 89 patients (10%) in the sildenafil group and 6 of 91 (7%) in the placebo group having an improvement of 20% or more in the 6-minute walk distance (P=0.39). There were small but significant differences in arterial oxygenation, carbon monoxide diffusion capacity, degree of dyspnea, and quality of life favoring the sildenafil group. Serious adverse events were similar in the two study groups.

Conclusions. This study did not show a benefit for sildenafil for the primary outcome. The presence of some positive secondary outcomes creates clinical equipoise for further research. (Funded by the National Heart, Lung, and Blood Institute and others; ClinicalTrials.gov number, NCT00517933.)

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Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. *Blood* 2002 Nov 15; 100(10): 3828-31. Epub 2002 July 5.

3. *Volume with supplement*

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short-and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; 42 Suppl 2: S93-9.

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Abend SM, Kulish N. The psychoanalytic method from an epistemological viewpoint. *Int J Psychoanal* 2002; 83 (Pt 2): 491-5.

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12. *Both personal authors and an organization as author* (This example does not conform to NISO standards).

Vallancien G, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; 169(6): 2257-61.

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21st century heart solution may have a sting in the tail. *BMJ* 2002; 325(7357): 184.

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Books and Other Monographs

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Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumours. In: Vogelstein B, Kinzler KW, editors. *The Genetic Basis of Human Cancer*. New York: McGraw-Hill. 2002; pp 93-113.

21. *Conference paper*

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In : Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: *Proceedings of the 5th European Conference on Genetic Programming;* 2002 Apr 3-5; Kinsdale Ireland. Berlin: Springer. 2002; pp 182-91.

22. *Personal author(s)*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical Microbiology;* 4th ed. St. Louis: Mosby. 2002.

23. *Editor(s), compiler(s) as author*

Gilstrap LC (3rd), Cunningham FG, VanDorsten JP, editors. *Operative Obstetrics*. 2nd ed. New York: McGraw-Hill. 2002.

24. *Author(s) and editor(s)*

Breedlove GK, Schorfheide AM. *Adolescent Pregnancy*. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services; 2001.

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Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. *Compendium of Nursing Research and Practice Development, 1999-2000*. Adelaide (Australia): Adelaide University; 2001.

26. *Conference proceedings*

Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. *Proceedings of the 5th Germ Cell Tumour Conference;* 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.

27. *Scientific or technical report*

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Yen GG (Oklahoma State University, School of Electrical and Computer Engineering, Stillwater, OK). Health monitoring on vibration signatures. Final report. Arlington (VA): Air Force Office of Scientific Research (US), Air Force Research Laboratory; 2002 Feb. Report No.: AFRLSRBLTR020123. Contract No.: F496209810049.

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Russelll ML, Goth-Goldstein R, Apte MG, Fisk WJ. Method for measuring the size distribution of airborne Rhinovirus. Berkeley (CA): Lawrence Berkeley Na-

tional Laboratory, Environmental Energy Technologies Division; 2002 Jan. Report No.: LBNL49574. Contract No.: DEAC0376SF00098. Sponsored by the Department of Energy.

28. *Dissertation*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

29. *Patent*

Pegedas AC, inventor; Ancel Surgical R& D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1.

Other Published Material

30. *Newspaper article*

Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. *The Washington Post*. 2002 Aug 12; Sect. A:2 (col. 4).

31. *Audiovisual material*

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

32. *Legal Material*

Public law:

Veterans Hearing Loss Compensation Act of 2002, Pub.L.No. 107-9, 115 Stat. 11 (May 24, 2001).

Unenacted bill:

Healthy Children Learn Act, S. 1012, 107th Cong., 1st Sess. (2001).

Code of Federal Regulations:

Cardiopulmonary Bypass Intracardiac Suction Control, 21 C.F.R. Sect. 870.4430 (2002).

Hearing:

Arsenic in Drinking Water: An Update on the Science, Benefits and Cost: Hearing Before the Subcomm. on Environment, Technology and Standards of the House Comm. on Science, 107th Cong., 1st Sess. (Oct. 4, 2001).

33. Map

Pratt B, Flick, P, Vynne C, cartographers. Biodiversity hotspots [map]. Washington: Conservation International; 2000.

34. Dictionary and similar references

Dorland's Illustrated Medical Dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic Material

35. CD-ROM

Anderson SC, Poulsen KB. *Anderson's Electronic Atlas of Hematology* [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

36. Journal article on the Internet

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. *Am J Nurs* [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6) : [about 3 p.]. Available from: http://www.nursingworld. org/AJN/2002/june/Wawatch.htm

37. Monograph on the Internet

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

38. Homepage/Web site

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

39. Part of a homepage/Web site

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http//www.ama-assn.org/ama/pub/category 1736.html.

40. Database on the Internet

Open database:
Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000- [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.asp

Closed database:
Jablonski S. Online Multiple Congential Anomaly/Mental Retardation (MCA/MR) Syndromes [database on the Internet]. Bethesda (MD): National Library of Medicine (US). c1999 [updated 2001 Nov 20; cited 2002 Aug 12]. Available from: http://www.nlm.nih.gov/mesh/jablonski/syndrome_title.html

41. Part of a database on the Internet

MeSH Browser [database on the Internet]. Bethesda (MD): National Library of Medicine (US); 2002 - [cited 2003 Jun 10]. Meta-analysis; unique ID: D015201; [about 3 p.]. Available from: http://www.nlm.nih.gov/mesh/MBrowser.html Files updated weekly.

MeSH Browser [database on the Internet]. Bethesda (MD): National Library of Medicine (US); 2002 - [cited

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