



RESEARCH ARTICLE

ASSESSMENT OF RESPIRATORY TRACT INFLAMMATORY MARKER WITH OVERNIGHT USE OF MOSQUITO REPELLENT IN COPD PATIENT AND NORMAL INDIVIDUAL

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ABSTRACT

Even after being aware of the various harmful effects of the mosquito repellents it is very commonly being used in the developing countries. Many studies in the past have been done on exploring the effects of smoke of mosquito repellants, emissions of irritating compounds and carcinogenic compounds and other pollutants, which suggests that the rate at which emission takes place can help predict the concentration in domestic setup and to assess the health risks. Human health is one of the biggest issues now a days, our aim of researching on this topic is to make people aware regarding the hidden health problems associated with the use of mosquito repellent. Much of our community doesn't know what hazards mosquito repellents can do to our body. In this study, we evaluated short term impact of mosquito mats on respiratory tract assess them through inflammatory markers (Tlc, neutrophils count, C-rp) PFT and other relevant investigation.

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INTRODUCTION

COPD is a widely prevalent disease, especially among adults all over the world. Prevalence of this disease has been studied in various countries. According to most recent study of WHO, 65 million people have moderate to severe COPD. Almost 3 million people died of COPD in 2005, which corresponds to 5% of all deaths globally. (Farokh. E. Udwardia *et al.*, 2011) Air pollution mainly from emission of pollutants from motor vehicles and industries has been significantly associated with lower FEV1 and FVC. Particulate pollutants, ozone and nitrogen dioxide has shown to produce bronchial hyper reactivity, airway oxidative stress, pulmonary and systemic inflammation. (Farokh. E. Udwardia *et al.*, 2011) Several bacterial infections have proven to be a causative factor in pathogenesis of COPD and have played a role in the clinical course of disease. These include the following: (1) development of lungs in childhood is impaired due to lower respiratory tract infection which was reflected as decreased FEV1 in adulthood; (2) bacterial accumulation in the lower respiratory tract can cause chronic inflammation in lung damage (the vicious circle hypothesis); (3) long standing infections in the respiratory tissues have contributed to the pathogenesis of COPD by intimidating the host response due to external irritants like cigarette smoke or by inducing a chronic inflammatory response; (4) infections have known to cause

exacerbations of long standing bronchitis, which have greatly increased the morbidity and mortality of COPD; and (5) immune response due to bacterial infections have shown to enhance airway hypersensitivity that greatly enhance the airway hyperreactivity. (Sethi, 2000) Mosquitoes transmit diseases to almost 700million people annually. The best known mosquito repellent is N, N-diethyl-m-toluamide, now called N, N-diethyl-3-methylbenzamide (DEET). Repellent use has often shown to offers individuals added protection against mosquito-borne diseases and it reduces the transmission of mosquito-borne diseases. Some people are allergic to mosquito repellents and some are not. It is commonly observed that mosquito repellents cause runny nose watery eyes and hoarseness. Certain rare complications like seizure, encephalitis and even cancer have also been associated with the use of mosquito repellent. (Weili *et al.*, 2003) Mosquito repellents are most commonly composed of pyrethrins, accounting for about 0.3–0.4% of coil mass. On burning a mosquito coil, release of insecticides takes place along with the particulate smoke. This in turn hampers the mosquito from entering the premises in which mosquito repellent is being used. Insecticides, especially pyrethrins have shown to have milder toxic effects in humans and lesser toxic effects on the reproductive system of animals. In clinics the most commonly observed complains are headache, nausea, and dizziness in male sprayers who were exposed to 0.01–1.98 $\mu\text{g}/\text{m}^3$ pyrethrins for 0.5–5 hr. (Weili *et al.*, 2003) Other than the insecticide mosquito repellents are composed of compounds organic in nature which act as fillers, binders, dyes, and other additives. On burning these repellants

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components produce large amount of particles submicrometer in sizes and gaseous irritants. Due to the smaller size particles they are capable of reaching the lower respiratory tract and usually get entrapped along with the already present particles which are organic in nature, some of which are known to be causative factor in cancer or are suspected carcinogens, such as polycyclic aromatic hydrocarbons (PAHs). Incomplete burning of these components usually generates such harmful particles (mosquito repellants). Various studies have found that the combustion of mosquito coils have generated which in turn has some carbonyl compounds which are known to be a strong irritation producing activity on the upper respiratory tract—for example, formaldehyde and acetaldehyde. As the mosquito repellent coils and mats have been very widely used and usually for longer duration throughout the year, their long term effects should also be of concern for the health of general population.⁴ Even after being aware of the various harmful effects of the mosquito repellents it is very commonly being used in the developing countries. Many studies in the past have been done on exploring the effects of smoke of mosquito repellants, emissions of irritating compounds and carcinogenic compounds and other pollutants, which suggests that the rate at which emission takes place can help predict the concentration in domestic setup and to assess the health risks.⁴ Human health is one of the biggest issues now a days, our aim of researching on this topic is to make people aware regarding the hidden health problems associated with the use of mosquito repellent. Much of our community doesn't know what hazards mosquito repellents can do to our body.

MATERIALS AND METHODS

Aim: Assessment of respiratory tract inflammation with overnight exposure to mosquito repellent mats in COPD patient and normal individual.

Objective

In this study, we evaluated short term impact of mosquito mats on respiratory tract assess them through inflammatory markers (Tlc, neutrophils count, C-rp) PFT and other relevant investigation.

1. Evaluate the change in total leucocyte count in normal individuals and COPD patients after overnight exposure with mosquito coil.
2. Evaluate the change in absolute neutrophil count in normal individuals and COPD patients after overnight exposure with mosquito coil.
3. Evaluate the change in cytological count in normal individuals and COPD patients after overnight exposure with mosquito coil.
4. Evaluate the change in FEV1/FVC in normal individuals and COPD patients after overnight exposure with mosquito coil.
5. Evaluate the change in Interleukin - 6 count in normal individuals and COPD patients after overnight exposure with mosquito coil.
6. Evaluate the change in C – reactive proteins in normal individuals and COPD patients after overnight exposure with mosquito coil.

Study Design

Prospective and comparative Study

Place of study

Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune-411011

Period of study

July, 2015 to September, 2017

Institute Ethics committee clearance was obtained before the start of study.

Sample Size

100 cases

The study subjects were divided into two groups:

Group A: 50 COPD patients

Group B: 50 normal individuals.

GROUP A

Inclusion Criteria:

1. COPD diagnosed as per GOLD guidelines
2. Age greater than 40 years
3. Both genders can be enrolled
4. Written and informed consent
5. Exposure to mosquito repellent mats

Exclusion Criteria:

1. Exacerbation in last 4 weeks
2. Upper respiratory tract infection/ lower respiratory tract infection in last 4 weeks
3. History of Bronchial Asthma
4. Regular use of mosquito repellent at home for atleast 2 weeks
5. Use of anti-inflammatory drugs for at least 2 weeks (Asprin, Naproxen)
6. On ICS for at least 2 days.
7. Systemic Inflammation due to other systemic disease (Rheumatoid Arthritis, CA of lung)

GROUP B

Inclusion criteria:

1. Sex, age matched with group A
2. No COPD a per GOLD guidelines
3. Non-smoker
4. Exposure to mosquito repellent mats.

Exclusion Criteria:

- 1 URTI/LRTI in last 4 weeks
- 2 History of Bronchial Asthma
- 3 On ICS for at least 1 week
- 4 Systemic inflammations due to other systemic disease

Methodology

- Patient of COPD coming to the OPD were be evaluated as potential study participant (Group A)
- Apparently healthy relative/ accompanying person were be evaluated as potential study participant (Group B)

Consent process

- Patient information sheet (PIS) were be given to both study groups
- Study was explained in detail to both groups
- Information consent form (ICF) were given to both study groups
- All related queries were be answered
- Enough time was given to both study groups to think about it
- If willing for the same, both study groups were called for screening visit.
- Consent were signed in presence of witness by both study groups.

Screening visit

- Screening number were given after obtaining consent
- Demographic, clinical examination and relevant investigation (PFT-DLCO, body box, chest X-ray) was done
- Evaluation for inclusion/exclusion criteria was done.
- Enrolment number was given to those who match the inclusion criteria.
- Group A and Group B patients were matched for Age and Gender.

Baseline Investigation (Markers of inflammation)

1. TLC, Absolute neutrophil count
2. Nasal lavage for cytology
3. IL-6 on nasal lavage and induce sputum
4. CRP (C-Reactive protein)

Study Visit

Study group were exposed to new mosquito mats (same brand) with an aberage room size of 20x10 feet with window closed at an average distance of 2-3 meters with an exposure period of 8 hours.

Next Day Morning

- Question were asked regarding any complain/symptoms
- Post exposure spirometry, DLCO, and Body Box was done.
- Baseline investigation markers were be repeated.
- TLC, absolute neutrophil count.
- Nasal Lavage- cytology
- Induced sputum-cytology
- IL-6 on nasal Lavage and induce sputum
- C-Reactive protein

RESULTS

1) Graph 1 - Bar diagram showing age group wise distribution of study sample.

Group Statistics

	Subjects	N	Mean	Std. Deviation	Std. Error Mean
Age	Normal	50	62.20	9.971	1.410
	COPD	50	62.34	8.649	1.223

Hypothesis	Levene's Test for Equality of Variances		Unpaired t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% CI of the Difference	
								Lower	Upper
Equal variances assumed	1.643	.203	-.075	98	.940	-.140	1.867	-3.844	3.564
Equal variances not assumed			-.075	96.0	.940	-.140	1.867	-3.845	3.565

Mean age of normal subjects and COPD subjects in study sample was approximately similar (62.2, 62.34 years respectively). There was no statistically significant difference among mean. ($p > 0.05$ null hypothesis accepted i.e. there was no difference among means)

2) Graph 2 – Pie chart showing sex - wise distributions of study samples

There were more males (Normal 98%, COPD 96%) in both the groups than females (Normal 2%, COPD 4%) in study samples.

3) All 50 samples in each group (Normal, COPD) were having negative nasal lavage cytology that is, they didn't showed negative results for acid fast bacilli staining and colony forming units and induced sputum cytology on pre & post exposure of mosquito repellent.

4) C Reactive Protein (CRP) tests wise distribution on pre & post exposure of mosquito repellent in study samples

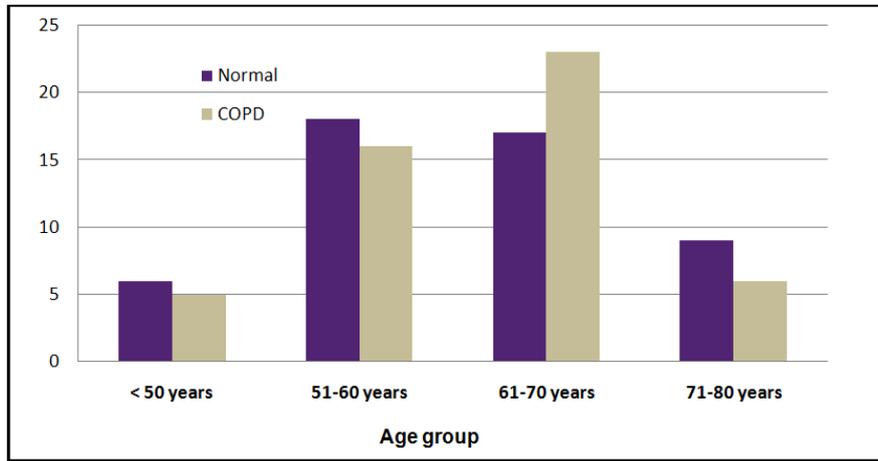
Subjects	Negative	Positive	Total
COPD	37	13	50
Normal	43	7	50
Total	80	20	100

Above table shows that were no change in frequency of CRP test on exposure of mosquito repellent. It remains as it was before the exposure of mosquito repellent. As the pre and post results for C – reactive protein testing showed no difference, no comparative statistical tests where applied.

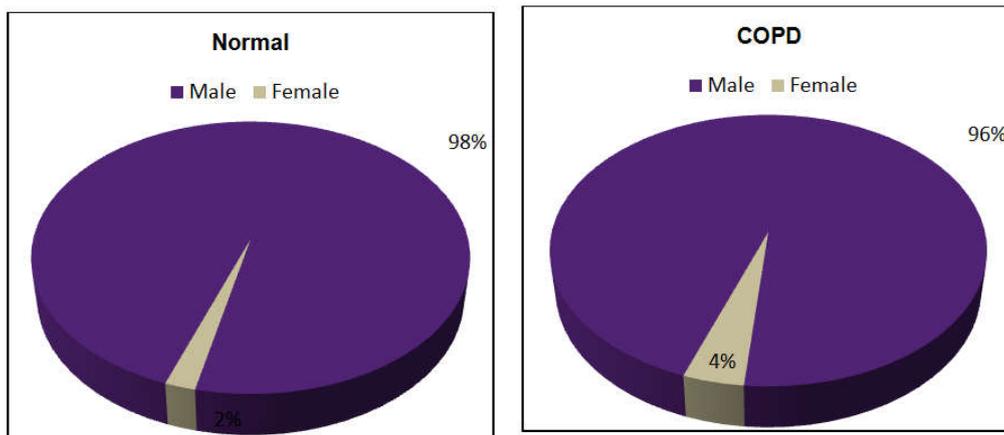
5) Effect of mosquito repellent on inflammatory markers in normal study subjects.

Paired Samples Test evaluating Total Leucocyte count.

Pair 1	TLC PRE Ex – TLC POST Ex	Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
		-1.658E3	1875.107	265.180	-2190.500	-1124.700	-6.251	49	.000



Graph 1. Bar diagram showing age wise distribution of study samples



Graph 2. Pie chart showing sex-wise distribution of study samples

Paired Samples Test evaluating Absolute Neutrophil count

	Paired Differences	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	Ancpre - ancpost	-1.376E3	1529.024	216.237	-1810.484	-941.396	-6.363	49	.000

Paired Samples Test evaluating Interleukin – 6 in Nasal Lavage

	Paired Differences	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	SIXNASALPRE - SIXNASALPOST	-.910480	1.788344	.252910	-1.418722	-.402238	-3.600	49	.001

Paired Samples Test evaluating Interleukin – 6 in Induced sputum

	Paired Differences	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	SIXSPPRE - SIXSPPOST	.115	.088	.012	.090	.140	9.310	49	.000

Statistical analysis comparing variables and their values

Paired Differences among Normal subjects							
Statistics Variables	Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference		T	Sig. (2-tailed)
				Lower	Upper		
TLC Pre & Post Exposure	-1.65E3	1875.10	265.18	-2190.50	-1124.70	-6.251	.000
ANC Pre & Post Exposure	-1.37E3	1529.02	216.23	-1810.48	-941.39	-6.363	.000
Interleukin - 6 Nasal Lavage Pre & Post Ex	-.91048	1.78834	.25291	-1.41872	-.40223	-3.600	.001
Interleukin - 6 Induced Sputum Pre & Post Ex	.115	.088	.012	.090	.140	9.310	.000

df 49

On application of paired t test, above table shows that, post exposure of mosquito repellent mean total leucocytes count, absolute neutrophils counts, interleukin 6 in nasal lavage was more than pre exposure value in normal subjects, and the difference of mean is statistically significant ($p < 0.05$). Mean of Interleukin 6 in induced sputum was more in pre exposure than post exposure, and the difference was statistically significant ($p < 0.05$).

Mosquito repellent makes significant increase in total leucocytes count, absolute neutrophils counts & interleukin 6 in nasal lavage among normal study samples. Significant decrease in interleukin 6 in induced sputum post exposure was observed.

more in post exposure than pre exposure, but the difference was not statistically significant ($p > 0.05$). Mean of Interleukin 6 in induced sputum was more in pre exposure than post exposure, and the difference was statistically significant ($p < 0.05$).

Mosquito repellent makes significant increase in total leucocytes count and absolute neutrophils counts in COPD study subjects. Significantly decreased in interleukin 6 count of induced sputum sample was observed in COPD subjects due to mosquito repellent.

Above tables shows that there was no significant difference in increased total leucocytes count, absolute neutrophils count

6) Effect of mosquito repellent on inflammatory markers in COPD study subjects

Paired Samples Test evaluating Total Leucocyte count

	Paired Differences	95% Confidence Interval of the Difference				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1 TLCPre - TLCPost	-1.658E3	1875.107	265.180	-2190.500	-1124.700	-6.251	49	.000	

Paired Samples Test evaluating Absolute Neutrophil count

	Paired Differences	95% Confidence Interval of the Difference				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1 ANCPre - ANCPPost	-1.376E3	1529.024	216.237	-1810.484	-941.396	-6.363	49	.000	

Paired Samples Test evaluating Interleukin – 6 in Nasal Lavage

	Paired Differences	95% Confidence Interval of the Difference				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1 SIXNASALPre - SIXNASALPost	-3.6945060E1	985.6749559	139.3954891	-317.0707830	243.1806630	-.265	49	.792	

Paired Samples Test evaluating Interleukin – 6 in Induced sputum

	Paired Differences	95% Confidence Interval of the Difference				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1 SIXSPUTUM Pre – SIXSPUTUM Post	.131	.123	.017	.097	.166	7.576	49	.000	

Statistical analysis Comparing Variables and their values

Paired Differences among COPD subjects	Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference		t	Sig. (2-tailed)
				Lower	Upper		
TLC Pre & Post Exposure	-1.65E3	1875.10	265.18	-2190.50	-1124.70	-6.251	.000
ANC Pre & Post Exposure	-1.37E3	1529.02	216.23	-1810.48	-941.39	-6.363	.000
Interleukin - 6 Nasal Lavage Pre & Post Ex	-3.69E1	985.67	139.39	-317.070	243.180	-.265	.792
Interleukin - 6 Induced Sputum Pre & Post Ex	.131	.123	.017	.097	.166	7.576	.000
df 49							

On application of paired t test, above table shows that total leucocytes count, absolute neutrophils counts having post exposure mean value was more than pre exposure value in COPD subjects, and the difference of mean is statistically significant ($p < 0.05$). Mean of Interleukin 6 in nasal lavage was

and interleukin 6 in nasal lavage due to mosquito repellent expose in COPD subjects in comparison with normal subjects ($p > 0.05$). There was no significant difference in decreased interleukin 6 in induced sputum due to mosquito repellent expose in COPD than normal subjects ($p > 0.05$).

7) Comparing effect of mosquito repellent on rise in inflammatory markers in normal & COPD study subjects

Statistics		Levene's Test for Equality of Variances		Unpaired t-test for Equality of Means					
		F	Sig.	t	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	95% CI of the Difference	
Hypothesis								Lower	Upper
Total leucocytes Count Difference	A	.000					375.02	-744.21	744.21
	B			.000	1.000	.000	375.02	-744.21	744.21
Absolute Neutrophils Count Difference	A	.000	1.000	.000	1.000	.000	305.80	-606.86	606.86
	B			.000	1.000	.000	305.80	-606.86	606.86
Interleukin 6 Nasal Lavage Difference	A	2.774	.099	-.259	.797	-.36.035	139.39	-312.66	240.59
	B			-.259	.797	-.36.035	139.39	-316.16	244.09
Interleukin 6 Induced Sputum Difference	A	.111	.740	.758	.450	.016	.021	-.026	.058
	B			.758	.450	.016	.021	-.026	.059

A = Equal variance assumed B = Equal variance not assumed

After evaluating the inflammatory changes in pre and post test results of TLC, ANC and interleukin 6 in nasal lavage it could be concluded that the response shown by the COPD patients and normal patients didn't show significant difference in their response.

8) FEV1 / FVC ratio

Group Statistics

Subjects	N	Mean	Std. Deviation	Std. Error Mean
Pre-exposure FEV1 / Normal	50	66.62	4.150	.587
FVC COPD	50	66.49	4.099	.580
Post-exposure FEV1 / Normal	50	67.48	2.837	.401
FVC COPD	50	67.36	2.771	.392

Ratio of FEV1 / FVC was approximately similar in normal as well as COPD subjects before and after the exposure of mosquito repellent.

DISCUSSION

This study was carried out with the intention to understand the inflammatory effects of inhalation of mosquito repellent mats fumes on the human respiratory system. A total of 100 cases were enrolled based on the inclusion and exclusion criteria described in materials and methods. Such study was thought to be essential as there is rampant use of mosquito repellent mats/ coils/ liquids, especially in the urban India where the prevalence of mosquito spread diseases such as malaria, dengue, chikenguniya etc. are on the rise since last few years. The safety of these mats has not been adequately evaluated. Many families plug-in the mats very near their beds and keep the windows closed as well. This leads to significant increase in the room air concentration of the insecticide (pralletherin) as well as the particulate matter, which are inhaled by the persons throughout the night. This may mean significant indoor air pollution and may be harmful to the family members that often include small children and neonates. On burning mosquito repellent mats, the particulate emissions are in the size range of 0.1–0.4 μm . In a small chamber study conducted at Japan, burning a mosquito mat in a room size of approximate 10 \times 20 \times 10 feet, the highest concentration of particulate matter were

of size range of 0.2–0.3 μm . No particles of size > 1 μm in diameter were created on combustion of mosquito mats. To assess the concentration and distribution of particles in that particular room, the room was measured to examine whether particle size distributions can be "artificially" affected by the nature of the small chamber (high surface-to-volume ratio). It suggested that it made no difference on particles > 0.1 μm . Ultrafine particles were not analysed in the chamber experiments. (Weili *et al.*, 2003) This study indicated that the particulate matter generated due to burning of a mosquito mats are of the respirable size i.e. they can be inhaled right upto small bronchi and alveoli without getting trapped by the large airway mucociliary clearance mechanism. The matters which are respirable particulate has been documented to be the main mechanism of COPD pathogenesis. Hence we decided to include both COPD and normal subjects in this study. C Reactive proteins are considered as important marker of acute systemic inflammation. There was no significant rise in the levels of CRP thus indicating that a single night exposure to mosquito repellent smoke did not cause significant systemic response. This was the case in both the groups i.e. COPD as well as normal subjects. The baseline pre-exposure CRP levels were also normal indicating absence of pre-existing significant systemic inflammation in stable COPD patients. This was expected as all the COPD patients in the study were in remission and had no recent exacerbation as per the inclusion/exclusion criteria.

An important objective of this study was to assess the impact of this overnight exposure on nasal mucosa as the nose takes most of the inhalation load as a first level of defence. In clinical practice, we frequently note people complaining of nasal stuffiness on using overnight mosquito mats. We determined this effect by monitoring the number of inflammatory cells and IL-6 levels which is considered as an important marker of acute inflammation. In accordance to our expectation, we found evidence of significant nasal mucosal inflammation on single overnight exposure, in both the normal subjects and COPD patients. On application of paired t test, post exposure of mosquito repellent (mats), total leucocytes count, absolute neutrophils counts, interleukin 6 in nasal lavage were more than pre exposure value in normal subjects, and the difference of mean is statistically significant ($p < 0.05$). Sputum analysis (induced sputum) gives useful information about airways inflammation and can be used to investigate

different aspects of airway inflammation. This is less invasive than obtaining BAL (Broncho Alveolar Lavage) and is often used as a research tool. Various researchers have concluded saying that collection of spontaneously expectorated and induced sputum may provide a viable alternative to bronchoscopy and BAL for assessing immune function in the lung, for both healthy individuals and patients with lung disease. Therefore, increasing IL-6 levels in induced sputum, which will more accurately reflects local immunity in the alveolar space, may be a first indicator of success of therapy while monitoring any disease. The presence of mononuclear phagocytes and epithelial cells in sputum may be the main source of IL-6 production during active inflammation. IL-6 is useful in serial monitoring of inflammatory markers in COPD or markers of neutrophilic airway inflammation like activation of neutrophils. In our study, no significant inflammatory changes were observed in the induced sputum after exposing the subjects to overnight exposure with mosquito repellent mats. There was no appreciable or statistically significant change in the neutrophils/ eosinophils or total WBC count on peripheral smear. Interleukin 6 in induced sputum was more in pre exposure than post exposure. It might be due to the dilution of the sputum done to convert it into serum for interleukin – 6 testing, but cannot be confirmed whether this is the exact reason.

After evaluating our study results it could be concluded that inflammatory response of nasal mucosa was similar in the normal subjects and COPD patients. As COPD is a chronic inflammatory disease of the respiratory tract the response of bronchial mucosa could have been more exaggerated than normal subjects but our study found no such difference. It will be appropriate to say that the host response developed in COPD as well as normal patients has same intensities. Usual response of the respiratory system to any kind of inflammation or external injury is narrowing of the pipes of respiratory system. Spirometry is a useful tool in assessing how much narrowing has taken place as well as the speed at which the lung can be filled or emptied. However when spirometry ratio of FEV1 / FVC was studied in normal as well as COPD subjects before and after the exposure of mosquito repellent no significant change was observed. It indicates that the mosquito repellent mat fumes did not trigger off airway hyper-responsiveness that is often found in COPD patients. The study indicates that the burning of mosquito mats causes significant nasal inflammation with a single overnight exposure. The effect on bronchial mucosa and the airway response is not seen on single exposure. The long term effects of regular exposure were not studied but there is a scope for future research.

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