

SPUTUM CYTOLOGY CULTURE HAEMATOLOGICAL CHANGES AND AIR QUALITY IN CHRONIC EXPOSURE TO SMOKE FROM BIOMASS FUEL IN RURAL AREA OF SOUTH INDIA

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ABSTRACT

BACKGROUND

Air pollution is generally perceived as an urban problem associated with automobiles and industries. However, half of the world's population in rural areas of the developing countries is exposed to some of the highest levels of air pollution due to burning of traditional biomass fuels. In view of this, the health impact of biomass fuel use in rural India has been evaluated in this study.

OBJECTIVES

To analyse the mass concentration in biomass fuel user and LPG user household and to investigate the effects of biomass smoke exposure in a group of rural women who cook regularly with biomass fuels and compare the results obtained from control group women who cook relatively cleaner fuel, liquefied petroleum gas (LPG).

METHODS

Respiratory health was evaluated from Questionnaire survey, Clinical examination, haematology, sputum cytology culture and serum C-reactive protein (CRP) levels are investigated in biomass and control users.

RESULTS

A total of 150 women were approached, of which only 70 non-smoking women without any history of any major chronic illness in the past were selected for this study. CRP levels differ significantly in biomass exposure than control users.

CONCLUSION

From our study it is clear that with increasing duration of exposure to biomass fuel combustion. Women who used to cook with traditional biomass fuels had low haemoglobin & Red Blood Cells values, increased neutrophil and allergic manifestations. Sputum cytology of majority biomass users revealed bacterial infections & chronic inflammation.

KEYWORDS

Rural Women, Biomass, C-reactive Protein (CRP), Liquefied Petroleum Gas (LPG).

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INTRODUCTION: Air pollution collectively describes the presence of a diverse and complex mixture of chemicals, particulate matter (PM), or biological material in the ambient air which can cause harm to humans. Humans have always been exposed to ambient air pollutants and have therefore adapted cellular defence mechanisms to protect against agents that may cause disease. Urbanisation, industrialisation and fossil fuel based transportation led to significant increase in air pollution in urban areas. World health organisation considers air pollution as a major public health threat that requires efforts in the areas of research

and policy making. An estimated number of 120,600 deaths are attributed to outdoor air pollution per year in India.^[1]

On the other hand, indoor air pollution also poses a significant health threat in rural India. Around 3 million people worldwide are exposed to smoke, the most important global risk factor for chronic obstructive pulmonary disease (COPD). In developing countries nearly 50% of deaths from COPD are attributable to biomass smoke, of which about 75% are of women.^[2] Inflammatory diseases in respiratory system, susceptibility to respiratory tract infections and cardiovascular diseases are linked to air pollution induced mortality and morbidity. Chronic obstructive pulmonary disease, lower respiratory tract infections and asthma are important lung diseases linked to air pollution.

Indoor air pollution is due to fuels in rural areas of the developing countries. The most significant issue that concerns indoor air quality in household environments of developing countries is that of exposure to emissions from combustion of fuels, particularly those used for cooking and heating. The use of open fires with simple solid fuels,

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biomass or coal, for cooking and heating exposes an estimated 2 billion people.^[3-4] in the world to enhanced concentrations of particulate matter and gases, up to 10-20 times higher than typical urban outdoor concentrations.^[5] About 74% biomass using rural household in India still rely on firewood or cow dung as their primary cooking fuel and large no. of household in urban areas depend on LPG (65%), Kerosene (7.5%) and coal (2.9%) for cooking purpose. Exposure to air pollution due to combustion of biomass fuels remains one of the significant risk factor for chronic respiratory diseases in the developing countries. It is estimated that an average woman in India may be subjected to 60,000 hours of exposure to smoke due to combustion of biomass fuels in her life time.

In COPD patients, increased CRP levels are associated with poor lung function, reduced exercise capacity and worse quality of life as well as being a significant predictor of all-cause mortality.^[6-8] The growing awareness of COPD being a complex disease involving several organs with a clearly established low grade systemic inflammation, biomarkers have been much of interest in clarifying the pathogenesis and progression of COPD as well as designing new therapeutic targets for the disease.^[9] The National Ambient Air Quality Standards of the U.S Environmental Protection Agency requires the daily average concentration of PM₁₀ (Particulate matter less than 10 µm in diameter) to be less than 150 µg/m³ and annual average to be less than 50 µg/m³. In contrast, concentration of PM₁₀ ranged above the average level during cooking in Indian households.^[10] The aim of the study to analyse the mass concentration in biomass fuel user & LPG user households and investigate the effects of biomass smoke exposure in a group of rural women who cook regularly with biomass fuels and compare the results obtained from control group women who cook relatively cleaner fuel, liquefied petroleum gas (LPG).

MATERIALS AND METHODS:

Measurement of Air Quality: Measurement of airborne pollutant concentration in cooking areas of rural households was measured by air monitor and measures particles load in the concentration range of 1 µg-100 µg/m³. We measured particulate matters with aerodynamic diameter of less than 2.5 µm (PM_{2.5}). Air quality measurement was done in biomass using and LPG-using household during study period. Monitoring was done for 8 hours/day in cooking hours of a single household of both users. We could not use the monitor for longer periods for the limitation of power. Since biomass using women cook in a sitting position 2-3 ft. away from the open chullah (oven), the monitor was placed in the breathing zone of the cook 2.5 ft. above the floor level and 3 ft. away from the chullah. LPG users, on the other hand, cook in a standing position. Accordingly the monitor was set 3.5 ft. above the floor level.

SUBJECTS: A total number of 150 women participated in this study. The biomass user group was represented by 46 women from Gangawara, Bangalore rural. They were in the age group of 25-79 years and cook regularly with wood, and agricultural refuse such as bamboo, jute stick, etc. Another group of 24 women from Gangawara who use LPG as

cooking fuel, aged 25-69 years, was enrolled as controls. Women in the study population and control groups were selected randomly. The study protocol was approved by the Ethical Committee of SDS-TRC and Rajiv Gandhi Institute of Chest Diseases Institute.

Inclusion Criteria:

- Females.
- Females who used to cook 3-5 hrs./day exposure regularly.
- Females having 5 yrs. or more than 10 yrs. of exposure to Chullah smoke using biomass fuel, wood, cow dung or crop residue.

Exclusion Criteria:

- Females having less than 5 yrs. of exposure to Chullah smoke.
- Smokers.
- Pregnancy.

Haematology: Venous blood was collected after informed consent from antecubital plexus. A sample was collected and used for routine haematology.

Sputum Cytology: The participants were instructed to wash their mouth with saline water and to cough vigorously to expectorate sputum. The samples were collected in a sterile plastic container. Smears were prepared on clean glass slides from thick viscous part of spontaneously expectorated sputum. The slides were immediately fixed in ethanol and stained with Papanicolaou (Pap) stain following the procedure of Hughes and Dodds.^[11] Smears were examined under a microscope and analysed according to cytologic criteria.^[12]

STATISTICAL ANALYSIS: Statistical analysis of the collected data was analysed by mean, percentages and correlation analysis by using Microsoft Excel and statistical tool.

RESULTS: A total of 150 women were approached from the village for the study out of which 70 women gave consent for the investigations such as haematology, sputum cytology, CRP levels. Further two groups were formed, biomass users and LPG users consisting of 46 and 24 women each of the group of 25-79 years (3-5 hr./day exposure). The mean of all parameters of biomass and LPG user:

Variables	Number (n=70)	
	Biomass (46)	Control (24)
Age Group In Years		
20-29	4	6
30-39	6	4
40-49	10	6
50-59	9	6
60-69	8	2
70-79	9	0
Marital Status		
Single	3	4
Married	43	20
Illiterate	30	14

Schooling	16	7
Duration of Cooking (hrs.) per day	3-5 hrs.	2-3 hrs.
Homes with separate kitchen	5	19

Table 1: Descriptive Characteristics of Biomass Fuel User and Control Women

Particulate Air Pollution in Biomass and LPG- Using Households: The concentration of PM_{2.5} level was 298 µg/m³ and 90 µg/m³ in biomass and LPG- using kitchen. Thus, biomass-using kitchen had about 3 times more than LPG users.

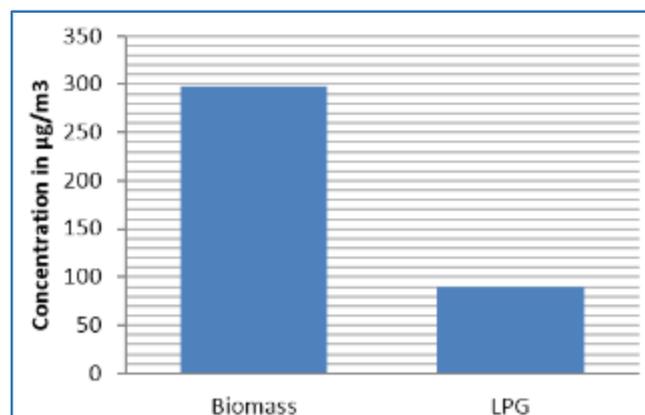


Fig. 1: Particulate Air Pollution in Cooking Areas of Biomass Fuel and LPG Use (Control) in Households during Cooking Hours

In biomass users, maximum percentage of biomass users showed increased Absolute eosinophil count than control as shown in Fig. 2.

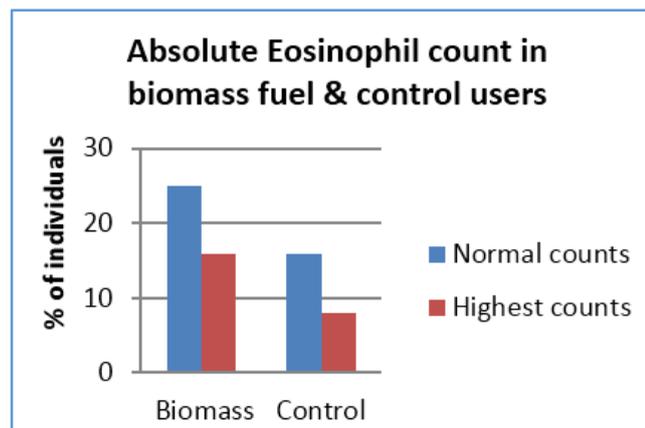


Fig. 2: Absolute Eosinophil Count in Biomass Exposure Who Never Smoked and in Control Users

*P<0.5 compared with control

Measurement of Serum CRP Levels: Venous blood samples were drawn from all subjects for CRP measurement and samples were immediately centrifuged and obtained serum samples were immediately frozen at -80°C until the time of analysis. High sensitive CRP measurements were performed. In biomass fuel user, the level of C-reactive protein was significantly high as compared to control shown in Fig. 3.

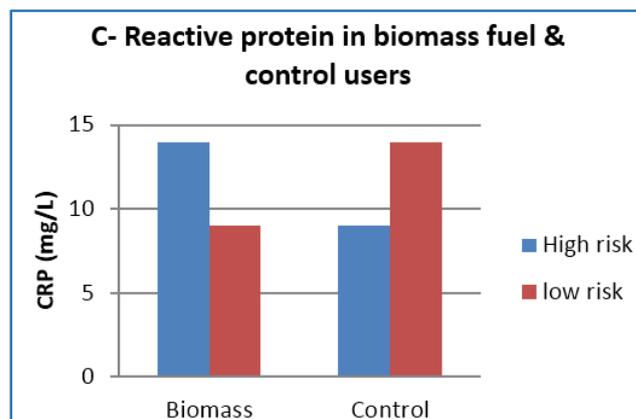


Fig. 3: C-reactive protein (CRP) Levels in Biomass Exposure who never smoked and In Control Users

*P<0.27 compared with control

Haematological Changes: Women who cook with biomass fuels had low haemoglobin and RBC values, but raised WBC, neutrophil and eosinophil counts (Table 2). Leucocytosis was observed in biomass fuel users when compared to control whose WBC counts were normal.

	Biomass User	LPG User
Haemoglobin	8	11
RBC	2.8	4.2
WBC	12000	8000
Platelets	2,00,000	2,82,000
Neutrophil (%)	82	70
Eosinophil (%)	5	4
Lymphocyte (%)	11	22
Monocytes (%)	2	4
AEC	500	385

Table 2: Haematological Changes in Biomass Fuel Users

*P<0.0001 compared with control

Sputum Cytology: Women who cook with biomass fuel had several cellular changes in their lungs. Sputum samples of these women showed significant number of alveolar macrophages, neutrophils, suggesting recurrent bacterial infection and inflammation. Cytological changes are presented in Table 3.

Parameters	Control	Biomass User
Alveolar macrophages/hpf	2.1	8
Neutrophil/hpf	54	65
Eosinophil/hpf	0.45	0.9
Lymphocyte/hpf	35	28
Monocytes/hpf	4	1.9

Table 3: Cytological Changes in Sputum-Differential Counts

Hpf, high power field (×40) of light microscope

DISCUSSION: The objective of this study was to assess the effect of biomass fuel and clean fuel smoke on respiratory parameters. We have shown that these women suffer from allergic manifestations and inflammatory changes in the airways. In our study, the female subjects taken in our study of biomass fuel users develop adverse effects on lungs in the 4th & 5th decade with average age of presentation of 40 yrs. The mean exposure in our study was 3-5 hrs./day with mean duration of exposure being 10 yrs. This depends on amount of exposures of particulate matter. A similar study conducted in Kanchipuram showed mean duration of cooking in the study was 3.5±1.3 hrs./day with mean cooking years being 12.2±5.2 years.^[13] A study conducted in Kolkata reported that PM_{2.5} level was 304±77 µg/m³ and 89±12 µg/m³ in biomass and LPG-using in kitchen.^[14] whereas our finding showed the concentration of PM_{2.5} level was 298±69 µg/m³ and 90±10 µg/m³ in biomass and LPG-using kitchen. Thus, biomass-using kitchen had about 3 times more than LPG users. The study conducted in Zaria, Nigeria shows haematological parameters have no significant differences between the two groups.^[15]; however, in our study, women who used to cook with traditional biomass fuels had low haemoglobin & RBC values, increased in neutrophil percentage.

Neutrophils are the most abundant leucocytes and one of the functions of eosinophils is in allergic reactions, which are important for phagocytosis and destruction of invading pathogens. Most evidence on effects of air pollution comes from studies of diesel exhaust (DE). Thus, the result of the present study agrees with the previous findings that short-term exposure to DE for 1 hr. induced an acute inflammatory response in the airways of healthy human volunteers.^[16-17] In previous study, most of the rural women (70.12%) cooked in separate but closed kitchens and were more exposed to biomass fuel smoke as compared to women who cooked in open non-separate kitchen and were less exposed. Ventilation status is known to influence pulmonary functions.

A study conducted in Porur, Chennai reported that cooks using an open outdoor kitchen had less exposure to fuel smoke than cooks using an enclosed kitchen as emissions are dispersed outdoors.^[1] The present study indicates CRP levels are raised in biomass users compared to control users. With regard to these findings we confirm that elevated CRP levels (9±4.5 mg/L) in our study when compared with study conducted in Turkey, the serum CRP levels were 7.97±11.11 mg/L.^[18] in users due to the ongoing inflammatory status and independent of biomass exposure. Sputum neutrophilia (neutrophil-65), (macrophage-8) was frequent in biomass users. The changes are commonly interpreted lung response to challenges to bacterial infections & chronic inflammations. Accordingly, these changes indicate biomass users are more affected from airborne pathogens than women using LPG. Sputum culture yield - biomass user (75%), LPG user (31%) and the most common growth isolated were *Staphylococcus viridans* followed by *Klebsiella* species and *Pseudomonas* species.

Exposure to biomass fuel leads to airway inflammation, mucociliary dysfunction and consequent airway structural changes.

Airway Inflammation: Chronic inflammation of the airways, lung tissue and pulmonary blood vessels is caused with exposure to biomass fuel. The exposure to biomass fuel causes inflammatory cells such as neutrophils, CD8⁺, T-lymphocytes, B cells and macrophages to accumulate. When activated, these cells initiate an inflammatory cascade that triggers the release of inflammatory mediators such as tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), matrix-metalloproteinases (MMP-6, MMP-9), C-reactive protein (CRP), interleukins (IL-1, IL-6, IL-8) and fibrinogen. These inflammatory mediators sustain the inflammatory process and lead to tissue damage as well as a range of systemic effects. The chronic inflammation is present from the onset of the disease and leads to various structural changes in the lungs which further perpetuate airflow limitation.

Structural Changes: Airway remodelling is a direct result of the inflammatory response associated with exposure to biomass fuel and leads to narrowing of the airways. Three main factors contribute to this: peribronchial fibrosis, build-up of scar tissue from damage to the airways and over-multiplication of the epithelial cells lining the airways. Parenchymal destruction is associated with loss of lung tissue elasticity, which occurs as a result of destruction of the structures supporting and feeding the alveoli (Emphysema). This means that the small airways collapse during exhalation, impeding airflow, trapping air in the lungs and reducing lung capacity.

Mucociliary Dysfunction: Exposure to biomass fuel leads to enlargement of mucous glands that line airway walls in the lungs, causing goblet cell metaplasia and leading to healthy cells being replaced by more mucus-secreting cells. Additionally, inflammation causes damage to the mucociliary transport system which is responsible for clearing mucus from the airways. Both these factors contribute to excess mucus in the airways which eventually accumulates, blocking the airflow and susceptible to infections. Biomass users suffered from airway inflammation and bacterial infection, as evident from sputum macrophage and neutrophilia respectively. In an earlier study, increase in the number of neutrophils has been reported in response to wood smoke exposure.^[19] and influx of neutrophils from circulation to the airways has been found following exposure to PM₁₀.^[20-21] Accumulation of more active neutrophils in the lungs could be helpful for better antimicrobial defence.^[22]

CONCLUSION: In present study, our findings validate the exposure to biomass fuel leads to respiratory disorders which can be avoided by adequate household ventilation, by improvement in stoves & change of the fuel type for cooking & heating.

The systemic inflammation is present and CRP is an important biomarker in means of reflecting disease severity and prognosis of patients. Cytology of sputum indicates presence of bacterial infection & chronic inflammation in biomass users compared to LPG users, in which we found *Staphylococcus viridans* was commonest microorganism isolated. Biomass exposure who never smoked had higher CRP serum levels compared to healthy controls results of the inflammatory nature of the disease itself. Exposure to biomass fuel leads to airway inflammation, mucociliary dysfunction and consequent airway structural changes resulting in impaired host defence mechanisms (susceptible to infections). In our study, biomass users acquired bacterial infections in which culture results showed mainly *Staphylococcus viridans* as a result of impaired host defence mechanism in biomass users, although even LPG users also showed similar culture results with similar infections which might be affecting their family members due to spread of infections. These studies will require the use of reliable but invasive methods of monitoring such as Bronchoscopy (bronchoalveolar lavage, biopsy, FNAC).

Future Directions for Research in this Area Include:

(a) Establishing methods for assessing remodelling so that it can be more readily related to clinical aspects of airway disease, lung function, airway inflammation and responsiveness to treatment; (b) Identifying the specific elements that contribute to airway remodelling, particularly with regard to the extracellular matrix; (c) comparing elements of remodelling in users with exposure to biomass fuel with those in users with control (LPG) who have fixed airflow obstruction.

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