

# Altered frequency of micronucleus in female COPD patients exposed to biomass smoke

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## ABSTRACT

*Chronic Obstructive Pulmonary Disease (COPD) is a major health problem. COPD is characterized by the presence of chronic bronchitis and emphysema. Smoking has been proposed as the main factor for the progression of COPD. The use of biomass fuel for cooking purposes also increases the risk of the COPD by three to four times. In the present investigation mean frequency of micronucleus (MN) was evaluated with the help of MN assay in biomass smoke exposed female COPD patients. Significant difference was observed in the mean frequency of micronucleus among COPD patients as compared to controls.*

**Key words:** COPD, MN, biomass.

## Introduction:

Chronic Obstructive Pulmonary Disease (COPD) remains a major global health problem. COPD is characterized by the presence of chronic bronchitis and emphysema (Macnee, 2006). Smoking has been suggested as the crucial factor for the progression of COPD (Repine *et al.*, 1997). The use of biomass fuel for cooking purposes also increases the risk of the COPD by three to four times (Malik, 1985; Dennis *et al.*, 1996). Salvi and Barnes. (2010) proposed that around 3 billion people are exposed to biomass fuel smoke, compared to 1 billion tobacco smokers; this data suggested that biomass smoke may become more significant risk factor for development of COPD than tobacco. Partial burning of biomass fuels such as cow dung crop residue in an open fire produces huge amounts of particulate matter as well as hydrocarbons, carbon monoxide, oxygenated and chlorinated organic products and free radicals, effects of these products have been studied and an increase in the incidence of COPD, asthma, low birth weight, interstitial lung disease, lower respiratory tract infections were reported (Fullerton *et al.*, 2008; Po *et al.*, 2011; Torres-Duque *et al.*, 2008; Noonan and Balmes, 2010; Smith *et al.*, 2000 ). Various investigators reported increased level of DNA damage in human subjects due to exposure of biomass smoke (Mondal *et al.*, 2011; Ceylan *et al.*, 2006; Pandey *et al.*, 2005; Mukherjee *et al.*, 2011). Micronucleus assay (MN) in buccal epithelial cells is a minimally invasive

technique for studying DNA damage, cell death, chromosomal instability, and the regenerative ability of the human buccal mucosal tissue. In the present study mean frequency of micronucleus (MN) was evaluated with the help of MN assay in biomass smoke exposed female COPD patients.

## Materials and methods:

For the present investigation 53 female subjects were taken out of which 33 were COPD patients and 20 subjects were biomass smoke exposed controls matched with respect to their age, dietary habits and biomass smoke exposure. Subjects were selected from different regions of Haryana. Prior to sampling an appropriate consent was taken from each subject. Ethical clearance was taken from Institutional Ethics Committee, Kurukshetra University, Kurukshetra.

The standard technique of Tolbert *et al.* (1992) was followed for micronucleus assay. Exfoliated buccal epithelial cells were collected gently from the inner cheek of the subjects with the help of moistened steel spatula. Cells were smeared on to pre-cleaned microscopic glass slides. Slides were stained with 2% Aceto-orcein (HIMEDIA, acetic acid RM5564, orcein RM277) for 20 minutes at 40°C and then washed in ethanol and distilled water, respectively. Then the slides were counter-stained with 0.1% Fast green solution (HIMEDIA RM 4266) for 12 minutes and

rinsed in ethanol and distilled water respectively for two to three times. Then the slides were air dried.

For each individual 1000 cells were scored for micronucleus and under Olympus CX-41 trinocular microscope at 1000 X magnification. In order to be considered as micronucleus the suspected nucleus is required to meet the following criteria : (a) rounded, smooth, perimeter suggestive of membrane and have less than third the diameter of the main nucleus (b) staining intensity similar to main nucleus; (d) same focal plane as that of main nucleus.

Statistical analysis was carried using ANOVA test.

## Results:

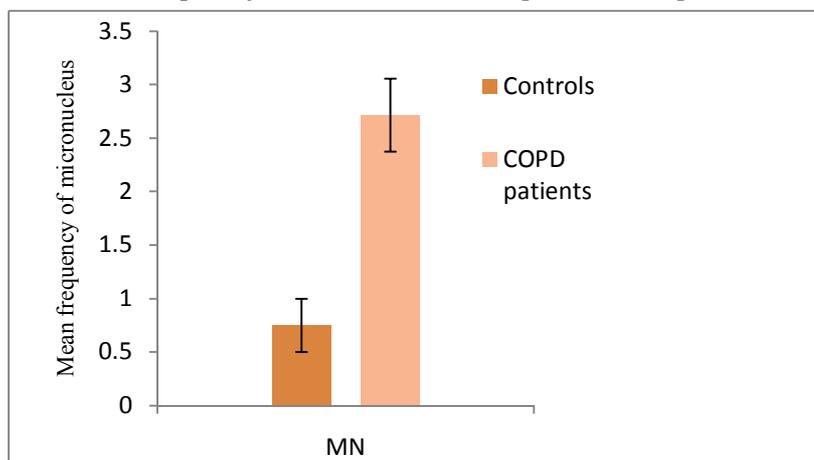
Mean frequency of micronuclei was analysed with the help of micronucleus assay in exfoliated buccal epithelial cells. Mean age of COPD patients was  $55.727 \pm 1.739$  years and for controls  $59.00 \pm 3.303$  years. The result of micronucleus assay showed markedly significant ( $p < 0.001$ ) increase in the mean frequency of micronucleus in biomass smoke exposed female COPD patients as compared to the control female subjects (Fig. 1) which indicates increase level of DNA damage in biomass smoke exposed COPD patients. The mean frequency of micronucleus in COPD patients and control subjects are given in Table 1.

**Table 1: Frequency of micronucleus in biomass smoke exposed female COPD patients and controls**

Micronucleus Frequency (MN)	Biomass smoke Exposed Subjects	N	Mean±S.E.
	Control	20	0.750±0.250
	COPD Patients	33	2.515±0.340*

\*Significant ( $P < 0.001$ ; 2-tailed) (Independent sample t test)

**Fig.1 Micronucleus frequency in biomass smoke exposed COPD patients and controls.**



## Discussion:

Although being a renewable and sustainable energy source, biomass causes major pollution (both indoors and outdoors) on combustion. Various epidemiological, clinical and experimental studies have shown that biomass smoke exposure is harmful to health and predisposes individuals and causes many chronic lung diseases, including COPD (Silva *et al.*, 2015). In the present research work, we observed increased micronucleus level in COPD patients when exposed to biomass smoke. Sisenando *et al.* (2012) studied

that exposed children to biomass combustion showed a significant difference in micronucleus frequencies as compared to non-exposed children in the school of Brazilian Amazon region. In another study, women using biomass fuels for cooking purposes compared with those using LPG, showed that women exposed to biomass fuel had higher levels of cytogenetic abnormalities such as chromosomal aberrations and formation of micronucleus in peripheral lymphocytes (Musthapa *et al.*, 2004). These studies also support

our data and results. In conclusion, exposure of biomass smoke can result to higher level of cytogenetic changes i.e. higher frequency of micronucleus.

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