

Effect Occupational Exposure to Rice Husk Dust on the Levels of Oxidative Stress Markers and Pulmonary Functions in Individuals Working in Rice Mills Around Raichur District Urban Area

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Abstract

Introduction: Air pollution to heart disease linkage involves the direct effects of pollutants on the CVS, blood/lung receptors, and/or indirect effects also mediated through inflammatory responses and pulmonary oxidative stress. The lung is an important target for pro-oxidant compounds mediated genotoxicity. The products such malondialdehyde (MDA) provide the evidence to support the involvement of free radical reaction in toxicology and disease. Increased levels of nitric oxide (NO) cause loss of local vascular regulation, vasoconstriction, and mechanical blockage of vessels cause a reduction in pulmonary vascular region. Both Vitamin C and Vitamin E are powerful antioxidants found in the lungs where they protect cells from oxidative damage.

Objectives: The objectives of the study were to evaluate the effect of rice husk dust on the levels of oxidative stress markers and pulmonary functions in the individuals working in rice mills by analyzing pulmonary function tests (PFTs) and estimating oxidative stress markers.

Materials and Methods: The study was carried on 134 rice mill workers. The PFTs and oxidative stress markers were estimated. Statistical analysis was carried out by applying Student's "t" test, ANOVA, and regression analysis.

Results: Forced expiratory volume, percentage was significantly reduced in elder age groups and long-term exposure groups compared to young age group and short-term exposure group individuals, respectively. MDA, NO levels were increased and Vitamin-C levels decreased in elder age groups and long-term exposure groups compared to young age group and short-term exposure group individuals, respectively. On regression analysis, there was an association with increased age and exposure duration with PFTs and oxidative stress markers.

Conclusion: The findings of the study clearly indicated the deleterious effect of long-term exposure to rice husk dust on pulmonary functions and also causing increased oxidative stress in the individuals. Furthermore, advancing age found to be additive factor for the levels of oxidative stress markers and decreased pulmonary functions.

Key words: Malondialdehyde, Nitric oxide, Oxidative stress, PFTs, Rice husk dust, Vitamin-C

INTRODUCTION

Many studies have found that increased cardiovascular morbidity and mortality are associated with exposure to

ambient air pollution, particularly particulate matter. There is no clear evidence whether such dust exposures increase the risk for ischemic heart disease or not. Till now, there is limited knowledge of the underlying mechanisms in pathogenesis.

A analytical study on carpenters to analyze the long-term occupational exposure to airborne wood dust particulate matter on oxidative stress showed a significant increase in serum malondialdehyde (MDA) with significant decrease in antioxidant level.

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According to the reputed biological mechanisms, air pollution to heart disease linkage involves the direct effects of pollutants on the CVS, blood/lung receptors, and/or indirect effects also mediated through inflammatory responses and pulmonary oxidative stress.^[1] The direct effects may possibly mediate through many varieties of agents which can readily cross the pulmonary epithelium into the systemic circulation. Within the systemic circulation, these direct effects stand for a probable explanation for the mechanism of rapid cardiovascular responses such as increased myocardial infarctions.^[2]

The lung is an important target for pro-oxidant compounds mediated genotoxicity because the bronchial epithelium being a physicochemical barrier plays a crucial role in initiating and augmenting defense as well as signaling systemic responses.

The detection and measurement of lipid peroxidation products such MDA provide the evidence to support the involvement of free radical reaction in toxicology and disease

Increased levels of nitric oxide (NO) cause loss of local vascular regulation, vasoconstriction, and mechanical blockage of vessels all together cause a reduction in pulmonary vascular region.^[3-5]

Each tissue has an antioxidant potential which is determined by the balance between factors promoting auto-oxidation and those exerting an antioxidant action. Multiple lines of antioxidant defense have evolved and serve to protect human body from oxidative stress including prevention, interception, and repair.^[6,7]

Vitamin C (ascorbic acid) is a principal and powerful antioxidant. It works in aqueous environment of the body. Both Vitamin C and Vitamin E are powerful antioxidants found in the lungs where they protect cells from oxidative damage.^[8] Vitamin C quickly identifies and removes many reactive species.^[9]

Many prospective studies so far have observed an inverse relationship between Vitamin C intake and cardiovascular diseases and also a strong protective effect of Vitamin E supplementation on coronary patients. Finnish and Swiss studies indicated that myocardial infarction is predicted in diminished nutritional status of vitamin and low blood levels of ascorbate. Low levels of Vitamin C increases 2.7 times the risk of myocardial infarction and this is independent of other risk factors.

MATERIALS AND METHODS

Study Design

It was cross-sectional analytical study.

1. Exposed
2. Unexposed.

Risk factor included was – exposure to dust.

Sample size

One hundred and thirty-four individuals were selected in each group.

Control – appropriate number of non-exposed subjects of same age group were selected as control.

Inclusion Criteria

- Volunteered rice mill workers aged above 20 and below 50 were included
- Only male individuals were selected
- Individuals working in rice mills having experience of 5 years or more were included
- Only rice mill workers who were working in dust and exposed to dusty environment were included in the study.

Informed consent was taken from all the individuals participated in the study. While the collection of data, structured questionnaire was used. It has helped to determine information in regard of general health, disease history, duration of exposure, and details of habits such as smoking and alcohol consumption.

Recording of lung function parameters: For the recording of lung function parameters, a computerized digital spirometer (SPIRO EXCEL 1.3) was used.

Before recording of pulmonary function tests (PFTs), individual participants were thoroughly explained regarding the procedure in their own language along with demonstration of the procedure.

For estimation of serum MDA and serum NO- 6 ml of intravenous blood was collected in plane tubes. Moreover, 2 ml of blood was collected in EDTA tube for estimation of ascorbic acid in plasma.

- Estimation of serum lipid peroxide (MDA)^[10]
- Estimation of serum NO as nitrite (NO)^[11]
- Estimation of serum ascorbic acid by phenylhydrazine^[12] spectrophotometry method.

RESULTS

Table 1 shows the Mean±SD of exposed and control groups. Student's "t" test was applied to know the significance between both the groups, $P < 0.05$ is considered as significant difference at CI of 95%. There were no significant differences in age, height, and weight between individuals of both the groups. Body mass index of both the groups has found in normal limits though groups show statistically significant difference ($P < 0.05$).

Table 2 shows the Mean ± SD of PFT parameters of study and control groups. Forced vital capacity (FVC) of exposed individuals has found significantly lower than control group individuals ($P < 0.0001$). Forced expiratory volume in first second (FEV₁) of exposed group individuals is also found significantly low compared to control group. Hence, FEV₁/FVC ratio is exposed group individuals, is also found significantly lower in comparison with control group individuals. Obtained values of PFT parameters of both the groups were in the lower normal limit.

Table 3 depicts the Mean±SD values of serum levels of MDA, NO, and also plasma level of Vitamin-C of exposed and control group. Oxidants level (MDA and NO) in exposed group was highly significant ($P < 0.0001$) compared to control group.

Exposed group was divided into three subgroups based on exposure duration to know the effect of duration of exposure. 5–9 years as exposed-I ($n = 82$), 10–18 years as exposed-II ($n = 31$), and 19–27 years as exposed-III ($n = 21$).

Table 4 represents Mean±SD of different groups and analysis of effect of duration of exposure to dust in rice mills on PFTs using ANOVA followed by *post hoc* "t" test. There was significant effect of exposure duration on FVC, FEV₁, and FEV₁/FVC of rice mill workers, exposed-III group showed below normal level of FEV₁/FVC ratio than exposed-I and exposed-II groups. *Post hoc* "t" test was performed to know the significantly affected group. There was significant decrease in FVC, FEV₁, and FEV₁/FVC ratio in all the groups of comparison.

Table 5 represents Mean ± SD of PFT parameters of exposed individuals with different age groups. Student's unpaired "t" test showed significant differences in FVC ($P < 0.001$) and FEV₁ ($P < 0.001$) among all the age groups of comparison whereas FEV₁/FVC ratio was significantly decreased in exposed individuals of Group-II and Group-III. There was no significance in FEV₁/FVC ratio of Group-I.

Table 1: Anthropometric parameters of the study and control groups

Parameters	Mean±SD of exposed (n=134)	Mean±SD of control (n=134)	P value
Age (years)	29.24±5.899	32.81±8.705	-
Height (cm)	165.0±4.799	164.6±6.168	0.562
Weight (kg)	59.21±8.777	60.87±6.830	0.084
Body mass index (kg/m ²)	21.75±3.019	22.43±1.541	0.022*
Years of exposure (years)	9.776±6.476	-	-

*- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

Table 2: Comparison of Mean±SD of pulmonary function test parameters of study and control groups

Parameters	Mean±SD of exposed (n=134)	Mean±SD of control (n=134)	P- value
FVC (forced vital capacity) (in liters)	2.890±0.465	3.233±0.322	$P < 0.0001$ ***
FEV ₁ (forced expiratory volume in first second) (in liters)	2.232±0.472	2.630±0.361	$P < 0.0001$ ***
Forced expiratory volume/forced vital capacity ratio (%)	76.88±8.937	81.16±5.914	$P < 0.0001$ ***

*- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

Table 3: Comparison Mean±SD of biochemical parameters of study and control groups

Parameters	Mean±SD of exposed (n=134)	Mean±SD of control (n=134)	P-value
Serum malondialdehyde (μmol/l)	7.009±1.892	5.639±2.091	$P < 0.0001$ *
Serum nitric oxide (μmol/l)	51.03±16.812	36.73±14.157	$P < 0.0001$ *
Plasma Vitamin C (mg/l)	0.863±0.324	1.173±0.262	$P < 0.0001$ *

*- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

Table 6 depicts the Mean ± SD of serum MDA, serum NO, and plasma Vitamin-C of exposed and control individuals in different age groups (Group-I, Group-II, and Group III). There was significant difference obtained in plasma Vitamin-C ($P < 0.01$) of exposed individuals in Group-I. No significant differences obtained in serum levels of MDA and NO in Group-I. There were high serum levels of MDA ($P < 0.01$), NO ($P < 0.001$), and low level of plasma Vitamin-C ($P < 0.001$) of exposed individuals in Group-II and there were higher serum levels of MDA ($P < 0.001$), NO ($P < 0.001$), and lower level of plasma Vitamin-C ($P < 0.001$) of exposed individuals in Group-III compared to Group I and II.

Regression analysis was done to know the association of different predictors on dependent variables.

Table 4: ANOVA followed by *post hoc* “*t*” test showing Mean±SD of pulmonary function test parameters of study group with duration of exposure

Parameters	Control (Mean±SD) n=134	Exposed-I (5–9 years) (Mean±SD) n=82	Exposed-II (10–18 years) (Mean±SD) n=31	Exposed-III (19–27 years) (Mean±SD) n=21	F value	P value
FVC (forced vital capacity) (in liters)	3.233±0.322	2.924±0.374	2.801±0.643	2.891±0.485	17.133	0.000***
FEV ₁ (forced expiratory volume in first second) (in liters)	2.629±0.361	2.335±0.369	2.091±0.665	2.037±0.389	25.370	0.000***
Forced expiratory volume ₁ /Forced vital capacity ratio (%)	81.161±5.914	79.816±6.855	73.339±11.478	70.018±7.863	20.763	0.000***

Post hoc “*t*” test

Parameters	Control versus exposed-I	Control versus exposed-II	Control versus exposed-III
	P-value	P-value	P-value
FVC (forced vital capacity) (in liters)	0.000***	0.000***	0.001**
FEV ₁ (forced expiratory volume in first second) (in liters)	0.000***	0.000***	0.000***
Forced expiratory volume ₁ /Forced vital capacity ratio (%)	0.434	0.000***	0.000***

*- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

Table 5: Comparison of Mean±SD of biochemical parameters of exposed individuals with different age groups

Parameters	Group I (21–30 years)			Group II (31–40 years)			Group III (41–50 years)		
	Control (n=68)	Exposed (n=68)	P Value	Control (n=32)	Exposed (n=33)	P value	Control (n=34)	Exposed (n=33)	P value
Serum malondialdehyde (µmol/l)	5.659±2.251	6.291±1.647	0.064	5.969±1.981	7.503±1.757	0.002**	5.288±1.848	7.994±1.941	$P < 0.0001$ ***
Serum nitric oxide (µmol/l)	37.61±16.26	42.18±14.09	0.082	36.56±9.591	59.63±15.22	$P < 0.0001$ ***	35.13±13.43	60.69±13.72	$P < 0.0001$ ***
Plasma Vitamin C (mg/l)	1.176±0.265	1.018±0.308	0.002**	1.241±0.201	0.709±0.257	$P < 0.0001$ ***	1.103±0.292	0.696±0.258	$P < 0.0001$ ***

*- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

On consideration of FEV₁/FVC ratio as dependent variable and exposure, age, MDA, Vitamin-C, and NO as constants, the R squared value (r^2) obtained was 28.2%. There was significant association of exposure ($P < 0.05$) and age ($P < 0.001$) on FEV₁/FVC ratio of exposed individuals. There was no significant association of MDA, Vitamin-C, and NO with FEV₁/FVC ratio of exposed individuals.

On consideration of MDA as dependent variable and exposure, age, Vitamin-C, and NO as constants, the R squared value (r^2) obtained was 70.5%. There was significant association of Vitamin-C ($P < 0.001$) on MDA of exposed individuals. There was no significant association of exposure, age, and NO on MDA of exposed individuals.

On consideration of NO as dependent variable and exposure, age, Vitamin-C, and MDA as constants, the R squared value (r^2) obtained was 85.8%. There was significant association of age ($P < 0.01$) Vitamin-C ($P < 0.001$) on NO of exposed individuals. There was no significant association of exposure and MDA on NO of exposed individuals.

On consideration of Vitamin-C as dependent variable and exposure, age, MDA, and NO as constants, the R squared value (r^2) obtained was 89.5%. There was significant association of MDA ($P < 0.001$) and NO ($P < 0.001$) on Vitamin-C in exposed individuals. There was no significant association of exposure, age Vitamin-C of exposed individuals.

There was no significant association of any of the dependent variables with the constants in control group individuals.

In the present study, we have obtained lower FVC ($P < 0.0001$), FEV₁ ($P < 0.0001$), and FEV₁/FVC ratio ($P < 0.0001$) compared to control [Table 2]. However, the obtained values are within the normal limits and are statistically significant. Our results are in agreement with the conclusions of Gildea and McCarthy (2003). They obtained a low but not a significant decrease in FVC relative to the control groups. However, absence of alteration in pulmonary function cannot be justified by FVC alone because in patients with obstructive lung diseases, FVC

Table 6: Regression analysis showing the effect of different predictors on dependent variables in exposed group

Dependent variable: FEV ₁ /FVC ratio				
Group	R Value	R Square (r ²)	Constants	Significance
Exposed	0.531 (53.1%)	0.282 (28.2%)	Exposure	0.028*
			Age	0.000***
			MDA	0.191
			Vitamin C	0.690
			Nitric oxide	0.786
Controls	0.121 (12.1%)	0.015 (1.5%)	Exposure	Not applicable
			Age	0.713
			MDA	0.232
			Vitamin C	0.515
			Nitric oxide	0.744

Dependent variable: MDA				
Group	R Value	R Square (r ²)	Constants	Significance
Exposed	0.840 (84.0%)	0.705 (70.5%)	Exposure	0.362
			Age	0.735
			Vitamin C	0.000***
			Nitric oxide	0.370
			Controls	0.121 (12.1%)
			Age	0.362
			Vitamin C	0.419
			Nitric oxide	0.584

Dependent variable: NO				
Group	R Value	R Square (r ²)	Constants	Significance
Exposed	0.926 (92.6%)	0.858 (85.8%)	Exposure	0.350
			Age	0.004**
			Vitamin C	0.000***
			MDA	0.370
			Controls	0.160 (16.0%)
			Age	0.425
			Vitamin C	0.127
			MDA	0.584

Dependent variable: Vitamin C				
Group	R Value	R Square (r ²)	Constants	Significance
Exposed	0.946 (94.6%)	0.895 (89.5%)	Exposure	0.895
			Age	0.820
			Nitric oxide	0.000***
			MDA	0.000***
			Controls	0.162 (16.2%)
			Age	0.457
			Nitric oxide	0.127
			MDA	0.419

*-P<0.05, **-P<0.01, ***-P<0.001. FVC: Forced vital capacity, FEV: Forced expiratory volume, MDA: Malondialdehyde

can be normal or slightly decreased.”^[13] Similar results have been seen in the study carried out by Anupriya *et al.*^[14] on saw mill workers.

Among the duration of dust exposure groups, we have obtained clinically significant lower values of FVC ($P < 0.0001$), FEV1 ($P < 0.0001$), and FEV1/FVC ($P < 0.0001$) ratio in higher exposure duration groups (1–9 years >10–18 years >19–27 years) compared to control group [Table 4]. Decrease in FVC and FEV1 may be due

to obstructive impairment which further increases with increase in number of years of exposure. In other words, there is a dose exposure relationship. Similar research studies conducted by Al-Neaimi *et al.* 2001 and Mwaiselage *et al.* 2004. The results also showed a significant reduction in FEV1%, which is an indication of obstructive impairment it is in turn may be due to mechanical irritation caused by dust exposure and individual susceptibility. These changes in pulmonary volume suggest an urgent need of improvement in dust control measures and health awareness toward dust preventive measures.^[15,16]

In comparison of FVC, FEV₁, and FEV₁/FVC ratio of study group with control group among different individuals with age groups [Table 5], we have obtained significantly lower values of higher age groups (21–30 years >31–40 years >41–50 years). This shows as the age advances there is decrease in flexibility hence loss of compliance and also increased airway resistance, which ultimately leads to decrease in FVC, FEV₁, and FEV₁/FVC ratio. Our results are in contrast to the results obtained by Ghotkar *et al.*^[17] They have shown as there is no significant decrease in FEV₁/FVC ratio, though there is decrease in values of FEV₁ and FVC in groups of individuals with increase in age.

Our study is in agreement with Hernberg *et al.* also shown that “there is a strong significant association between MDA level and oxidative stress. He also proved that ascorbic acid has significant role in reducing the oxidative stress.”^[18]

In the present study, we have categorized the exposed individuals into three groups based on years of exposure. We have noticed that as the duration of exposure increased, there were marked increase in oxidative stress markers such as MDA and NO. Ascorbic acid level was significantly reduced in groups of individuals with longer duration of exposure. The present study is supported by the previous study conducted by Debra *et al.*, they observed as duration of exposure was increased, oxidative stress effect was also enhanced. They showed long-term exposure to cotton dust results in inflammatory cell migration into the air spaces. This will generate reactive oxygen species by opsonization, thereafter appear to precede increased lung permeability and reflect a loss of integrity of epithelial tight junction.^[19]

On regression analysis for effect of different predictors on dependent variables, we have obtained positive correlation between age and FEV₁/FVC percentage, negative correlation between exposure duration and FEV₁/FVC percentage. Other predictors showed no significant effect in exposed group. There was a negative correlation between serum MDA, serum NO, and antioxidant Vitamin C. Age has shown positive correlation on serum level of NO

Table 7: ANOVA showing Mean±SD of biochemical parameters of study group with duration of exposure

Parameters	Control (Mean±SD) n=134	Exposed-I (5-9 years) (Mean±SD) n=82	Exposed-II (10-18 years) (Mean±SD) n=31	Exposed-III (19-27 years) (Mean±SD) n=21	F value	P value
Serum malondialdehyde (µmol/l)	5.639±2.091	6.627±1.749	7.600±2.103	7.629±1.797	13.425	0.000***
Serum nitric oxide (µmol/l)	36.729±14.157	48.316±17.620	54.251±15.252	56.899±13.817	21.591	0.000***
Plasma Vitamin C (mg/l)	1.173±0.262	0.915±0.331	0.798±0.330	0.752±0.248	27.708	0.000***

Post hoc "t" test

Parameters	Control versus exposed-I	Control versus exposed-II	Control versus exposed-III
	P-value	P-value	P value
Serum malondialdehyde (µmol/l)	0.001**	0.000***	0.000***
Serum nitric oxide (µmol/l)	0.000***	0.000***	0.000***
Plasma Vitamin C (mg/l)	0.000***	0.000***	0.000***

*-P<0.05, **-P<0.01, ***-P<0.001

Table 8: Comparison of Mean±SD of pulmonary function test parameters of exposed and control individuals with different age groups

Parameters	Group I (21-30 years)			Group II (31-40 years)			Group III (41-50 years)		
	Control (n=68)	Exposed (n=68)	P value	Control (n=32)	Exposed (n=33)	P value	Control (n=34)	Exposed (n=33)	P value
FVC (forced vital capacity) (in liters)	3.272±0.30	3.033±0.346	P<0.0001***	3.280±0.302	2.906±0.439	0.0002***	3.111±0.344	2.580±0.560	P<0.0001***
FEV ₁ (forced expiratory volume in first second) (in liters)	2.644±0.334	2.443±0.349	0.0008***	2.712±0.370	2.198±0.387	P<0.0001***	2.522±0.389	1.831±0.510	P<0.0001***
Forced expiratory volume ₁ /Forced expiratory volume ratio (%)	80.72±5.946	80.55±7.002	0.885	82.49±6.485	75.70±6.708	P<0.0001***	80.80±5.236	70.47±10.58	P<0.0001***

*-P<0.05, **-P<0.01, ***-P<0.001

among exposed group. There was no significant correlation between predictors and dependent variables in control group [Tables 7 and 8].

CONCLUSION

- FVC, FEV₁, and FEV₁/FVC percentage were significantly decreased in exposed group compared to control group. This indicates the adverse effect of dust exposure on lung function parameters
- Decrease in values of PFT parameters was observed in group with exposure duration of 19-27 years compared to groups with exposure duration of 10-18 years and 5-9 years. It is concluded that the duration of exposure is increased, the vital capacity of lungs decreased significantly. Expiratory capacity also decreased significantly which indicates that there is an increase in airway obstruction as the duration of exposure to dust is increased
- Significant decrease in PFT parameters was observed in group III (age 41-50 years) compared to group II (age 31-40) and Group I (age 21-30). It signifies that

as age advances, there is decrease in vital capacity along with increase in airway resistance. Therefore, abnormal decrease in efficiency of lungs may occur

- On comparison with control group, we found significant increase in serum levels of MDA and NO which are oxidants present in blood and are regarded as oxidative stress markers. We found significant decrease in plasma level of ascorbic acid which is a first line of defense among the antioxidants. These findings show that there is an increased oxidative stress which could be the causative factor for cardiovascular dysfunction and hematological abnormalities in rice mill workers on longstanding exposure.
- When individuals are grouped according to age and exposure duration, elderly age group and long-term exposure groups have shown mild decrease in PFTs. These groups have also shown increased stressor level to upper normal values and decreased anti-stressor to lower normal values. As the age advances adverse effect of dust on serum NO level is enhanced. This is an alarming message for individuals working in rice mills that continued and longstanding exposure may lead to increased oxidative stress and oxidative damage.

- It may be advisable to estimate levels of oxidative stress markers such as serum MDA, serum NO, and plasma ascorbic acid as routine investigations for industrial workers.
- Supplementation of Vitamin C may be recommended for rice mill workers.
- Dietary supplementations of citrus fruits and vegetables may be advised for such workers.

Limitations

- We have not estimated the chemical and physical properties of dust to which workers were exposed
- We have not estimated the total antioxidant level
- Female individuals were not included in the study.

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Self.

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