

Occupational Health Hazards among Sewage Workers: Oxidative Stress and Deranged Lung Functions

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ABSTRACT

Background: Sewage workers, because of their occupation, are exposed to different types of dusts, bio-aerosols, fumes and gases like methane, hydrogen sulfide, sulphur dioxide, etc, which contribute towards oxidative stress and detrimental effects on various body functions, especially lung functions.

Aims and Objectives: This study was carried out on sewage workers (who had been working for more than five years). We wanted to study the role of oxidative stress in development of impaired lung functions among sewage workers.

Materials and Methods: This cross sectional study was done in a tertiary care hospital (J.N. Medical College) in Aligarh, U.P. Study was done from March 2008 to December 2009. The study group comprised of 62 sewage workers who had been working for more than five years (32 non-smokers and 30 smokers) and 60 control subjects (30 smokers and 30 non-smokers). The pulmonary functions of these workers were assessed by using a MIR (Medical International Lab) Spiro Lab II Spirometer, with subjects in sitting position. Valid written consents were obtained from all the subjects. Malondialdehyde (MDA) is produced as a result of

the action of reactive oxygen species (ROS) on the lipids present in the membranes of the cells, especially, contracting muscle cells. Serum MDA levels were assessed as an indirect measure of oxidative stress in these sewage workers and they were compared with serum MDA levels of control subjects. Appropriate statistical tests were applied for analysis of the data which was generated.

Observation and Results: There were statistically significant decreases in Peak Expiratory Flow Rate (PEFR), Forced Expiratory Volume in first second (FEV1) and FEV1/FVC percent ratio (<80%) and Forced Expiratory Flow at 25%-75% of volume as percentage of Vital Capacity (FEF 25%-75%). Also, we found statistically significant increased levels of serum MDA in these sewage workers as compared to those in control subjects (with a p-value of <0.05 with a confidence interval of 95%).

Conclusion: Our study found that the occupational exposure of the sewage workers to harmful dust, fumes, gases and bio-aerosols contributed to oxidative stress among them. This oxidative stress was one of the mechanisms which led to the development of obstructive impairment of lung functions in these sewage workers.

Keywords: Sewage workers, Oxidative stress, Lung functions

INTRODUCTION

Oxidative stress is a condition in which the delicate balance which exists between production of pro-oxidants (free radicals) and their subsequent destruction via the antioxidant defense system comes in favour of free radical expression [1]. A free radical is any species which is capable of existence, which contains one or more unpaired electrons [2]. There are many types of free radicals, like hydrogen atoms, transition metal ions, carbon centered radicals and sulfur centered radicals (e.g., Thiyl). But those which are derived from either oxygen and/or nitrogen represent the most important class of radicals which are generated in living systems [3-5]. Indirect assessment of oxidative stress involves the measurement of the more stable molecular products which are formed via the reaction of RONS (reactive oxygen and nitrogen species) with certain biomolecules like lipids. Common lipid peroxidation end products are isoprostanes, malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOHs), conjugated dienes (CDs), etc. [6].

The study of respiratory mechanics is done by doing Pulmonary Function Tests (PFTs) [7]. Sewage workers are exposed to dust, bio-aerosols and various gases and these dust, bio-aerosols and gases are implicated as one of the causes which lead to development of asthma, COPD and other obstructive changes in the lungs [8-11].

MATERIALS AND METHODS

This cross sectional study was done in the Department of Physiology of a tertiary care hospital, J.N. Medical College, A.M.U. in Aligarh, U.P. Approval for conducting the study was obtained from the

institutional ethical committee of the hospital. Also, valid written consents were taken from all the subjects.

Study group: Sixty two sewage workers who were working since more than five years (32 non-smokers and 30 smokers); males who were aged between 18-50 years.

Control group: Sixty matched subjects (among which 30 were non-smokers and 30 were smokers) of similar sex (males), age (between 18 to 50 years), height, race and socioeconomic strata.

Inclusion Criteria

- Those who were apparently healthy.
- Those who gave their consents for the study.

Exclusion Criteria

- Those who had a recent history of acute upper respiratory tract infections.
- Those who had/having chronic medical illnesses like Diabetes mellitus, hypertension, chronic renal diseases and, chronic respiratory ailments.

PFT (pulmonary function test)/lung function test was done by using a computerized Spirometer MIR (Medical International Research) Spiro Lab II. The technique of spirometry was demonstrated to the subjects before they were asked to perform the test. The test was done 3 times and best among 3 readings was taken as final reading. Recommendations of American Thoracic society (ATS)/ERS task force series were followed while spirometry was performed [12].

Serum Malondialdehyde (MDA) was estimated by the method which was adopted by Philpot [13].

For estimating serum malondialdehyde, 5 millilitres of the blood sample was collected from peripheral veins of the subjects. This sample was centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was used for serum malondialdehyde (MDA) estimation as follows:

MDA Estimation

Principle: One molecule of MDA reacts with two molecules of Thiobarbituric acid (TBA) at a pH of 3.5. The pink coloured chromogen is measured spectrophotometrically at 532 nm.

Procedure: For the assay, 1 ml. of serum was mixed with 2.5 ml. of 20% Trichloroacetic acid (TCA) and 1 ml of 0.67% of aqueous solution of TBA. The mixture was heated for 30 minutes in a boiling water bath. The pink pigment was extracted with 2 ml. of n-Butanol and its absorbance was read at 532 nm against n-Butanol which was used as a blank.

MDA= (OD of sample/OD of standard) X concentration of standard MDA in nanograms/ml of serum.

STATISTICAL ANALYSIS

Unpaired 't' test was applied for statistical evaluation of the data which was generated. SPSS (Statistical package for Social Sciences), version 17.0 software was used.

The statistical significance level was set at a 'p' value of <0.05 and a confidence interval (CI) of 95%. Statistically significant changes have been shown in tables as values which are marked by *.

Nonsmoker sewage workers were compared with nonsmoker control subjects for lung functions [Table/Fig-1] and serum MDA [Table/Fig-3]. Smoker sewage workers were compared with smoker control subjects for lung functions [Table/Fig-2] and serum MDA levels [Table/Fig-4].

RESULTS

As has been shown in [Table/Fig-1], there were statistically significant decreases in PEFR, FEV₁, and FEF 25%-75% among non-smoker

	Control Subjects (non-smoker) (n=30) Mean ± SD	Sewage Workers (non-smoker) (n=32) Mean ± SD	p-value
Age (years)	36.50 ± 8.96	36.23 ± 9.20	NS
Height (meters)	1.70 ± 0.05	1.70 ± 0.06	NS
Weight (kilograms)	61.50 ± 10.20	60.70 ± 9.20	NS
FEV ₁	88.50 ± 18.80	60.72 ± 11.98	<0.01*
FVC	86.66 ± 12.92	85.87 ± 15.16	NS
FEV ₁ / FVC %	>80%	<80%	\$
PEFR	90.16 ± 14.30	64.40 ± 8.54	<0.01*
FEF _{25%-75%}	84.00 ± 18.20	63.40 ± 17.12	<0.01*

[Table/Fig-1]: Comparison between non-smoker sewage workers with non-smoker control subjects
NS means Non Significant change. *means statistically significant (p-value <0.05) change with Confidence Interval (CI) of 95%. \$ FEV₁/ FVC % ratio indicates obstructive pattern of Lung Functions impairment among non- smoker Sewage Workers

	Control Subjects (smoker) (n=30) Mean ± SD	Smoker Sewage Workers (smoker) (n=30) Mean ± SD	p-value
Age (years)	37.40 ± 7.66	38.70 ± 8.20	NS
Height (meters)	1.68 ± 0.06	1.67 ± 0.05	NS
Weight (kilograms)	58.82 ± 7.20	57.32 ± 8.39	NS
FEV ₁	81.90 ± 10.17	56.80 ± 11.91	<0.01*
FVC	84.25 ± 11.20	85.00 ± 15.96	NS
FEV ₁ / FVC	>80%	< 80%	\$
PEFR	78.16 ± 5.10	58.53 ± 7.42	<0.01*
FEF _{25%-75%}	60.10 ± 17.22	51.00 ± 7.69	<0.05*

[Table/Fig-2]: Comparison between smoker sewage workers with smoker control subjects

sewage workers as compared to the values in non-smoker control subjects, while FVC was not decreased significantly among these groups. FEV₁/FVC per cent ratio was <80%, which indicated obstructive pattern of impaired lung functions. These findings were in accordance with findings of previous studies [8-11].

[Table/Fig-2] shows similar changes in pulmonary function tests of smoker sewage workers as compared to those seen in smoker control subjects.

Also, [Table/Fig-3] shows a statistically significant increase in serum MDA in non-smoker sewage workers as compared to its value which was seen in non-smoker control subjects. [Table/Fig-4] shows similar significantly increased serum MDA values among smoker sewage workers in comparison with values seen in smoker control subjects.

DISCUSSION

Kogevinas M, in his population based study which was done on more than 15,000 individuals across Europe, found the highest risk of asthma, which was defined as bronchial hyper-responsiveness and reported.

That asthma symptoms were seen in farmers (odds ratio 2.62 [95% CI 1.29-5.35]), painters (2.34 [1.04-5.28]), plastic workers (2.20 [0.59-8.29]), cleaners (1.97 [1.33-2.92]), spray painters (1.96 [0.72-5.34]), and agricultural workers (1.79 [1.02-3.16]). The most consistent results found across countries were seen among farmers and cleaners. Excess asthma risk was associated with high exposure to biological dusts, mineral dusts, gases and fumes. In our study, we also found an obstructive pattern of lung function impairment among sewage workers.

Johncy et al., in their study done on 25 street sweepers who were working for more than 5 years, found statistically significant decreases in FEV₁, FVC, PEFR, FEF_{25-75%} like we found in our study, but they found the ratio of FEV₁/FVC to be increased, while in our study, we found it to be increased. So, they found both restrictive and obstructive patterns of lung function impairment, while we found only obstructive pattern of lung function impairment [14].

Mohan et al., in their study done on 150 non-smoker carpenters who are exposed to wood dust, found a statistically significant decrease in PEFR, like we found in our study [15].

Saha et.al. conducted a study on 59 dry cell battery factory workers who were exposed to cadmium, nickel, cobalt, potassium hydroxide, sodium hydroxide. They found a trend of decrease of lung volumes with increment of age and duration of exposure. The pulmonary function abnormalities which were found among the dry battery factory workers were found to be obstructive (18.5%), restrictive (5.6%) and mixed (4.5%) [16].

Skeletal muscles generate free radicals during rest and their production is increased during performance of contractile activities [17,18]. Overproduction of free radicals may result in oxidative

	Mean value Serum MDA (nanograms /millilitre)	Standard Deviation (SD) (nanograms/millilitre)
Control Subjects (non-smoker) [n=30]	3.53	2.45
Sewage Workers (non-smoker) [n=32]	25.42*	3.77

[Table/Fig-3]: Comparison of serum MDA level in non-smoker sewage workers and non-smoker control subjects

	Mean value Serum MDA (nanograms /millilitre)	Standard Deviation (SD) (nanograms/millilitre)
Control Subjects (smoker) [n=30]	7.33	2.23
Sewage Workers (smoker) [n=30]	34.45*	5.26

[Table/Fig-4]: Comparison of serum MDA level in smoker sewage workers and smoker control subjects

stress. Oxidative stress increases the relative risk of development of COPD [19,20].

Previous researches have shown that oxidative stress impairs skeletal muscle contractions [21-25]. Decreased function of the respiratory muscles, especially the diaphragm, is known to occur in patients with severe chronic obstructive pulmonary disorder (COPD) [26-28].

The results of our study were in accordance with those of the above mentioned studies, which indicated increased serum MDA levels (indicating oxidative stress), along with impaired lung functions (COPD) among sewage workers.

Free radicals can interfere with excitation contraction coupling at several sites at the molecular level, which contribute to respiratory muscle dysfunctions like Ryanodin Receptor (RyR) channel activity [29] and nitric oxide (NO) inhibited Ca^{2+} release which occurs via the RyR channel [30]. Free radicals reduce the amplitude of action potentials, which contribute to decreased muscle contractions [31].

LIMITATIONS OF THE STUDY

- This was a cross-sectional study and so, a cause and effect relationship could not be established.
- The sample size or the number of the subjects in each group was small

CONCLUSION

Finally, on the basis of our study, we can conclude that there were oxidative stress and impaired lung functions among sewage workers and that this oxidative stress could be one of the mechanisms that could contribute to development of obstructive impairment of lung functions in these sewage workers.

REFERENCES

- [1] Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect*. 1994;102:5-12.
- [2] Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med*. 1991;91:14S-22S.
- [3] Miller DM, Buettner GR, Aust SD. Transition metals as catalysts of "autoxidation" reactions. *Free Radic Biol Med*. 1990;8:95-108.
- [4] Bogdan C, Rollingshoff M, Diefenbach A. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Curr Opin Immunol*. 2000;12:64-76.
- [5] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39:44-84.
- [6] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem*. 2006;52:601-23.
- [7] Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med*. 1995;153(3):1107-36.
- [8] Kogevinas M, Anto JM, Sunyer J, Tobias A, Kromhout H, Burney P. Occupational asthma in Europe and other industrialised areas: a population based study. European Community Respiratory Health Survey Study Group. *Lancet*. 1999;353(9166):1750-4.
- [9] Rao NM, Kashyap SK, Kulkarni PK, Saiyed HN, Purohit AK, Patel BD. Pulmonary function studies in 15-18 years of age workers exposed to dust in industries. Indian. *J Physiol Pharmacol*. 1992; 36(1):51-4.
- [10] Bechlake MR. Chronic airflow limitation: its relationship to work in dusty occupations. *Chest*. 1985;88(4):608-17.
- [11] Hameed AAA, Shakour AA, Yasser HI. Evaluation of bio-aerosols at an animal feed manufacturing industry: A case study. *Aerobiologia*. 2003;19:89-95.
- [12] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of Spirometry. *Eur Respir J*. 2005;26:319-38.
- [13] Philpot J. Assay for MDA levels. *Rad Res*. 1963 :3:55-80.
- [14] Johncy SS, Dhanyakumar G, Samuel VT, Ajay KT, Bondade SY. Acute Lung Function response to dust in Street Sweepers. *Journal Clinical Diagnostic Research*. 2013;7(10):2126-9.
- [15] Mohan M, Aprajita, Panwar NK. Effect of Wood dust on Respiratory Health Status of Carpenters. *J Clinical Diagnostic Research*. 2013;7(8):1589-91.
- [16] Saha K, Sarkar S, Bandopadhyay A, Mallik MK, Banerjee A, et al., Pulmonary Function Impairments among Dry Cell Battery factory Workers. *J Clinical Diagnostic Research*. 2012;6(3):342-5.
- [17] Davies KJ, Quintanilha AT, Brooks GA, et al. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun*. 1982;107:1198-205.
- [18] Reid MB, Haack KE, Franchek KM, et al. Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol*. 1992;73:1797-804.
- [19] Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med*. 1991;91:14S-22S.
- [20] Shindoh C, Di Marco A, Nethery D, et al. Effect of PEG-superoxide dismutase on the diaphragmatic response to endotoxin. *Am Rev Respir Dis*. 1996;145:1350-4.
- [21] Reid MB, Haack KE, Franchek KM, et al. Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol*. 1992;73:1797-804.
- [22] Supinski GS, Stofan D, Ciuffo R, et al. N-acetylcysteine administration and loaded breathing. *J Appl Physiol*. 1995;79:340-7.
- [23] Ciuffo R, Nethery D, Di Marco A, et al. Effect of varying load magnitude on diaphragmatic glutathione metabolism during loaded breathing. *Am J Respir Crit Care Med*. 1995; 152:1641-7.
- [24] Kobzik L, Reid MB, Bredt DS, et al. Nitric oxide in skeletal muscle. *Nature*. 1994;372:546-8.
- [25] Barclay JK, Hansel M. Free radicals may contribute to oxidative skeletal muscle fatigue. *Can J Physiol Pharmacol*. 1991;69:279-84.
- [26] Polkey MI, Kyroussis D, Hamnegard CH, et al. Diaphragm strength in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1996;154:1310-7.
- [27] Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. *Am J Respir Crit Care Med*. 1996;153:976-80.
- [28] Bernard S, LeBlanc P, Whittom F, et al. Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1998;158:629-34.
- [29] Favero TG, Zable AC, Abramson JJ. Hydrogen peroxide stimulates the Ca^{2+} -release channel from skeletal muscle sarcoplasmic reticulum. *J Biol Chem*. 1995;270:25557-63.
- [30] Meszaros LG, Minarovic I, Zahradnikova A. Inhibition of the skeletal muscle ryanodine receptor calcium release channel by nitric oxide. *FEBS Lett*. 1996;380:49-52.
- [31] Courtois M, Maupouil V, Fantini E, et al. Correlation between direct ESR spectroscopic measurements and electromechanical and biochemical assessments of exogenous free radical injury in isolated rat cardiac myocytes. *Free Radic Biol Med*. 1998;24:121-31.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 20, 2013**

Date of Peer Review: **Jan 15, 2014**

Date of Acceptance: **Feb 26, 2014**

Date of Publishing: **Apr 15, 2014**