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OXIDATIVE STRESS IN HUMAN POPULATION EXPOSED TO OUTDOOR AIR POLLUTION AT HYDERABAD

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ABSTRACT

Introduction: Air pollution is environmental risk to public health. As urban air quality declines, the risk of stroke, lung cancer, cardiovascular and respiratory diseases increases. Oxidative stress from air pollution exposure may be an important mechanism for these deleterious effects. Therefore, effect of outdoor air pollution on oxidative stress in humans was evaluated.

Methods: In this community based, cross-sectional study, adults of either gender exposed to outdoor pollution were included. Subjects with history of intake of any medication or co-morbidity known to affect oxidative stress were excluded. Subjects exposed to outdoor pollution > 5hrs/day at least 5days/week were considered as cases and those exposed to outdoor pollution < 2hrs/day more than 5days/week as controls. Serum levels of Malondialdehyde, Total Nitric oxide and Sulfhydryl (Total) were estimated by UV spectrophotometry and compared between cases and controls.

Results: Total of 28 subjects were enrolled, from two areas in Hyderabad of which area 1was afflicted with vehicular pollution and area 2 with industrial pollution. Serum level of Malondialdehyde was significantly high and sulfhydryl (total) and total nitric oxide were significantly low in cases when compared to controls. Mean serum concentration of sulfhydryl (total) was significantly high in cases of Area 1, location with vehicular pollution.

Conclusion: This study demonstrates oxidative stress in population with high exposure to ambient air pollution when compared to population with lower exposure.

Keywords: oxidative stress; ambient air pollution; malondialdehyde; total nitric oxide; glutathione; particulate matter

INTRODUCTION

Air pollution is a great environmental risk to public health. According to World Health Organization (WHO) 2016 report on ambient air pollution, the population of Africa, Asia and the Middle East are exposed to higher levels of air pollutants when compared to rest of the world. [1] Amongst the air pollutants like Nitrogen oxides, ozone, carbon monoxide, sulfur dioxides and particulate matter (PM), that are associated with significantly high mortality or morbidity; $PM_{2.5}$ (PM with diameter < 2.5 μm) is a pollutant that has been well studied and is considered as an indicator of exposure to air pollution. [1] Both short-term and long-term exposure to PM with a diameter of 10 μm or less, exert deleterious effect on health, as they can penetrate and lodge deep inside the lungs. Therefore, WHO Air quality guidelines recommend to worldwide countries to aim and achieve the lowest concentrations of PM possible. The permissible standards suggested are annual mean of 10 μg/m3 and 24-hour mean of 20 μ/m3 for $PM_{2.5}$ and annual mean of 25 μg/m3 and 24-hour mean of 50 μg/m3 for PM_{10}. [1] In an effort to achieve the standards, WHO compiles data related to annual mean concentrations for $PM_{2.5}$ and PM_{10} in cities worldwide since 2011. [1] Annual median exposure to ambient (outdoor) $PM_{2.5}$ in μg/m3, in urban population in 2014 was reported to be 9, 15,16 in high income countries of America, Europe and Western Pacific regions respectively while in India, the annual mean of $PM_{2.5}$ in μg/m3, was reported to be 122 in Delhi in 2013 and 59 in Hyderabad in 2014. [2] In 2012, one out of every nine deaths was attributed to air pollution-related conditions. The WHO Western Pacific

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and South East Asian Regions bear most of the burden with 1.1 million and 799 000 deaths, respectively. As urban air quality declines, the risk of stroke, heart disease, lung cancer, and chronic and acute respiratory diseases, including asthma, increases in the population. [1] The risk of cardiovascular disease (CVD) has been consistently associated with increased exposure to airborne particulate matter. [3] A study done in Netherlands, to evaluate the effect of long-term exposure to traffic related air pollution revealed significantly high relative risk in population exposed to air pollution related to traffic variables for respiratory mortality. [4] Oxidative stress and depressed antioxidant capacity are known to be associated with the risk of cardiovascular disease, hypertension, and chronic kidney disease. Reactive oxygen species (ROS) at physiological levels act as signaling molecules in cardiovascular system and contribute to the maintenance of cardiovascular homeostasis. However, excess ROS generation plays a role in the initiation and progression of CVD. [5] Oxidative stress responses to ROS and subsequent inflammation from air pollution exposure may be an important mechanism for the deleterious health effects. [3] Following exposure to pro-oxidant air pollutants, oxidative stress can occur, due to biochemical imbalance between ROS generation and antioxidant defense mechanism in body. The uncontained ROS is known to attack and denature lipids, proteins, carbohydrates, DNA, RNA, NO, etc. damaging the tissues. It also activates nuclear factor kappa-light-chain- enhancer of activated B cells (NF□B), which increases transcription of pro-inflammatory molecules like chemokines, cytokines, and acute phase proteins that contribute to coronary artery disease risk. [3] Particulate matter, especially ultrafine PM<0.1 μm, can cause excessive generation of ROS in the lungs, blood and vascular tissues. This uncontained ROS leads to oxidative stress that appears to play a central role in the respiratory and cardiovascular effects of air pollution through its immunomodulating, inflammatory and thrombogenic effects. [3] Taking these findings into consideration, this study was taken up to evaluate the effect of outdoor air pollution on oxidative stress in human population and compare with controls.

MATERIALS AND METHODS

This study was a community based, cross-sectional study designed in accordance with Good Clinical Practice guidelines. The study received Institutional Ethics Committee approval and all the subjects provided written informed consent before the start of the study. Adults of either gender aged above 18 years exposed to outdoor pollution were included. Subjects with history of smoking, diabetes, hypertension, hyperlipidemia, renal dysfunction or hepatic dysfunction were excluded from the study. In addition, subjects with history of intake of medications / over the counter products that can affect oxidative stress, or having history of any other disorder known to affect oxidative stress were also excluded from the study. Subjects who were exposed to outdoor pollution more than 5hrs/day at least 5days/week were considered as cases and those who were exposed to outdoor pollution less than 2hrs/day more than 5days/week were considered as controls. Subjects were recruited from general population of Hyderabad residing in Jubilee Hills (Area 1, a location mainly afflicted with vehicular pollution) and Jeedimetla (Area 2, a location mainly afflicted with industrial pollution), the areas that were reported to have moderate Air Quality Index (AQI) as per National Ambient Air Quality Monitoring Programme (NAMP), Central Pollution Control Board (CPCB), India. [6] On the study day, demographic details and medical history of subjects was documented, physical and clinical examination was done and vitals were recorded and the eligible subjects were recruited into study. Details of duration of exposure to outdoor pollution, which included how many years of exposure, how many days in a week and how many hours in a day was recorded. Thereafter, a blood sample of 15ml was collected and transported to the site in half an hour where it was centrifuged at 3000 rpm for 20 min and the serum was stored at -80°C till further analysis. Samples were analyzed to estimate serum levels of oxidative stress markers, malondialdehyde (MDA) by TBARS assay, Total NO (Nitrite/Nitrate) using Greiss reagent and Sulfhydryl (Total) using Ellman reagent by UV spectrophotometry. Serum concentrations of oxidative stress markers were compared between cases and controls using unpaired student 't' test. Serum concentrations of oxidative stress markers were also compared between cases of two areas and controls of two areas from which subjects were recruited using unpaired 't' test. Statistical significance was considered at $p < 0.05$. All statistical analysis were computed using Graphpad. The sample size was estimated to be 34 subjects, to enroll 28 subjects (14/ group) with an anticipated difference between means of serum malondialdehyde (MDA) levels as 2.5 nanomol/ml with a SD of 2 [7], considering power of the study to be 90% at 5% level of significance and 20% screen failure rate.

RESULTS

Of the 31 subjects screened, 28 were enrolled with 14 subjects as cases and 14 as controls. 3 subjects were excluded, as they did not meet eligibility criteria. The demographic data of the cases and controls was comparable as can be observed from Table 1. The mean serum concentration of malondialdehyde, a pro-oxidant stress marker was observed to be significantly high in cases when compared to controls ($p<0.001$) (Figure 1), while the mean serum sulfhydryl levels and total NO levels, the anti-oxidant stress markers were found to be significantly high in controls when compared to cases ($p<0.05$ and $p<0.001$ respectively). (Figure 2) The mean serum concentration of the oxidative stress markers when compared between cases of Area 1 and Area 2, it was observed that mean serum malondialdehyde level was high in cases of Area 1, though non-significant; mean serum sulfhydryl level was significantly high in cases of Area 1, while mean serum total nitric oxide level was significantly high in Area 2. (Figure 3 & 4). There was no significant difference in serum malondialdehyde levels, sulfhydryl levels and total NO levels between controls of Area 1 and Area 2. (Figure 5 & 6). None of the subjects had any history of respiratory discomfort or allergic symptoms.

DISCUSSION

In this study, it was observed that the serum level of pro-oxidant marker, malondialdehyde was significantly high and anti-oxidant markers sulfhydryl and total NO were significantly low in cases when compared to controls. As oxidative stress with inflammation is thought to mediate both the pulmonary and extra-pulmonary health effects of PM, and measurement of oxidative stress reflects the true exposure to PM [8], our study results appear relevant and assume significance. A study done by Y Suresh et al., on traffic police in Hyderabad in the year 2000 to determine whether exposure to air pollution increases tissue antioxidant levels, reported that lipid peroxides by TBARS assay were significantly high and nitrite levels were reduced in police when compared to normal controls, as were the results of our study. The activities of catalase, superoxide dismutase and glutathione peroxidase in RBC, were also significantly lower in police than in controls. [9] Similar findings were reported in a study done at Indonesia by Kahar et al., to determine the effect of $PM_{2.5}$ on serum MDA levels in workers at a bus station. The serum MDA levels in bus station workers was reported to be significantly high when compared to administration workers (9.44±2.94 nmol/ml vs 5.6±0.6; $p<0.01$). [10] Another study done by Rundell et al., to investigate whether inhalation of PM was associated with adverse respiratory health, also reported that MDA in exhaled breath condensate (EBC) increased by 40% after exercise when compared to baseline (p=ns) in a location with low PM exposure while MDA increased by 208% after exercise when compared to baseline ($p = .06$) after exercise in a location with high PM exposure. [11] A study done in Turkey, to investigate the levels of markers of oxidative stress and vitamin E in diesel exposed toll collectors, also found higher serum MDA in cases than controls. (5.76 ± 2.15 μmol/L vs. 3.07 ± 0.76 μmol/L, p=0.0001). However, they observed higher serum nitrite+nitrate levels in cases than controls (96.50 ± 45.54 μmol/L vs. 19.32 ± 11.77 μmol/L, p=0.0001) respectively and the authors attributed the observed high plasma nitrite+nitrate levels in toll collectors to the probable penetration of the gaseous components of diesel exhaust (DE) which include nitrogen oxides (NO, NO2), and lack of facilities for measuring the level of the gaseous components of DE in manual tollbooths was projected as a major limitation of the study.[12] In our study, as expected the serum total nitrate was found to be significantly high in controls than cases. In a study done by Suyatna FD et al. in Indonesia, to investigate the oxidant and antioxidant status of policemen, reported that the plasma MDA content of country policemen either non smokers or smokers was significantly lower ($p < 0.05$) than that of administrative policemen and traffic policemen of Jakarta (city). They also observed that MDA content of non-smoker country policemen was significantly lower than that of the smoker ones ($p < 0.05$), thereby demonstrated the harmful effects of smoking to the antioxidant defense mechanism of the body. [13] There are many studies, [10,12,13] in which it was observed that smoking increased oxidative stress. But, in our study, smokers were excluded to negate the confounding effect of smoking on outcome. Not just smoking, subjects with history of any co-morbidity like diabetes, hypertension or any intake of any concomitant medication that is known to effect oxidative stress were excluded, which was the strength of our study. A study done in Kurnool, to evaluate oxidative stress in traffic police, also reported significantly elevated plasma MDA level in cases (traffic police on duty) when compared to controls (administrative traffic police). [7] A panel study, nested within a quasi-experimental design with "high-low-high" pollution levels, was designed in healthy

young adults according to the timelines of the Olympic pollution control measures in Beijing in 2008, to determine the changes in biomarker levels with changes in concentrations of pollutants. In this study, smokers were excluded. They observed significant increases in fractional exhaled nitric oxide (FE_{NO}) (ranging from 10–75%) associated with interquartile range (IQR) increases in $PM_{2.5}$. EBC nitrite was positively associated with all pollutants; however, the largest effect estimates per IQR increase in pollutant concentration was observed to be 21.9% for $PM_{2.5}$. The authors concluded that exposure to air pollution adversely affects health through acute changes in pulmonary inflammation and oxidative damage to lipids and DNA. [14] In our study, when the mean serum concentration of the oxidative stress markers were compared between cases of Area 1 and Area 2, it was observed that serum concentrations of sulfhydryl were significantly high in cases of Area 1. Area 1 was a locality, which is mainly afflicted with vehicular pollution whereas Area 2 was an industrial area, in which pollution was due to industrial effluents. Similar results were reported in a study done to evaluate airway antioxidant responses to diesel exhaust exposure by Behndig et al., who evaluated glutathione levels at 18 hr post 2 hr exposure to air vs post 2 hr diesel exhaust (DE) exposure in the alveolar lavage, and reported a significant increase in glutathione levels post diesel exhaust exposure when compared to post air exposure (0.46 microM post DE vs 0.33 post air; $p < 0.05$) and opined that in the alveoli, where tissue particulate matter deposition is low, no inflammatory changes were observed at 6 hr and 18 hr of DE exposure and this was attributed to protective antioxidant response as higher levels of reduced glutathione. [15] In an other study, done by Weidong et al., to determine whether Glutathione-S-transferase M1 (GSTM1) deficiency could modulate diesel exhaust particles (DEP)-induced pro-inflammatory response, as individuals with the GSTM1 null genotype completely lack the GSTM1 enzyme activity and are susceptible to asthma. They observed that exposure of human bronchial epithelial cells to 25-100 µg/ml DEP for 24 h induced a significant increase in IL-8 protein expression, which indicates GSTM1 null genotype is associated with aggravation of DEP-induced airway inflammation in human subjects. [16] The findings from these studies demonstrate that diesel exhaust causes bronchial inflammation. Therefore, whenever there is exposure to diesel exhaust, glutathione, a sulfhydryl, moves onto lung surface and protects against inflammation in the alveolar region, as an antioxidant defense mechanism. [15] This is in agreement with the findings of our study, that the glutathione levels were significantly high in cases of area 1 (location with vehicular pollution) than in cases of Area 2 (area with industrial pollution). According to Citizens' report - Air quality and mobility challenges in Hyderabad, ambient air pollution exposure causes oxidative stress, lung function impairment, and respiratory ailments. Hence, State pollution control board scientists' caution that the city needs aggressive and sustained action to prevent ambient air pollution to protect public health, as most of the particulates come from combustion sources that are more toxic in nature.[17] As the city of Hyderabad is getting expanded with number of vehicles and industries increasing proportionally, the results of our study which demonstrate an imbalance between pro-oxidant and anti-oxidant markers in population exposed to ambient air pollution, indicating oxidative stress, emphasize the importance of controlling ambient air pollution, reducing morbidity and mortality on long term. However, long term, prospective cohort studies comparing population with high exposure to ambient air pollution with population with low exposure and studies comparing population from locations with high Air Quality Index with those living in locations with low Air Quality Index are necessary to establish the causal link of oxidative stress with morbidity and mortality related to ambient air pollution.

CONCLUSION

This study demonstrates that population with high exposure to ambient air pollution have high levels of pro-oxidant markers and low levels of anti-oxidant markers when compared to population with low exposure indicating oxidative stress in population with high exposure. Oxidative stress being one of the markers that predict the risk of development of cardiovascular and respiratory disease, this study in this city of Hyderabad, that is rapidly developing and getting polluted from combustion sources assumes importance.

Conflicts of Interest: The authors declare that there is no conflict of interest. The study has been done for academic interest.

Table 1: Comparison of baseline characteristics of cases and controls.

	Parameter	Cases (n=14)	Controls (n=14)	p value
1	Age (yrs)	32 ± 6.3	36.24 ± 8.2	ns
2	BMI (kg/m^2)	24.3 ± 3.1	24.5 ± 3.1	ns
3	Duration of Exposure (yrs)	8.1± 7.2	7.4 ± 3.3	ns
4	Duration of Exposure (hrs/day)	7.1 ± 0.9	1.2 ± 0.4	N/A

Data presented as Mean ± SD; ns- non-significant p>0.05; N/A- Not Applicable

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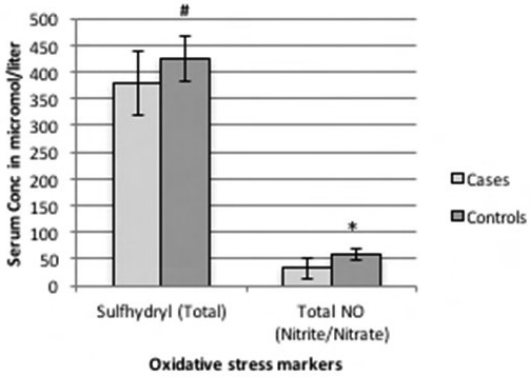
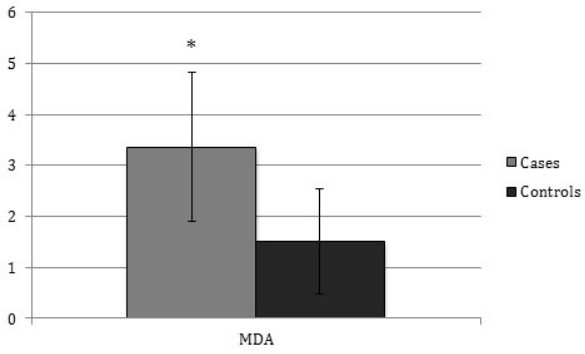
Figure 1: Comparison of the serum levels of anti-oxidant stress markers, Sulfhydryl (Total) and Total NO (Nitrite/ Nitrate) between cases (n=14) and controls (n=14)



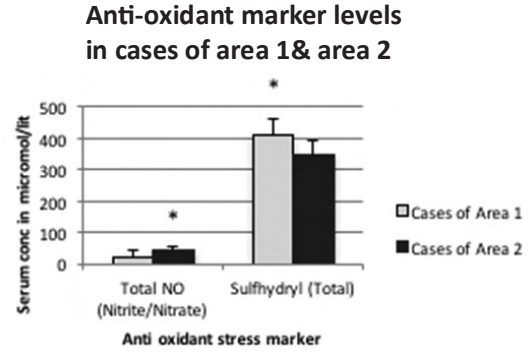
* indicates p < 0.001 and # indicates p < 0.05

Figure 2: Comparison of the serum levels of pro-oxidant stress marker, MDA between cases (n=14) and controls (n=14)



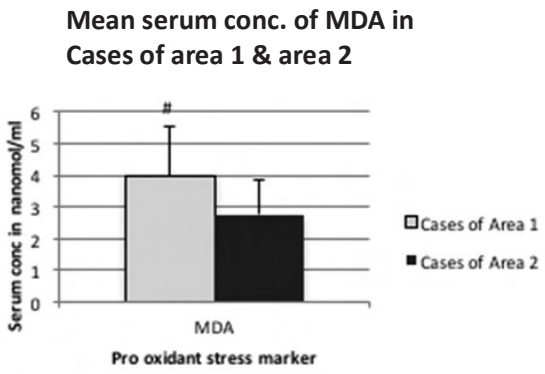
* indicates p < 0.001

Figure 3: : Comparison of the serum levels of anti-oxidant stress markers, Sulfhydryl (Total) and Total NO (Nitrite/ Nitrate) between cases of Area 1(n=7) and Area 2 (n=7)



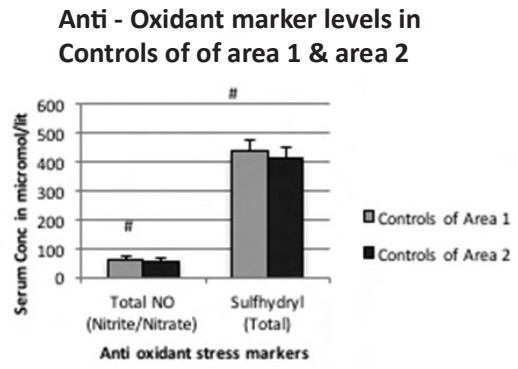
* indicates p < 0.05

Figure 4: Comparison of the serum levels of pro-oxidant stress marker, MDA between cases of Area 1(n=7) and Area 2 (n=7)



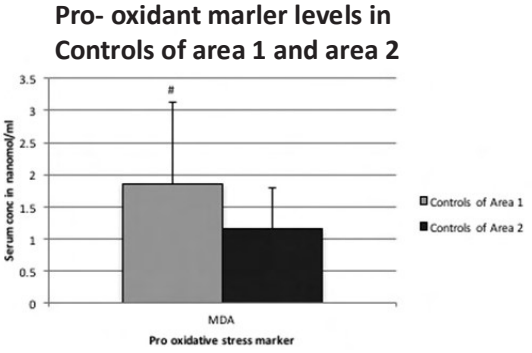
* indicates p < 0.001 and # indicates p < 0.05

Figure 5: Comparison of the serum levels of anti-oxidant stress markers, Sulfhydryl (Total) and Total NO (Nitrite/ Nitrate) between controls of Area 1(n=7) and Area 2 (n=7)



* indicates p < 0.001

Figure 6: Comparison of the serum levels of pro-oxidant stress markers, MDA between controls of Area 1(n=7) and Area 2 (n=7)



* indicates p < 0.05