Effects of Diesel Combustion Nanoparticles on Oxidative Stress Markers among the Exposures

DEVANUR RAJASHEKARAR MURTHY MAHADESWARA PRASAD1,2, SHASHANK KUMAR2, SUTTUR SRIKANTANAIK MALINI3,*, MANJULA SHIVANAGAPPA4

1Assistant Professor, Department of Forensic Medicine & Toxicology, Mysore Medical College and Research Institute, Mysore, Karnataka, India
2Research Scholar, Department of studies of Zoology, Manasa Gangothri, Mysore, Karnataka, India
3Assistant Professor, Department of studies of Zoology, Manasa Gangothri, Mysore, Karnataka, India
4Reader, Department of Oral Surgery, JSS Dental College & Hospital, Mysore, Karnataka, India

Abstract

Background: Although studies are available on lipid peroxidation products and the antioxidant status in experimental animals, a detailed report on human exposed to diesel combustion nanoparticles is meagre. We aimed to study the variation in oxidative stress markers among exposures.

Method: A cross-sectional study during the period between 2015-2017 was conducted among 500 male garage workers of age group 25-40 years with history of exposure for 6-8 hrs a day without using any protective aids during work, for 6-12 years and 300 controls, who live in hilly areas where they were sparsely exposed. Serum oxidative stress markers were estimated and compared.

Results: A significant variation was observed among the oxidative stress markers in exposures with surge in melanaldehyde (MDA) and reduction in superoxide dismutase (SOD) and catalase (CAT).

Conclusion: Unprotected exposure to diesel combustion products induces oxidative stress which can alter recordable change among the markers. Oxidative stress, being the route cause for cell damage, can be marked in the initial stages and is a good biomonitoring factor in primary care.

Keywords: Diesel Combustion Nanoparticles; Lipid Peroxidation; Melanaldehyde; Oxidative Stress Markers; Superoxide Dismutase; Catalase

INTRODUCTION

In the present scenario, of industrialization and modernization, the living cells are subjected to enormous stress, to which they respond by altering their cellular metabolism and activating their defence mechanisms. Biochemical system is the first to respond to any stress. The stress response consists of stress proteins, both enzymatic and non-enzymatic, which are the primary protective responses that are highly conserved components of cellular stress (1). In some, overproduction of cellular oxidants or failure of endogenous protective mechanism disrupts oxidative metabolism, cellular antioxidant defences or damage macromolecules like proteins, lipid, DNA and mitochondria. This cellular patho-physiology has received attention in recent years, in attributing to the understanding of various disorders. Such changes can result in simple to complex illnesses like early aging, chronic disorders, degenerative disorders, carcinomas, generalized weakness, lethargy, infertility, polycystic ovarian diseases, and recurrent pregnancy loss. The time has come to find the potential candidates for predicting cellular toxicity against environmental chemicals. The commonest is the diesel combustion nanoparticles, the powerful agent which can trigger cellular-toxicity and subsequent geno-toxicity among the exposures. In such, the management of forthcoming disorders may require the benefits of personal medicine. Even though cells are endowed with protective responses, any enhancement in the stress beyond the capacity to cope up with may result in cellular damage leading to cell death and is genetically regulated.

The key to aerobic life is molecular oxygen which can be converted into cytotoxic byproducts referred to as reactive oxygen species (ROS). ROS have been reported to play a major role in enhancing the toxicity of the triggering agent (2). ROS produced during oxidative stress have been implicated in apoptosis as possible signalling molecules (3). Although several stress markers have been proposed for the evaluation of oxidative stress, in the current study, oxidative stress markers are evaluated by the estimation of super oxide dismutase, catalase and lipid peroxidation as ROS includes hydrogen peroxide and super oxide, the cytotoxic byproducts responsible for the cellular injury in the form of damaged

Correspondence to: Dr. Suttur Srikanthaik Malini; MSc, PhD. Assistant Professor, Department of studies of Zoology, Manasa Gangothri, Mysore, Karnataka, India.
Tel: +91 990 050 55 67, E-mail: manudrp@gmail.com
Received 20 January 2018; Accepted 01 March 2018
DNA, and complex molecules like lipids and proteins (Figure 1). In the initial stages or in the acute phases, the ROS can be reduced by the antioxidant enzymes. Naito et al has described the phase-wise activity of antioxidant network in biological system which counteracts the cell damage (Figure 2) (4).

The ill effects of the diesel combustion nanoparticles among the exposures usually mimic that of a common cold or acute viral infection in the initial phase. But in long-term exposure it can account for the wide range of disorders like fleeting aggravation of chronic disorders such as bronchitis, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, psoriasis, dermatitis, dacryocystitis, behavioural changes including memory, attention deficits, depression, anxiety and irritability. Oxidative stress has been proposed as the patho-physiology in relation to nanoparticle exposure and health hazard. Measurement of oxidatively modified substances has been established as new markers by mass spectrography. Thus, we aimed at estimating oxidative stress markers among the exposures to diesel combustion nanoparticles that suffer from recurrent simple illness in the initial phase of their occupation and acute exacerbation of chronic illness.

**METHODS**

After ethical clearance, IHEC-UOM No. 123 PhD/2016-17, and consent, a cross-sectional study was conducted in the Molecular Reproductive and Human Genetics Lab, ManasaGangothri, University of Mysore, Mysore, Karnataka, India during the period between 2015-2017. The study population and sample size was 500 male garage workers of age group 25-60 years who were exposed to diesel combustion nanoparticles for 6-8 hrs a day without using any protective aids during work, for 6-12 years without any diseases and disorders. The controls were 300 males of age 25-60 years, apparently healthy, who lived in hilly areas and had negligible or nil exposure to diesel combustion nanoparticles. The subjects were non-alcoholic, non-smoker, non-diabetic and non-hypertensive. Questionnaires were administered to characterize the work practices, exposure history and use of protective equipment.

Estimation of SOD, CAT and lipid peroxidation (LP) product melanaldehyde (MDA) was done by spectrophotometric
method from the aseptically drawn 5 ml of venous blood, as per Kazri et al 2000; Sinha 1972; Kobayashi et al, 2001; Ohkawa et al, 1979; and Prieto et al, 1999 (5-7). LP was measured by the malonaldehyde (MDA) level estimation. The results were tabulated and analysed statistically.

**Statistical analysis**

The data obtained were analyzed using statistical software ‘Statistical Package for the Social Sciences (SPSS) version 14’. Independent 't' test was performed between case and control groups. P < 0.05 was considered as a significant value.

**RESULTS**

The results of both control and cases were tabulated and analysed. Among the study population, 77.8% (n= 389) did not reveal any symptoms, whereas 22.2% (n= 111) revealed clinical ailments like generalised weakness 8% (n= 40), respiratory system disorders like respiratory distress, COPD and asthma 2.4% (n= 12), conjunctival features like watery, congested and dry conjunctivae 4.4% (n= 22), abdominal symptoms like pain abdominal, diarrhoea and nausea 3% (n= 15), skin disorder 2.6% (n= 13), type II diabetes mellitus (DM) 1.2% (n= 6) and hypertension (HT) 0.6% (n= 3) (Table 1). There were no obvious causative factors causing these ailments apart from exposure to diesel combustion nanoparticles.

Majority of cases complained of generalized weakness and loss of endurance which can be attributed to hypoxic status. Respiratory symptoms of COPD and chronic disorders like DM and HT were found among the workers who had more work experience. Acute features of inflammatory response like respiratory distress and asthma, allergic conjunctivitis, and abdominal manifestations like nausea and abdominal pain were found in few corners.

Among the exposures, the activity of oxidative stress markers, like SOD and CAT, as well as the level of LP were increased. (Table 2). In chronic disorders like COPD, DM and HT, these parameters were significantly reduced when compared with the cases with simple clinical ailments (Table 1). MDA levels were significantly increased reflecting the more amount of LP. The oxidative stress markers activities among the asymptomatic cases were also low when compared with those of the cases (Table 1). All these parameters were at a satisfactory level among the controls (Table 2).

In this study, the significant mean difference was observed in SOD (p=0.000*), CAT (p=0.001) and LP (p=0.000*) levels between case and control.

**DISCUSSION**

The diesel combustion nanoparticles trigger the inflammatory response. Whenever there is cell insult the cells respond with changes in the basic metabolism which reflects in the form of cell protective mechanisms and generation of oxidative stress factors. In health promotion and improvement of life quality, it is of paramount important to

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>No. of Cases</th>
<th>SOD%</th>
<th>CATU/20</th>
<th>LPnmol MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM+HT</td>
<td>6 + 3</td>
<td>25.33 ± 6.14</td>
<td>0.25 ± 0.02</td>
<td>6.14 ± 2.12</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD, Asthma</td>
<td>12</td>
<td>23.47 ± 4.11</td>
<td>0.2 ± 0.06</td>
<td>5.33 ± 1.98</td>
</tr>
<tr>
<td>Conjunctiva watery, Conjunctiva</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>congested, Conjunctiva dry</td>
<td>22</td>
<td>39.65 ± 2.15</td>
<td>0.33 ± 0.008</td>
<td>4.21 ± 1.06</td>
</tr>
<tr>
<td>Abdominal pain Diarrhoea</td>
<td>04</td>
<td>41.73 ± 11.21</td>
<td>0.35 ± 0.07</td>
<td>3.94 ± 0.88</td>
</tr>
<tr>
<td>Nausea</td>
<td>11</td>
<td>42.22 ± 10.41</td>
<td>0.34 ± 0.097</td>
<td>3.87 ± 1.03</td>
</tr>
<tr>
<td>Skin disorders</td>
<td>13</td>
<td>45.34 ± 6.13</td>
<td>0.3 ± 0.004</td>
<td>3.96 ± 1.02</td>
</tr>
<tr>
<td>Generalised weakness</td>
<td>40</td>
<td>41.73 ± 5.11</td>
<td>0.41 ± 0.092</td>
<td>4.11 ± 1.94</td>
</tr>
<tr>
<td>No clinical features</td>
<td>389</td>
<td>74.758 ± 12.496</td>
<td>0.08 ± 0.261</td>
<td>3.27 ± 1.547</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>62.901 ± 16.32</td>
<td>0.0077 ± 0.0147</td>
<td>2.0832 ± 0.42965</td>
</tr>
</tbody>
</table>

**Table 2. Group statistics of SOD, CAT and LP among the exposed (n= 500) and control group (n=300)**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP(nM MDA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>500</td>
<td>3.5380</td>
<td>1.51751</td>
<td>0.06787</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>2.0832</td>
<td>0.42965</td>
<td>0.02476</td>
</tr>
<tr>
<td>CAT(U/20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>500</td>
<td>0.0134</td>
<td>0.02617</td>
<td>0.00117</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>0.0077</td>
<td>0.01470</td>
<td>0.00085</td>
</tr>
<tr>
<td>SOD%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>500</td>
<td>68.0360</td>
<td>12.49630</td>
<td>0.55885</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>61.9010</td>
<td>16.32698</td>
<td>0.94421</td>
</tr>
</tbody>
</table>
Diesel Combustion Nanoparticles and Oxidative Stress Markers
D. R. M. Prasad et al.

### Independent Samples ‘T’ Test

<table>
<thead>
<tr>
<th></th>
<th>p value</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>LP</td>
<td>0.000</td>
<td>1.45482</td>
<td>1.27900</td>
</tr>
<tr>
<td>CAT</td>
<td>0.001</td>
<td>0.00576</td>
<td>0.00252</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>6.13497</td>
<td>4.11856</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Detect feeble etiologies responsible for disease ailments as early as possible and to undertake aggressive interventions to fight them. Oxidative stress is a high-profile element among the risk factors for aging (4). Oxidative stress induces ROS generation. Proteins in the body undergo a variety of post-translational modifications. Among these modifications, oxidative modifications are substantially involved in aging and disease.

In the present study, there is convincing evidence that oxidative stress markers are significantly altered among the exposures which can be declared as due to the diesel combustion nanoparticle. The antioxidant scavenging enzymes SOD and CAT activities in blood were reduced due to the oxidative stress among the exposures along with increase in the LP. This reveals significant plasma membrane destruction due to the nanoparticle-induced inflammation. The exposure to diesel combustion nanoparticles increased the MDA concentration by 81% in Moller study. In the present study, the MDA level was increased by 91.88% (Table 2). As can be seen, the MDA level continuously increased among exposures where the subjects were suffering from clinical ailments (Table 1). The increase is proportionately high as the severity of illness raised. The exposure to diesel combustion nanoparticles has altered the SOD and CAT activity by 20.89% and 72.07% (Table 2). As can be seen, the MDA level continuously increased among exposures where the subjects were suffering from clinical ailments. The increase is proportionately high as the severity of illness raised. This was in consistent with Moller where the effects of diesel combustion nanoparticles on the activities of antioxidant enzymes, SOD and CAT in blood were altered by 21.2% and 35.1%, respectively when compared with the controls (8). The exposure to diesel combustion nanoparticles increased the MDA concentration by 81%. Moller and Radu et al. concluded that there exists a reliable relationship between exposure to diesel combustion-derived nanoparticles and oxidative markers in urine, blood and exhaled-breath condensate (8, 9). Diesel combustion-derived nanoparticles and their constituents generate oxidative stress in a number of cell types crucial to the development of disorders. Pretreatment or co-administration of free radical scavengers or other antioxidant compounds like SOD, NAC, tiron, and cysteine prodrugs prevent particulate matter-induced cardiovascular impairment and early cell degeneration and aging and have the capacity to reverse some aspects of the particulate matter-induced cardiovascular impairment suggesting that oxidative stress is an important mechanism in the cardiovascular disorders (4, 10–14).

The three biomarkers used indicate higher levels of oxidative stress among exposures than those observed in a control population. Even though various factors may be involved in influencing stress markers which can cause oxidative stress and DNA damage, in the present study, apart from diet and body mass, all other probable causes have been ruled out. Diesel combustion nanoparticles can be categorized and discussed according to their ability to produce LP or alter body antioxidant status. Abdollahi et al. describe that the toxicity of a particle depends on its capability to stimulate free radical production, induction of LP, and disturbance of the total antioxidant capacity of the living (15). Radu et al. showed the depletion of intracellular glutathione and increased LP mediate cytotoxicity of nanoparticles (9). In their study, they investigated the effects of nanoparticles on LP and the antioxidative system in MRC-5 lung fibroblast cells following exposure for 24, 48 and 72 hours. Exposure to α-Fe2O3 nanoparticles increased LP by 81%, 189% and 110% after 24, 48 and 72 hours, respectively (9). Conversely, the reduced glutathione concentration decreased by 23.2% and 51.4% after 48 and 72 hours of treatment, respectively. Apart from this, they revealed the increase in activities of SOD, CAT, glutathione peroxidase, glutathionetransferase and glutathione reductase within the interval between 48-72 hours (9).

The superoxide anions that escape dismutation can react with hydrogen peroxide according to Haber-Weiss reaction to form hydroxyl radicals or become prorogated to hydroperoxyl radicals. Hydroperoxyl and hydroxyl radicals are able to abstract hydrogen atom from a methylene group adjacent to double bonds of polyunsaturated fatty acids forming carbon centered radicals that react with molecular oxygen to form lipid peroxides (16). The significant increase of MDA concentration suggests that the antioxidative system adaptation was not sufficient to prevent the damage of membrane lipids, in turn, the LP products could affect the structure of DNA bases proteins and carbohydrates (17). Similar results were obtained in human bronchial epithelial BEAS-2B cells exposed to titanium di oxide nanoparticles and zinc oxide whereas in A549 human lung epithelial cells
treated with maghemiteno-sized particles, the change in lipid peroxidation variation was statistically insignificant (18–20).

The diesel combustion nanoparticle-inflicted health hazards are directly in relation to the impact of exposure to ambient air particulates on the human respiratory system. Nemery et al. (2001) reported 63 fatalities due to ambient air pollution in the Meuse valley, Belgium (21). The ambient air particle mass concentration also contributes to the proportionate increase in the severity of respiratory illnesses. There is evidence that the mass of particle’s (≤1μm) mass median aerodynamic diameter has demonstrated exacerbation of respiratory diseases and increases human mortality as well (22–24). In the present study also we could find that the long-term exposures presented with COPD when compared with acute exposures. The particulate size of 2.5μm increases the incidences of cardiovascular diseases and related mortalities (25, 26). The surface composition of the nanoparticles plays a dominant role in the generation of ROS and causing oxidative stress. This is the most accepted mechanistic paradigm as a reason for the toxicity of the nanoparticles (27, 28).

These nanoparticles can cause their interaction with molecular dioxygen (O2) by redox–cycling and the electron capture can lead to the formation of superoxide radicals (O2-•) and can generate ROS (29, 30). The ROS has got the capacity to react with biomolecules and produce byproducts like lipids-isoprostanes, age pigment, or lipofuscin; proteins-carbonyl and nitrotyrosine derivatives; DNA causing its fragmentation and base oxidations such as (8-hydroxy-2-deoxyguanosine (8-OHdG)). 8-OHdG are the profuse base oxidation product in DNA having altered base-pairing properties, with adenine replacing cytosine in the daughter strands formed after replication of the affected DNA strand (31).

Oxidative stress reduces the potency of the endogenous antioxidant system whereas the redox active antioxidants from food or food supplements enhance this depleting antioxidant defences and help prevent oxidative damages. Antioxidants can affect the gene expression in two general ways, either by changing the redox status of the cell, or directly by specific interactions with molecular targets in a manner partially independent of their antioxidant / radical-scavenging ability. Cell signalling pathways are very sensitive to oxidants and cellular redox status changes. Hence, free radicals and antioxidants must regulate gene expression. Activation of the plasma membrane nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), auto-oxidation of polyphenols or bioflavonoids and mitochondrial metabolism can lead to the generation of hydrogen peroxide, sometimes referred to as a second messenger. The presence of hydrogen peroxide in the cytosol changes the glutathione / glutathione disulfide ratio (important for cell signalling systems) and provides an oxidizing environment, which is needed for the binding of activated transcription factors such as nuclear factor kappa B and inhibits protein phosphatases, causing hyper phosphorylation of enzymes and proteins of cell regulatory processes(30, 32). Any of or all these events can modulate gene expression thus making the antioxidants critical components of cell growth, development, differentiation and function which are in turn responsible for simple to complex illnesses.

**CONCLUSION**

Oxidative stress is a central mediator of adverse systemic effects of diesel combustion nanoparticle exposure known to insult cell cultures, isolated tissues, and animal models and now evidence is beginning to emerge in humans also. The alteration in cellular metabolism-induced stress among exposures can aggravate chronic diseases and disorders. Thus, the measurement of oxidative stress markers can be a good biomonitoring and is recommended to be performed in a regular manner as a primary preventive measure. Apart from treating the aggravatd symptoms, the treating doctor should consider these factors to reduce the oxidative stress.

**LIMITATION**

The limitation is that this study was conducted among a limited population, while it is demanding in large scales.

**Conflict of interest:** None to be declared.

**Funding and support:** None.

**REFERENCES**