Orginal Article

OXIDATIVE STRESS INDUCTION AND HEPATO-RENAL MALFUNCTION AMONG CHRONIC EXPOSURE CASES TO THE DIESEL COMBUSTION NANOPARTICLES.

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ABSTRACT

Introduction: Although studies are available on hepato-renal toxicity, lipid peroxidation products and the antioxidant status in experimental animals, a detailed report on exposed individuals is meagre. We aimed to study the variation in oxidative stress markers and correlate with hepato-renal parameters among exposures.

Method: Oxidative stress status and hepato-renal activity were studied in blood samples obtained from 50 individuals who were exposed to diesel combustion nanoparticles with a minimum work history of 8 year in the age range of 32–60years. Controls were age-matched university students and people living in hilly and forest areas. They were evaluated for oxidative stress markers, including superoxide dismutase, catalase, lipid peroxidation in blood and hepato-renal activity parameters like serum levels of bilirubin, SGOT, SGPT, urea and creatinine.

Results: The results show increased oxidative stress markers and variation among the hepato-renal parameters. It is concluded that exposures to diesel combustion nanoparticles are exposed to more oxidative stress and associated with a significant stress on hepatic and renal tissues.

Conclusion: The measurement of serum levels of bilirubin, SGOT, SGPT, urea and creatinine among chronic exposures and correlating with oxidative stress markers can be a good monitoring factor, and is recommended to be performed in a regular manner.

Keywords: diesel combustion nanoparticles; oxidative stress; superoxide dismutase; catalase; lipid peroxidation; hepato-renal stress

INTRODUCTION

Most of the ill effects of exposure to diesel combustion nanoparticles have been attributed to the accumulation of oxidative stress markers and inflammatory response. Exposures to chemical irritants like particulate matter have also been shown to induce oxidative stress and DNA damage both in vivo and in vitro.[1, 2, 3]However, mere exposure in healthy individuals or exposures with recommended protection by itself cannot account for the wide range of disorders. Chronic exposure among the persons can result in hepato-renal toxicity as these are the end organs targeted by the metabolic and excretory products. The toxicity of the nanoparticles can cause various range of disorders as they are readily absorbed by the skin, oral mucous membrane, and conjunctiva, gastrointestinal and respiratory routes. This results in reactive oxygen species (ROS) generation and subsequent damage of cellular respiration and damage to genetic material. The health effects can cause short-term adverse health effects following acute exposure as well as long-term effects as a result of chronic low-level exposures. The health effects from acute exposure include irritation of the nose, throat, and skin causing burning, stinging and itching as well as rashes and blisters. The ability and severity to produce altered body antioxidant status, stimulation of free radical production, induction of lipid peroxidation, and disturbance of the total antioxidant

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capability of the body decides the diseased status and the quality and quantity of nanoparticle toxicity effects.[4]In many cases, the symptoms mimic those of a cold or flu. Health effects from chronic exposure include hepato-renal manifestation, behavioral changes including memory and attention deficits, depression, anxiety and irritability which many a times hamper the quality of work among the mechanics. In view of all these, we aimed to explore the relationship among the ROS markers and hepato-renal markers which provides a strong foundation for determining the mechanism by which diesel combustion nanoparticles induce oxidative stress and disease, and to determine whether tissue damage in humans is brought about by direct or by indirect action of nanoparticles.

MATERIALS AND METHODS

The study was conducted in the Molecular Reproductive and Human Genetics Lab, Manasa Gangothri, University of Mysore, Mysore, Karnataka, India after consent. The ethical clearance was taken from Institutional Ethical Committee, IHEC-UOM No. 123PhD/2016-17. The study population and sample size being 50 male ggarage workers of age group 32-60years who had exposure to diesel combustion nanoparticles for 6-8hrs a day for 6-12years without using any protective aids during work were subjected to evaluation of ROS markers and compared with that of the control population. The control population being 50 males of same age group who are apparently normal university students and live in hilly areas where there is very minimal or negligible exposure to diesel combustion nanoparticles and other stress triggering factors. The subjects were non-alcoholic, non-smoker, not diabetic and hypertensive. Some sample subjects suffered with clinical ailments like weakness, respiratory distress, watery/congested conjunctiva, diarrhoea, nausea, irritation, and lethargy. Questionnaires were administered to characterize the work practices, exposure history and use of protective equipment. They were evaluated for oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LP) in blood and hepato-renal activity parameters like serum levels of bilirubin, SGOT, SGPT, urea and creatinine. Lipid peroxidation was measured by the melanaldehyde (MDA) level estimation. The results were tabulated and analysed statistically.

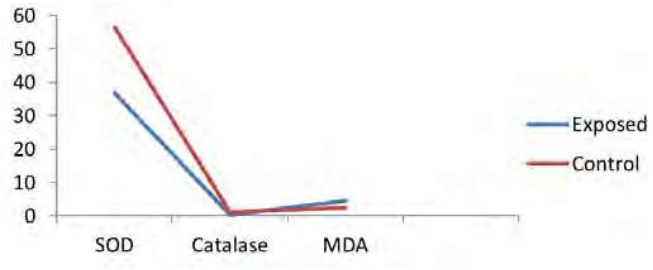
Table 1: Clinical features and mean serum levels of SOD, CAT, MDA, bilirubin, SGOT, SGPT, blood urea and serum creatinine among the exposed group (n=50)

Clinical Feature	No. of Cases	SOD %	CAT u/ml	MDA Nmol/ml	Serum Bilirubin	SGOT U/L	SGPT U/L	Blood Urea	Serum Creatinine
Generalised weakness	02	25.33± 6.14	0.25± 0.02	6.14± 2.12	0.7± 0.12	28± 2.4	45.47±3.4	38±1.2	0.8± 0.12
Respiratory distress COPD, Asthma	10	23.47± 4.11	0.2± 0.06	5.33± 1.98	0.94± 0.13	30± 1.6	54.1±2.4	32±2.2	0.98± 0.13
Conjunctiva watery	06	34.65± 2.9	0.33± 0.008	4.43± 1.06	1.2± 0.17	30± 2.3	42.6±3.8	27±3.3	0.82± 0.17
Conjunctiva congested, Conjunctiva dry, Abdominal pain	07	39.21± 2.15	0.31± 0.07	4.21± 1.21	0.9± 0.12	25± 2.4	38.17±4.4	31±2.6	0.74± 0.12
Diarrhoea	05	41.73± 11.21	0.35± 0.07	3.94± 0.88	1.1± 0.23	34± 3.4	48.12±3.3	36±2.4	1.1± 0.13
Nausea	08	42.22 ±	0.34 ±	3.87± 1.03	1.3± 0.12	40± 2.3	35.45±1.3	38±1.4	1.2± 0.12

RESULTS

Among the study population, we could find 5 cases of Type II diabetes mellitus (DM), and 6 cases with increased blood pressure (Hypertension) were detected. (Table 1) The oxidative stress markers showed significant variation like rise in the level of SOD and CAT. More the severity of illness more the SOD and CAT level. In chronic disorder like DM and hypertension (HTN), these parameters were significantly increased when compared with simple clinical ailments. MDA levels were significantly reduced reflecting the more amount of LP. All these parameters were at satisfactory level among the controls (Figure 1).

Figure 1: Mean serum levels of SOD, CAT and LP among the exposed (n=50) and control group (n=50)



DISCUSSION

Organisms are subjected to a large number of stresses and the cells respond by altering their cellular metabolism and activating their defence mechanisms. The first response of an organism to any environmental alteration inducing stress is at the biochemical level. Stress response and anti-oxidant defence system consists of stress proteins, termed as heat shock proteins and anti-oxidants which are the primary protective responses.[5] Molecular oxygen is the key to aerobic life and is also converted into cytotoxic by-products referred to as ROS. In addition to their involvement in the normal metabolic activities, ROS have been reported to play a major role in enhancing the toxicity of several xenobiotics including metals and pesticides. Though cells are endowed with protective responses, however, an enhancement in the stress beyond the capacity of a cell to cope up may result in cellular damage leading to cell death. One of the major forms of cell death, apoptosis, has been shown to be genetically regulated. Recently, ROS produced during oxidative stress have been implicated in apoptosis as possible signaling molecules.[6]Animal study in rats reveals that certain chemicals produce hepato-renal toxicity and has been reported to induce oxidative stress.[7] One of the mechanisms the chemicals could elicit toxicity is by inhibiting mitochondrial ATP production through the uncoupling of oxidative phosphorylation that could lead to the generation of ROS.[8] In the present study, the diesel combustion nanoparticles have been shown to cause adverse effect on non-target organ, liver and kidney. The expressions observed in the exposed may be due to attainment of its threshold limit in the cell, and is supported by study of Domenico et al.[9] It has been noticed that ROS induction and impairment in hepato-renal parameters is generally correlated with early cytotoxic events, and is a secondary consequence of damages that affect cellular integrity. The lipophilic nature of the compounds easily allows them to pass plasma membranes, alter vital cellular functions before interacting with cellular proteins denaturing them and triggering stress protein induction. [10] Oxidative stress evoked is evident by a significant alteration in ROS generation and anti-oxidant enzyme activities. A significant upregulation of ROS generation and a strong positive correlation is drawn between ROS generation and MDA inducing LP and the enzyme modification among the exposed. A significant increase in SOD and CAT activities observed in the exposed may be an attempt by them to abate the adverse effect of free radicals generated by the test chemical which correlates with Lin et al study.[11] The hepato-renal parameters show alteration among the subjects whose ROS markers are significantly high. ROS have been reported previously to play an important role in the regulation of gene expression by activating transcription factors that, in turn, mediate induction of proteins involved in cellular response to environmental conditions.[12] Hence, a possibility that ROS generated following chemical exposure could activate transcription factors that may trigger variation in hepato-renal parameters among exposed cannot be ruled out in the absence of any other triggering factors. A positive correlation was drawn among ROS generation and hepato-renal parameters expression. This type of expression in stanozolol-treated rats was correlated with a marker of oxidative damage to proteins.[13] Cell death has been reported to occur during development and also in response to various stress events[14] by apoptosis. Implication of oxidative stress in chemical induced apoptosis was not addressed in the study. We clearly demonstrated that inhibitors of SOD and CAT significantly increase ROS generation in concurrence with a significant enhancement in hepato-renal impairment in chronic exposures. This correlation was significant among the people with metabolic disease like diabetes and

hypertension. Some of them showed significant variation among the subjects with nausea and vomiting, the basic clinical feature in hepato toxicity (Table 1). Various studies like use of model organisms especially lower eukaryotes like *Caenorhabditis elegans* and *Drosophila*, have generated much interest after the unravelling of the genome sequences of these organisms.[15,16] With the advent of bioinformatics' tools, it is now clearly evident that the majority of *Drosophila* genes have orthologues in humans. Thus, laboratory based experimental evidences using *Drosophila* are useful to generate information that could be of use for their efficient extrapolation to higher mammals. Oxygen, an element indispensable for life, under certain situations acts deleteriously on the humans. It is mainly due to free radicals and oxidative stress.[17] Free radicals are unstable and highly reactive molecules with one or more unpaired electrons having various chemical structures, such as hydroxyl, superoxide, nitric oxide and lipid peroxyl radicals.[18] To get the molecular stability these molecules seek nearby molecules to obtain another electron, and this damages the structure and function of the molecule. Thus if free radicals are not inactivated, their chemical reactivity can damage all cellular macromolecules, including proteins, carbohydrates, lipids and nucleic acids.[19, 20] The radicals in turn have the ability to change the structure of DNA and serve as a precursor of cancer by inducing genotoxicity.[21, 22] These free radicals and reactive oxygen species are derived either from normal essential metabolism in the human body or from external sources, such as exposure to rays, ozone, cigarette smoking, certain drugs, pesticides, air pollutants and industrial chemicals. Free radical formation occurs continuously in cells as a consequence of both enzymatic and non-enzymatic reactions.[19, 20] The balance between the production of free radicals and antioxidant defenses in the body has important health implications. If there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage.[23]

CONCLUSION

The study suggests involvement of ROS in modulating hepato-renal impairment following exposure to diesel combustion nanoparticles. Based on the relative sensitivities of various end points examined, we favour ROS markers and its correlation with hepato-renal parameters as an early indicator of cellular hazard against diesel combustion nanoparticles.

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