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ORIGINAL ARTICLE

Effects of Particulate Matter Exhaust on Functional Parameters of Organ Systems and Reactive Oxygen Species: A Cross-Sectional Study

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Abstract

Background: From the day of industrial revolution and urbanization, exposure to diesel exhaust particles has become an environmental and occupational health concern as they contain a variety of nanoparticles which interfere with cellular function. Every organism on Earth has been exposed to minute foreign particles which enter the organ system and disrupt the cellular processes, interact with membranes, cell organelles, DNA, and other proteins to establish a series of dynamic bio-physico-chemical interactions. We aimed to study the variation in inflammatory cellular components and oxidative stress markers among exposures along with the variation in functional parameters of the organs involved in detoxifying these nanoparticles, the renal and hepatic system.
Methods: After the ethical clearance from Institutional Ethical Committee, IHEC-UOM No. 123PhD/2016-17, a cross-sectional study was undertaken in Molecular Reproductive and Human Genetics Lab, Manasa Gangothri, University of Mysore, Mysore, Karnataka, India during 2015-2019, among 500 male garage workers of age group 18-60years with history of exposure for 6-8 hours a day without using any protective aids during work, for past 6-12 years and 300 controls, who live in areas where they were sparsely exposed. Serum oxidative stress markers, hemogram, renal and liver functional parameters were analysed.
Results: A significant variation was observed among the oxidative stress markers among the exposures with surge in malondialdehyde and reduction in superoxide dismutase and catalase with variation in cell count, renal and liver functional parameters ($p \leq 0.05$).
Conclusion: Diesel combustion nanoparticles induces oxidative stress which reflects hepato-renal aberration.

Key words: Diesel Combustion Nanoparticles, Particulate Matter, Oxidative Stress, Reactive Oxygen Species

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INTRODUCTION

The ill effects of exposure to diesel nanoparticles have been attributed to the inflammatory response, responsible for a series of health hazards, like simple rhinorrhea to genetic dysfunction. Exposures to chemical irritants like particulate matter have also been shown to induce oxidative stress and DNA damage both in vivo and in vitro (1-5). The end organs in metabolism and excretion, the liver and kidneys are likely to be damaged and can result in hepato-renal toxicity. The toxicity of the nanoparticles will be sensed by different tissues which responds in various ways to cause various range of disorders. The particulate matter (PM) and its role in induction of ROS and organ damage is mainly by oxidative stress and single nucleotide polymorphisms (SNPs), the most common type of DNA variation in humans, and occur when a single DNA building block, or nucleotide, is replaced with another. Thus the reactive oxygen species (ROS) generation and subsequent damage of cellular respiration and genetic material (6). The chronic low-level exposure can cause long-term adverse effects whereas acute exposure results in short-term adverse effects.

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 capability of the body decides the disease status and the quality and quantity of nanoparticle toxicity (7). In many acute cases, the symptoms mimic a cold or flu. Chronic exposure can result in hepato-renal manifestations, behavioral changes including memory and attention deficits, depression, anxiety and irritability which many a times hamper the quality of work. (5, 6, 8). In view of all these, we aimed to explore the total and differential count in blood, hepato-renal toxicity and also the relationship among the

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ROS markers and hepato-renal functional markers.

METHODS

After the 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, Manasa Gangothri, University of Mysore, Mysore, Karnataka, India during 2015-2019.

Study Area and Sample Population

A 500 male garage workers of age group 18-60 years who were occupationally exposed to diesel combustion nanoparticles for 6-8 hours a day without using any protective aids during work, for 6-12 years. The control subjects comprising of 300 males of age 18-60 years were from in and around locality of Mysore, were apparently healthy having negligible or nil exposure to diesel combustion nanoparticles. The control subjects were non-alcoholic, non-smoker, without any associated metabolic disorders. The subjects were informed about the study objectives and a set of questionnaires were used to characterize the work practices, demography, exposure history and use of protective equipment.

Blood Collection and Serum Separation

Post phlebotomy plain blood collected in vacutainers and maintained at 4ºC. 3ml of blood was allowed to coagulate and centrifuged at the rate of 3000rpm for 3min.

Analysis of Total and Differential Count

The total and differential counts were estimated by using Fully Automated Hematology Analyser (Trivitron Health Care), a quantitative, automated hematology analyzer used for *in vitro* diagnostic use in screening patient whole blood based on volumetric impedance analysis method. The cells counted was in terms of white blood cells, (WBC) = N x 10^3 cells/µL

The normal range for WBC lineage is as follows: Neutrophils - 2.0–7.0×10^9/l (40–80%); Basophils: 0.02-0.1×10^9/l (0.5–1.0%); Eosinophils: 0.02-0.1×10^9/l (1–4%); Lymphocytes - 1.0–3.0×10^9/l (20–40%) and Monocytes- 0.2–1.0×10^9/l (2–10%).

Analysis of hepato-renal functional parameters

Hepto-renal parameters like, blood urea, serum creatinine, glutamic oxaloacetic transaminase (SGOT), glutamic Pyruvic Transaminase (SGPT), alkaline phosphatase (ALP) [9] were measured using automated biochemistry analyzer (Cobas c 311 analyser; Roche diagnostics, USA).

RESULTS

Analysis of Total and Differential Blood Count

The total blood count and differential cell count along with their indices among exposures and control group. Results showed that mean total count was high in exposures. The total count being 8143 cells which was within normal range but when compared with the control group (5600 cells) it was increased. It was observed that among the exposures the macrophages (6.12%), eosinophils (6.5%) and monocytes and basophils (3.62%) showed an increased cell rate. No significant difference among neutrophil and lower lymphocytes (13%) was noted. A mean increase in macrophages, eosinophils, monocytes and basophils were found and were in direct proportion with exposure duration. The combined data for exposed versus controls showed that diesel combustible nanoparticle was a significant predictor of this variation. In cases of disorder like diabetes mellitus (DM), hypertension (HT), respiratory distress, chronic obstructive pulmonary disease (COPD), asthma and allergic conjunctivitis the counts showed significant increase.

Analysis of Hepato-Renal Functional Biomarkers

The functional parameters of both liver and renal system showed significant variations (p = 0.0001). All the biochemical markers were elevated (Table 1) which signifies the hepato and nephro-toxicity. Among the study population we could find 6 diabetics (Type II) 3 hypertensives and majority with impaired ROS parameters, suffering from some clinical manifestations (Table 1). Cases with clinical features like nausea, vomiting, pain abdomen showed increased level of serum creatinine with increase in SGOT and SGPT levels signifying the hepato-renal toxicity of various degrees and slight elevations of bilirubin (Table 1).

The statistical analysis of the parameters showed a significant variation which were clinically also significant. (Table 2 and 3).

DISCUSSION

Environmental exposure to air pollutants that could be routinely met in urban areas can trigger subtle changes in the inflammatory response [13]. In the current scenario exposure to diesel combustion nanoparticles has created an alarming situation. It is observed that diesel combustion can cause a wide range of large single doses up to diffused chronic exposures leading to physiological stress and dysfunctions of organ systems in inhaled exposures [14]. This statement came true in present study. A mean increase in hemogram, suggests inflammatory response. The observations of this study were found to be similar to the finding of Steenhof *et al.,*[15] where a significant count and monocyte recruitment were affected in healthy individual who were exposed to short-term air pollution [16]. Poursafa *et al.,* [17] concluded a positive significant correlation with total count and platelets among children and adolescents in Isfahan city of Iran. Thus the inflammatory cell count was found to have an increasing trend.

In the present study, the exposures (n = 500) had a significant higher total count (p = 0.01), neutrophils (p = 0.07) and monocytes (p = 0.03) when compared with the control group (n = 300), but no contributory differences were noted in the numbers of total lymphocytes which consensuses with Rhodes *et al.,*[18] However, the literature has mixed results for the relationship between total cell counts and exposure to diesel combustion nanoparticles. Diesel exhaust did not record any effect on total count and differential count [19]. In our study the cases with local inflammatory clinical features had demonstrable neutrophilia which correlates with Salvi *et al.* [20], Behndig *et al.* [21],S tiegel *et al.* [13], and Alabi [22] but in contrary to Mills *et al.,*[23]. There was no rise in immature cells but Bleck [24] obtained immature lymphocytes and monocyte predominantly among exposures which may be a response to the diesel exhaust nanoparticles.

Table 1. Clinical features and mean serum levels of oxidative stress and hepato-renal markers among exposures (n=500) and controls (n=300)

Sample	Clinical Feature	No. of Cases	Oxidative stress parameters				Liver function tests						Renal function tests	
			SOD (13.46 ± 1.61 units/m)	CAT (0.0505 +/- 0.0018 u/l)	MDA (1.076 µmol/L)	TAC 300-460µmol/l.	Serum Bilirub (1-1.2mg/d)	SGOT (8 and 45 u/L)	SGPT (7-56u/L)	ALP (44-117 u/L)	Albumin (3.5-5.5 g/L)	Total prote (35-52g/dl)	Blood Urea (6-24 mg/dL)	Serum Creatinine (0.7-1.3mg/dL)
Exposures	DM HT	6 3	25.33±6.14	0.25±0.02	6.14±2.12	108.4±23.2	0.9±0.1	52±3.2	91.24±6.4	81.4±4.4	3.6±0.8	8.5 ±0.24	42±3.7	1.04±0.23
	Respiratory distress COPD, Asthma	12	23.47±4.11	0.2±0.06	5.33±1.98	114.6±20.2	0.94±0.13	48±1.6	54.1±2.4	44.1±1.4	5.3±0.76	8.2 ±0.18	32±2.2	0.98±0.13
	Conjunctiva watery, Conjunctiva congestion Conjunctiva Dry	22	39.65±2.15	0.33±0.008	4.21±1.06	148.3±10.8	0.9±0.12	45±2.4	48.17±4.4	38.0±4.1	5.8±0.46	7 ±0.25	31±2.6	0.74±0.12
	Abdominal pain & Diarrhoea	04	41.73±11.21	0.35±0.07	3.94±0.88	160.6±9.6	1.1±0.23	52±3.4	78.12±3.3	68.2±3.0	5.5±0.23	6 ±0.45	36±2.4	1.1±0.13
	Nausea	11	42.22±10.41	0.34±0.097	3.87±1.03	183.96±14.5	1.3±0.12	48±2.3	35.45±1.3	32.5±0.3	5.4±0.9	7.4 ±0.34	38±1.4	1.2±0.12
	Skin disorders	13	45.34±6.13	0.3±0.004	3.96±1.02	218.42±12.3	0.7±0.03	38±1.8	42.25±2.4	52.25±4.4	5.4±0.45	6.4 ±0.14	26±3.5	0.9±0.08
	Generalised weakness	40	41.73±5.11	0.41±0.092	4.11±1.94	230.34±10.32	0.7±0.12	46±2.4	55.47±3.4	51.7±2.4	5.1±0.68	7.7 ±0.32	38±1.2	0.9±0.12
	No clinical features	389	51.70±3.4	0.81±0.16	3.12±0.16	258.96±14.34	0.86±0.42	39±7.4	48.14±6.4	67.24±5.4	5.2±0.14	7.8 ±0.45	24±5.43	0.94±0.34
Controls		300	57.60±5.4	1.11±0.16	2.34±0.34	290.45±40.43	0.92±0.02	32±4.2	43±3.4	63±3.4	5.45±0.62	6.2 ±2.3	24±2.3	0.8±0.06

*Normal values in bracket

Table 2. Mean and t-test for Equality of Means of hepato-renal markers among exposures (n=500) and controls (n=300)

Parameters	Samples	N	Mean (SD)	Std. Error Mean
Total Bilirubin	Controls	300	0.9574 (0.6683)	0.03859
	Exposures	500	0.9636 (0.6272)	0.04435
Direct bilirubin	Controls	300	0.2859 (0.2352)	0.01358
	Exposures	500	0.8561 (0.4951)	0.02214
SGOT	Controls	300	31.2367 (13.3797)	0.77248
	Exposures	500	38.4172 (15.3109)	0.68472
SGPT	Controls	300	28.3700 (13.0486)	0.75336
	Exposures	500	41.2608 (15.6427)	0.69956
ALP	Controls	300	99.2553 (71.2029)	4.11090
	Exposures	500	101.3519 (82.9475)	3.70953
Total proteins	Controls	300	7.0992 (0.2419)	0.01397
	Exposures	500	9.9421 (1.8436)	0.08245
Blood urea	Controls	300	29.7433 (4.9354)	0.28495
	Exposures	500	39.6857 (16.7869)	0.75073
Serum creatinine	Controls	300	0.7533 (0.0935)	0.00540
	Exposures	500	1.4167 (1.4085)	0.06299

*Note the increasing trends of hepato-renal parameters among the cases

Table 3. T-test for Equality of Means

Independent Samples Test		t-test for Equality of Means				
		T	Df	Sig. (2-tailed)	95% Confidence Interval of the Difference	
					Lower	Upper
Total bilirubin	Equal variances assumed	0.104	498	0.917	0.12319	0.12319
Direct bilirubin	Equal variances assumed	18.713	798	0.000	0.62995	0.62995
SGOT	Equal variances assumed	6.727	798	0.000	9.27600	9.27600
SGPT	Equal variances assumed	11.988	798	0.000	15.00157	15.00157
ALP	Equal variances assumed	0.365	798	0.716	13.38595	13.38595
Total proteins	Equal variances assumed	26.565	798	0.000	3.05299	3.05299
Blood urea	Equal variances assumed	10.000	798	0.000	11.89401	11.89401
Serum creatinine	Equal variances assumed	8.145	798	0.000	0.82334	0.82334

The exposures exhibited mild to moderate eosinophilia. Alvarez-Simon *et al.,*[25] and Timmerman *et al.*[26] revealed an increase production in eosinophilia as primary inflammatory (p = 0.004) response. The monocytes and basophils were observed to have a rise in number among the cases when compared with that of the controls. Eosinophilia triggers allergic manifestations [27,28] were reflected in having allergic manifestations like dry itchy skin, conjunctival congestion, watery conjunctiva, rhinorrhea, sneezing, dry irritant cough, bronchial asthma and respiratory distress. This was similar to the conclusion Bruske *et al.,*[29] that ambient air pollution with combustible particles impacts the hemogram among the COPD patients.

The first response of an organism to any environmental alteration inducing stress is at the biochemical level. Stress response and anti-oxidant defense system consists of stress proteins, termed as heat shock proteins [30]. The anti-oxidant process is the primary protective response of the cell, contributes to increase in total protein, which is evident in the present study. 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, 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. ROS produced during oxidative stress have been implicated in apoptosis as possible signaling molecules [31].

Hepato-renal toxicity

Animal study in rats reveals that certain chemicals produce hepato-renal toxicity and has been reported to induce oxidative stress [32]. One of the mechanisms through 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 [33]. Diesel exhaust contains variety hepato-toxic nanoparticals. Studies indicated that diesel exhaust particles exposure and mortality due to atherosclerosis and cirrhosis of the liver are directly related. The results indicated that nanoparticle-rich diesel exhaust induced hepatic inflammation and dyslipidemia [34, 35]. Most of the studies on hepatotoxicity have focused on genotoxicity or mutagenicity [35]. A significant increase was noted in SGOT, SGPT and ALP markers among the exposures with DM, HT and abdominal pain subjects. These cytoplasmic marker enzymes are known to be released from damaged cells due to abnormal cellular dynamic process following the exposure to hydrocarbon fractions present in the exhaust [36].

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 Nemmer *et al.*[37] who also demonstrated acute kidney injury in his experiments with pulmonary exposure to nanoparticles. It has been noticed that ROS induction and impairment in heato-renal parameters are 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 [38, 39]. Oxidative stress evoked is evident by a significant alteration in ROS generation and anti-oxidant enzyme activities. A significant up regulation of ROS generation and a strong positive correlation is drawn between ROS generation and MDA inducing LP and the enzyme modification among the exposed. In the present study the hepatic enzyme SGPT and alkaline phosphotase show a significant increasing pattern (p = 0.000), which is in direct proportion to ROS enzymes. However, the variation in SGOT was not statistically significant (p = 0.904). A significant change in SOD and CAT activities observed in the exposed may be an attempt to abate the adverse effect of free radicals generated by the test, correlates with Lin *et al.,*[36]. The hepato-renal parameters show alteration among the subjects whose ROS markers are significantly high. However, a notable increase in the blood urea and serum creatinine could be determined among the exposures and controls (Figure 1). 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 [39]. 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. So, it is evident that the increase in ROS generation in concurrence with enhanced hepato-renal impairment (Table 1 and Table 2) in chronic exposures. This correlation was significant among the people with metabolic disease and associated disorders.

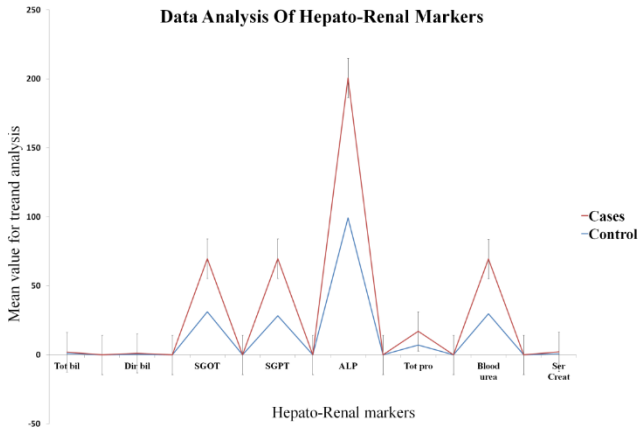

Figure 1. Trend of hepato-renal markers among exposures (n=500) and controls (n=300)

The cases with increased oxidative stress recorded increase in hemogram also. As a part of inflammation, the total protein and albumin content in serum showed mild elevation (Table 1). Increase in blood urea and serum creatinine with an associated increase in bilirubin reveals hepato-renal stress. Similar result was showed by Sarah *et al.,* [5] who demonstrated in a dose-dependent study which was in direct proportion to the amount of exposure and was reversible if the exposure was withdrawn.

On perusal of the hemogram, hepato-renal functional parameters and the clinical features it becomes evident that the ROS generation and organ system toxicity is significant among the exposures. Neutrophil counts were not correlated with functionalization of the lung in the initial stages, but when it escalated to chronic condition the inflammatory cells increased and perfusion capacity of lung reduced leading to COPD and infiltration of inflammatory cells to kidney and liver cause's glomerulonephritis and hepatitis which will be of idiopathic in nature. In the present scenario a significant correlation, was noted among the serum protein concentration and neutrophil counts among the exposed which correlates with Sarah and Kyjovska [5, 38]. Thus, diesel combustion nanoparticles are considered to induce a serious pathological consequence on hematological, hepato-renal parameters in occupational exposures.

CONCLUSION

The present study suggests the involvement of ROS in modulating hepato-renal impairment following exposure to diesel combustion nanoparticles. Based on the relative

sensitivities of various end points examined, the study favors ROS markers and its correlation with hepato-renal parameters as an early indicator of cellular hazard against diesel combustion nanoparticles. The liver and kidney will be affected either directly and/or indirectly in chronic exposure with the elevated changes observed in the biochemical profiles. An in-depth understanding of the mechanisms activated by diesel combustion nanoparticles will enable in the future for improving knowledge in the field risk assessment as well as the setting of new strategies towards diesel combustion nanoparticles health protection.

LIMITATIONS

The limitation is that this study was conducted among a limited population, while it is demanding in large scales. The PM concentration and contents in the workplace air of exposed individuals and determination of PM's type (e,g. PM2.5, PM5, PM10) could not be analysed.

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