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Saliva as an important body fluid in the detection of oxidative stress in community based studies: Preliminary study with police personnel's exposed to automobile exhaust

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Abstract
The aim of this study was to evaluate if occupational exposure to automobile exhaust could cause alterations in the levels of antioxidants and in relevant salivary parameters in traffic police. Saliva was collected and assayed for lactate dehydrogenase, amylase, malondialdehyde, glutathione and total antioxidant activity in healthy traffic police and their non-traffic counterparts. The collection of saliva is easy, non-invasive and does not need the services of trained staff, factors useful in large population based studies. The results showed that when compared to the non-traffic police, the biochemical changes in saliva were more pronounced in traffic police and was significant ($p< 0.0001$; $p< 0.002$). Our study indicates noteworthy oxidative stress and enhanced cell health in police personnel chronically exposed to automobile exhaust. The results also indicate that saliva could be used as alternative to blood and is useful to assess oxidative stress in people working in environmentally hazardous conditions.

Keywords: automobile exhaust; salivary antioxidants; oxidative stress; glutathione; trolox-equivalent antioxidant capacity; thiobarbituric acid-reactive substances; amylase; lactate dehydrogenase, police

1. Introduction
Pollution from traffic emissions is a major problem in urban areas and several epidemiological studies have conclusively shown a direct association between the increased levels of exposure to automobile exhaust with premature morbidity and mortality from cardiovascular disease, cancer, asthma and allergic diseases [1-9]. The exhausts emitting from the combustion of petrol- and diesel-fueled vehicles are a complex mix of pollutants and consist predominately of carbon monoxide, nitrogen oxides, particulates and hydrocarbons – especially the polycyclic aromatic hydrocarbons (PAHs), nitroaromatics, benzene, 1,3-butadiene, sulfur dioxide, lead, volatile organic compounds, ozone, and many other chemicals such as trace toxics and greenhouse gases [10, 11]. According to the International Agency for Research on Cancer, the "heavy diesel engines like buses, lories and trucks contribute approximately 50–60% of the trace emissions in urban areas, and the exhaust from diesel and gasoline powered engine have been classified as a Group 2A and 2B carcinogens, respectively [10, 11].
Traffic police officials are an important component of the society and play a pivotal role in maintaining law and order in the society. When compared to any other occupational group, the traffic police are frequently exposed to environment polluted by fumes, exhaust of vehicles, use of blowing horns, blow of dust in the air by a speeding vehicle and exposure to UV radiation from the sun. All these events increases the physical and mental stress and published reports indicates that the traffic police have increased incidence of diabetes mellitus, hypertension and cardiovascular disease [12, 13]. Accordingly, the Occupational Disease Intelligence Network system for Surveillance of Occupational Stress and Mental Illness, has classified traffic police profession to be amongst the top three stressful occupations [14].

Mangalore (Figure 1) is the trade and commercial center of the Western Region of the Karnataka State, in southern part of India and according to the 2011 census of India, the urban area of Mangalore has a population of 619,664; while the city metropolitan area has a population of 484,785 [15]. Mangalore is recognized as one of the rapidly growing two tire cities of India and this has lead to an enormous increase in vehicular traffic. Unlike most other Indian cities, the proper city of Mangalore does not have any industries that can be blamed for air pollution and the city's pollution is therefore entirely due to automobile exhaust. Information from the police department indicated that there are 60 male and 4 female traffic police personnel to coordinate the traffic movement of the city and that their duty hours were from 8 am to 8 pm.

Constant exposures to pollutants emitted from the automobile exhaust increases vulnerability for respiratory, cardiovascular, reproductive and neurological problems in the exposed individual and mechanistic studies performed mostly with blood have shown that superfluous generation of free radicals contributes immensely to the pathogenesis [3, 16-20]. Although useful, blood drawl involves stress to the volunteer and needs the services of an experienced phlebotomy staff, which in compromised conditions and in large population based studies is a major problem. This has necessitated a need for alternative biofluid that is easy to collect and can meet the criteria of a biomarker to assess oxidative stress in large population based studies.

Studies have shown that saliva is a useful body fluid in evaluating and studying oxidative stress markers in diabetes mellitus, oral diseases and in alcoholics [21-26]. Recently, we have also observed that a significant correlation existed between the between the activities of amylase, LDH, GSH and antioxidant capacity in the blood and saliva, and also that saliva could be a useful diagnostic tool alternate to blood [27]. Additionally, reports also suggest that saliva can be a useful biofluid to assess oxidative stress in people working in hazardous condition like in mine workers [28] and in copper-melting factory workers [29]. In lieu of all these observations, the present study was performed to evaluate the salivary oxidant-antioxidant status and salivary enzymes in the male traffic police personals and compared with age matched non-traffic police working in the police head office.

Materials and Methods

This was a single-centre, investigator blinded purposive sampling study, and was conducted between March 2012 and April 2012 at Father Muller Medical College, Mangalore, India. The study was approved by the Institutional Ethics Committee of Father Muller Medical College, and written informed consent was taken from all the willing volunteers after explaining to them the reason, objective, drawbacks, benefits and usefulness of the study in both English and in Kannada (the local and also the state language of Karnataka). The study aimed to recruit subjects into two groups giving 80% power to detect an estimated 50% difference between the two groups. It was required to calculate the difference in proportions between two groups for which nMasterTM 1.0 software was used. Each group was calculated to require at least eight subjects. However for better results we collected samples from twenty individuals.

The study subjects were grouped as follows: Group-I: Male police personnel in Mangalore, who were in non-traffic duties, age group of 21 to 58 years; Group-II: Male police personnel on traffic duties with regular exposure to emissions from vehicles, age group of 23 to 53 years. The inclusion and exclusion criteria are enlisted in table 1. The volunteers were requested to expectorate in to a pre weighed collecting tube as previously described by Navazesh [30]. This was to ensure that the variability in salivary flow rate and composition, be minimized. Individual subjects were asked by the investigators to rinse the mouth with clean water thoroughly to remove any food debris and then after 10 minutes, directed to expectorate into a sterile plastic container by not exerting any form of force. The collected saliva samples were then centrifuged at 3000 rpm for 10 minutes, and the supernatants were used for assay of biochemical parameters. The samples were analyzed by one of the investigator who was not aware of the cohorts.

All the biochemical assays were done in UV-visible spectrophotometer (Shimadzu, Japan). The saliva was assayed for total protein levels by the method of Lowry *et al*. 1954. The levels of Total antioxidant capacity [31], GSH [32]. LPx [33]. amylase [34]. and LDH [35]. were estimated in saliva using the internationally accepted standard procedures with suitable quality

Statistical analysis: The values were expressed as mean with standard deviation. Significance of the difference of the values between the groups was evaluated by using Student's *"t"* test.

Results

The years of service between the non traffic and traffic police was observed to be 3.28±1.2 and 3.23±1.07 and was not significant. The results of the biochemical parameters estimated in the saliva are presented in figure 2. When compared to non traffic police, the salivary level of malondiadehyde, LDH and amylase was significantly higher in traffic police (P<0.001). The traffic police staff also had significantly lower salivary levels of GSH and total antioxidant capacity than the non traffic police staff (P<0.002).

Figure 1: Geographical location of Mangalore in India

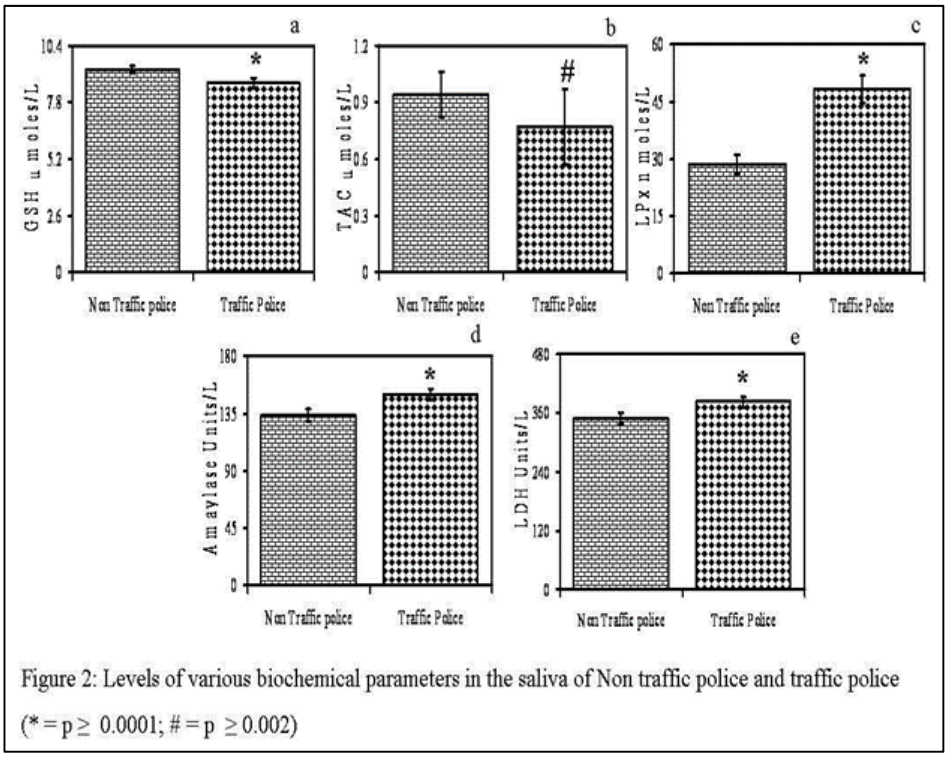

Figure 2: Levels of various biochemical parameters in the saliva of Non traffic police and traffic police

(* = p ≥ 0.0001; # = p ≥ 0.002)

Table 1: Details on the criteria used for the selection of volunteers for the study

Inclusion criteria	Exclusion criteria
Group I (Non Traffic police)	**1.** Smokers, tobacco chewers and alcoholics
1. Age (18-60 years) **2.** Working as non-traffic police staff for a minimum of 2 years.	**2.** Had chronic illness like diabetes, hypertension, cancer, renal failure, inflammatory diseases (IBD) or mental illness like bipolar disorder or schizophrenia. **3.** Had acute illness like fever, malaria, jaundice in the past one month **4.** Were on medications (like antibiotics, anti malarial drugs, analgesics etc) for the past one month. **5.** Volunteers with poor oral hygiene (carries and periodentoitis). **6.** Regularly consumed anti-inflammatory medications, antioxidant supplements and multivitamins for the past one month. **7.** Regular users of alcohol based dental products for gargling.
Group II (Traffic Police)	
1. Age (18-60 years) **2.** Working as traffic police staff for a minimum of 2 years and exposure was ≥ 45 hours / week.	

Discussion

Epidemiological studies have shown that constant exposure to automobile exhaust, which contains a range of toxic compounds, causes a variety of ailments, especially in the bus drivers, street policemen and street vendors [10, 36-41]. The adverse health effects of these toxic chemicals are reported to be due to the surfeit generation of ROS, oxidative stress, mutagenesis and inflammation and previous studies mostly with blood have substantiated these observations [8, 9, 42]. Recent studies have conclusively shown that saliva is a useful body fluid in studying oxidative stress markers in chronic alcoholics, smokers, oral cancer, diabetes, periodontitis, mine workers and chronic renal failure patients [21-26, 43] and in people working in occupationally hazardous industries [28, 29].

Free radicals generated cause autoxidation of polyunsaturated fatty acids and initiate the lipid peroxidation whose cumulative damage is far in excess of their initial reaction products. Malondialdehdye, one of the end products of lipid peroxidation is a sensitive and convenient marker of lipid peroxidation, and is measured as thiobarbituric acid-reactive substances (TBARS). In this study, it was observed that when compared to the non traffic police, the levels of MDA was greater in the traffic police personnel's regularly exposed to automobile exhausts. These observations are in accordance to the earlier reports where increased the levels of MDA was seen in the blood of traffic police [16, 20]. Further the levels of salivary amylase were also increased indicating the adverse effect of exposure to automobile exhaust. With regard to LDH, a general marker of cellular health, we also observed elevated levels in the saliva of the traffic police, clearly indicating that the exposure to automobile exhaust increases cell damage.

Saliva is equipped with enzymatic and non-enzymatic antioxidants, to protect against the free radical-inducing agents [44]. and previous studies have shown that their levels are decreased or impaired in various pathological conditions [22, 45]. In this study, we observed a decrease in the level of salivary total antioxidants in the traffic police and are in agreement to the previous reports with mine workers [28]. and smokers [46]. Additionally the levels of and GSH, the predominant cellular antioxidant involved in scavenging of electrophilic free radicals [47]. were also decreased in the traffic police personals and supports the earlier observations [48]. Together both these observations clearly indicate that reduced levels of GSH will compromise the cell's ability to negate the toxic effects of free radicals generated from automobile exhausts.

Conclusions and future directions:

The present study shows for the first time that saliva can be a valuable body fluid to evaluate the oxidative stress in traffic police personnel's continuously exposed to automobile exhaust. Collection of whole saliva is cost effective, non-invasive and does not require skilled technical person. Saliva has similarity with the blood regarding many of its components and the non-invasive collection methods involved in its collections offers a possibility of it being a replacement to blood as a biological product for large epidemiological studies in constrained situations. Considering the distinct advantages of saliva collection, further studies employing larger sample size and correlating the salivary changes with the biochemical changes in blood, especially for cardiac, liver, kidney and lung function are required as this can help in adopting of physical and protective means to prevent/reduce occupational hazards of automobile exhaust in police and other risk groups.

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***Dedication:** The authors dedicate this study to the police personnel's for their service and contribution to the society

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