# "STUDY ON OCCUPATIONAL HEALTH OF PETROL PUMP WORKERS AND AUTOMOBILE MECHANICS IN WESTERN MAHARASHTRA WITH SPECIAL REFERENCE TO CYTOGENETIC ALTERATIONS".

A thesis submitted to Faculty of Medicine, BLDE University, Vijaypur, Karnataka, India for the award of the Degree of

## Doctor of Philosophy (Medical)

### Subject: Physiology


**BY**

**Miss. Smita Vitthal Patil**

Ph. D. Research Scholar

Batch 2012-13

Registration NO: 12PHD013

Department of Physiology

BLDE (DU), Shri B. M. Patil Medical College, Hospital & Research Centre

Vijaypura, Karnataka, India.


**September– 2019**

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**Dr. Sumangala M. Patil**
Prof. and Head
Dept. of Physiology
BLDE (Deemed to be University).
Shri B. M. Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka.

**Dr. Sampada S. Kanitkar**
Co-Guide
Ex-HOD & Professor
Department of Oral Pathology
Bharati Vidyapeeth (Deemed to be University). Dental College & Hospital, Sangli. Maharashtra

**Dr. Arvind A. Patil**
Principal
BLDE (Deemed to be University).
Shri B. M. Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka.

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR- 586103

# BLDE (DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College, Hospital & Research Centre, Vijaypur, Karnataka, India

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**Date:** 9/9/2019

**Place: Vijaypura**

Seal and signature of the Principal

**(Dr. Aravind A. Patil)**
PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR- 586103

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***Date:*** 9|9|2019

***Place: Vijaypura***

***Signature of the candidate***

**Miss. Smita Vitthal Patil**

Ph. D Research Scholar
Registration No: 12PHD013.
Department of Physiology
BLDE (Deemed to be University),
Shri B. M. Patil Medical College,
Hospital &Research Centre, Vijayapura,
Karnataka.

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Shri B. M. Patil Medical College, Hospital & Research Centre, Vijaypur, Karnataka, India

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Signature of Candidate

**Miss. Smita Vitthal Patil**

Ph. D Research Scholar

Registration No: 12PHD013.

Signature of the Guide

Prof. and Head
Dept. of Physiology
Dr. Sumangala Patil,
HOD & Professor

Department of Physiology,

BLDE (Deemed to be University),

Shri B M Patil Medical College, Hospital

Research Centre, Vijayapura, Karnataka, India.

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Place: Vijayapura Mrs. Smita V. Patil (Gaikwad)

Date:

# DEDICATION

*I dedicate this thesis to*

**My Parents, Family**

Husband Pandurang, Daughters: Ishita, Avani

&

**My Teachers**

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| Abbreviations | |
|---|---|
| WHO | World Health Organization |
| PPW | Petrol Pump Workers |
| AM | Automobile Mechanics |
| PMS | Premium Motor Spirit |
| $NO_2$ | Nitric oxide |
| $CO_2$ | Carbon dioxide |
| CO | Carbon monoxide |
| ($C_6H_6$) | Benzene |
| PPE | Personal Protective Equipment |
| Ht | Height |
| Wt | Weight |
| Cms | Centimeters |
| Kg | Kilogram |
| BMI | Body Mass Index |
| BSA | Body Surface Area |
| TBW | Total Body Water |
| Bpm | Beat Per Minute |
| BP | Blood Pressure (mmHg) |
| SBP | Systolic Blood Pressure (mmHg) |
| DBP | Diastolic Blood Pressure (mmHg) |
| PP | Pulse Pressure (mmHg) |
| MAP | Mean Arterial Pressure (mmHg) |
| mmHg | Millimeters of Mercury |
| HR | Heart Rate (bpm) |

| Abbreviations | |
|---|---|
| CVS | Cardiovascular System |
| CNS | Central Nervous System |
| PEFR | Peak Expiratory Flow Rate |
| Hb | Hemoglobin (gm%) |
| RBC | Red Blood Corpuscles (millions/cumm) |
| WBC | White Blood Cells (Thousand/cumm) |
| TLC | Total Leucocytes Count (thousand/cumm) |
| DLC | Differential Leucocytes Count |
| PLT | Platelet |
| PCV | Packed Cell Volume (%) |
| MCV | Mean Corpuscular Volume (fL) |
| fL | Femtolitre |
| MCH | Mean Corpuscular Hemoglobin (pg) |
| Pg | Picogram |
| MCHC | Mean Corpuscular Hemoglobin Concentration (%) |
| HCT | Haematocrit |
| LFT | Liver Function Tests |
| RFT | Renal Function Tests |
| SGOT | Serum Glutamate Oxalate Transaminase |
| AST | Aspartate Transaminase |
| SGPT | Serum Glutamate Pyruvate Transaminase |
| ALT | Alanine Transaminase |
| ALP | Alkaline Phosphatase |
| GGT | Gamma GlutamylTransferase |
| IU/L | International Units per Liter |
| $Na^{++}$ | Sodium |

| Abbreviations | |
|---|---|
| $K^+$ | Potassium |
| EDTA | Ethylene DiamineTetraacetic Acid |
| NAD | Nicotinamide Adenine Dinucleotide |
| NADH | Nicotinamide adenine Dinucleotide |
| MDH | Malate Dehydrogenase |
| LDH | Lactate Dehydrogenase |
| GLDH | Glutamate Dehydrogenase |
| mEq/L | Miliequivalent per Liter |
| Pb | Lead |
| OD | Optical density |
| BLL | Blood lead level |
| MDA | Malondialdehyde |
| ROS | Reactive Oxygen Species |
| TCA | Trichloroacetic Acid |
| TBA | Thiobarbituric Acid |
| MN | Micronuclei |
| NA | Nuclear Abnormalities |
| BNC | Binucleate cell |
| KRC | Karyorrhetic cell |
| KLC | Karyolitic cell |
| RNA | Ribonucleic acid |
| SD | Standard Deviation |
| > | Greater than |
| ≥ | Greater than or equal to |
| % | Percentage |
| g/dl | Gram per deciliter |

| Abbreviations | |
|---|---|
| gm% | Gram percentage |
| µl | Micro liter |
| µm | Micrometer |
| mmol/L | Millimole per Liter |
| nmol/L | Nanomoles per Liter |
| Ml | Mililiter |
| $mm^3$ | Cubic millimeter |
| $m^2$ | Square meter |
| ANOVA | Analysis of variance |

## ABSTRACT

**Title:** "Study on occupational health of petrol pump workers and automobile mechanics in western Maharashtra with special reference to cytogenetic alterations".

**Background:** Occupational health hazard is of great concern for workers. Particularly the petroleum and automobile exhaust derived air pollutants have become a major health hazard which occurs in different fuel handlers such as petrol pump workers (PPW) and automobile mechanics (AM).

**Aim:** The main aim of this study was to assess the occupational health status in petrol pump workers & automobile mechanics, who are continuously exposed to petroleum and exhaust fumes during their working hours & compare it with the control group.

**Materials and Methods:** A human cross-sectional study was carried out in Western Maharashtra (India) on 70 petrol pump workers (PPW) and 70 automobile mechanics (AM), between the age group of 20–40 years which were working on petrol pumps and in automobile garages for more than 1 year.70 healthy males with the same socioeconomic status were chosen as controls. petrol pumps and in automobile workers were divided into three groups based on their duration of exposure, i.e. 1–5 years, 6–10 years, and more than 11 years of exposure. Anthropometric, socio-demographic parameters were recorded. Blood lead level (BLL) was measured by using Atomic Absorption Spectrophotometer (AAS). Further evaluation of oxidative stress, cardio-respiratory, hematological, biochemical parameters (LFT, RFT, electrolytes) and cytogenetic alteration (MN assay) was done by using standard methods. Statistical analysis was done by using one way ANOVA and Pearson correlation.

**Results:** Occupationally exposed groups had significantly high BLL (group-II 25.58µg/dL, group-III 15.43µg/dL) as compared to control group (9.730µg/dL). Oxidative stress parameters MDA ($P<0.001$), GGT ($P<0.02$) were significantly increased, in group-II and III. Systolic blood pressure ($P<0.016$), MAP ($P<0.018$) was significantly raised, whereas PEFR ($P<0.000$) and 40 mmHg endurance ($P<0.000$) was significantly decreased in group-II and III as compared to control group. Hemoglobin ($P<0.019$), RBC count($P<0.000$), PCV($P<0.011$), blood indices MCV($P<0.000$), MCH ($P<0.000$), MCHC($P<0.000$) were significantly decreased in study group, whereas total WBC count ($P<0.000$), differential WBC count and platelet count ($P<0.000$) was significantly increased in group-II and III as compared to control group. SGOT ($P<0.002$), SGPT ($P<0.040$), ALP ($P<0.0000$) and total bilirubin ($P<0.000$), direct ($P<0.000$), indirect ($P<0.036$), blood urea ($P<0.003$), serum

Creatinine (P<0.000), and electrolyte ($Na^+$ and $K^+$) were significantly increased, whereas serum total proteins(P<0.000), albumin (P<0.001), globulin (P<0.041) were significantly decreased in group-II and III as compared to control group. Blood lead showed a significant positive correlation with serum SBP (r = 0.397), MAP (r = 0.399), MDA (r = 0.646), whereas negative correlation with PEFR (r = -0.246), Hb% (r = -0.257), RBCs count (r = -0.340) in study group. Significant increase in frequencies of micronuclei (MN) (P<0.01), binucleate cells (BNC) (P<0.012) karyorrhectic cells (KRC) (P<0.030) karyolytic cells (KLC) (P<0.001) in group-II and III as compared to control. Duration of exposure has a significant impact on most of the parameters in the study group.So poor oral health was observed in the study group. Less number of group-II (AM) and III (PPW) workers aware about the occupational hazards of lead. The higher percentage of group-II and group-III workers did not use personal safety measures.

**Conclusion:** This study clearly indicates an adverse effect of heavy metal lead and other pollutants present in petroleum and exhaust fumes. It increases oxidative stress that causes adverse changes in hematological, renal, and hepatic function and cytogenetic alterations in AM and PPW of western Maharashtra (India). Therefore, it is important to create better awareness of occupational hazards among these workers. Promote them to use personal safety measures at the workplace for better health.

**Keywords:** Occupational health, Automobile mechanics (AM), petrol pump workers (PPW) BLL, oxidative stress, duration of exposure, micronuclei (MN).

# INTRODUCTION

***"Money is worthless if one looses the most valuable of everything: one's HEALTH''***

This sentence was phrased back in the 18th century by the famous Italian physician ***Bernardo Rammazini*** centuries by 1613-1714, He is considered as the father of occupational medicine which is still true and relevant even today.

Globally, it has been well recognized that 'ill health' directly arises as a result of occupational exposure to a large number of hazardous chemicals. Occupational exposure has been well recognized by the 'World Health Organization (WHO)' as an important contributor to mortality and morbidity (International Labour Organization 2011).

**1.1: Occupational Health:**

"Occupational Health is the branch of medicine which deals with all aspects of 'health' and 'safety' at the workplace and it has a strong focus on primary prevention of occupational health and hazards (Thangaraj S *et al.* 2017)".The main aim of occupational health is to 'promote and maintain the highest degree of physical, mental and social well-being of workers' in all occupations (K. Park 2017). Occupational health not only deals with work-related diseases or disorders but also it encircles all other aspects that affect a worker's physical and mental health. Recently with the changing scenario, there is a need to find out other risk factors associated with occupational health and hazards at the workplace.

**1.2: Occupational Hazards:**

In recent years, 'occupational health hazards' have grown as one of the major public health problems worldwide. From the Indian perspectives, vital occupational diseases are silicosis, asbestosis, byssinosis, musculoskeletal diseases, pneumoconiosis, pesticide poisoning, and noise induced hearing loss.

An occupational hazard that experienced by the workers at the workplaces. Occupational hazard can cause severe health issue amongst workers due to, unhygienic conditions and absence of safety at the workplace. It not only harms worker's health but also it minimizes the work productivity. Economic growth and productivity of the nation can be well achieved by promoting workers 'health and safety' as well as by improving the quality of work.

**1.3: Types & causes of occupational hazards:** Several studies have reported that automobile mechanics (AM) & petrol pump workers (PPW) are exposed to many hazards such as physical, chemical, biological (biohazards), psychosocial, and ergonomic hazards at their workplace. These hazards can cause different occupational diseases.

**Physical Hazards**- Common physical hazards at the workplaces are heat (heat burns, heatstroke, heat exhaustion and heat cramp) and cold (frostbite, chilblains, immersion foot and erythrocyanosis), light (poor illumination or excessive brightness leads to eye strain, fatigue, headache, frequent lacrimation, redness and pain in or around the eye), noise (temporary or permanent hearing loss), vibration (usually affect hands and arms), occupational exposure to ultraviolet radiations happen mainly in 'arc welding' that mainly affects the eyes (K. Park 2017).

**Chemical hazards** caused by exposure to chemicals in the workplace**.** These chemicals may be found in the working environment as gases, vapours/fumes (petrol/diesel and welding), mists/ aerosols, dusts (silica, asbestos, cotton), metals (lead, cadmium, mercury, arsenic, chromium, zinc, antimony, beryllium, cobalt, manganese, phosphorous and other) and liquids or solids. There are different types of harmful chemicals, including dermatologic agents, asthmagens, systemic toxins, reproductive toxins, neurotoxins, immune agents, carcinogens, pneumoconiotic agents, and sensitizers (CDC-Chemical Safety 2015). Occupational exposure to these chemicals in the workplace can leads to dermatitis, silicosis, and anthracosis, etc.

**Biological hazards (biohazards)** caused due to biological agents, including microorganisms (bacteria, fungi, viruses) and toxins released by living organisms. These biological agents can cause health-related issues such as skin irritation, allergies to infections (e.g. tuberculosis, AIDS) and cancer in occupationally exposed workers. 'Influenza' is the best example of a biological hazard (CDC - Seasonal Influenza (Flu) in the workplace 2015).

**Psychosocial hazards** have an impact on social life or psychological health. Psychosocial hazards experienced at the workplace involve work-related stress (overtime work, overwork, burnout, etc.), bullying – emotional, sexual and verbal harassment, as well as violence at the workplace, can cause burnout.

**Ergonomical hazards** mainly occur due to uneven working postures, frequent bending, the time interval between work shifts, working surface height, frequent lifting heavy loads, repetitive & monotonous work for a longer duration. It can cause musculoskeletal problems such as neck and back strain, backache, repetitive strain injuries (RSI) and 'carpel tunnel syndrome'(K. Park 2017).

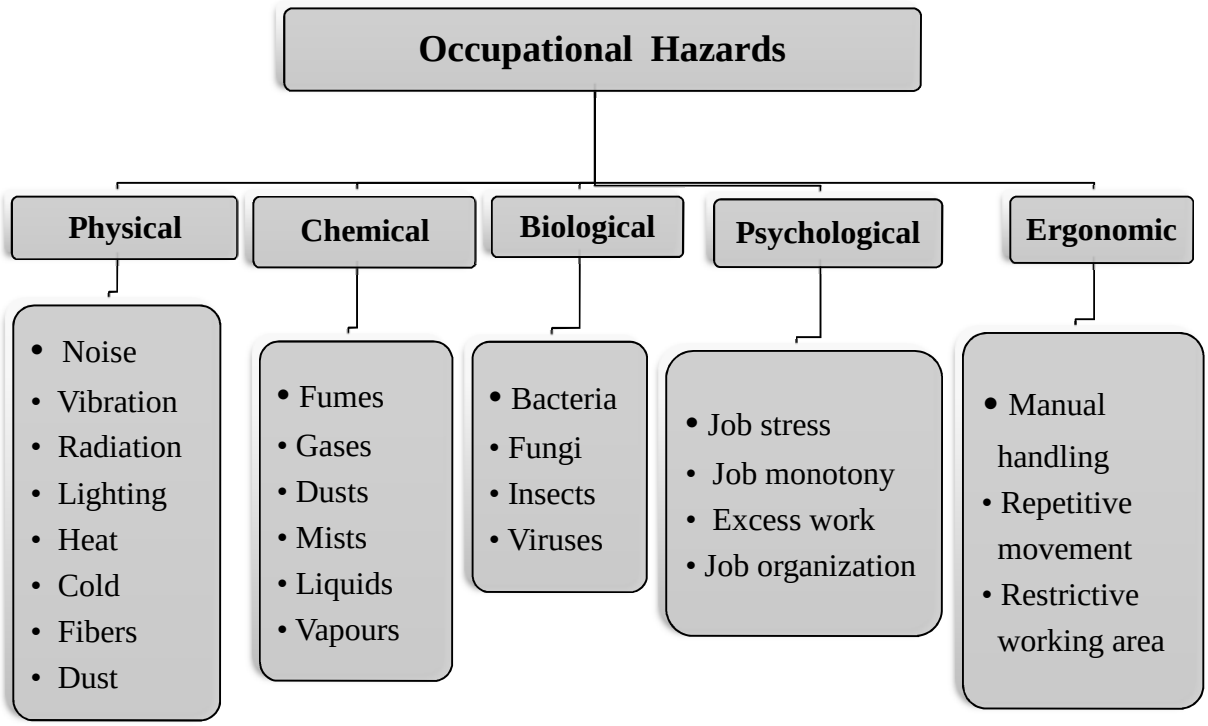



**Figure 1.1:–** Types and causes of occupational hazards.

An increasing number of vehicles with the emission of burnt fuel is a major cause of air pollution. Pollution from automobile emissions is a vital problem in urban areas. Several epidemiological studies have 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 (Manjeshwar Shrinath Baliga *et al*. 2017; Kelly FJ 2011).

Petroleum fuels are petroleum-derived liquid mixtures used as fuels. Petroleum fuel contains different types of saturated and unsaturated hydrocarbons obtained by fractional distillation of crude oil. It contains volatile aromatic compounds such as 'Benzene, Toluene, Ethylbenzene and Xylenes' (BTEX compounds). It also contains some heavy metals like arsenic, nickel, cadmium, lead, mercury, and chromium. Benzene and ethylbenzene are classified as group 1 and 2b carcinogens according to IARC (IARC 2000). Benzene is a cytotoxic, hematoxic, immunotoxic and genotoxic aromatic hydrocarbon that is used in many fields in the industry. It is also a common environmental contaminant and component of cigarette, gasoline and automobile emissions (Ayfer Beceren *et al*. 2016).

Recently there has been rapidly growing automobiles and its exhaust. Automobile exhaust mainly contains polycyclic aromatic, aliphatic, alicyclic and halogenated hydrocarbons. These are 'benzene, toluene, octane, xylene, styrene, naphthalene, pyrene,

benzo α-pyrene, butane, hexane and trichloroethylene, particulate matters and heavy metals like lead'. It also contains gases like nitric oxide ($NO_2$), carbon dioxide ($CO_2$) and carbon monoxide (CO). These gases are pro-oxidant in nature. Thus prolonged exposure to these gases could lead to an increase in levels of oxidative stress in the body (Ankita Salvi *et al.* 2017). It is well-identified that the vehicular exhaust is the major source of polycyclic aromatic hydrocarbons (PAHs) (Ghimire Dayaram *et al.* 2018).

The IARC has also classified diesel exhaust as a Group 1, known human carcinogen, with sufficient evidence for lung carcinogenicity (IARC 2014). Health effects of gasoline exhaust are less well understood because gasoline and diesel exhausts occur together for many exposed populations. The IARC has classified gasoline exhaust as a possible human carcinogen (Group 2B) based on sufficient evidence in animals, but inadequate evidence in humans (IARC 2014).

Millions of workers working in different occupational settings have the potential to get exposed to many types of hazardous substances at the workplace. Till today no alternative has been found in the Indian automobile industry for petrol or gasoline or premium motor spirit (PMS). Modern-day life in cities is associated with an enormous increase in vehicular traffic that emitting exhausts and polluting the environment. In India, there is no provision for self-service on petrol stations, the petrol pump workers (PPW) are employed for fueling of the vehicles. Apart from filling tasks, they are engaged in works like unloading of fuel and daily checking of fuel levels in the storage tanks.

Automobile mechanics (AM) belong to an 'informal sector' of a developing country like India. Automobile garages are a small-scale industry composed of skilled workers, such as mechanics, spray-painters, panel beaters, welders, battery recyclers, and radiator and air-conditioner repairers. Most of the petrol pumps and automobile workshops have been identified as major sources of environmental pollution, as they are located on the busy roads so, therefore, there are more chances of exposure to exhaust and air pollutants as well.

Automobile mechanics does routine maintenance & repair of motor vehicles by performing various activities such as spray painting, welding, vehicle repairing, and servicing and general work including vehicle washing and test driving. Most of the time they suck petrol or diesel with the mouth through a tube from the vehicle tank, while repairing the vehicles. They also often wash vehicle parts with petrol without wearing gloves.

Petrol pump workers and automobile mechanics are a group of professionals who are continuously exposed to petrol or diesel fumes, auto exhaust and other pollutants at their

workplace. Exhaust gases from motor engine enter into the bloodstream through the nose, mouth, skin and digestive tracts and have been shown to produce harmful effects, on the bloodstream, bones, and lymph nodes (Onuoha Moses *et al.* 2016).

Occupational health has been gaining importance for the fact that long term exposure to vehicle exhaust, vapors of petrol and dust can lead to permanent morbidity (Afshan Afroz *et al.* 2013). There is evidence from research in India and abroad the Netherlands, United Arab Emirates, Africa, and Bangkok) which indicates that petrol pump workers and automobile mechanics have been suffering from many adverse health effects due to exposure to petroleum fumes and automobile exhaust.

Many harmful effects have been reported after exposure to the different chemical constituents present in petrol. Inhalation of small amounts of petroleum fumes and automobile exhaust are known to cause symptoms like headache, nose, throat and eye irritation, dizziness, nausea, vomiting, low appetite, confusion, difficulty in breathing and pain in different regions of the body (Rahul *et al.* 2017). Some effects of petrol on the skin in previous studies, which include rashes and redness over the skin. Allergic (hypersensitivity) reactions have been observed in many studies but these are rare in occurrences (Rahul *et al.* 2017, Mahmood NM *et al.* 2012, Nurka Pranji *et al.* 2002).

According to the ATSDR (2007) acute exposure to benzene present in automobile, exhaust can cause 'central nervous system (CNS) disorder' like headache, drowsiness, dizziness, tremors, and convulsions. It also causes pulmonary and gastrointestinal disorders (nausea, vomiting). Chronic exposure to benzene may have an impact on the hematopoietic system and cause leukemia (Johannes burg 2015).

Previous studies indicate that major respiratory hazards were observed in workers exposed to petrol are due to air pollution from petrol or diesel exhaust. Automobile exhaust produces pollutants such as 'hydrocarbons, oxides of nitrogen and carbon' these are a major benefactor for particulate matter. Petrol and diesel exhaust particles are very small sized, by the virtue of their larger surface area they can transport larger fraction of harmful compounds like different hydrocarbons and heavy metals on their surface. They can persist for a longer period of time and finally deposit in greater number deeper into the lungs than large-sized particles. Thus chronic exposure to such compounds can cause chronic inflammation of respiratory tract and lung parenchyma (N Anupama *et al.* 2012).

Many epidemiological and experimental studies reveal that the hydrocarbons present in fuel vapors and automobile exhaust adversely affect the organs (heart, lungs, liver, skin, and kidneys) and **s**ystems **(**respiratory, hematological cardiovascular, reproductive, immune

and nervous system) both in children and adults. Recent studies show that 'acute' and 'chronic' exposure to petroleum compounds is related to various systemic health effects, such as hypertension, hematological (aplastic anemia and pancytopenia), respiratory (shortness of breath, upper respiratory tract irritation and bronchoconstriction), reproductive, immunological, dermatological, renal, neurological problems such as blurred vision and central nervous system (CNS) pathologies in human beings. Toxic substances present in petroleum fumes and automobile exhaust can cause various forms of nephrotoxicity, hepatotoxicity, genotoxicity and they have mutagenic, immunotoxic, carcinogenic and neurotoxic potential (Rahul *et al.* 2017; Nurka Pranji *et al.* 2002; Christopher E *et al.* 2017).

Automobile exhaust is the main source of a large number of health-damaging pollutants. Some recent studies have also found automobile exhaust can create hydrocarbons. Most components of the vehicular exhaust are oxidant in nature and are highly reactive oxygen species (ROS). These ROS are free radicals that cause injury in tissues through membrane damaging processes leading to cell dysfunctions. Although these pollutants may interact with other organ systems, their most obvious effects are in the lungs because lungs present the largest exposed surface to the atmosphere (Pakkala A *et al.* 2014). Some recent studies have also found automobile exhaust can create hydrocarbon-based free radicals, these free radicals are believed to cause lung cancer and respiratory and cardiovascular diseases (A.V. Anuja *et al.* 2014).

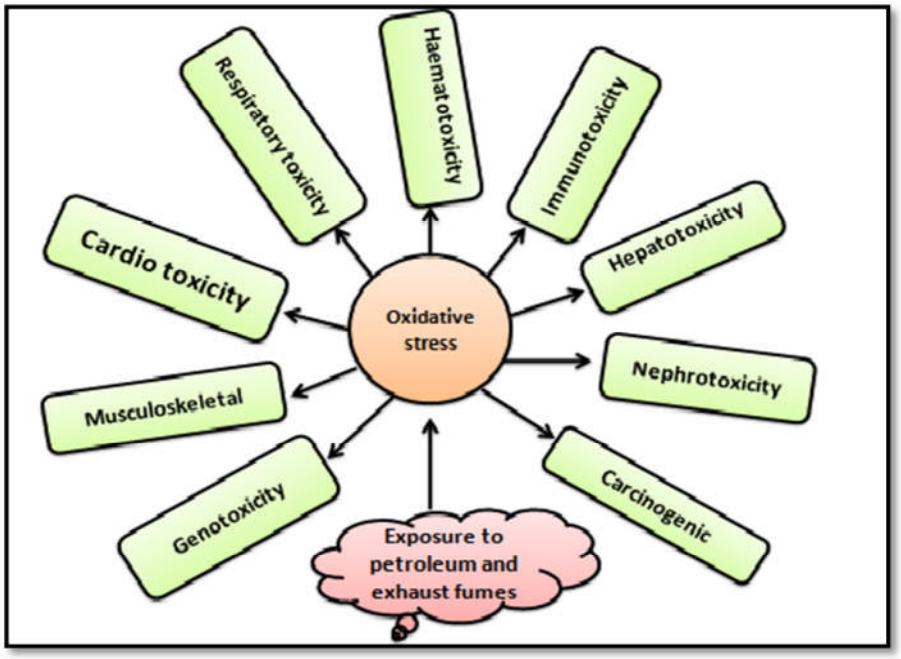



**Figure 1.2:** Multi-systemic effect of petroleum fumes and its exhaust on occupationally exposed workers.

Occupational health hazards associated with petroleum exposure have been recorded for many decades. Following facts taking into consideration, we have planned to study the health status in occupationally exposed petrol pump workers & automobile mechanics of Western Maharashtra.

1. The effect of an occupational hazard in the unorganized workforce (AM and PPW) is not adequately studied in Western Maharashtra.
2. There is no data available on the general health status of the people who are routinely exposed to petroleum and exhaust fumes because of their occupation.
3. Also to create awareness of health hazards associated with petroleum and exhausts fumes exposure in occupationally exposed petrol pump workers & automobile mechanics.

Such type of extensive study has not been carried out on petrol pump and automobile workers, especially in Western Maharashtra in relation to cytogenetic alterations. Hence we have selected this topic to study.

# Review of Literature

## 2.1 Western Maharashtra:

Maharashtra state is situated in the western part of the country and has 35 districts and carved out as a linguistic entity of Marathi-speaking people. Maharashtra has a long coastline stretching nearly 720 kilometers along the Arabian Sea. It is the 2nd largest state in India in terms of population (112.4 million = Male: 58.4 million and Female: 54 million (9.4% of India's population). The geographical area of this state is 3.08 lakh Sq. Km. This state has four biggest cities of the country such as Mumbai (12 million people), Pune (2.5million people), Nagpur (2 million people) and Nashik (1.1 million people).

Maharashtra state is positioned between 16° N and 22° N latitude and 72° E and 80° E longitude, Maharashtra has a 720 km long coastline stretching from Daman in the north to Goa in the south and is encircled by the five States such as Gujarat, Madhya Pradesh, Chhattisgarh, Andhra Pradesh, and Karnataka. It falls in the resource development zone called the 'Western Plateau' and 'Hill Regions'.

Topographically Maharashtra state is classified into five broad regional groups historically evolved as socio-cultural units like greater Mumbai, Western Maharashtra, Marathwada, Konkan, and Vidarbha. Maharashtra has been recognized as the best industrial state of India, and a pioneer for small-scale industries. Maharashtra has also become a leading 'automobile production hub' and a major IT growth center and having 1141 petrol pumps.

"Western Maharashtra" is considered as a heartland of Maharashtrian culture for many reasons. Pune is considered as the cultural capital of the Maharashtra state. Western Maharashtra covers seven districts namely Kolhapur, Sangli, Satara, Solapur, Pune, Ahmednagar, and Nashik (Economic Survey of Maharashtra, 2011-12). Western Maharashtra is surrounded by four rivers such as Krishna, Bhima, Sina, and the Godavari. The total geographical area of this region is approximately 89853 Sq. Km. and the total population of western Maharashtra is 34.09 million (Male: 17.61 and Female:16.48 million). Literacy rate of the population aged seven years and above is 82.23 percent (Male 89.95% and Female 74.20%) in 2011. Western Maharashtra has a typical monsoon climate, with hot, rainy and cold weather seasons (maximum time hot and dry climate). This area experiences three seasons: summer,

monsoon, and winter. The winter in January and February is followed by summer between March and May and the monsoon season between June and September. The average rainfall in this region is 608-635 mm. This region has a prosperous belt and is famous for its sugar factories. A farmer in this region is economically well off due to fertile land and good irrigation system. Sugarcane is the most important commercial cash crop in this area. Another recent cash crop that increases the economy of western Maharashtra is grapes, pomegranate, tobacco, banana, onion, and vegetables. But in the central dry plateau region of western Maharashtra jowar, bajara, groundnuts, cereals, and pulses are the important crops.

Lots of occupations have been found in this area (Western Maharashtra). But in the present study, we choose automobile mechanics and petrol pump workers as an occupationally exposed study group. Automobile repair workers are the informal sector and are exposed to so many hazards at their working environment like asbestos, mineral oils, solvents, paint pigments, anticorrosive substances, heavy metals and automobile exhaust (Richard Amfo-Out *et al.* 2016). These workers are low-income earners with less or no basic education and limited knowledge of occupational health and hazards. Most of the occupationally exposed workers live in urban areas; they remain untouched by civilization, and remain backward, particularly in health, education and socio-economic aspects.

## 2.2: History of occupational Health and Safety.

Occupational health can trace its roots back as far as ancient Greece when 'Hippocrates' a Greek physician and the 'father of medicine' (Hippocratic Oath) had observed lead poisoning among miners. 'Bernardino Ramazzini', an Italian physician was considered as the **father of occupational medicine.** Pliny the Elder, a Roman Senator, was the first who recommended that miners should use respiratory protection.

In nineteenth-century industrial machines were seriously dangerous. Workers did not have any kinds of basic safety practices and compensation schemes that we took for granted nowadays. "Harry McShane", a 16 years old young American factory worker, is a prime example. He got caught in the belt of a machine in a spring factory in the month of May 1908.

That time no compensation was paid. He had already been working in the factory for two years when the accident has happened. According to a statement made by Harry's father, his employers did not pay attention while he was at the hospital, or after he came home. As a result of this in 1833, the factory act came into force in an attempt to improve working conditions and sees the appointment of the first factory inspectors. Sir "Thomas Marison Legge" in 1898 became the first factory inspector in England (William Cowie 2013).

In the 20$^{th}$ century (1919) to improve the working condition and protect employees from work-related health issues, the foundation of the International Labour Organization (ILO) has been done, which is made up of representatives of Government, employers and the workers.

Traditionally, occupational health has been focused on protecting employees from work-related ill health. Now a day's there is increasing focus not only on work but also on the physical, social and mental well-being of workers by preventing ill health and all potential causes of ill health in the workplace.

Recently, in India, the following major research institutes are active in the field of occupational health (K. Park 2017).

1. The central mining and research station, Dhanbad under the Council of Scientific and Industrial Research (CSIR)
2. Industrial Toxicology Research Centre (ITRC), Lucknow under the CSIR
3. Occupational Health Research Institute, Ahmedabad under the Indian Council of Medical Research.
4. National Environmental Research Institute at Nagpur.
5. All India Institute of Hygiene and Public Health, Kolkata.
6. Indian Institute of Technology Kanpur.

## 2.3: Historical background of petroleum fuel.

The modern history of petroleum began in the 19th century with the refining of paraffin from crude oil. The Scottish chemist ‘James Young’ in 1847 noticed a natural petroleum seepage in the Riddingscolliery at Alfreton, Derbyshire from which he distilled a light thin oil suitable for lamp lighting and at the same time he obtaining a thicker oil suitable for lubricating machinery that is gasoline or petrol. The term "gasoline" was first used in North America in 1864, which refers to fuel used for automobiles. The term ‘Petrol’ was first used as the name of a refined petroleum product around 1870 by British wholesaler Carless, Capel& Leonard, who marketed it as a solvent (Carless, Capel & Leonard 2012). The name "petrol" is used in place of "gasoline" in most Commonwealth countries. The term ‘Petrol’ is an abbreviation of ‘petroleum’ derived from the Greek words ‘Petros’ (meaning rock or stone) and ‘oleum’ (oil) (Savita Kittad *et al.* 2015). The term 'petrol' is used in the UK, India and a few other places, 'gasoline' or 'gas for short' is used in the United States, while in Nigerian English it is termed as ‘premium motor spirit’ or ‘petroleum spirit’ and ‘mogas’. Petrol or gasoline is a volatile, flammable liquid. At room temperature, ‘petrol’ is a clear colorless to pale brown or pink in color with a distinctive odor.

Diesel fuel originated from experiments conducted by German scientist and inventor “Rudolf Diesel” for the compression-ignition engine he invented in 1892. For decades, diesel was an ‘unwanted byproduct’ or ‘distillate’ of this crude oil refining, but it was not considered important and called diesel fuel until 1894. Before it was coined as diesel fuel, it was referred to as ‘distillate’. In Australia, diesel fuel still goes by the term distillate. When it was named in 1894, it was due to diesel inventing the diesel engine and using this distillate to power it.

In India, the petrol filling station is commonly known as a petrol bunk or a petrol pump. The term "gas station" is mostly used in the Canada and United States, where the fuel is known as "gasoline" or "gas". In some regions of Canada, the term "gas bar" is used. Elsewhere in the English-speaking world, where the fuel is usually known as "petrol", the form "*petrol station*" or "*petrol pump*" is used. In the United Kingdom and South Africa, "garage" is still commonly used, even though the petrol station may not have service facilities. Similarly, in Australia, the

term ‘servo’ describes any petrol station, while in Japanese English; it is called a "gasoline stand".

### 2.3.1:- What is Petroleum fuel?

Petroleum is a thick, flammable, yellow-to-black mixture of gaseous, liquid, and solid hydrocarbons that occurs naturally beneath the earth's crusts and can be separated into fractions such as natural gas, gasoline, naphtha, diesel, kerosene, fuel, and lubricating oils and paraffin wax (Christian Serekara Gideon *et al.* 2016). Petrol or gasoline is a colorless petroleum-derived flammable liquid that is used primarily as a fuel in spark-ignited internal combustion engines. Gasoline is a volatile, flammable liquid. At room temperature, ‘petrol’ is a clear colorless to pale brown or pink in color with a distinctive odor.

Diesel fuel is any liquid fuel used in diesel engines, originally obtained from crude-oil distillation (petrodiesel), but nowadays many alternatives have been developed for partial or total substitution of petrodiesel, such as biodiesel (from vegetal oils), and synthetic diesel (usually from a gas fuel coming from coal reforming or biomass, also named gas to liquid fuels, GTL).

### 2.3.2: Composition of petroleum fuel:

Petrol is a petroleum-derived complex liquid mixture of petroleum hydrocarbons. The four main types of hydrocarbons present in gasoline are aliphatic, aromatic, an asphaltic hydrocarbon. Gasoline vapors contain about 15-60% alkanes (paraffin) and 30-60% cycloalkanes (naphthenes), 2–30 % aromatic and asphaltic (remainder) hydrocarbons. It contains light-chain volatile aromatic compounds such as benzene, toluene, ethylbenzene and xylene (so-called BTEX). It also contains many trace elements such as Carbon (93-97%), hydrogen (10-14%) nitrogen (0.1-2%), oxygen (1-1.5%) and sulfur (0.5–6%) with trace amounts of heavy metals (0.1 %) such as iron, nickel, copper, vanadium, cadmium and lead (Pb). Most of the hydrocarbons in petrol are insoluble in water and soluble in some organic solvents (Anne Marie H. 2018).

Diesel is a mixture of hydrocarbons blended to meet some specific properties. It contains about 75% saturated hydrocarbons (primarily paraffin including n, iso, and cycloparaffins), and 25% aromatic hydrocarbons (including naphthalenes and alkylbenzenes).

Normally paraffin (straight-chain hydrocarbons) between carbon numbers C12-C20 are the predominant components of diesel (Owen K *et al.* 1995). Cetane number is the most important property of diesel. Cetane number is a measure of the tendency of diesel fuel to knock in a diesel engine.

### 2.3.3: Uses of Petroleum fuel:

Petroleum fuels are mainly used as a fuel for motor vehicles for internal combustion of engines in automobiles, trucks and in light aircraft.

1. Petrol is primarily used to run petrol engines in cars, motorbikes, etc. while Diesel is used for transportation in cars, trucks, and motorbikes (on-road uses), etc.
2. Diesel fuel is used for mining, construction, and logging (off-road uses). It also used for farming, rail transportation, electric power generation, marine shipping and for military transportation (Owen K *et al.* 1995).
3. Petrol is used as a solvent, mainly well known for its ability to dilute the paints.
4. Historically, petrol was used as a cleaning fluid to remove grease stains from clothing, and metal surfaces. Also, it was used for the treatment of head lice (during the late Victorian era). But this treatment method was not used for a longer time because of the inherent fire hazard and the risk of dermatitis.
5. Petrol is also used in kitchen ranges and for lighting and is still available in a purified form, known as camping fuel, white gas, Coleman fuel, for use in lanterns and portable stoves.

## 2.4: Composition of Exhaust Fumes:

Exhaust fumes are a complex mixture of pollutants. It predominately consists 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 toxins and greenhouse gases (Manjeshwar SB *et al.* 2017). Automobile exhaust is made up of two main parts such as gases and soot (particles). The gases present in the automobile exhaust are "carbon dioxide, carbon monoxide, nitric oxide, nitrogen dioxide, sulfur oxides, and hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs)" (WHO 2003-2004). The soot (particulate) portion of automobile exhaust is made up of particles such as carbon, organic materials (including PAHs), and traces of metallic compounds. Particulate matter is the complex mixture of organic and inorganic particles, such as dust, pollen, soot, smoke, and liquid droplets[13]. Based on the size, particulate matter is often divided into two main groups,

1. The coarse particles with a diameter between 2.5 to 10 μm ($PM_{10}$) - a coarse fraction of the particulate matter can include dust, Pollen grains, mold spores and plant and insect parts.

2. Fine particles with a diameter of 2.5μm or less ($PM_{2.5}$) - they are formed from gases.

3. Ultrafine particles with a diameter smaller than 0.1 μm. Ultrafine particles (up to 0.1 μm) are formed by nucleation, which is the initial stage in which gas becomes a particle.

## Table 2.1: Composition and health effect of exhaust fumes.

| Composition of exhaust Fumes | Health effect of exhaust fumes |
|---|---|
| Nitrogen ($N_2$) | No adverse health effects |
| Oxygen ($O_2$) | No adverse health effects |
| Water ($H_2O$) vapors | No adverse health effects |
| Carbon Dioxide ($Co_2$) | Nontoxic greenhouse gas can cause global warming |
| Carbon monoxide (CO) | Produced due to incomplete combustion of fuel. It decreases the oxygen-carrying capacity of blood and cause headache, respiratory problems and at high concentration even death of the person. |
| Nitrogen Oxides (NOx) | They react with hydrocarbons to produce low-level ozone which can cause inflammation of the airways, reduced lung function, and trigger asthma. They also contribute to the formation of particulate matter. |
| Sulphur Dioxide (SO2) | It forms acids on combustion leading to acid rain and engine corrosion. It also contributes to the formation of ozone and of particulate matter. |
| **Particulate matter (PM) or soot –** | |
| Particulate matter | Smaller particles can pass deep into your lungs causing respiratory complaints and contributing to the risk of developing cardiovascular diseases. |
| Hydrocarbons (HC) | HCs are emitted from vehicle exhausts cause breathing problems and increased symptoms in those with asthma. |
| Benzene(C6H6) | Benzene is emitted from vehicle exhausts as unburnt fuel and also through evaporation from the fuel system. Benzene is toxic and carcinogenic and long-term exposure has been linked with leukemia. |
| Metals | Metallic elements in automobile emissions are silicon, copper, calcium, zinc and phosphorous, lead, manganese, chromium, zinc, and calcium. |

## 2.5: Heavy Metal Lead (Pb):

The lead was one of the earliest metals discovered by the human race and was in use by **3000 B.C.**The original use of lead plumbs the depths of history. In fact, the oldest known lead article is a figurine found in Egypt that dates back to 4,000 BC. Later, because of its malleability and resistance to corrosion, lead was used extensively by the ancient Romans for water pipes, aqueducts, tank linings and cooking pots and plumber who joins and mends pipes takes his name from the Latin word **plumbum**, meaning lead. Plumbum is also the origin of the terms '**plumb bob**' and '**plumb line**' used in surveying and also the chemical symbol for lead is (**Pb**).

It is one of the earliest metal discovered by the human being and has been used over 6000 years. Lead mining probably predated the Bronze or Iron Ages, with the earliest recorded lead mine in Turkey about 6500 BC. Heavy metal Lead is mentioned in the five thousand years old Indian epic *Mahabharat* and is found in the excavation of its contemporary town 'Mohenjo-Daro', Older scriptures Chandogya, Upanishad, and Yajurveda also clearly refer to lead (Patil Arun J. 2004).

### 2.5.1: Uses of heavy metal lead:

Recently lead has been used in acid battery manufacturer, pesticides, pottery, boat building, printing press, silver jewellery making, for protective covering of the telephone wires, making containers for storing corrosive liquids, soldering cans, traditional practices such as folk remedies, cable sheathing, in color pigments, petrol additives, soldering water distribution pipelines, ceramic glazes, paper industries, casting toys, in ornaments, vibration damping materials under machines and buildings, lining tanks, evaporating pans, sinks, cisterns, manufacture of lead compounds e.g. white lead, chrome yellow etc. coils and pump valves etc. It is used for protection against gamma radiation while working with radioactive substances. The amount of lead used in these products has been reduced in recent years to minimize its harmful effect on human and animal health (Patil Arun J. 2004; ATSDR 2005; WHO 1995; Patil Arun J *et al.* 2007, Debasish Bandyopadhyay *et al*. 2014).

## 2.5.2: Sources and occurrence of lead:

There are four major sources by which human being get exposed to lead are air, water, soil, and food.

Lead is obtained in the combined state from the earth crust. Metallic lead does occur in nature, but it is rare. Lead is usually found in ore with zinc, silver, and copper. Lead is extracted together with these metals. The important ores of the lead are Galena (PbS), Anglesite (Pb $SO_4$), Cerussite ($PbCO_3$), Canarkite ($PbOPbSO_4$), Crocoisite ($PbCrO_4$) (Debasish Bandyopadhyay *et al*. 2014). Lead reacts with moist air, water containing dissolved oxygen, acids, alkalis, chlorine, sulfur, etc. Lead exhibits bivalency and has the power of oxidation. Metallic lead is tasteless and odorless, but some of the oxides and salts of lead give a sweet taste, which can cause a major health problem, especially in children. Lead is insoluble in water, but it dissolves in some of the salts, hence lead salts can be carried long distances in water supplies.

Heavy metal lead can be found in two forms,

1) Inorganic lead can be found in higher lead content paint, soil, dust, and various consumer products.
2) Organic lead found in gasoline (Tetraethyl and tetramethyl lead) to increase octane rating (Debasish Bandyopadhyay *et al*. 2014).

## 2.5.3: Absorption of Lead

The inhalation and ingestion are the primary roots of absorption of lead compounds. Approximately 40% of lead oxide fumes are absorbed through the respiratory tract and only 5-10% absorbed from the gastro-Intestinal tract via food, beverages, soil, and dust. Soluble salts of lead are easily absorbed into the bloodstream from the intestine. Lead is not absorbed from the skin surface (Dermal absorption) until the skin is broken or damaged. Dietary factors, nutritional status, the chemical form of the metal and patterns of food intake affect lead absorption.

### 2.5.4: Distribution of Lead in the body:

After absorption lead is distributed throughout the body, mostly it is stored in three compartments of the body such as blood, soft tissues (liver, kidney, and brain) and bone. In blood, approximately 99% of the lead is found in the erythrocytes and only 1% of absorbed lead is found in the plasma and serum then distributed to bones (affect the process of erythropoiesis), teeth and soft tissues (liver, kidney, aorta, brain, lungs, spleen, skeletal muscles) where it is readily exchangeable (Debasish Bandyopadhyay *et al*. 2014; Needleman HL *et al.* 1996).

### 2.5.5: Half Lifetime of lead in the body:

Lead in blood has an estimated half-life of 35 days, in soft tissue approximately 4 to 6 weeks, and in bones 20 to 30 years (Lyn Patrick 2006).

## 2.6: Symptoms, Occupational hazards and use of personal safety measures at the workplace in petrol pump workers and automobile mechanics.

A study on the effect of chronic exposure to petroleum products on some hematological and biological parameters carried out by Akintonwa A *et al.* (2005); on 200 male petrol station attendants in Ibadan, Nigeria. The symptoms in petrol station attendants due to inhalation and skin exposures were abdominal pain (52.4%), headache (47.7%), eye irritation (33%), dizziness (26.4%) and chest pain (21.2%). The other symptoms include itchiness and difficulty in breathing. The symptoms due to oral exposure to petrol include constipation (42.6%), nausea (32.9%), watery stool (33.1%), cough (26.9%) and chest pain (25.2%). The other symptoms include itching throat, increased sputum production, and dizziness.

Another cross-sectional study was done by Yassin M *et al.* (2009); assessed knowledge, attitude, practice, and self-reported symptoms associated with leaded gasoline exposure among 200 gasoline station workers (mean age 30.7 ± 9.4 years) in the Gaza Strip. A low level (7.5%) of illiteracy was recorded. One hundred and fifty workers (75%) reported a relatively high level of knowledge on the health impact of leaded gasoline. A higher proportion of workers (147) were aware of the inhalation of leaded gasoline as a route of exposure than skin (18.0%) or

mouth (3.0%). Knowledge concerning the accumulation of lead in the body was low (26.0%). The higher the education level, the more knowledge the workers had, particularly knowledge about the health effects of leaded gasoline. Protective measures were poorly used. Common self-reported symptoms among leaded gasoline exposed workers were a headache (32.5%), nausea (27.5%), and pallor (23.0%). There was a significant increase in the prevalence of self-reported symptoms with increasing years of work in the station ($p = 0.001$). The prevalence of symptoms was generally higher among workers who did not use the protective gear than those who did not use it.

A study was done by Uzma N. *et al*. (2008); and Singhal M. *et al.* ( 2007); showed inhalation of petroleum fumes and automobile exhaust can cause significant health problems and respiratory symptoms like chronic cough, breathlessness, and wheezing.

Kesavachandran *et al.* (2006); studied the lung function abnormalities among petrol pump workers of Lucknow, North India. They found that a higher prevalence of respiratory symptoms was primarily a consequence of exposure to the petrol vapors found in the workplace in the petrol filling stations.

A descriptive cross-sectional study carried out among petrol station attendants in Nigeria by Johnson OE *et al.* (2018); commonest health problems reported were headache (53.6%), low back pain (33.3%), eye irritation (29.5%), dizziness (24.6%), cough (18.6%) and nausea (18.6%) in Nigerian petrol pump attendants. They also observed a statistically significant association between having headache, nausea, cough, and inhalation of petrol vapors ($p<0.05$) or car exhaust fumes ($p<0.05$).

Sambo M.N. *et al.* (2012); studied the occupational health hazards among roadside automobile mechanics in northwestern Nigeria**.** They found that 53.5% of automobile mechanics had secondary education, 29.5% had primary education while 3.5% were illiterate and 12.5% had informal education. Majority of the mechanics (44.5%) were involved in general vehicle repairs, (26.5%) were motor engine mechanics, (15%) were auto electricians and 9% were welders. The commonest injuries were burns (86%), bruises (64.5%), crushed digits (62%) and cuts (59%). Forty-nine percent of automobile mechanics had experienced low backaches, 15% had joint pains and 7% had a hernia. Eighty-two percent (82%) were aware of

the protective devices. The commonest known safety devices were overalls (85%), boots (82.5%) and rubber gloves (80%).

A study carried out by Okafoagu Nneka *et al.* (2017); on knowledge of occupational hazards and safety practices among petrol station attendants in Sokoto metropolis, Sokoto State, Nigeria. They observed 59.0% petrol station attendants had poor knowledge of occupational hazards, while only 15.1% had a negative attitude. 72.4% of petrol station attendants knew that volatile organic compounds (VOCs) were harmful to health. Good proportion 92.4% knew about work safety practices such as not to smoke cigarettes at the workplace. Only 2.8% of petrol station attendants used hand gloves while 19.4% used apron always.

A cross-sectional study was carried out by Thangaraj S *et al.* (2017); on occupational health hazards among automobile mechanics working in an urban area of the Bangalore. They observed that musculoskeletal diseases were the most common health problems by mechanics (62%), followed by cuts and injuries (58%). The commonest known personal protective equipment (PPE) was eye goggles (87.3%) and hand gloves (80%).

Elenwo E.I. (2018); reported occupational hazards like back pain (18%), burns (16%), headaches (15%), hearing problem (6%), fatigue (16%), respiratory problems (4%) and have dizziness (13%) in automobile mechanics. They observed 57% of automobile mechanics had secondary education, 75.4% were not aware of their job was a hazardous one. They noticed 73.9% automobile mechanics not aware of using personal protective equipment, but about 59.7% used one type of personnel protective equipment or the other.

Marahatta SB *et al.* (2018); studied the awareness of occupational hazards and associated factors among automobile repair artisans in Kathmandu Metropolitan City, Nepal. Their study revealed that 56% automobile repair artisans were aware of occupational hazards and 44.3% workers were used PPE and 31.5% automobile repair artisans receiving training prior to joining the work, in Kathmandu. This level of awareness and PPE use is insufficient, and probably it highlights an unsafe worksite environment for the workers.

## 2.7: Effect of petroleum and exhaust fumes on blood lead level.

Lead is recognized as one of the major environmental toxins, most widely distributed in nature. Lead-induced toxicity has been an important and well-studied issue of public health concern around the globe for years. The difficulty with lead is that once it is mined from the earth, there is no known way to destroy or make it harmless.

Dongre NN *et al.* (2011); found that blood lead and urinary lead levels were significantly increased in automobile workers as compared to controls. This indicates more absorption of lead in automobile mechanics than the controls. Blood lead level (BLL) generally reflect acute (current) exposure because of the short half-life of lead in blood (28–36 days), Estimation of blood lead is the best and most sensitive biomarker for identifying human exposure to lead and its adverse effect on human health. This increased blood lead level in automobile mechanics indicates that the release of lead fumes, particles, dust and fuel vapors were more in automobile garages. Poor hygiene and inappropriate protection increase the risk of exposure.

Adela Yalemsew *et al.* (2012); revealed automotive-garage workers had significantly higher BLL than that of the controls. The BLLs of all the lead-exposed individuals were found to be over10 μg/dL, and 53% of them had BLLs ranging 12–20 μg/dL, with the remaining 47% having over 20 μg/dL. The BLL of the workers increased with the duration of working in an automotive garage. Individuals involved in manual car painting comprise a larger percentage (58%) of those with the highest BLLs (≥ 20 μg/dL). The BLLs of the workers are influenced by their occupational practices and roughly paralleled with the duration of occupational lead exposure.

Amah UK *et al.* (2014); noticed that the mean blood lead level was significantly higher in automobile repairers than in the control group. Another study was done by E.Chukwu Onyeneke *et al.* (2016), and M. Adejumo *et al.* (2017); also observed individuals who are occupationally exposed to lead had significantly elevated blood lead levels (BLL) as compared to controls. They observed that the prevalence of lead toxicity in occupationally exposed subjects was 57% with no toxicity found in the controls. The highest prevalence of lead toxicity (64%) was found in the study group.

A study done by Sirwan M. Mohammed *et al.* (2014); revealed a statistically significant increase in the mean BLL among gasoline station workers, as compared to mean BLL of controls. Among the studied sample of workers, the smoking individuals had a higher mean of BLL as well as, the symptoms were more intensive among them when compared to non-smoking workers.

Abdulsalam Saliu *et al.* (2015); studied comparative assessment of blood lead levels of automobile technicians in organized and roadside garages in Lagos. They observed higher blood lead level in 40.3% of the organized and 34.3% of the roadside groups. The organized group had statistically significant higher blood lead level (66.0$\mu$g/dl) than the roadside (43.5$\mu$g/dl) group. Higher prevalence of elevated blood lead level was observed in Automobile technicians in organized garages in Lagos than the roadside group.

Nur Kusuma Dewi *et al.* (2017); studied the blood lead level among fuel station workers. The maximum limit of blood lead levels according to WHO (1995) was 25 μg/dl. Based on these criteria, they found that 59.6% of respondents had average blood lead levels (10-25 μg/dl) which were still within the normal limit, 32.7% had low lead levels (<10 mg/dl) and 7.7% of respondents had high blood lead levels (>25 mg/dl).

Assessment of some heavy metals among petrol station attendants in Ekpoma and its Environs had done by Airhomwanbor O *et al.* (2018); The analysis showed a significant increase ($p<0.05$) in the serum level of the lead of petrol station attendants when compared with controls. There was no significant difference ($p>0.05$) in the level of iron, zinc, and copper of petrol station attendants when compared with controls. The results generated in this study have shown that the petrol station attendants in the study area may be prone to lead toxicity. Therefore they recommend that petrol station attendants should avoid direct inhalation of the fume from petrol by covering their nose with a nose mask.

## 2.8: Effect of petroleum and exhaust fumes on oxidative stress.

Oxidative stress is a relatively new term in biology that was first introduced by Sies in 1991. Oxidative stress is defined as "an imbalance of free radicals and antioxidants in the body, which can lead to cell and tissue damage". In the review of Mark R. Miller 'oxidative stress' is defined as the generation of reactive oxygen species (ROS) over and above that which the body's defenses can remove (Mark R Miller *et al.* 2012). The body's cells produce free radicals during normal metabolic processes. However, cells also produce antioxidants that neutralize these free radicals. A balance between free radicals and antioxidants is necessary for proper physiological function.

Production of ROS is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides like superoxide anion ($\bullet O_2^-$), Hydrogen peroxide ($H_2O_2$), Hydroxyl radical (•OH), Organic hydroperoxide (ROOH), Peroxynitrite (ONOO-), Alkoxy (RO•) and peroxy (ROO•) (Toshikazu Yoshikawa *et al.* 2002).

Various markers exist for oxidative stress including erythrocytes glutathione (GSH) levels, plasma malondialdehyde (MDA), nitrite/nitrate (NOx) and homocysteine (Hcy) levels, as well as serum ceruloplasmin (Cp), total antioxidants (TAO), endothelin-1 (ET-1) levels and γ-glutamyl transferase (GGT).

Malondialdehyde (MDA), is the final product of lipid peroxidation and is a good marker of free radical mediated damage and oxidative stress. It is known that oxidative stress is caused by either increase production of reactive oxygen or nitrogen species or decrease antioxidant defenses or both. It has been reported that MDA content increases with increasing heavy metal concentration, as a result of inhaled petroleum and exhaust fume, thus indicating a concentration dependent free radical generation (Idoko Alexander *et al.* 2017).

The primary role of 'cellular gamma-glutamyl transferase' (GGT) is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. Paradoxically, recent experimental studies indicate that cellular GGT could also be involved in the generation of reactive oxygen species (ROS) in the presence of iron or other transition metals. Although the relationship between cellular GGT and serum GGT is not known and serum GGT activity has been commonly used

as a marker for excessive alcohol consumption or liver diseases. Epidemiological studies consistently suggest that serum GGT within its normal range might be an early and sensitive enzyme related to oxidative stress (Lee DH *et al.* 2004).

Lead induces oxidative stress by producing free radicals and lowering the antioxidant defense system. The mechanism of lead-induced oxidative stress involves an imbalance between generation and removal of ROS (reactive oxygen species) in tissues and cellular components. Although reactive oxygen species (ROS) are important in signaling processes, their excessive production leads to cellular and tissue damage. ROS attack lipid membranes, proteins, and nucleic acids (Ghanwat G.H. *et al.* 2015). Reactive oxygen species play important roles in cell signaling, a process termed reduction-oxidation signaling.

Systematic studies on heavy metal lead-induced oxidative stress have been based mostly on in vitro experiments (Ziad Shraideh *et al.* 2018). Lead may directly be attached to a cell membrane, thus increasing the sensitivity of the membrane to the process of lipid peroxidation (Flora G *et al.* 2012). The biological mechanisms behind lead-induced oxidative stress have been reviewed by Ahamed and Siddiqui (Ahamed M *et al.* 2007). These mechanisms include the inhibiting of the enzymes involved in the heme biosynthetic pathway and thereby generating free radicals, increasing the susceptibility of cell-membrane to peroxidation, depleting glutathione (GSH) and changing the activities of the antioxidant enzymes.

Numerous types of particulate matter (PM) derived from vehicle exhausts have the capacity to generate oxygen free radicals (Particles as a direct source of free radicals). The oxidative properties of particulate matter present in automobile exhausts trigger the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals. Particulate matter-induced oxidative stress and inflammation are dependent on particle toxicity and the capability to mobilize antioxidant defenses, which might offer a possible explanation to the increased sensitivity displayed by asthmatics to air pollutants (Magnus Lund back, 2009).

The lung because of its large surface area is clearly a primary target organ for oxidative injury (Mark R Miller *et al.* 2012). The endothelial cells lining the vessel lumen are a major target for oxidative stress leading to endothelial dysfunction. ROS are a central source of

oxidative stress and are secreted by leukocytes as well as by the endothelium itself. At low doses, ROS serve as an important participant in cell-signaling. However, at high doses, ROS can cause cellular injury (Magnus Lund back 2009).

Animal and in vitro studies have demonstrated increased airway oxidative stress and inflammation triggered by particulate matter air pollution. Several studies in healthy individuals as well as in asthmatics, utilizing experimental chamber exposures to diesel exhaust, suggest a diesel exhaust-induced oxidative stress and airway inflammation (Magnus Lund back, 2009).

Many researchers have conducted studies on occupational exposure to petroleum hydrocarbons (BTEX) and heavy metals like lead on human body systems. These hydrocarbons and its reactive metabolites can cause the continuous generation of reactive oxygen species (ROS), which can cause lipid peroxidation and damages DNA, RNA which leads to genetic modification and alterations in the functions of key enzymes and proteins. Reactive oxygen species plays a very important role in a number of cellular functions like cell signaling, apoptosis, gene expression and the activation of cell signaling cascades (Ray PD *et al.* 2012; Amrin Shaikh *et al.* 2017).

Benzene is a major component of petrol. Evaporation of petrol is common during its handling, distribution, and storage which intern releases benzene along with vehicle exhaust. Activation of benzene and its reactive metabolites leads to the continuous production of reactive oxygen species (ROS), which leads to lipid peroxidation and damages DNA, RNA, leading to genetic modification and alterations in the functions of important enzymes and proteins (Malini SSN *et al.* 2017).

A study was done by Ragia M. Hegazy *et al.* (2014), and Olufunsho Awodele *et al.* (2014); evaluated oxidative stress in benzene exposed fuel station workers and petroleum tanker drivers. They observed a highly significant increase in MDA level in fuel station workers compared to controls. Also, they found serum MDA and SOD levels were significantly decreased in the petroleum tanker drivers as compared to controls.

Owagboriaye F.O. *et al.* (2016); studied the effect of gasoline fumes on stress hormones, antioxidant status and lipid peroxidation in the albino rat. They found that MDA level was significantly increased with increasing daily exposure time to gasoline fume as compared to control. Level of ACTH was significantly reduced in the gasoline fume exposed

rats when compared to control, while aldosterone and corticosterone levels were significantly increased with increase in the daily period of gasoline fume exposure relative to the control.

Another study by Adnan J. M. *et al.* (2017); on the effects of some heavy metals on Oxidant / Antioxidant Status in gasoline station workers. They found a highly significant increase in heavy metals (Pb, Hg, and Cd) and MDA level in gasoline station workers. Also, there was a significant decrease of biochemical parameters (TAC, SOD, CAT, GRx, GST, GPx, ALAD and GSH) in the gasoline station workers as compared to the control group. There was a significant positive correlation between MDA and blood lead level. As the duration of the exposure of work increased which lead to a significant increase in levels of lead (Pb), mercury (Hg), cadmium (Cd) and MDA in filling station workers.

Akinosun O.M. *et al.* (2017); studied toxic metals induced oxidative stress in auto mechanics. They noticed that there was a significant increase in oxidative stress index in automobile mechanics as compared to the control group. There were no significant differences observed between blood Pb levels, estimated glomerular filtration rate and albumin: creatinine ratio in both groups.

In the review of the prevalence of exposure of heavy metals and their impact on health consequences by Rehman K *et al.* (2018); mentioned Pb induced oxidative stress is the major mechanism responsible for its toxicity. Changes in membranes fatty acid composition are the major intrinsic mechanism for Pb induced oxidative damage to membranes. The lateral phase separation induced by Pb affects the processes related to membranes such as exocytosis and endocytosis and processes of signal transduction. Recently, researchers have found that Pb cause gene expression alterations. An important human DNA associated protein protamine is involved in Pb toxicity because of gene expression alterations induced by it and then it interacts with zinc-binding sites on protamine.

In Hilla city of Iraq, Safa W. Azize *et al.* (2018); studied the heavy metals and their effects on oxidant / antioxidant status in workers of the fuel station. The results revealed that Pb and Cd and MDA levels significantly higher in the blood of workers than healthy controls ($p<0.01$) respectively. This study also found a significant decrease in the levels of TAC, SOD, Zn, Cu, Mg, Vit. C and Vit.K in the petrol pump workers than healthy controls ($p<0.05$). This

study suggests that exposure to heavy metal pollution in the workplace (fuel stations) led to an increase in the oxidative stress in petrol pump workers which decreased the antioxidant levels.

Ziad Shraideh *et al.* (2018); studied the associations between occupational lead exposure and various markers of oxidative stress in Jordanian automobile occupational workers. The results showed that the concentration of malondialdehyde (MDA) in plasma was increased in the case of workers than controls being the highest in automobile electronics and the lowest in automobile mechanics. This suggests that lead may have induced oxidative damage to automobile workers by generating free radicals and lipid peroxidation and impaired their enzymatic and non-enzymatic antioxidants defenses, and this damage was enhanced as the duration of exposure/work duration was increased.

## 2.9: Effect of petroleum and exhaust fumes on different systems:

It is quite clear that the major occupational health hazards of a petrol pump and automobile workers are due to continuous inhalation of petroleum fumes and its exhaust. Petroleum fumes and automobile exhaust induced oxidative stress, it is multifactorial and it affects various organ and organ systems. Both petrol and diesel fuel undergo combustion in automobile engines and forms combustion-derived nanoparticles. These particles are highly respirable and have a large surface area which can carry a larger fraction of toxic, hydrocarbons, and metals on their surface (Mehta JN *et al.* 2018).

Automobile exhaust consists of a mixture of soot, gasses including oxides of sulfur and of nitrogen, carbon dioxide, carbon monoxide, and liquid aerosols and particles including heavy metal lead. It leads to various lung disorders, carcinogenesis, and changes in hematological parameters (Mehta JN *et al.* 2018). These gases and heavy metal are pro-oxidant in nature and prolonged exposure to it can cause the development of oxidative stress in the body (Ankita Salvi *et al.* 2017). These gases and heavy metal like lead causes the generation of free radicals inside cells and that leads to impairment of cellular functions thereby affecting the health of automobile mechanics and petrol pump workers.

### 2.9.1: Effect of petroleum and exhaust fumes on the cardiovascular system (Heart).

On the basis of numerous population studies in different settings, including prospective studies, it has been well documented that automobile exhaust and heavy metal lead induces arterial hypertension. Hydrocarbons and heavy metal lead present in fuel vapors and exhaust acts at multiple sites within the cardiovascular system, it exerts direct effects on the excitability and contractility of the heart, also they alter the compliance of vascular smooth muscle tissue and acts directly on the parts of the central nervous system which are responsible for blood pressure regulation **(**Yao Lu *et al.* 2015).

Magnus Lund back (2009); said that exhaust particles with a diameter of >10 μm are filtered out in the nose and larynx and rarely reach the lower airways. However, a fine and

ultra-fine particle can penetrate deep into the lower airways and reach the peripheral regions of the bronchioles. These small particles may translocate into the systemic circulation.

An experimental study carried out by I. P. Odigie *et al.* (2004); showed that significant increase in systolic, diastolic and mean arterial blood pressures and insignificant increase in heart rate in lead treated rats compared to control rats. Interestingly, they also found that significant increase in the concentration of sodium, potassium and chloride ions in lead treated rat compared to control rats. They observed that chronic exposure to low levels of lead resulted in electrolyte retention and elevation of blood pressure.

Heavy metal lead is known to affect the cardiovascular system in occupationally lead-exposed populations. Dongre NN. *et al.* (2011); found slightly increased systolic and diastolic blood pressure in automobile workers with respect to controls. This indicates that increased blood lead level does not alter blood pressure severely but hypertension is prominent in automobile workers those were chronically exposed to lead. So possibly "lead-induced nephrotoxicity is a probable cause of secondary hypertension" in these workers. Also lead has both direct and indirect effects on the blood vessel and the smooth muscle contractility and thereby it affects blood pressure.

Cardiotoxicity induced by inhalation of petroleum products studied by O.M. Azeez *et al.* (2015); observed that inhalation of petroleum products leads to a significant rise in SBP, DBP, PP, MAP, and heart rate in petrol, kerosene, and diesel exposed rats. These findings revealed that the cardio-toxic effect of petroleum products is due to "the ability of petroleum hydrocarbon to enhance the sensitization of myocardium to catecholamines, impair vagal activity and increased baroreceptor activity with resultant vasoconstriction and increased arterial blood pressure".

In a recent review of Fioresi M. *et al.* (2016); several deleterious effects of lead exposure had been observed on the cardiovascular system (blood pressure) from different researchers. Some of them noted that lead interferes with cardiac contractility (Fioresi M *et al.* 2013); it increases vascular tone and peripheral resistance, also it stimulates of the renin-angiotensin system (Simoes MR *et al.* 2011); it reduces the availability of nitric oxide and increased oxidative stress (Fiorim J *et al.* 2011); and interference in the cardiac autonomic

control. Researchers point out that occupational lead exposure is reasoned as a cardiovascular risk factor.

Another study conducted by Tiu DN *et al.* (2017); on the effects of chronic inhalation of petrol fumes on white cell count s in a population of male petrol filling workers occupationally exposed to the solvents in petrol and air pollutants for short and long durations. Long term exposure to petrol fumes has deleterious effects on human hematopoietic system leading to bone marrow depression. There was a significant increase in SBP, MAP, PP and pulse rate in petrol pump workers due to the adverse effect of automobile exhaust and pollutants. This is associated with an increased WBC count. Recently, it has been suggested that 'immune mechanisms' may play a major role in the development of some forms of hypertension.

Kristen E. *et al.* (2012); observed inhalation of diesel exhaust was associated with a rapid, measurable increase in systolic blood pressure without changes in DBP and HR. This is possibly due to the interaction of $PM_{2.5}$ with nociceptive or noradrenergic receptors stimulate the sympathetic nervous system (SNS), either directly via vasoconstrictive effects of norepinephrine on a blood vessel or indirectly by increasing circulating levels of the vasoconstrictor agent Angiotensin-II.

According to Yuanyuan Cai *et al.* (2016); short-term exposure to pollutants like $SO_2$, $PM_{2.5}$, $PM_{10}$ and long-term exposure to pollutants like $NO_2$ and $PM_{10}$ were significantly associated with the risk of hypertension. Short-term exposure to other air pollutants such as $NO_2$, $O_3$, and CO and long-term exposure to NOx, $PM_{2.5}$, and $SO_2$ had positive relationships with hypertension. They suggest that short-term or long-term exposure to these pollutants may increase the risk of hypertension. The mechanism by which air pollution could contribute to the development of high blood pressure includes inflammation and oxidative stress, which may lead to changes in the arteries.

## 2.9.2: Effect of petroleum and exhaust fumes on the respiratory system (Lungs):

Previous studies indicate that major respiratory hazards found in workers exposed to petroleum fumes are due to air pollution from petrol and diesel exhaust.

Mehta JN *et al.* (2018); studied pulmonary function in petrol pump workers in Anand district. They found that statistically significant decrease in forced expiratory volume in the first second ($FEV_1$), $FEV_6$, $FEV_1/FEV_6$, and peak expiratory flow rate (PEFR) in the study group than the control group ($P<0.001$). They said this decline in lung functions was due to the accumulation in peribronchial lymphoid and connective tissues along with varying degrees of wall thickening and remodeling in terminal and respiratory bronchioles arising from each pathway. Bronchiolar walls with marked thickening contained moderate to heavy amounts of carbon and mineral dust, and wall thickening is associated with an increase in collagen and interstitial inflammatory cells including dust-laden macrophages. It was suggested that exposure to fuel smell leads to the restrictive type of lung disease in petrol pump worker.

A few studies of Aparajta Neerajkant Pawar *et al.* (2011); Sandip M Hulke *et al.* (2012); and Aparajta, Neerajkant Pawar *et al.* (2012); have shown the effect of petroleum fumes on lung function tests. They found a statistically significant decrease in FVC (Forced vital capacity), $FEV_{0.5}$, $FEV_1$, $FEV_3$, FEF50%, FEF25-75%, PIFR & PEFR in petrol pump workers. They observed a statistically significant decrease in FVC, $FEV_1$ in petrol pump workers who were exposed to more than 5 years. Flow rates FEF25-75%, PEFR & PIFR was significantly decreased in petrol pump workers who were exposed to more than 10 years.

Analysis of dynamic pulmonary function in automobile mechanics had done by Anupama N. *et al.* (2012); observed a significant decrease in dynamic pulmonary function tests like FVC, $FEV_1$, $FEV_1$/FVC ratio, PEFR, FEF 25-75, FVC, $FEV_1$ and FEF 25-75 in the automobile mechanics. They said that Petrol or diesel exhaust particles are very small sized, by the virtue of their larger surface area they can transport larger fraction of harmful compounds like different hydrocarbons and heavy metals on their surface. They can persist for a longer period of time and finally deposit in greater number deeper into the lungs than large-sized particles. Thus chronic exposure to such compounds can cause chronic inflammation of

respiratory tract and lung parenchyma. Their results point out that there was more of restrictive disease and small airway disease developing in the automobile mechanics.

Another study conducted by Siddanagoudra SP *et al.* (2012); to study the respiratory morbidity in spray paint workers in the automobile sector. Their study revealed that PEFR and FEF25–75% were insignificantly decreased in spray painters, suggesting smaller airway obstruction in the lung. There was a strong correlation observed between duration of exposure and pulmonary functions.

In the study of Batta M. *et al.* (2015); found the effect of automobile exhaust on pulmonary function tests in petrol pump workers. They found that FVC, $FEV_1$, $FEV_3$, PEFR, FEF25%, FEF50%, FEF75%, FEF25-75%, and MVV were significantly reduced in petrol pump workers as compared to controls. Also, there was a statistically insignificant difference observed in $FEV_1$/FVC% and $FEV_3$/FVC% between the two groups.

According to T. Shonga *et al.* (2015); higher prevalence of lung function impairment observed among fuel attendants (29.0%) than the controls (7.4%) with a significant reduction in FEV1, FVC, and the PEFR among the exposed subjects. They observed this is due to the cumulative effect of benzene in petrol and $SO_2$ in diesel. These substances may cause respiratory impairment in fuel attendants.

A Dynamic lung function tests in occupationally exposed petrol pump workers of Western Maharashtra was carried out by Patil SV *et al.* (2016); They found a statistically significant decrease in $FEV_1$, FVC, PEFR, $FEV_1$/FVC ratio, PEFR, FEF 25%, FEF 50 %, FEF 75 % and FEF 25-75 in petrol pump workers as compared to control. Santhalingam B. *et al.* (2017); showed a significant decrease in lung function tests ($FEV_1$, FVC, and PEFR) among petrol pump workers as compared to the control group.

Sapna S Bhardwaj *et al.* (2018); showed a statistically significant decrease in FVC, PEFR, $FEV_1$, $FEV_1$/FVC ratio <80% and Forced Expiratory Flow at 25%-75% of volume as a percentage of vital Capacity (FEF 25%-75%). Smoker garage workers had a higher frequency of chronic respiratory symptoms and lung function impairment as compared to nonsmoker garage workers. They suggest that people who are exposed to auto exhaust emission for more

than 15 years are likely to develop a mixed type of lung disease. This suggests that chronic exposure to air pollution in the automobile garages due to automobile exhausts, fuel vapors, etc. adversely affected pulmonary function and increased the frequency of respiratory symptoms.

Akintunde AA *et al.* (2018); studied the lung functions abnormalities among auto mechanics in Nigeria. They found that the mean values of $FEV_1$, FVC and PEFR were decreased less than 80% of expected values. Restrictive, obstructive and mixed type of lung function abnormalities were observed among 53%, 10% and 2% of automobile mechanics. The mean values of $FEV_1$ and FVC reduced significantly with increasing job duration.

### 2.9.3. Effect of petroleum and exhausts fumes on hematological parameters:

In human, petroleum and exhaust fumes can cause a wide range of biological effects depending upon the level and duration of exposure. It has effects at the subcellular level, as well as effects on the overall functioning of the body and range from inhibition of enzymes to the production of marked morphological changes and death, can be observed.

Olufunsho Awodele *et al.* (2014); evaluated the hematological, hepatic and renal functions of petroleum tanker drivers in Lagos, Nigeria. They showed a significant increase in WBC count and a significant decrease in the lymphocytes of the petroleum tanker drivers as compared to the control group. Also, there was an insignificant increase in RBC count, neutrophil count and hematocrit value. A decrease in platelet count was observed in petroleum tanker drivers as compared to the control group.

A comparative cross-sectional study carried out by Mahmoud S. El-Saadawy *et al*. (2011); on 105 car mechanics in car repair workshops in Zagazig City. They found the majority of car mechanics had contact dermatitis (25.7%), followed by wounds & burns (10.5%) and oil acne (4.8%). Hematological parameters showed that car mechanics had decreased erythrocyte, hemoglobin, hematocrit values, lymphocytes and platelets levels, but increased neutrophil levels.

Sirwan M. Mohammed *et al.* (2011); carried out a study on hematological, biochemical and blood lead level among gasoline station workers in Sulaimaniya city. There was a significant increase in hemoglobin content observed in the gasoline station workers as compared to controls. Oppositely, there were no significant differences observed in RBC count, hematocrit value, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count, mean platelet volume, total and differential WBC count between gasoline station workers and control group.

The results of the study conducted by Hala Samir Abou-EIWafa *et al.* (2015); showed that the mean hemoglobin level and RBCs count were significantly lower in petrol station attendants than the control group while the non-significant increase in WBC and platelets counts were observed among petrol station attendants and control group. Mean hematocrit

value was significantly lower in the petrol station attendants than the control group while MCV, MCH, and MCHC were similar in both groups.

Another study done by Ahmed Abdalla Agab Eldour *et al.* (2015); showed no significant difference between mean values of the hemoglobin, RBC, WBC, and platelets count among petrol station workers. They conclude that workers exposed to benzene may develop bone marrow depression, as it was evidenced by a drop in reticulocytes, HCT and red cell indices in workers. They suggest that WBC and platelets count were not sensitive indicators of benzene-induced hematotoxicity.

Tiu DN *et al.* (2017); carried out research on the study of WBC count and its association with blood pressure in petrol filling workers. They observed a significant increase in total leucocytes, granulocyte and monocytes count while a decrease in lymphocyte counts was observed in petrol station workers. They conclude that long term exposure to petrol fumes has deleterious effects on human hematopoietic system leading to an increase in blood pressure which was also associated with an increased WBC count.

Effect of fuel inhalation on blood indices in gasoline station workers studied by Mashael Bin-Mefrij *et al.* (2017); studied They showed that WBC count was significantly higher among gasoline fuel station workers than the controls. The mean value of MCH and MCHC were significantly lower in study participants as compared to the control group. All other blood indices such as RBC count, hemoglobin%, hematocrit, MCV, and platelet count were not showing significantly different between the study and the control group.

Al Jothery A.H. *et al.* (2017); studied the changes in the hematological profile among workers at petrol stations in Babil Province/Iraq. No significant differences were noticed in the means value of RBC counts, Hb%, PCV, MCV, MCH, MCHC, RDW, WBC counts, differential counts of WBC, platelet counts between workers exposed to benzene (filling workers) and non-exposed (office's workers). However, only one biomarker was significantly influenced by exposure to benzene, in which the mean of platelet volume (MPV) was significantly increased in the filling workers. However, Hb% and PCV were significantly increased in smoker workers compared to non-smokers at petrol stations, while the total platelet count was significantly reduced.

Zerihun Ataro *et al.* (2018); studied the occupational health risk in workers working in automobile garages. They studied blood pressure and hematological parameters between garage workers, they observed significant decreases in RBC count, hemoglobin%, hematocrit value, and MVC among garage workers compared to the control group. In contrast, total WBC and platelet count were significantly higher in garage workers than controls. There were no significant differences observed in MCH and MCHC between the two groups.

A cross-sectional study carried out by Patrick Adu *et al.* (2018); in automobile mechanics of Ghana. They observed the reduced hematopoietic output in automobile mechanics and automobile sprayers. Significant decrease in RBC count was observed in mechanics while compared to controls. Significantly a higher hemoglobin level was observed in sprayers compared to controls. Significantly increased MCH and MCHC were found in both mechanics and sprayers as compared to controls. Additionally, the total WBC counts and absolute lymphocyte count was significantly reduced both in mechanics and sprayers compared to controls. Moreover, platelet counts were significantly reduced in both mechanics and sprayers compared to controls.

### 2.9.4. Effect of petroleum and exhausts fumes on liver and kidney:

The liver and Kidney maintains a unique position within the circulatory system. The liver is extremely active in oxidizing triglycerides to produce energy. The primary function of the liver is the production and excretion of bile. It excretes bilirubin, cholesterol, hormones, and drugs. It plays a major role in the metabolism of fats, proteins, and carbohydrates. The liver is the major site for converting excess carbohydrates and proteins into fatty acids and triglyceride, which are then exported and stored in adipose tissue. A kidney is a paired bean-shaped organ whose functions includes removing metabolic waste products from the blood, maintains the body fluids, formation of urine, and plays important functions of the body.

Most dangerous aromatic hydrocarbons found in unleaded petrol are benzene, toluene, ethylbenzene, and xylenes (BTEX). Hepatotoxicity and nephrotoxicity have been reported following human and animal exposure to unleaded petrol (Benson JM *et al.* 2011). Kidney adenoma, elevated serum activity of liver enzymes, urea, creatinine, and potassium, and

decreased chlorine and sodium has been reported in laboratory animals (Uboh FE *et al.* 2009). Similarly, proteinuria, increased serum activity of liver enzymes (aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase), total bilirubin and fatty liver changes have been reported in petrol pump workers following exposure to unleaded petrol (Masoud Neghab *et al.* 2015).

Assessment of hematological, hepatic and renal functions of petroleum tanker drivers in Lagos, Nigeria had done by Olufunsho Awodele *et al.* (2014). They noticed that there was a significant increase in the levels of serum alanine aminotransferase (ALT), albumin (ALB) and alkaline phosphatase (ALP) but insignificant decrease in aspartate aminotransferase (AST) in petroleum tanker drivers as compared to control subjects. There was a significant increase in the levels of blood urea and serum creatinine and lipid peroxidation level (MDA) in the petroleum tanker drivers as compared to controls. The increased lipid peroxidation level may be responsible for the renal and hepatic damage observed in the petroleum tanker drivers.

A cross-sectional study was conducted by Masoud Neghab *et al.* (2015); in which the hepatotoxic and nephrotoxic potentials of occupational exposure to unleaded petrol were assessed in a group of workers employed in Shiraz private petrol stations. They observed that direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea and plasma creatinine was significantly increased in petrol station workers than the controls. Conversely, serum albumin, serum total protein, and serum concentrations of calcium and sodium were significantly decreased in petrol station workers than the controls.

Gali RM *et al.* (2012); studied the liver enzymes and protein among 35 petrol pump attendants, 25 petrol hawkers and 40 healthy control subjects in a Nigerian population. They found a significant increase in the levels of plasma liver enzyme such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in petrol hawkers when compared with controls. Only alkaline phosphatase level was raised in petrol-pump attendants when compared to controls.

A study was conducted by Amah UK *et al.* (2014); on the nephrotoxic effect of lead exposure among automobile repairers. They found a significant increase in blood lead level, the serum level of creatinine, urea and uric acid in automobile repairers than in the controls.

Whereas, there was no significant change observed in the serum level of electrolytes ($Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$) among study and control groups. There was a significant positive correlation observed between duration of exposure and blood lead level. However, no significant correlations observed between the duration of exposure and serum concentration of creatinine, urea, uric acid, and electrolytes ($Na^+$, $K^+$, $Cl^-$ and $HCO3^-$) in the automobile repairers. They suggest that high blood lead level among automobile repairers may be responsible for raised levels of renal markers which may eventually lead to their renal damage.

Kapil Soni *et al.* (2016); studied the impact of petroleum fumes on the liver and kidney functioning of petrol filling attendants and garage attendants. A total of 125 adult males (18-40 years) were divided into five groups such as control group, petrol filling station attendants with occupational exposure up to 8 years, petrol filling station attendants with occupational exposure more than 8 years, garage attendants with occupational exposure up to 8 years and garage attendants with occupational exposure more than 8 years. There was a significant increase in SGOT and SGPT level in petroleum fumes exposed groups indicating a predisposition for hepatotoxicity. Susceptibility of nephrotoxicity was observed, as there was a sharp elevation in serum creatinine level in petroleum fumes exposed groups than the control group.

Adejumo B. I. *et al.* (2016); assessed liver functions of selected automobile professionals in the South region of Nigeria. There was no significant difference in total and conjugated bilirubin, serum albumin, total protein, ALT, AST and ALP level in auto workers. In contrast, auto mechanics showed a significant increase in γ–glutamyltransferase (GGT) level as compared to control. Similarly, a significant decrease in serum albumin concentration was observed in spray painters compared to control group.

In the study of E.Chukwu Onyeneke *et al.* (2016); included 32 mechanics, 30-panel beaters, 24 petrol attendants, battery chargers and motorcycle riders grouped as ‘others’ and 30 not occupationally exposed control subjects. They studied the effect of occupational exposure to lead on liver function parameters. They noticed that the activity of ALT, AST, and ALP in mechanics, panel beaters and others were significantly increased as compared to controls. There were no significant changes in the serum levels of total protein, albumin, conjugated and total bilirubin in exposed subjects compared to controls.

Another study done by Mashael Bin-Mefrij *et al.* (2017); shows the effect of fuel inhalation on the kidney and liver function and blood indices in gasoline station workers. They found that blood urea and serum creatinine level was significantly higher among workers who were exposed to gasoline and fuel fumes for more than 5 years. There were no significant differences found in the serum ALP, ALT, AST and GGT levels between participants working less than 5 years and those who work for more than 5 years.

A study carried out by Ayobola A Iyanda *et al.* (2017); on biomarkers of hepato-renal damage in fuel filling station attendants using protective gears. They found that serum creatinine, blood urea, ALP, AST, ALT levels were significantly higher in fuel filling attendants, whereas total protein, albumin, and uric acid were significantly lower in fuel filling attendants compared with control. On the other hand, the activity of ALP, AST, ALT, creatinine, urea, uric acid, and globulin were significantly higher in fuel filling station attendants who did not use protective gears than controls but total protein and albumin were lower, while γ-glutamyl transferase activity was not significantly different in fuel filling station attendants who did not use protective gears and in the control group.

A study was done by Holkar Shrirang *et al.* (2017); to find out the effects of long term exposure assessment of benzene in petrol pump workers. They noticed that all liver enzymes such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were significantly increased in petrol pump workers compared with control subjects.

Another study was conducted by Adejumo B.I. *et al.* (2016); to assess the renal biomarkers in commercial automobile workers in Benin City, Edo State, Nigeria. They found that the levels of serum uric acid, blood urea, nitrogen, creatinine, and electrolytes such as chloride and bicarbonate were elevated and higher in the automobile workers compared with the controls. Apart from blood urea, the values of all the other biomarkers of renal function fell within the normal reference range. However, there were no significant differences in all the renal biomarkers when compared among all the exposed groups.

## 2.10: Effect of petroleum and exhaust fumes on cytogenetic changes (MN & other NA):

Buccal cells symbolize a preferably first target site for early genotoxic events that are induced by carcinogenic agents such as benzene entering in the body. Cytogenetic damage is probably the most important and fundamental cause of the development of anomalies and degenerative changes in human and animals.

### 2.10.1: Mechanism of Formation of Micronuclei (MN):-

Micronuclei (MN) are also known as Howell–Jolly bodies first identified at the end of 19$^{th}$century in red cell precursors by William Howell, an American and Justin Jolly, a Frenchman (Sears D.A *et al.* 2011). At that time, Howell–Jolly bodies were described as remnants of nuclei of red blood cells circulating in organs with pathological features (Sears D.A *et al.* 2011).

Micronuclei (MN) are small chromatin bodies that appear as small nuclei in the cytoplasm by the condensation of acentric chromosome fragments, acentric chromatid fragments or whole chromosomes that lagging behind in the daughter nuclei at the completion of telophase during mitosis, because they did not attach properly with the spindle during the segregation process in anaphase during cell division at telophase (Holland Nina *et al*. 2008; M. Fenech *et al.* 2011). These cells later mature and are exfoliated. According to Holland, the Baseline frequencies for micronucleated cells in the buccal epithelium are usually within the baseline range of the 0.05-11.5 MN per 1,000 cells. Cells with multiple micronuclei are rare in healthy subjects but become more common in individuals exposed to radiation or other genotoxic agents (Harshvardhan S. *et al.* 2010).

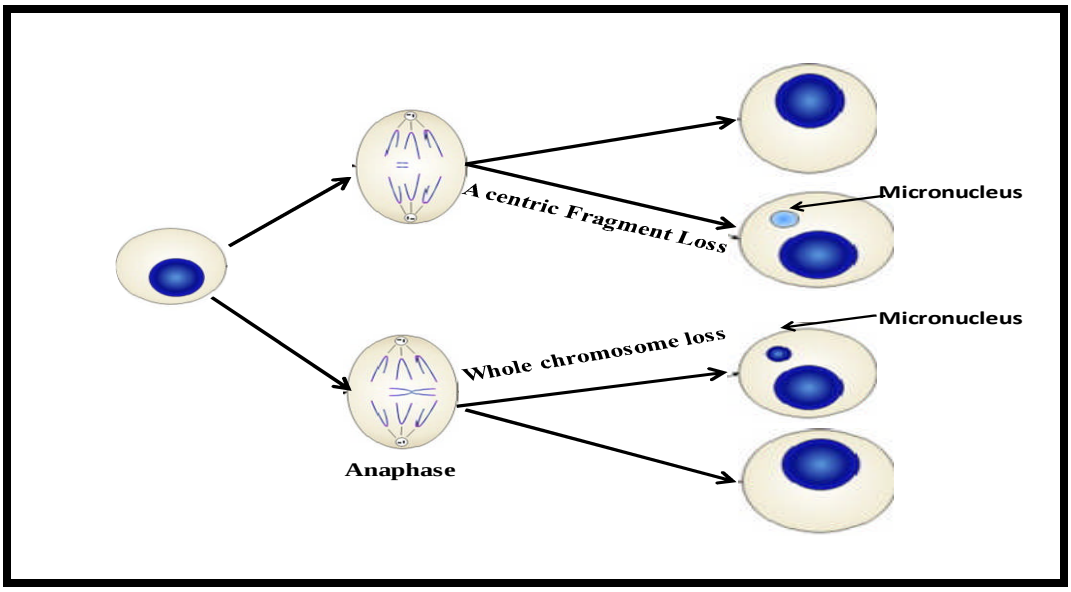



**Figure 2.1:** Mechanism of Micronuclei formations in oral buccal epithelial cells.

**Source:** Holland Nina, Claudia Bolognesi, Micheline Kirsch-Volders, Stefano Bonassi, et al. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on the current status and knowledge gaps. Mutation Research. 2008; 659: 93–108.

Thus, MN is the only biomarker that allows the simultaneous evaluation of both clastogenic (chromosome breakage) and aneugenic (whole chromosome) effects in a wide range of cells, which are easily detected in interphase. MN is a reliable biomarker, which shows the ability of agents to cause chromosome loss, it is not repairable. It could be used as a sensitive cytogenetic biomarker and rapid indicator of genetic damages in the exfoliated buccal epithelial cells as results of occupational exposure (Sima Ataollahi Eshkoor *et al.* 2011).

## 2.10.2: Other nuclear abnormalities:

Exfoliated cells of buccal mucosa are good indicators of chromosomal damage and other nuclear abnormalities such as binucleates, karyorrhexis, and karyolysis.

**Binucleated cells (BNC)** are most commonly present in cancerous cells. BNC or the presences of two nuclei within a cell are probably an indication of the failure of cytokinesis following the last nuclear division in the basal cell layer. Recently it has been observed that chromosomal

non-disjunction occurs with a higher frequency in binucleated cells that fail to complete cytokinesis, rather than in cells that have completed cytokinesis (Sabharwal R *et al.* 2015)

**Karyorrhexis:** Karyorrhexis or nuclear disintegration involves the loss of integrity of the nucleus. It is characterized by the extensive aggregation of nuclear chromatin. It exhibits a densely speckled nuclear pattern which indicates nuclear fragmentation and can eventually lead to the disintegration of the nucleus (Sabharwal R *et al.* 2015).

**Karyolysis:** Karyolysis or nuclear dissolution represents an advanced stage of apoptosis and necrosis. It is angular and flat in shape and is Feulgen and aceto-orcein negative. It has a cytoplasmic area that of the size of terminally differentiated cell and only ghost-like image of the nucleus remains (Sabharwal R *et al.* 2015).

MN is induced by genotoxic stress such as clastogens or aneugens. The agents that cause chromosomal breaks are called clastogens (radiation) and which act on the spindle are called aneuploidogens or aneugens (vincristine) (Sabharwal R *et al.* 2015).

Karryorhetic (KR) and karyolitic (KL) cells showed the cytotoxic state of the cell and may appear as a result of an apoptotic or necrotic form of cell death due to increasing age and exposure to mutagenic or carcinogenic agents. The MN count is increased in potentially malignant disorders like oral submucous fibrosis, leukoplakia, erythroplakia, lichen planus, and squamous cell carcinoma.

Studies have shown that the diesel exhaust particles and benzene in petroleum fumes can induce micronuclei formation. The evidence from population-based studies is very limited and not yet conclusive. Recently, Benzene and ethylbenzene are classified as group 1 and 2b carcinogens according to IARC (IARC, 2000). IARC also classified DEE as a Group 1 carcinogen mainly. The IARC has also classified diesel exhaust as a Group 1, known human carcinogen, based on its association with lung cancer in epidemiological studies (IARC, 2014).

Rafiq Khan M *et al.* (2012); studied the genotoxicity in automobile mechanics occupationally exposed to polycyclic aromatic hydrocarbons using micronuclei and other nuclear abnormalities. They showed that significantly higher frequencies of MN and other NA in occupationally exposed automobile mechanics than those of the control group. In addition, a higher degree of NA was observed among the exposed subjects with smoking, drinking, tobacco chewing habit, which is indicative of cytogenetic damage in these individuals.

Genotoxicity in exfoliated buccal epithelial cells of foundry workers occupationally exposed to polycyclic aromatic hydrocarbons ($PAH_S$) was assessed by Saranya Ramalingam Singaravelum *et al.* (2013); observed a significant increase in the frequency of MN in foundry workers compared to controls. This means genetic damage in the buccal cells of foundry workers was significantly higher than that in controls.

Xiao Zhang *et al.* (2015); shows that exposure to diesel engine exhaust (DEE) induces a significant increase in the frequencies of micronucleus (MN), Nucleoplasmic Bridge (NPB), and Nuclear Bud (NBUD) frequencies in PBLs and further suggests that DEE exposure level was positively associated with MN frequencies. Previous studies have shown that increased MN, NPB, and NBUD frequencies were associated with increased cancer risk; accordingly, Xiao Zhang *et al*. (2015); suggest that the DEE exposed workers are at higher cancer risk than non-DEE exposed workers.

In the study of Umegbolu EI *et al.* (2016); detected micronuclei formation in petrol station attendants in Awka, Nigeria. Their study revealed a significant difference in MN frequency between the exposed and control groups; also MN occurrence was higher in those exposed individuals with a duration of exposure more than 2 years than in those exposed for less than 2 years. It was also observed that the frequency of micronuclei formation was highest in the age range of 40-49 years in both the exposed and control groups.

Another study conducted by Sunanda Paul *et al*. (2016); studied nuclear abnormalities in exfoliated buccal epithelial cells of petrol pump attendants of Southern Assam. They observed the higher frequency of micronuclei (MN) among petrol pump workers than the control group. Significant increase in nuclear buds and binucleate cells were observed in petrol pump workers than the control group.

Shilpi *et al.* (2016); studied cytogenetic biomonitoring among petrol filling station workers; a hematological and micronucleus study. They noticed that the MN frequency in Giemsa stain was significantly higher among the cases compared with controls. Similarly, MN frequency in PAP was also significantly higher among the cases compared with controls. There was no significant difference in the frequency of MN in Giemsa and PAP on the basis of duration of exposure and working hours/day of the cases.

Faiza Butt *et al.* (2017); studied the cytogenetic bio-monitoring in fuel station attendants of Gujarat, They observed the higher frequency of buccal cells with nuclear anomalies such as micronuclei (MN), binucleated (BN), Karyorrhetic (KR), and karyolitic (KL) cells was observed in the exposed group as compared to control samples and this might be due to the exposure to benzo-a-pyrene and other carcinogenic agents present in gasoline and vehicular exhaust.

Sridhar Reddy Erugula *et al.* (2017); studied micronuclei frequency in petrol pump workers. In his study, it was found that an average number of micronuclei (MN) was directly proportional to the years of exposure, in subjects with 14 years of exposure and least in less exposed subjects (3-5 years). The average number of micronuclei (MN) in subjects who worked for more than 12 hours a day was high. A higher frequency of micronuclei was observed in petrol pump workers when exposed for a longer duration and much higher MN were seen in workers with tobacco use.

Another study carried out on genotoxic effects of exposure to gasoline fumes on petrol pump workers by Shaikh A. *et al.* (2018); noticed that the frequencies of micronucleated cells, nuclear bud, condensed chromatin cells, karyorrhectic cells, pyknotic cells, and karyolytic cells were significantly higher in the exposed workers compared to the comparison group.

Micronuclei - as a biomarker of genotoxicity in automobile mechanics of western Maharashtra studied by Smita *et al*. (2018); found a significant increase in frequencies of MN, BN, KR, and KL cells in automobile mechanics compared to controls. MN and other NAs reflect genetic changes and events associated with carcinogenesis so it is considered as biomarkers of genotoxicity. Therefore, the results of this study indicate that AMs exposed to PAHs are under the risk of significant cytogenetic damage.

Azubuike NC *et al.* (2019); evaluated micronuclei from exfoliated buccal epithelial cells in automobile spray painters. The results obtained showed a statistically significant increase in MN frequency in buccal epithelial cells of automobile spray painters when compared with the control group. Elevated MN frequency was also observed with increased age, smoking and alcohol consumption habits. MN frequency was significantly affected by the duration of working experience (years) of the spray painters whereas no difference was observed with a number of working hours/day.

# AIM AND OBJECTIVES

## 3.1: Aim.

To assess the occupational health status in petrol pump workers & automobile mechanics, who are continuously exposed to petroleum and exhaust fumes during their working hours & compare it with the control group.

## 3.2: Objectives.

1. To evaluate anthropometric and sociodemographic profile in automobile mechanics & petrol pump workers (study groups) and compared it with the control group of Western Maharashtra.
2. To study the percentage frequency of self-reported symptoms amongst study groups.
3. To evaluate heavy metal lead (Pb) level in the study and control group, compare it with the duration of exposure in study groups.
4. To study the impact of heavy metal (lead) in petroleum and exhaust fumes on oxidative stress by evaluating biomarker of oxidative stress malondialdehyde (MDA) and γ-glutamyl transferase (GGT) among study and control group, compare it with a duration of exposure in study groups.
5. To study the impact of petroleum and exhaust fumes on the cardiovascular and respiratory system in the study and control group, compare it with a duration of exposure in study groups.
6. To study the effect of petroleum and exhaust fumes on hematological parameters in the study and control group and compare it with the duration of exposure in study groups.
7. To study the impact of petroleum and exhaust fumes on biochemical parameters (LFT, RFT, and Electrolytes) in the study and control group, compare it with a duration of exposure in study groups.
8. To find out the correlation between possible heavy metals (blood lead level) with blood pressure, PEFR, Hb%, RBCs count and MDA.

9. To study cytogenetic alterations (Nuclear abnormalities) in exfoliated buccal cells in the study and control group, compare it with a duration of exposure in study groups.

## 3. 3: Hypothesis

Occupational exposure to petroleum and exhaust fumes and heavy metals like lead can cause significant changes in cardiorespiratory, hematological, biochemical and cytogenetic parameters. Occupational exposure may increase oxidative stress, biomarkers like malondialdehyde **(**MDA) and gamma glutamyl transferase (GGT) in petrol pump workers & in automobile mechanics with an increase in the duration of exposure of work.

# Materials & Methods

## 4.1: Study Design:

**Study Design** - A human cross sectional study.

**Study population –** Workers occupationally exposed to petroleum products were taken as study group (Automobile Mechanics, petrol pump workers) and healthy occupationally unexposed subjects with same socioeconomic status were taken as a control group.

**Study Place -** Western Maharashtra (Sangli, Satara and Kolhapur District)

**Study Duration -** 3 yrs. (From July 2014 to July 2017).

## 4.2. Classification of Participants and sample size:

Distribution of participants who participated in this study was as follow,

**Table 4.1: Classification of study groups.**

| Groups | Subjects | Number of participants |
|---|---|---|
| I | Control | 70 |
| **Study Groups** | | |
| II | Automobile mechanics (AM) | 70 |
| III | Petrol pump workers (PPW) | 70 |
| Total | | 210 |

Depending on the duration of exposure study groups were further divided into three groups,

**Table 4.2: Classification of study groups (workers) according to the duration of exposure.**

| **Study Groups** | | |
|---|---|---|
| Duration of Exposure | Group-II<br>Automobile Mechanics (AM) | Group-III<br>Petrol Pump Workers (PPW) |
| 1-5 years of exposure. | Group-IIA | Group-IIIA |
| 5-10 years of exposure. | Group-IIB | Group-IIIB |
| >10 years of exposure. | Group-IIC | Group-IIIC |

### 4.2.1: Sample size calculation:

The estimated minimum sample size required for the present study according to the equation was 70 per group. The total sample size for this study was 210 subjects. (Petrol pump workers-70, Automobile mechanics - 70 & Control -70)

The following formula was used to calculate the sample size (Aprajita *et al.* 2011)

$$n = \frac{4 * \sigma^2 \left(Z_\alpha + z_{1-\beta}\right)^2}{\delta^2}$$

α = 1%, $Z_\alpha$ = 2.576, 1-β (Power) = 95%, $z_{1-\beta}$ = 1.645, σ = ± SD = 1.32

δ= Mean difference = 5.17 – 3.78 = 1.39 $\therefore n$ = 64.11 ≅ 70

### 4.3. Inclusion and exclusion criteria:

**Inclusion criteria:**

- The employees working as the automobile mechanics, petrol pump workers in western Maharashtra (study groups).
- Healthy participants with the same age and socioeconomic status and who were occupationally not exposed to petroleum products were considered as a control group.
- Only male participants between 'age group of 20 – 40' years.
- Non- smokers, Non-Alcoholics, Nontobacco/Gutakha/ Mawa chewers were included in the study.

**Exclusion criteria:**

- Participants with a duration of exposure less than 1 year were excluded.
- Participants who were doing yoga or any other kind of regular physical exercise.
- Participants with a known history of major illness were excluded.

## 4.4. Ethics

**Informed consent:**

A 'Written informed consent' was obtained from all the study participants before their participation in the study. (Appendix I)

**Institutional approval:**

An Institutional Ethical Clearance (IEC ref. No. 104/2014-2015, dated 5/12/ 2014) was taken to carry out this study from Institutional Ethical Committee of BLDE (Deemed to be University), Sri B.M. Patil Medical College, Hospital and Research Center Vijaypur.

**Declaration of Helsinki:**

We followed the 'declaration of Helsinki' during the entire study.

## 4.5. Study Protocol:

For this work, prior permission was taken from the president of petrol pump association Sangli district & owners of automobile workshops. With prior appointment, a meeting was arranged with owners of each petrol pump & automobile mechanics to explain to them the need for the study & get permission from them for study subjects.

With prior permission & appointment, automobile mechanics & petrol pump workers interacted with the help of questionnaire & interview. Prior to biological specimen collection, socio-demographic, occupational and clinical data were collected from the participants. The socioeconomic status of all the study participants and controls was average. None of the participants had a 'past history of major illness'. Dietary intake and food habits of all the participants were normal.

The present study was conducted in the Department of Biochemistry, Bharati Vidyapeeth Medical & Dental College, Sangli and B.L.D.E.A's Shi B.M. Patil Medical College and Hospital Vijaypur.

## 4.6. Anthropometric parameters:

All the recordings were done in the morning between 9:00 am to 11:00 am after the rest of 10 minutes.

a. **Height (cm):** Height was measured using a device (BIOCONTM) mounted on the wall with the subject in standing posture without footwear nearest to 0.1cms.
b. **Weight (Kg):** weight was measured using a standard weighing machine, with a minimum of clothing nearest to 0.1 kgm.

c. **Body Mass Index (BMI)**: 'Body Mass Index' was estimated from weight and height by dividing 'weight in Kilograms (Kg)' by 'height in meters square ($m^2$)' and the formula was expressed as $Kg/m^2$ (Ab Latif Wani et al. 2015).

d. **Body Surface Area (BSA):** 'Body Surface Area' was calculated by using formula (Mosteller RD 1987)

BSA = 'SQR RT ([Height (cm) x Weight (Kg)] / 3600' and was expressed in $m^2$

e. **Total Body water (TBW)** = was calculated by using formula (Watson PE *et al.* 1980)

TBW = '2.447-0.09156 X Age + 0.1074 X height + 0.3362 X Weight'.

## 4.7: Sociodemographic Factors:

We have recorded the following sociodemographic factors like age, educational status, and income. We have also recorded diet and working pattern in the study participants.

## 4.8: Physiological Parameters:

'Before the recording of physiological parameters, individual participants were thoroughly explained regarding the procedure in their own language along with a demonstration of the procedure'.

**Blood Pressure and Heart Rate:**

a) **'Systolic and Diastolic Blood Pressure' (mmHg) –** Blood pressure was measured by using 'Diamond mercury sphygmomanometer' in sitting posture & was expressed in mmHg.

b) **'Pulse Pressure (PP)' (mmHg):** It is the pressure difference between systolic and diastolic blood pressure and was expressed in mmHg.

c) **'Mean Arterial Pressure (MAP)' (mmHg):** It is the pressure existing in the arteries. It was calculated by formula = DBP + 1/3 $^{rd}$ of PP and was expressed in mmHg.

d) **'Heart Rate' (bpm):** It was measured clinically from the radial artery and was expressed as beats per minute (bpm).

## 4.9: Measurement of pulmonary function tests

1. **'Peak Expiratory Flow Rate (PEFR)':-**

"It is the maximum velocity in liters/minute with which air is forced out of the lungs." PEFR was measured with the help of Mini Wright's peak flow meter. It is a very light and convenient instrument, as it is portable and independent of electrical supply. The subjects were asked to take deep inspiration and blow out fast and forcefully into the peak flow meter by closing the nose tightly.

'PEFR' measurement is very popular in primary health care centers and is commonly applied as a 'quick screening method' for assessing lung function in the clinic or at the bedside (Brand P L. *et al.* 2003). It is a 'valuable tool in lung functions studies' for diagnosis, treatment and in epidemiological and occupational studies for identifying the presence of airflow limitation, assessing its severity and variation (Jain P *et al.* 1998).

**Significance: '**PEFR' measurements help to assess the degree of large airway obstruction. This is highly effort dependent and hence many clinicians now use PEFR in addition to FVC and FEV1. PEFR depends upon age, sex, build, etc. (Begum sadiqua 2011).

2. **40 mmHg endurance time:-**

40 mmHg endurance time was measured with the help of Diamond mercury sphygmomanometer. The subjects were asked to take deep inspiration and then close the nose. Then subjects were asked to blow in the rubber tube which was attached to mercury manometer to increase the mercury level up to '40 mmHg' without blowing the cheeks. The subjects were asked to maintain this mercury level up to 40 mmHg as long as possible. The time interval between the beginning of blow and breaking point was noted as 40 mmHg endurance time.

## 4.10: Sample Collection:

For hematological and biochemical parameters (LFT, KFT & Electrolytes) 10 ml blood sample was collected by using aseptic precaution from venipuncture of each subject & immediately transferred into sterile potassium EDTA anticoagulant bulb and plain bulb. The blood sample obtained was analyzed on a daily basis at B.V.D.U.D.C.H Sangli. The other biochemical parameters (blood lead & MDA) were analyzed within one week after sample collection.

**Table 4.3: List of the standard methods used for hematological & biochemical parameters.**

| Sr. No. | Name of the Hematological & Biochemical Parameters | Name of the Method |
|---|---|---|
| **Hematological parameters** | | |
| Hemoglobin (Hb), RBC count, Total leucocytes count, Platelet count, Haematocrit (PCV), Blood indices like 'Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC)'. | | By using 'fully automated Hematology computerized cell counter' (NIHON CODON – Tulip, Japan). |
| **Biochemical Parameters** | | By using autoanalyzer (Coralizer 200 Tulip group fully automated clinical chemistry analyzer). For GGT used semiautoanalyzer (Biosystem BTS-350) |
| **Special Parameters** | | |
| 1 | Blood Lead (Pb) | Parson P.J. Salvin W. (1993) |
| 2 | Melanodiaaldehyde (MDA) | Kei Satoh method |
| 3 | Gamma Glutamyl Transferase (GGT) | Szasz method , Kinetic |
| **Liver Function Tests (LFT)** | | |
| 4 | 'Serum Glutamate Oxalate Transaminase (SGOT)' or 'Aspartate Transaminase (AST)' | IFCC, Kinetic Method |
| 5 | 'Serum Glutamate Pyruvate Transaminase (SGPT)' or 'Alanine Transaminase (ALT)' | IFCC, Kinetic Method |
| 6 | Alkaline Phosphatase | Modified IFCC, Kinetic Method |
| 7 | Serum Total Protein | Biuret Method, Endpoint |
| 8 | Serum Albumin | BCG Dye Method, Endpoint |
| 9 | Serum Bilirubin (Total, Direct & Indirect) | DIAZO Method |
| **Renal Function Tests (RFT)** | | |
| 10 | Blood urea | Urease – GLDH, Fixed Time |
| 11 | Serum Creatinine | Jaffe's Method, Initial rate |
| **Electrolytes** | | |
| 12 | Na+ (mEq/L) and K+ (mEq/L) | Flame- photometry |

All the above mentioned hematological & biochemical parameters were measured by using standard kit methods.

## 4.11: Estimation of hematological parameters:

All hematological parameters were analyzed by using 'fully automated hematology computerized cell counter' (NIHON CODON – Tulip, Japan).

**Analysed parameters:**

1. **Red Blood Corpuscles (RBC) count:**
   Data of RBC Count represents the total number of red blood cells in 1µl of whole blood.
2. **White Blood cell (WBC) count:**
   Data of WBC count represents the total number of white blood cells in 1µl of whole blood.
3. **Platelet Count (PLT):**
   Data of platelet count represents the total number of platelets in 1µl of whole blood.
4. **Hemoglobin (Hb):**
   Hemoglobin value represents hemoglobin concentration in the 1dl of blood.
5. **Hematocrit or Packed Cell Volume (HCT or PCV):**
   It represents the true relative percentage volume of red blood cells.
6. **Mean Corpuscular Volume (MCV):**
   It represents the average volume of red cells. It is expressed in $\mu^3$.
7. **Mean corpuscular Haemoglobin (MCH):**
   It represents the average weight of hemoglobin in a single red blood cell. It is expressed in µ or pg.
8. **Mean Corpuscular Haemoglobin Concentration(MCHC):**
   It represents the amount of hemoglobin per 100 ml of red cells. It is expressed in percentage (%).

**Table 4.4: Normal values of hematological parameters**

| Sr. No. | Analyzed Parameters | Normal Values |
|---|---|---|
| 1. | R.B.Cs Count | 4.5 - 6.5 million/ cumm. |
| 2. | WBC Count ( /Cu mm) | 4000 -11000 /cumm. |
| 3. | Platelet count ( Lakh / Cu mm) | 1.5 – 4 lac/cumm. |
| 4. | Hb % | 14-18 gm/dl |
| 5. | PCV (%) | 40 – 54 % |
| 6. | M.C.V. (fL) | 76 – 96 fL |
| 7. | M.C.H. (pg) | 27 – 32 pg |
| 8. | M.C.H.C % | 30– 36 % |
| 9. | Poly morphs (%) | 40 – 75 % |
| 10. | Lymphocyte. (%) | 20- 40 % |
| 11. | Eosinophil. (%) | 1 – 6 % |
| 12. | Basophil (%) | 0 – 1 % |
| 13. | Monocyte (%) | 2 – 10 % |

## 4.12: ESTIMATION OF SPECIAL BIOCHEMICAL PARAMETERS:

### 4.12.1:- Estimation of blood Lead level (BLL).

**Method** - Parson P.J. Salvin W. 1993

**Principle for blood lead estimation:** Whole blood or urine sample was mixed with the diluents and matrix modifier i.e. ammonium dihydrogen phosphate and Triton X 100. The proteins from the samples were precipitated by 2.0 M Perchloric acid and the clear supernatant was obtained after 10 minutes by ultracentrifugation at 10,000 RPM. The lead concentration from the supernatant was measured by using Atomic Absorption Spectrophotometer (AAS) analytical gena (novAA 350). The final step of analysis causes vaporization of lead, which absorbs energy at the 283.2nm line emitted from a hollow cathode lamp. The absorbance of energy at this wavelength is specific for heavy metal lead and proportional to its concentration. Exposure of the specimen to the high chloride concentration is to be avoided because the chloride salt of lead is volatile at the charring temperatures used in atomic absorption analysis.

**Reagents Preparation for Lead Estimation:**

1. Nitric acid (0.2 %): 0.2 ml ultrapure concentrated nitric acid was diluted to 100 ml with double distilled water.

2. Diluents: The diluents and matrix modifier was 2.0 g/l ammonium dihydrogen phosphate and 10.0 ml/L Triton X-100 in 0.2 % nitric acid.

3. Perchloric acid (2.0 M): 40.18 ml of 70% Perchloric acid was diluted to 200 ml with deionized water.

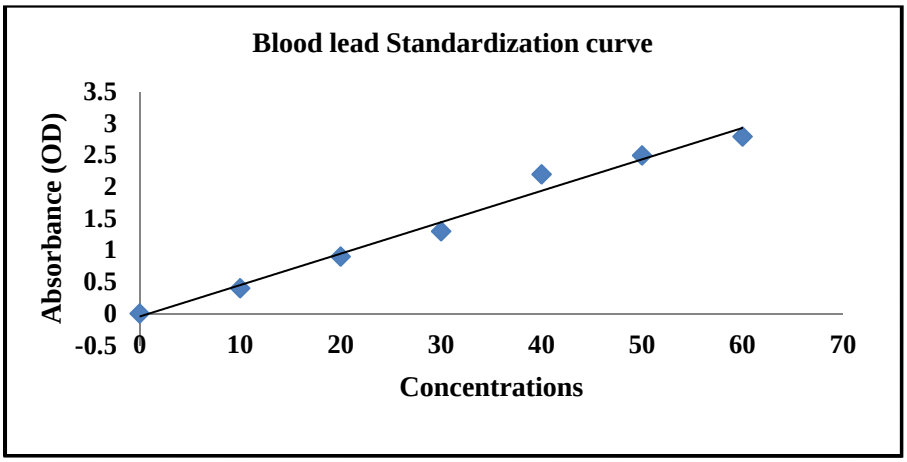
4. Calibrators: The calibrators are prepared from stock reference solution containing lead at 1000 µg/ml (Baker Intra analyzed atomic absorption standard). The stalk reference solution was diluted with 0.2 % nitric acid to 10µg/ml and then diluted further with diluents to achieve final concentrations of 10, 20, 40 and 60µg/dl which were used to calibrate the instrument.

**Standardization of Method**:

Following calibrators were prepared from the stalk reference solution of lead

| Sr. No. | Std No. | Concentrations of stalk reference solution of lead | OD |
|---|---|---|---|
| 1 | S1 | 10 | 0.4 |
| 2 | S2 | 20 | 0.9 |
| 3 | S3 | 30 | 1.3 |
| 4 | S4 | 40 | 2.2 |
| 5 | S5 | 50 | 2.5 |
| 6 | S6 | 60 | 2.8 |

**Figure: 4.1: Blood lead standardization curve**




**Procedure:**

1. 1 ml heparinized whole blood or 1ml urine samples were taken in clean polypropylene test tubes.
2. 4.0 ml of diluent and 1.5 ml 2.0 M perchloric acid was added and the solution was mixed well and kept it for 10 minutes for protein precipitation.
3. The cell debris and protein precipitate were separated by using ultracentrifuge (Hitachi Japan SCP 85 H) machine at 10,000 RPM for 10 minutes.
4. Then the clear supernatants were collected in another clean polypropylene test tubes and

the lead concentration was measured by using AAS.

5. Reagent blank consist of 1 ml triple distilled water, 4ml diluent and 1.5 ml perchloric acid.

   Reagent blank solutions were prepared in bulk in the same proportion.

**Normal value of lead**:

In Adults : >10µg/dl

In children - > 5µg/dl

## 4.12.2: Estimation of Lipid peroxidation (MDA).

Method- Kei Satoh 1978.

**Principle:** Auto-oxidation of unsaturated fatty acids involves the formation of semi-stable peroxides, which then undergo a series of reactions to form MDA. Malondialdehyde reacts with Thiobarbituric acid (TBA) to form pink-colored chromogen. The resulting chromogen was extracted with 4.0 ml of n-butyl alcohol and the absorbance of which was measured at 530 nm.

**Reagent Preparation:**

1. Trichloroacetic acid (TCA) reagent: 20g of TCA was dissolved in100 ml of distilled water to prepare 20% TCA.
2. Sodium sulfate solution (2M): 28.4 gm of anhydrous sodium sulfate was mixed in 90 ml of distilled water by heating and stirring. Then distilled water was added to make the final volume of 100 ml.
3. Thiobarbituric acid (TBA) reagent: 670 mg of TBA in 100ml of 2M sodium sulphate solution.
4. Sulphuric acid (0.05M) - Dilute 4.904 ml of concentrated sulphuric acid with distilled water to make the volume of one liter.
5. N-butyl alcohol was available in the market.
6. Standard MDA solution: 164.2 mg malondialdehyde (1,1,3,3 tetra methoxy propane, Mw = 164.2) was dissolved in 1 liter of distilled water that 1 mol solution. Using this serial dilution was made to solutions of concentration1nmol to 10 nmol.

**Procedure:**

1. In 0.5 ml of serum, 2.5 ml of TCA was taken in a test tube and was mixed and allowed to stand for 10 min at room temperature.
2. Centrifugation at 3500 rpm for 10 min was done.
3. The supernatant was discarded after centrifugation and the precipitate obtained was washed 2 times with 0.05 M Sulphuric acid.
4. 2.5 ml of 0.05 M Sulphuric acid and 3 ml of TBA reagent were added to the precipitate & the test tube containing the mixture was kept in a boiling water bath for 30 min.
5. Then the tube was cooled in cold water followed by addition of 4 ml of n butyl alcohol with vigorous shaking to extract the chromogen.

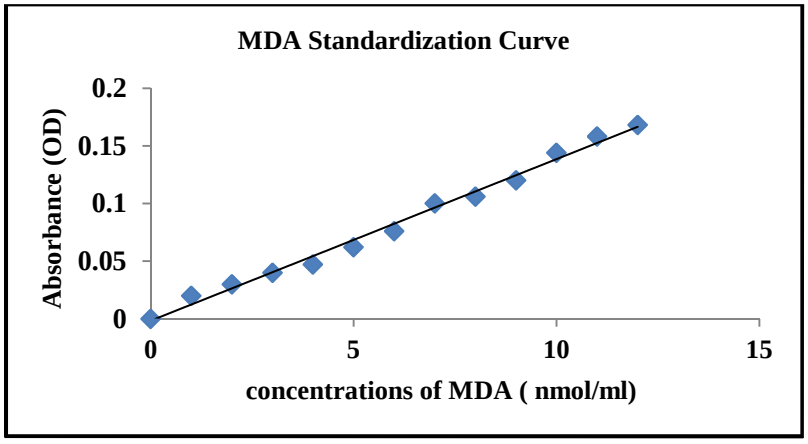
6. The colored organic phase was separated by centrifugation at 3000 rpm for 10 minutes and the absorbance (OD) was read at the 530 nm wavelength using a colorimeter. By using the above procedure absorbance of standard solutions with different concentrations were measured and the graph was plotted with concentrations in nmoles of MDA per ml against absorbance. From the graph, the values of lipid peroxidation (MDA) in the serum were determined.

**Standardization of Method**:

Following calibrators were prepared from the working standard solution.

| Sr. No. | Std No. | Standard MDA solution (10 nmol /ml ) | Sulphuric acid (0.05M) | Total Volume (ml) |
|---|---|---|---|---|
| 1 | S1 | 0.1 | 2.9 | 3.0 |
| 2 | S2 | 0.2 | 2.8 | 3.0 |
| 3 | S3 | 0.3 | 2.7 | 3.0 |
| 4 | S4 | 0.4 | 2.6 | 3.0 |
| 5 | S5 | 0.5 | 2.5 | 3.0 |
| 6 | S6 | 0.6 | 2.4 | 3.0 |
| 7 | S8 | 0.7 | 2.3 | 3.0 |
| 8 | S9 | 0.8 | 2.2 | 3.0 |
| 9 | Blank | ----- | 3.0 | 3.0 |

**Figure: 4.2: MDA standardization curve.**




The normal value of MDA**:** 4 - 8 nmol/ml.

## 4.12.3: Estimation of γ Glutamyl Transferase (GGT):

Method - Szasz method (Kinetic) (Szasz G 1976; Scand 1976; Tietz 1986).

Used semiautoanalyzer

**Principle:**

GGT catalyzes the transfer of amino group between L–γ Glitamyl-3-carboxy-4nitroanilide and glycylglycine to form L – γ Glutamylglycylglycine and 5-amino-2 nitrobenzoate. The rate of formation of 5-amino-2nitrobenzoate is measured as an increase in absorbance which is proportional to GGT activity in the sample.

Kinetic determination of Gamma GT according to the following reaction.

$$\text{GLUPA-C} + \text{Glycylglycine} \xrightarrow{\text{Gamma GT}} \text{L} - \gamma \text{ Glutamyl-Glycylglycine} + \text{5-Amino- 2-nitrobenzoic acid.}$$

GLUPA-C = L – Gamma-Glutamyl-3-carboxy-p-nitroanilide

**Assay Kit Components for GGT contain:**

$R_1$ - Reagent 1 – Tris buffer pH (8.25)-133 mmol/L, Glycylglycine- 138 mmol/L.

$R_2$ – Reagent 2 - GLUPA-C -23 mmol/L.

**Preparation of working reagent:** Working reagents was prepared by 'mixing 4 volume of reagent 1 ($R_1$) with 1 volume of reagent 2 ($R_2$)'.

**Procedure:**

After mixing serum sample with the working reagents, incubated it for one minute at $37^0$ C. 'the change in absorbance per minute was measured (△OD/min.) during 3 minutes'.

The program was set for the semi-automated instrument as follow,

| **Addition sequence for the test** | **For GGT** |
|---|---|
| Mode of reaction | Kinetic |
| Wavelength | 405nm |
| Temperature | $37^0$C |
| The volume of working reagent | 1000 µl |
| The volume of serum sample | 100 µl |
| Incubation time | 60sec. |
| Unit | U/l |

**Calculation:** Gamma Glutamyl Transferase (GGT) activity (U/L) = ( △OD/min.) X 1158

**Normal value for GGT:**

Normal value of GGT in Female –5-32 U/L.

Normal value of GGT in Male – 10-45 U/L.

## Estimation of Liver Function Tests

**4.12.4: Estimation of Serum Glutamic Oxaloacetate Transaminase (SGOT)** or **Aspartate Transaminase (AST) and 4.12.5: Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT).**

**Method - IFCC** Method, kinetic (Burtis C.A 1994:790 -795)

The SGOT (AST) and SGPT (ALT) were measured by using reagents of Meril Diagnostic kit.

**Principle of SGOT:**

'Serum glutamic oxaloacetate transaminase (SGOT)' catalyzes the transfer of amino group between L-Aspartate and α–Ketoglutarate to form 'Oxaloacetate' and 'glutamate'. Oxaloacetate formed reacts with 'nicotinamide adenine dinucleotide (NADH)' in presence of malate dehydrogenase and lactate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to SGOT (AST) activity in the sample.

$$\text{L- Aspartate} + \alpha\text{–Ketoglutarate} \xrightarrow{\text{AST}} \text{'Oxaloacetate'} + \text{'L-Glutamate'}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{'L-Malate'} + NAD^+$$

**Principle of SGPT:**

Serum Glutamic Pyruvate Transaminase (SGPT) catalyzes the transfer of amino group between L-Alanine and α–Ketoglutarate to form 'pyruvate and glutamate'. Pyruvate formed reacts with 'nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase to form L-lactate & NAD'. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to 'SGPT' (ALT) activity in the sample.

$$\text{L-Alanine} + \alpha - \text{Ketoglutarate} \xrightarrow{\text{ALT}} \text{'Pyruvate'} + \text{'L-Glutamate'}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{'L-Lactate'} + NAD^+$$

**Assay kit components for SGOT and SGPT contain:**

$R_1$ - Reagent 1

$R_2$ – Reagent 2

**Reagent Preparation:** reagent $R_1$ and $R_2$ are ready for use.

**Table 4.5: showing composition in the test for 'SGOT & SGPT'.**

| Composition in test | For SGOT | For SGPT |
|---|---|---|
| Tris buffer | 80 mmol/l pH 7.8 | 80 mmol/l pH 7.5 |
| L- Aspartate | 200 mmol/l | --------- |
| L- Alanine | -------- | 200 mmol/l |
| 2 oxoglutarate | 12 mmol/l | 12 mmol/l |
| NADH | 0.18 mmol/l | 0.18 mmol/l |
| MDH | ≥ 600 U/l | -------- |
| LDH | ------- | ≥ 2000 U/l |

**Procedure:**

The program was set for the fully automated instrument as follow,

| Addition sequence for the test | For SGOT | For SGPT |
|---|---|---|
| Reagent 1 ($R_1$) | 240 µl | 160 µl |
| Reagent 2 ($R_2$) | 60 µl | 40 µl |
| Serum sample | 30 µl | 20 µl |
| Main Filter | 340 | 340 |
| Incubation time | 60 | 120 |
| Unit | U/l | U/l |
| Check time | 180 sec | 140 sec. |

**Calculation:**

Auto Quant automatically calculates the SGOT & SGPT activity of each sample.

SI Conversion factor: 1 U/L X 0.017 = 1µkat/l

**Normal reference values of SGOT & SGPT:**

**SGOT:** 5 - 45 IU/L at $37^0$ C.

**SGPT:** 7 - 55 IU/L at $37^0$ C.

## 4.12.6: Estimation of Serum Alkaline Phosphatase:

Method -Modified IFCC, Kinetic (Burtis CA 1994:830-884)

**Principle:**

The enzyme alkaline phosphatase hydrolyzes the '4-Nitrophenolphosphate' to release 4-nitrophenol under alkaline condition. The 4-nitrophenol obtained is detected spectrophotometrically at 405 nm to give a measurement of alkaline phosphatase activity in the sample.

$$\text{2-amino-2methyl – 1 propanol + p- nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{4-nitrophenol} + \text{2 – amino-2- methyl-1- propanolphosphate.}$$

**Assay Kit Contain:**

$R_1$- Alkaline phosphatase reagent

Composition: '2-amino-2-methyl–1 propanol buffer 0.35 mol/l pH 10.40, magnesium acetate 2mmol/l, zinc sulfate 1mmol/l, HEDTA 2 mmol/l, 4-NPP 16 mmol/l'.

**Reagent Preparation: -** Reagent ($R_1$) is ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| **Addition sequence for the test** | **For Alkaline Phosphatase** |
|---|---|
| Reagent 1 | 200 µl |
| Serum Sample | 4 µl |
| Main Filter | 405 |
| Incubation time | 60 |
| Unit | U/l |
| Check time | 120 sec |

**Calculation:**

Auto Quant automatically calculates the alkaline phosphatase activity of each sample.

SI Conversion factor: 1 U/l X 0.017 = 1µkat/l.

**The normal reference value of Alkaline Phosphatase:**

Alkaline phosphatase**:** 41 – 137 IU/L at $37^0$ C.

## 4.12.7: Estimation of Serum Total Proteins:

Method- Biuret (End Point) **(**Burtis CA.1994:695-700)

**Principle:**

Proteins peptidic bonds react with cupric ion (Cu) in alkaline solution to form a blue-purple colored complex, the absorbance of which is measured at 546 nm. The intensity of the color formed is directly proportional to the amount of protein present in the sample. Each cupric ion can complex up to 6 peptidic bonds.

$$\text{Protein} \xrightarrow[\text{Alkaline Medium}]{Cu^{2+}} \text{Blue- purple color complex}$$

**Assay Kit Contain:**

$R_1$- Total protein reagent

Composition – Cupric sulphate 15 mmol/l, sodium potassium tartrate 40 mmol/l, potassium iodide – 30 mmol /l, NaOH 0.6 mol.

**Reagent Preparation:** Reagent ($R_1$) is ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| **Addition sequence for the test** | **For Total protein** |
|---|---|
| Reagent 1($R_1$) | 200 µl |
| Serum Sample | 4 µl |
| Main Filter | 546 |
| Incubation time | 560 sec |
| Unit | gm/dl |
| Check time | 36 sec |

**Calculation:**

Auto Quant automatically calculates the protein concentration of each sample.

SI Conversion factor: 1 gm/dl X 10 = 1gm/l

**Normal Reference Values of Total protein:**

Total protein**:** 6 – 8.3 gm/dl OR 60 -83 gm/l.

## 4.12.8: Estimation of Serum Albumin:

Method - Biuret (End Point) (Burtis CA.1994:700-704)

**Principle:**

Albumin complexes with 'Bormo Cresol sulfonpthalein (BCG)' dye at an acidic pH of 4.2. The blue-green colored complex is formed; the absorption of Albumin-BCG complex is directly proportional to the concentration of albumin present when measured between 580 – 630 nm with absorbance maxima at 620 nm.

Acidic Medium

Albumin + BCG ⟶ 'Albumin-BCG complex'

**Assay Kit Contain:**

$R_1$- Albumin reagent

**Composition:** 'Succinate buffer mmol/l pH 4.2, bromocresol green 0.1 mmol/l, surfactant'.

**Reagent Preparation:** Reagent ($R_1$) is ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| **Addition sequence for the test** | **For albumin** |
|---|---|
| Reagent 1 | 200 µl |
| Serum Sample | 2 µl |
| Main Filter | 620 |
| Incubation time | 60 sec |
| Unit | gm/dl |
| Check time | 120 sec |

**Calculation:**

Auto Quant automatically calculates the serum albumin concentration of each sample.

SI Conversion factor: 1 gm/dl X 10 = 1gm/l.

**Normal reference values of serum albumin:**

Albumin**:** 3.2– 5gm/dl OR 32 -50 gm/l.

**Globulin:** Concentration of globulin was measured by using the following formula,

Globulin = Total protein–albumin.

## 4.12.9: Estimation of Serum Bilirubin (Total & Direct):

Method – DIAZO (End Point) (Burtis CA. 1994: 1458 -1470)

**Principle:**

Bilirubin is coupled with 'diazotized sulphanilic acid' in acidic medium to form the pink-colored azobilirubin. The intensity of the color produced is directly proportional to the bilirubin concentration present in the sample.

Bilirubin + Diazotized sulphanilic acid ⟶ Azobilirubin compound.

**Reagent Preparation:** Reagents are ready for use.

**Assay Kit for total bilirubin contains:**

$R_1$ - Total Bilirubin Reagent 1

$R_2$ – Total Bilirubin Reagent 2

These reagents contain 'sulphanilic acid 0.5mmol/l, Cetrimide 30 mmol/l, hydrochloric acid 100 mmol/l and sodium nitrate 150mmol/l.

**Assay Kit for direct (Conjugated) bilirubin contains**:

$D_1$ – Direct Bilirubin Reagent 1

$D_2$ – Direct Bilirubin Reagent 2

These reagents contain sulphanilic acid 0.5 mmol/l, hydrochloric acid 100 mmol/l and sodium nitrate 150 mmol/l.

**Reagent Preparation:** Reagents $R_1$ and $R_2$ are ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| Addition sequence for the test | For Total Bilirubin | For Direct (Conjugated) Bilirubin |
|---|---|---|
| Reagent-1($R_1$) | 210 µl | 210 µl |
| Reagent-2($R_2$) | 70 µl | 70 µl |
| Serum sample | 30 µl | 30 µl |
| Main Filter | 546 | 546 |
| Incubation time | 250 sec | 250 sec |
| Unit | mg/dl or µmol/l | mg/dl or µmol/l |
| Check time | 36 sec | 36 sec |

**Calculation:**

Auto Quant automatically calculates the Total Bilirubin concentration of each sample. SI Conversion factor: 1 mg/dl X 17.1 = 1µmol/l.

**Indirect (Unconjugated) bilirubin** was calculated by using the following formula,

Indirect Bilirubin (Unconjugated Bilirubin) = Total Bilirubin – Direct Bilirubin (Conjugated)

**Normal reference values of total bilirubin:**

Total Bilirubin**:** Adults: 0.1 –1.2 mg/dl OR 1.7 – 20.5 µmol/l.

Infants: 1.2 – 12 mg/dl OR 20.5 – 205 µmol/l.

Direct (Conjugated) Bilirubin**:** Adults and Infants: 0 - 0.3 mg/dl OR 0 – 5.1 µmol/l.

Indirect (Unconjugated) Bilirubin: 0.2 – 0.7 mg/dl.

## ESTIMATION OF KIDNEY FUNCTION TESTS

### 4.12.10: Estimation of Urea:

Method - Urease–GLDH, Fixed time (Burtis CA.1994: 1528-1531)

**Principle:**

Blood urea is decomposed by urease enzyme to form ammonia and carbon dioxide. Ammonia combines with α-ketoglutarate in presence of glutamate dehydrogenase (GLDH) and NADH to form Glutamate and NAD. The rate of formation of NAD is measured at 340 nm and it is directly proportional to blood urea. Each molecule of urea hydrolyzed causes the production of two molecules of $NAD^+$.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

**Assay Kit Contain:**

$R_1$ - Urea Reagent 1

$R_2$ –Urea Reagent 2

**Composition:** Tris buffer 100 mmol/L pH 7.9, α-Ketoglutarate-Glycylglycine-7.5mmol/L, Urease >8KU/l, GLDH >800 U/l, NADH 0.32 mmol/L & stabilizers.

**Reagent Preparation:** Reagents $R_1$ and $R_2$ are ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| **Addition sequence for the test** | **For Urea** |
|---|---|
| Reagent 1 | 160 µl |
| Reagent 2 | 40 µl |
| Serum Sample | 2 µl |
| Main Filter | 340 |
| Incubation time | 60 sec |
| Unit | mg/dl |
| Check time | 120 sec |

**Calculation:**

Auto Quant automatically calculates the Urea concentration of each sample.

SI Conversion factor: 1 mg/dl X 0.357 = 1mmol/l.

To convert mg/dl of urea to mg/dl of BUN divide the results by 0.467 (Urea= 2.14 X BUN).

**Normal reference value of urea:**

Urea: 13 – 45 mg/dl OR 4.6 -16 mmol/l.

## 4.12.11: Estimation of Creatinine:

Method - Jaffe's Method, Initial rate (Burtis CA.1994: 1532-1537)

**Principle:**

Creatinine reacts with Picric acid in alkaline medium to form an orange-red color complex. This color absorbs light at 510 nm (500-520nm). The rate of increase in absorbance is directly proportional to the concentration of creatinine in the sample.

$$\text{Creatinine} + \text{Picrate} \xrightarrow{\text{Alkaline medium}} \text{Orange-red complex.}$$

**Assay Kit Contain:**

$R_1$ – Creatinine Reagent 1 - NaOH 0.3 mmol/l.

$R_2$ – Creatinine Reagent 2 - Picric acid 11 mmol/l.

**Reagent Preparation:** Reagents $R_1$ and $R_2$ are ready for use.

**Procedure:**

The program was set for the fully automated instrument as follow,

| **Addition sequence for the test** | **For Urea** |
|---|---|
| Reagent 1 | 150 µl |
| Reagent 2 | 150 µl |
| Serum Sample | 30 µl |
| Main Filter | 510 |
| Incubation time | 60 sec |
| Unit | mg/dl |
| Check time | 120 sec |

**Calculation:**

Auto Quant automatically calculates the creatinine concentration of each sample.

SI Conversion factor: 1 mg/dl X 88.4 = 1µmol/l.

**Normal reference value of creatinine:**

Men**:** 0.6 – 1.4 mg/dl OR 53 -124 µmol/l.

Women: 0. 6 – 1.2 mg/dl OR 53 -106 µmol/l.

## 4.12.12: Estimation of Electrolytes Sodium ($Na^+$) & Potassium ($K^+$)

Method - Flame photometry (John D. Baur (Ed) 1982; Nobert Tietz (Ed) 1976).

**Principal**:

At higher temperature atoms of various elements dissociate from their salts to become higher energy state and emit specific spectral bands which absorbs through proper interference filter or spectral barriers in a photodetector. The absorbance is proportional to the element's concentration. The emitted light of sodium absorbs maximum at 589 mµ, potassium at 404.5 and 766.5 mµ and calcium at 620, 554 and 422.7 mµ.

**Reagents supplied:**

Ready to use standards are diluted 1 to 100. Therefore, the values are comparable only when the test sample is similarly diluted 1 to 100 with distilled water or 1mMol/L lithium base.

**Assay Kit Contain:**

| I. Cat. No. SC 405……..500ml | II. Cat.No. C 409……..500ml | II. Cat.No. SC 413……..500ml |
|---|---|---|
| Sodium – 120 mmol/l | Sodium – 140 mmol/l | Sodium – 160 mmol/l |
| Potassium - 2 mmol/l | Potassium - 4 mmol/l | Potassium - 6 mmol/l |

**Procedure:**

The concentration of sodium and potassium ions in the serum was determined by flame photometric estimation of sodium and potassium, using compressed air. Diluted serum 1:100 in glass distilled water (BIOLAB Cat No.AR 556/AR557) OR 1mmol/L lithium base. Adjusted required pressure. Released gas from the cylinder. Released gas knob of the machine and light

the burner and adjust the flame about 5 to 6 cms in size. Feed electrolyte free distilled water. Feed Standard 120/2 and adjust the DPM reading $Na^+$ 120, $K^+$ 20 Using appropriate knob. Now feed diluted sample and it was sprayed as a fine mist of droplets (nebulized) into a non-luminous gas flame which emits the characteristic golden or lilac color. Light of a wavelength corresponding to the metal being measured (sodium or potassium) was selected by a light filter and allowed to fall on a photosensitive detector. The amount of light emitted is proportional to the concentration of metallic ions (sodium or potassium) present.

**Normal Values of Sodium and Potassium:**

Sodium = 133-146 mmol/l. and Potassium = 3.8 – 5.4 mmol/l.

## 4.13: MN and other nuclear abnormalities (Cytogenetic changes):

Study subjects were instructed to rinse their mouth with water before sampling. Exfoliated cells of the buccal mucosa were obtained by a sterile wooden spatula. For each individual, the slides were prepared in triplicate by smearing the cells onto pre-cleaned slides. The slides were then air-dried and fixed with methanol for 10 min. Then air-dried & stained it with Giemsa (Biolab diagnostic Pvt. Ltd.) stain for 15-20 min. 1000 cells of each subject were counted.

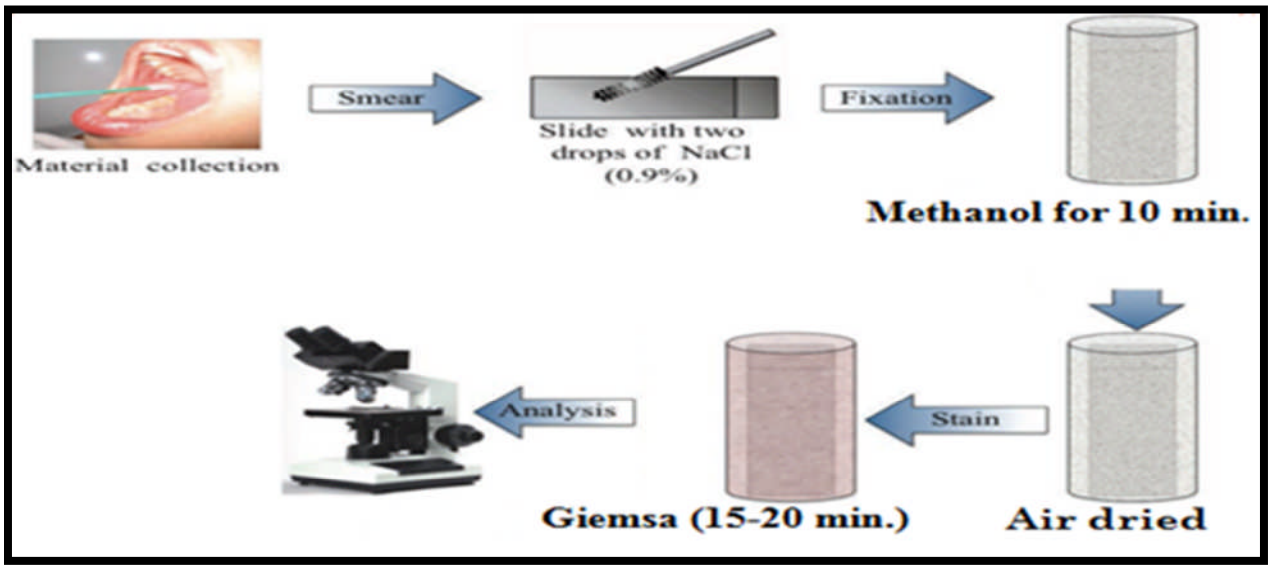



**Figure 4.3: Method used for scoring frequencies of MN and other nuclear abnormalities.**

Slides were evaluated using classification for nuclear abnormalities by Tolbert PE *et al.* (1991); to determine the micronucleus (MN) frequencies. Briefly, the following criteria were used for the analysis of micronuclei and other nuclear abnormalities,

- A micronucleus must have less than one-third the diameter of the main nucleus. It should be on the same plane of focus; must have a smooth, oval or round shape with same color, texture, and refraction as the main nucleus. It should be clearly separated from the main nucleus.
- Cells with two nuclei were considered as binucleates.
- Nuclei fragmented into irregular pieces were considered as karyorrhexis.
- Nuclear dissolution, in which a Geimsa-negative, ghost-like image of the nucleus remains, was considered as karyolysis.

## 4.14: Statistical analysis:

- Data were analyzed using SPSS software Version -16.0.
- Data obtained was expressed in terms of mean ± SD.
- One way analysis of variance (ANOVA) was used for between-group analysis & it was followed by post hock't' test.
- Level of statistical significance was established at $p<0.05$. Frequencies & percentages (%) were given for qualitative variables.
- Pearson correlation was done to find correlations between quantitative variables.

# Results and Discussion

## 5.1: Anthropometric Parameters

### 5.1.1: Results.

**Table-5.1: Anthropometric parameters amongst the study and control group.**

| Parameters | Group-I (N=70) | Group-II (N=70) | Group-III (N=70) | F-value | P-value |
|---|---|---|---|---|---|
| Height (cm) | 168.84 ± 8.18 | 161.51 ± 6.39 [a] | 165.11 ± 6.66 [b,c] | 18.538 | 0.000* |
| Weight (Kg) | 68.13 ± 17.79 | 57.90 ± 12.03 | 63.51 ± 13.692 [c] | 8.492 | 0.000 * |
| BMI($Kg/m^2$) | 23.96 ± 6.83 | 22.22 ± 4.71 | 23.16 ± 4.06 | 1.851 | 0.160 NS |
| Total Body Water | 40.95 ± 6.20 | 36.87 ± 4.071 | 38.721 ± 5.16 [b,c] | 10.758 | 0.000 * |
| Body Surface area ($m^2$) | 1.78 ± 0.22 | 1.60 ± 0.18 [a] | 1.69 ± 0.21 [b] | 12.955 | 0.000* |

Group I - Control Group, Group II-Automobile mechanics (AM),Group III-Petrol pump workers (PPW). *depicts 'P' value≤0.05 is significant. While 'P' value> 0.05 = not significant (NS).Data are presented as Mean ± SD; Analysis of variance (ANOVA) followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference amongst (Group-I and II), (Group-I and II), (Group-II and II) respectively.

Table 5.1 shows a statistically significant decrease in weight, total body water and body surface area amongst group-II (AM) and III (PPW) compared to group-I (control). BMI was insignificantly decreased in group-II and group-III as compared to group-I (control).

### 5.1.2: Percentage change impact on anthropometric parameters:

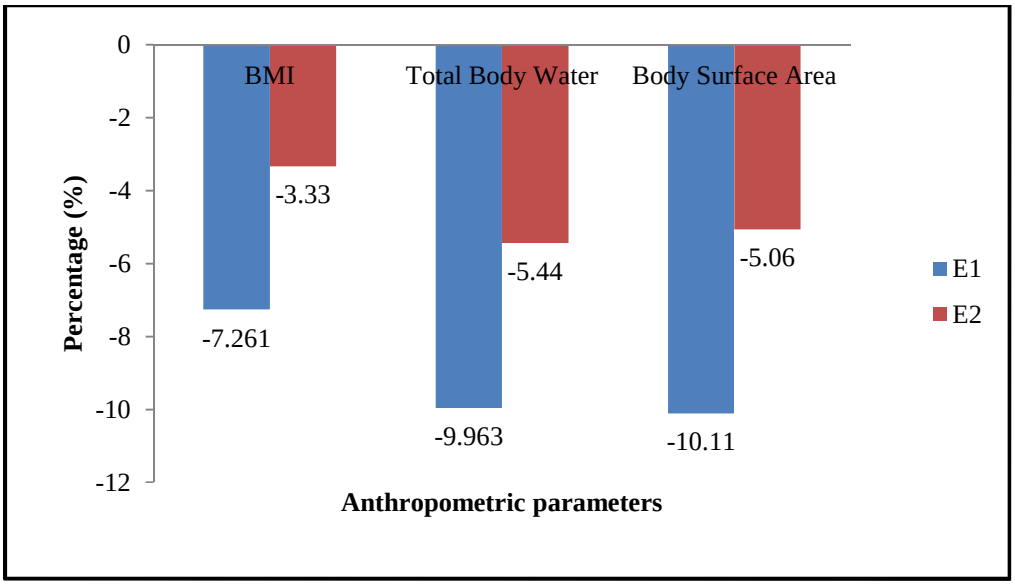



**Figure 5.1:** Percentage change in anthropometric parameters in group-II (AM) and group-III (PPW) workers. $E_1$= (Group I- Group II). $E_2$ = (Group I- Group III) considering group-I as a control group.

Figure 5.1 depicts the percentage difference in anthropometric parameters amongst the study group with respect to control group. We observed the difference in percentage change of BMI, total body water and body surface area all are decreased to a greater extent in group-II (E1) compared to group-III (E2).

### 5.1.3: Discussion

This decrease in body weight and BMI among these workers indicates that there is significant occupational stress induced by long term exposure to heavy metal lead at their workplace (Wani Ab Latif *et al.* 2016). This also could be due to severe gastritis and malabsorption caused by heavy metal lead (Pb), which ultimately leads to less caloric consumption and decreases body weight (Das KK *et al.* 2015). It also might be due to complex interactions between various gasoline components and a signaling pathway involving intercellular and molecular mechanisms that result in the suppression of growth stimulatory signals and the stimulation of growth stimulatory pathways and that subsequently causes growth retardation and weight loss (Murtala BA *et al.* 2015). This decrease in body weight contributes to a decrease in body surface area (BSA) and total body water in group-II and III workers.

Our results correspond with the results of other studies which mention that occupational exposure to petroleum hydrocarbon and heavy metal lead is associated with a decrease in height, weight, BMI in occupationally exposed petrol pump and automobile garage workers (Tiu DN *et al.* 2017, Wani Ab Latif *et al*. 2016).

A similar result has been observed in another study that shows a decrease in mean values of height, weight and BSA in automobile spray paint workers (Gupta G. *et al.* 2016). Similarly Rahul *et al.* (2016); noticed a decrease in anthropometric parameters like age, height, weight, BMI in petrol pump workers of Jaipur City, Rajasthan.

Our findings do not correlate with the findings of research done by Sandip M Hulke *et al.* who reported that there was an increase in body weight and BMI in petrol pump workers (Sandip M Hulke *et al*. 2012).

## 5.2: Socio-demographic profile of the study participants:

### 5.2.1: Results.

**Table 5.2: Socio-demographic characteristics amongst the study and control group.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | P–value |
|---|---|---|---|---|
| **Age Range (Yrs)** | | | | |
| 20-30 (n=143) | 25.54 ± 2.88 | 23.45 ± 2.99[a] | 25.20 ± 2.99 | 0.001* |
| 31- 40 (n=67) | 36.52 ± 3.27 | 35.53 ± 3.36 | 35.68 ± 2.96 | 0.518 NS |
| **Education** | **Group-I (n=70)** | **Group-II (n=70)** | **Group-III (n=70)** | |
| Primary | 14(20.0%) | 55 (78.6%) | 60 (85.7%) | |
| Secondary | 34 (48.6%) | 11 (15.7%) | 6 (8.6%) | |
| Higher | 22 (31.4%) | 4 (5.7%) | 4 (5.7%) | |
| **Monthly Income** | | | | |
| ≤ 6000/month | 5 (7.1%) | 49 (70%) | 48 (68.6%) | |
| >6000/month | 65(92.9%) | 21(30.0%) | 22(31.4%) | |

Group-I–Control, Group-II - Automobile mechanics (AM), Group-III - Petrol pump workers (PPW). * depicts P≤0.05 is significant, while 'P' value> 0.05 - not significant (NS).Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript an express the significant difference amongst (Group-I and II).

Table 5.2 depicts the socio-demographic characteristics of the participants. Our findings revealed that all the participants were males and the majority of workers were in the age group of 20- 30 years. Statistically significant changes observed in the mean ages of age group 20-30 years. This finding showed that more young populations are working on petrol pumps and in automobile garages.

With regard to education, Majority of group-II (78.6%) and group-III (85.7%) workers have a primary level of education and a few have secondary (15.7% AM and 8.6% PPW) and degree level education (5.7%). This revealed that petrol pump workers and automobile mechanics had a basic level of education and none of them were illiterate. Most of the group-II (70%) and group-III (68.6%) workers have a monthly income of ≤6000, whereas very few group-II (30%) and group-III (31.4%) have monthly income >6000.

**5.2.2: Discussion.**

Automobile mechanics & petrol pump workers are the individuals from the low socioeconomic background and this could be evidenced by their educational background and a monthly income as shown in the present study.

Similar findings were shown by Thomas SJ (2013), who reported majorities (65.2%) of petrol pump workers were in the age group of 18-27 years. Majority of workers (49.37%) had a high school education and a few (6.33%) had degree-level education. Most of the workers (43.04%) had a monthly income between Rs.2001-Rs.6000; whereas very few (6.33%) had monthly income above Rs.8000.

## 5.3: Diet pattern, working pattern, and duration of exposure in study and control groups.

### 5.3.1: Results

**Table 5.3: Percentage frequency of diet pattern, working pattern, and duration of exposure in the study and control group.**

| Characteristics | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) |
|---|---|---|---|
| **Diet** | | | |
| Vegetarian | 63 (91.3% ) | 7 (10.0%) | 22 (31.4%) |
| Mixed | 7 (8.7% ) | 63 (90.0% )* | 48 ( 68.6% ) * |
| **Working Time** | | | |
| Day | 70 (100.0%) | 70 (100.0%)* | 58 (82.9%) |
| Night | 0 (0.0%) | 0 (0.0% ) | 12 (17.1 % )* |
| **Working Hours/Day** | | | |
| 8 Hr./ Day | 69 (98.6%) | 67 (95.7%)* | 65 (92.9%) |
| **Years of Exposure** | | | |
| 1-5 years | -- | 38 (54.3%)* | 30 (42.9%) |
| 6-10 years | -- | 17(24.3%) | 28 (40.0%)* |
| More than 10 years | -- | 15 (21.4%)* | 12 (17.1%) |

Group I–Control Group, Group II-Automobile mechanics (AM), Group III–Petrol pump workers (PPW). * depicts maximum percentage frequencies.

Table 5.3 depicts the percentage frequency of diet pattern, working pattern, and duration of exposure in the study and control group.

### 5.3.2: Discussion:

Among study population majority of group-II (90%) & III (68.6%) workers consumed a mixed diet. Majority of group-I workers (91.3%) were vegetarians.

Maximum numbers of group-II (95.7%) workers were working 8 hr/day as compared to group-III (92.9%) workers. Almost all the group-I and II workers had day time

duty, but very few group-III workers had a night shift. This indicates that a small proportion of workers worked as part-time workers and continuing their education along with the duty.

Considering the years of experience, present study findings showed nearly half of the group-II (54.3%) and group-III (42.9%) workers had 1-5 years of exposure. Less number of group-II workers (24.3%) has 6-10 years of exposure and very few group-III workers (17.1%) exposed for more than 10 years. This could be due to the fact that many of the petrol pump workers and automobile mechanics were young and may just have started working after recently completing their secondary education. Another possible reason could be that workers quit the job frequently; they were not considering their jobs in petroleum pump and automobile garages as a permanent job.

Our findings correspond with the findings of Kesavachandran C *et al*. (2006); which depicted that most of the petrol pump workers (69.5%) had experience of fewer than 5 years and few had experience of >15 years. Johnson OE *et al.* (2018); also observed 109 (50.7%) petrol pump workers had worked for a year, followed by 94 (43.7%) workers had 1-5 years of exposure and very few 15 (7%) had more than 10 years of exposure.

## 5.4: Self-reported symptoms amongst study and control groups.

### 5.4.1: Results.

**Table-5.4: Percentage (%) distribution of self-reported symptoms in all groups.**

| Symptoms | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) |
|---|---|---|---|
| **Respiratory Symptoms** | | | |
| Chest pain | 18 (25.7%) | 30 (42.9%)* | 20 (28.6%) |
| Cough | 16 (22.9%) | 29 (41.4%)* | 29 (41.4%)* |
| **Skin Related Symptoms** | | | |
| Skin Irritation | 1 (1.4%) | 26 (37.1% )* | 20 (28.6%) |
| **Eye Related Symptoms** | | | |
| Eye Irritation | 6 (8.6%) | 38 (54.3%)* | 24 (34.3%) |
| **GIT related Symptoms** | | | |
| Low appetite | 1 (1.4%) | 8 (11.4%) | 26 (37.1%)* |
| Nausea | 1 (1.4%) | 3 (4.3%) | 23 (32.9%)* |
| Vomiting | 6 (8.6%) | 32 (45.7%)* | 26 (37.1%) |
| Diarrhea | 7 (10.0%) | 34 (48.6%)* | 20 (28.6% ) |
| Constipation | 9 (12.9%) | 35 (50.0%) | 34 (48.6%) |
| Abdominal Pain | 13 (18.6%) | 37 (52.9%)* | 29 (41.4%) |
| **Muscle and CNS related Symptoms** | | | |
| Headache | 14 (20.0%) | 23 (32.9%)* | 17 (24.3% ) |
| Insomnia | 3 (4.3%) | 18 (25.7%) | 26 (37.1%)* |
| Mental confusion | 4 (5.7%) | 10 (14.3%) | 31 (44.3%)* |
| Muscle Pain | 6 (8.6%) | 27 (38.6%)* | 17 (24.3% ) |
| Dizziness | 1 (1.4%) | 9 (12.9%)* | 3 (4.3%) |
| Weakness | 6 (8.6%) | 7 (10.0%) | 35 (50.0%)* |

Group I – Control Group, Group II - Automobile mechanics (AM), Group III - Petrol pump workers (PPW).* depicts maximum percentage frequencies.

Table 5.4 indicates frequent symptoms concerning different systems like respiratory, skin, eye, muscle, GIT and CNS. Higher percentage of cough (41.4%), chest pain (42.9%), skin irritation (37.1%), eye irritation (54.3%), muscle pain (38.6%), vomiting (45.7%), diarrhea (48.6%), constipation (50.0%), abdominal pain (52.9%), dizziness (12.9%) and headache (32.9%) observed in group-II (AM) workers, while higher percentage of weakness (50.0%), low appetite (37.1%), nausea (32.9%), insomnia (37.1%), and confusion (44.3%) were reported by group-III (PPW) workers.

**5.4.2: Discussion.**

The maximum percentage of all these symptoms were observed in group-II and group-III as compared to group-I. This could be due to an increased blood lead level in them.

Many epidemiologic studies have shown that cough, sputum production, dyspnoea, chest pain were associated with exposure to motor vehicle exhaust emissions (Bener A *et al.*1998; Nakai S *et al.* 1999). Notably, we observed such type of symptoms in previous studies, which showed that inhalation of petrol vapours or car exhaust fumes causes health problems like headache (53.6%), low back pain (33.3%), eye irritation (29.5%), dizziness (24.6%), cough (18.6%) and nausea (18.6%) in petrol pump workers (Johnson OE *et al.* 2018).

Similar symptoms like abdominal pain (15%), diarrhea (19%), vomiting (3%), muscle pain (73%) loss of appetite (3%) and headache (80%) were observed in automobile mechanics (Amah UK *et al.* 2014). It was reported by Thangaraj S *et al*. (2017); that musculoskeletal diseases were common work-related illness (62%).

This suggests that certain automobile workers were involved in welding; it makes them vulnerable to several eye-related symptoms such as eye irritation and conjunctivitis. The most commonly reported occupational health problems by the mechanics were muscle pain (38.6%), and this could be due to the discomforting positions they are forced to adapt in the process of their work. Increased frequency of symptoms related to the digestive system (abdominal pain, nausea, vomiting, diarrhea, constipation, and low appetite) may

indicate the direct effect of occupational lead exposure at the contaminated workplace environment (Wani Ab Latif *et al*.2016).

## 5.5: Heavy Metal Lead (Pb)

## 5.5.1: Results

**Table 5.5: Blood lead (Pb) level in study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F-value | P-value |
|---|---|---|---|---|---|
| Blood Lead (Pb) (µg/dl) | 9.730 ± 5.32 | 25.58 ± 13.97 [a] | 15.43 ± 8.49[b,c] | 45.776 | 0.000* |

Group I–Controls, Group II - Automobile Mechanics (AM), Group III - Petrol Pump Workers (PPW). * depicts 'P' value ≤0.05 is significant. Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.5 indicates results of blood lead level (BLL) in all the groups. Surprisingly we found a statistically significant increase in blood lead level amongst group-II (AM) and group-III (PPW) as compared to group-I (control group). The elevated BLL in the study group indicates that lead absorption was more pronounced in Group-II (AM) and Group-III (PPW) than Group-I (control group). This means that they have been exposed to inhalation of petroleum fumes and automobile exhausts for a longer duration.

### 5.5.2: Impact of percentage change on blood lead level (BLL).

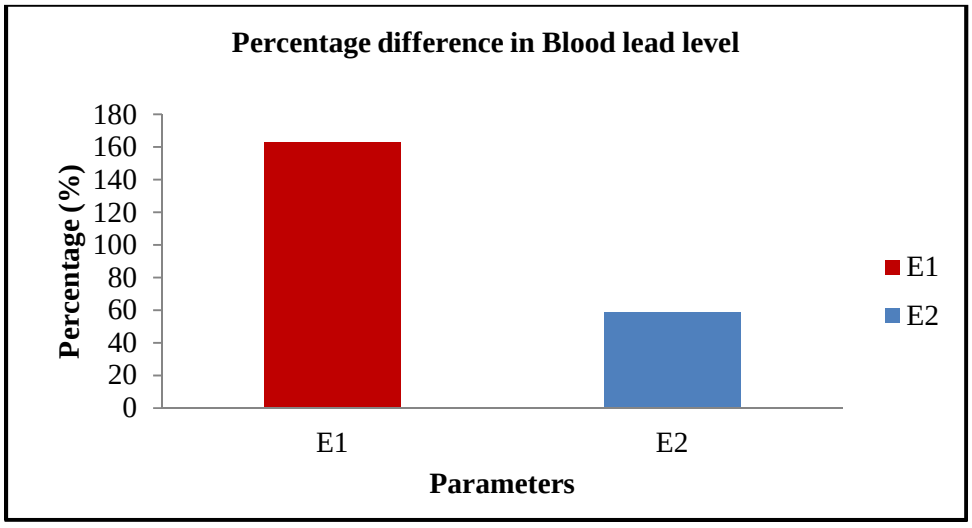



**Figure: 5.2**

**Figure-5.2:** Percentage change impact on BLL of automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II). $E_2$= (Group I- Group III) considering group-I as a control group.

Figure 5.2 depicts the percentage change impact on blood lead level (BLL) in study groups. There was a greater increase in percentage difference of BLL among group-II (AM) as compared to group-III (PPW) with respect to the control group.

**Table-5.6: Effect of duration of exposure on blood lead (Pb) level amongst study groups.**

| **Study Groups** | **Parameters** | **Group-IIA (n = 38)** | **Group-IIB (n = 17)** | **Group-IIB (n = 15)** | **F-value** | **P-value** |
|---|---|---|---|---|---|---|
| Group-II (AM) (n=70) | Blood Lead (Pb) (µg/dl) | 16.08 ± 9.01 | 32.19 ± 6.73 [a] | 42.11 ± 10.30 [b,c] | 53.039 | 0.000* |
| Group-III (PPW) (n=70) | **Parameters** | **Group-IIA (n = 30)** | **Group-IIB (n = 28)** | **Group-IIC (n = 12)** | **F- value** | **P-value** |
| | Blood Lead (Pb) (µg/dl) | 10.01 ± 3.42 | 17.26 ± 6.91 [a] | 24.73 ± 11.04 [b,c] | 22.687 | 0.000* |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics. Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB -6 to10 yrs. of exposure, Group-IIIC->10 yrs. of exposure in petrol pump workers. * depicts 'P' value ≤0.05 significant, while 'P' value >0.05 not significant (NS). Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c expresses the significant difference amongst groups (IIA and IIB), (IIA and IIC), (IIB and IIC) and (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively.

Table 5.6 represents the effect of duration of exposure on blood lead level in AM and PPW. It seems that as the duration of exposure increases, there was a significant increase in BLL amongst group-II (AM) and group-III (PPW) workers. There was a significant increase in BLL in group-IIB and group-IIC as compared to group-IIA in group-II (AM). We found a significant increase in BLL in group-IIIB and IIIC as compared to group-IIIA in group-III (PPW). There was a significant increase in BLL in group-IIB as compared to the group-IIC in group-II (AM) and in IIIB as compared to IIIC in group-III (PPW).

### 5.5.3: Discussion.

The observed higher BLL in the AM than in the PPW indicate a greater exposure of the AM relative to the PPW. This could be due to,

1. The AM have possible sources associated with lead exposure at the workplace, like repairing car batteries, car painting, radiators and most probably due to closer contact with automobile exhaust fumes.
2. Moreover, AM those does routine maintenance and repair of motor vehicles are commonly found in India and are exposed to petroleum and exhaust fumes, a likely source of lead by sucking with their mouth through a tube in an attempt to siphon petrol from the vehicle tank. They also often wash vehicle parts with petrol and diesel without wearing gloves.
3. Improper ventilation and lack of exhaust fans observed in the automobile workshops, while petrol pumps are situated in open space.
4. It also might be due to a lack of knowledge about the hazardous effect of lead and ineffective quality control measures to check lead toxicity, since this study was done in the developing area of western Maharashtra.

Blood lead measurement has been used as a reliable marker of recent exposure to this toxic heavy metal. Increased blood lead level in the auto-mechanics and petrol pump workers observed in this study indicates that these groups of workers are more exposed to heavy metal lead than the control group because they come in contact with heavy metal lead in their daily activities at their workplace. The increased BLL in the study groups indicates that despite modern technical advancements, the rate of lead absorption was definitely greater in all AM and PPW than the control group.

Previous studies carried out an analysis of automobile exhaust emissions and they have been reported that 21-28% of petrol lead is retained in the exhaust system. In all these studies, traffic exhaust emission has been implicated as the source of lead in the atmosphere (Jost D. *et al.*1989, Nriagu J.O. 1978 and Simmonds PR. *et al*. 1983)

Results of Dongare NN *et al.* corroborate with the results of our study. They reported significantly increased BLL in automobile workers as compared to controls. Increased BLL in these workers indicates that the release of lead fumes, particles, dust, and vapors was more in those places. Poor hygiene and inappropriate protection increase the risk of exposure (Dongare NN *et al.* 2011).

Similar to our study noticeably high BLL with a range of 11.73–36.52 μg/dL observed in automotive garage workers of Jimma town. Individuals involved in manual car painting had a larger percentage (58%) of those with the highest BLL (≥20 μg/dL) than other automotive garage workers. These painters, in addition to the oral exposure routes, are more likely exposed to inhalation of lead fumes found in the dyes than those workers engaged in other auto-repairing activities. This could be a possible reason for the observed BLL difference between the two groups. They also showed that as the duration of exposure (1–3, 3–6 and above 6 years of exposure) increases BLL (15 μg/dL, between 15-20 μg/dL, above 20 μg/dL) were significantly increased in automotive garage workers. This clearly shows the direct relationship between BLL and years of service years which are in accordance with the findings of the duration of exposure of the present study (Adela Y. *et al*. 2012).

Similarly, high BLL observed in gasoline station workers, possibly due to the inhalation of air contaminated with lead, eating contaminated foods as a result of some bad habits like not washing contaminated hands before eating and not use the protective clothes (Adnan J. M. Al-Fartosy *et al.* (2017). In another study done on petrol pump workers showed only 7.7% petrol pump workers had the BLL above normal (>25 μg/dL) and 92.3% still had BLL within the normal limit, this might be because of less occupational exposure in petrol pump workers. (Nur Kusuma Dewi *et al.* 2017). It has been observed that a significant increase in the blood lead level amongst petrol station attendants as compared to control subjects. This significantly increased BLL may be the result of its use in octane rating of petrol that is still in use in some developing countries (Airhomwanbor O.K. *et al.* 2018). These results are in accordance with our findings.

Increased BLL in subjects occupationally exposed to heavy metal lead. Increase in the blood lead level observed in the non-occupationally exposed subjects. This could be from other environmental contamination and exposures including inhalation of exhaust fumes or contaminated dust particles or inadvertent ingestion of contaminated foods or drinks which is a possible indication of the extent of lead pollution in Nigeria which is in accordance with results of the present study (Obi-Ezeani CN *et al.* 2019).

## 5.6: Oxidative Stress

### 5.6.1: Results

**Table 5.7: Oxidative stress biomarker (MDA and GGT) in the study and control group.**

| Parameters | Group- I (N=70) | Group-II (N=70) | Group-III (N=70) | F-value | P-value |
|---|---|---|---|---|---|
| MDA (nmol/ dl) | 3.344 ± 1.69 | 6.628 ± 2.77 [a] | 4.894 ± 3.047[b,c] | 28.553 | 0.000* |
| Serum GGT (IU/L) | 18.87 ± 5.755 | 23.12 ± 9.135[a] | 21.62 ± 11.378 [b] | 3.96 | 0.020* |

Group I– Control, Group II- Automobile Mechanics (AM), Group III- Petrol Pump Workers (PPW). Malondialdehyde-MDA, γ-GlutamylTransferase-GGT.* depicts 'P' value ≤0.05 is significant. Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.7 indicates results of oxidative stress biomarkers (MDA and GGT) in all groups. Surprisingly we found a statistically significant increase in MDA and GGT activity amongst group-II (AM) and group-III (PPW) workers as compared to group-I (control group). Statistically significant increase in MDA level observed in group-II (AM) compared to group-III (PPW).

### 5.6.2: Percentage change impact on MDA and GGT level.

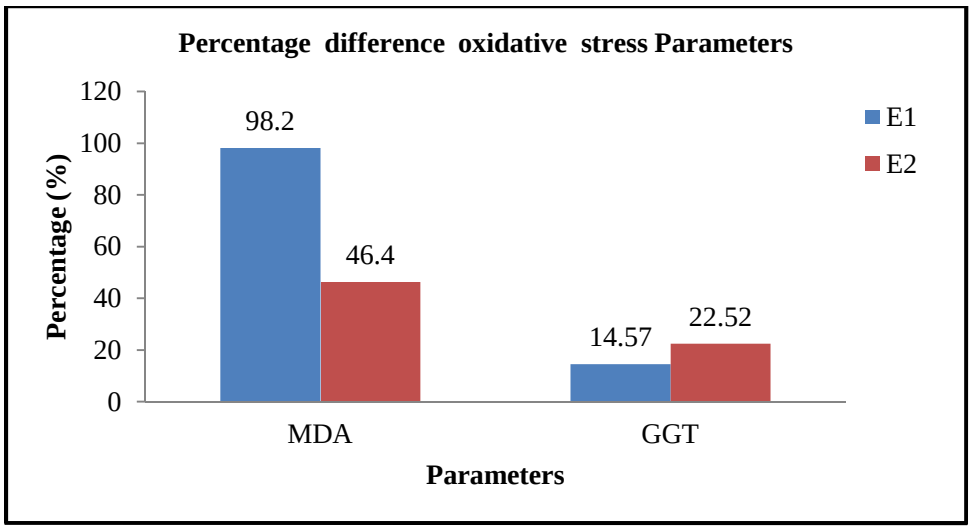



**Figure 5.3**

**Figure-5.3:** Percentage change impact on MDA and GGT level in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure 5.3 depicts the percentage change impact on MDA and GGT level in study groups. There was a greater increase in percentage difference of MDA level in group-II (AM) and GGT activity in group-III (PPW) with respect to the control group.

**Table-5.8: Effect of duration of exposure on MDA and GGT level amongst study groups.**

| **Study Groups** | **Parameters** | **Group-IIA (n = 38)** | **Group-IIB (n = 17)** | **Group-IIB (n = 15)** | **F-value** | **P-value** |
|---|---|---|---|---|---|---|
| Group-II (AM) (n=70) | MDA (nmol/dl) | 5.11 ± 2.35 | 8.55 ± 1.87 [a] | 8.89 ± 2.36[b,c] | 18.951 | 0.000 * |
| | Serum GGT (IU/L) | 17.43 ± 9.75 | 21.80 ± 7.37 | 32.01 ± 12.70[b,c] | 11.524 | 0.000* |
| Group-III (PPW) (n=70) | **Parameters** | **Group-IIIA (n = 30)** | **Group-IIIB (n = 28)** | **Group-IIIC (n = 12)** | **F- value** | **P-value** |
| | MDA (nmol/dl) | 2.71 ± 1.23 | 5.78 ± 1.56 [a] | 8.29 ± 4.56[b,c] | 30.041 | 0.000* |
| | Serum GGT (IU/L) | 22.78 ± 9.04 | 21.95 ± 8.63 | 26.70 ± 10.35 | 1.180 | 0.314 NS |

Malondialdehyde-MDA, γ-Glutamyl Transferase-GGT. Group-IIA-1 to 5 yrs. of exposure, Group-IIB- 6 to10 yrs. of exposure, Group-IIC- >10 yrs. exposure in automobile mechanics. Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB -6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in Petrol pump workers. * depicts 'P' value ≤ 0.05 significant, while 'P' value > 0.05 - not significant (NS). Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c expresses the significant difference amongst the group (IIA and IIB), (IIA and IIC), (IIB and IIC) and (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively.

Table 5.8 represents the effect of duration of exposure on MDA and GGT activity in automobile mechanics and petrol pump workers. Duration of exposure has a significant impact on MDA and GGT level. With the increased duration of exposure, there was a significantly increased MDA level amongst group-II (AM) & group-III (PPW). As the duration of exposure increases, there was a significant increase in GGT activity in group-II, while the duration of exposure has an insignificant impact on GGT activity among Group-III. There was a significant increase in MDA and GGT level in group-IIB and IIC as compared to group-IIA in group-II. We found a significant increase in MDA level in group-

IIIB and IIIC as compared to group-IIIA in group-II (PPW). We also observed a significant increase in MDA level in group-IIB as compared to the group- IIC in AM and in IIIB as compared to IIIC in PPW.

### 5.6.3: Discussion

Oxidative stress is an imbalance of free radicals and antioxidants in the body, which can lead to cell and tissue damage.

The MDA and GGT level was significantly increased in the occupationally exposed group (group-II and III) which could be due to lead-induced oxidative stress. Petroleum and exhaust fumes have been recognized as factors which can enhance peroxidative processes and oxidative stress within cells (Owagboriaye F.O *et al.* 2016). Numerous types of particulate matter (PM) derived from vehicle exhausts have the capacity to generate oxygen free radicals (Particles as a direct source of free radicals). The oxidative properties of particulate matter present in automobile exhausts trigger the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Magnus Lund back 2009).

A GGT mediated oxidative stress has been reported by Lee DH *et al.* (2004); it is capable of inducing oxidation of lipids, protein thiols, alterations of the normal protein phosphorylation patterns and biological effects such as activation of the transcription factor. Also, it is involved in the glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water and certain L-amino acids and peptides leaving cysteine products to preserve intracellular homeostasis of oxidative stress.

Serum GGT is proposed as an early and sensitive marker for oxidative stress. It plays an important role in maintaining intracellular glutathione transport into cells, thus mediating intracellular protective antioxidant mechanisms. As it has a role in increasing glutathione transport into the cell, an increase in GGT level was thought to be a response to oxidative stress (Yavuz B. B. *et al.* 2008). However recent experimental studies indicate that ectoplasmic GGT may also be involved in the generation of oxidative stress. This effect of GGT seems to occur when GGT is expressed in the presence of iron or other transition metals (Dongare NN *et al.* 2010).

Lipid peroxidation results from the release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes to form malondialdehyde. Malondialdehyde (MDA) is one of the end-products of the peroxidation

of membrane lipids caused by ROS formation, especially by the superoxide ion. It is currently considered to be a basic marker of oxidative stress (Odewabi *et al.* 2014).

Oxidative stress is one of the mechanisms which are involved in the pathogenesis of various diseases such as neurodegenerative diseases, cardiovascular diseases, hepatobiliary diseases, and renal disease. Lead-induced oxidative stress is multifactorial and affects various organ systems (Emmanuel OG *et al.* 2018).

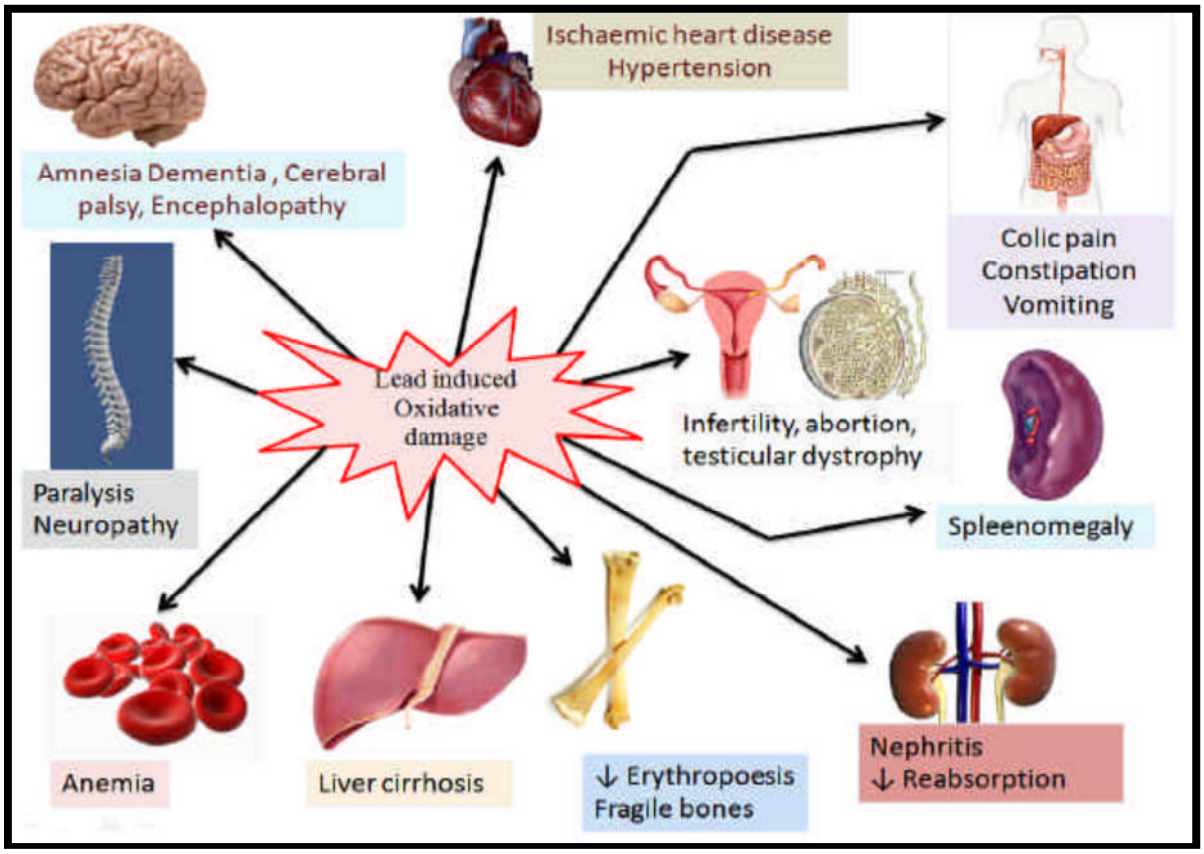



**Figure-5.4:** Heavy metal lead-induced oxidative damage in different organs and organ Systems.

**Source:** Debasish Bandyopadhyay *et al.* Lead-induced oxidative stress: a health issue of global concern. Journal of Pharmacy Research 2014; 8(9):1198-1207.

Increasing evidence indicates that multi-factorial mechanisms might be involved in metal-induced toxicity and it is suggested that one of the well-known mechanisms is a metal-induced generation of reactive oxygen species (ROS). Recently, oxidative stress has been proposed as a possible mechanism involved in lead toxicity (Debasis B *et al. 2014*).

Lead does not directly induce the peroxidation of lipids. It, however, makes the formation of free oxygen radicals easier. The intrinsic mechanism underlying lead-induced oxidative damage to membranes is associated with changes in its fatty acid composition

(Patra RC. *et al.* 2011). The fatty acid chain-length and unsaturation are the determinants for membrane susceptibility to peroxidation, and lead-induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation of the membrane. Thus, lead affects membrane-related processes such as the activity of membrane enzymes, endo and exocytosis, transport of solutes across the bilayer, and signal transduction processes by causing lateral phase separation. Lead accumulation in tissues causes oxidative DNA damages including strand break, although the evidence of lead-induced oxidative damage to DNA is less conclusive (Patra RC. *et al.* 2011).

Exhaust fumes generate ROS in two different ways. Firstly, ROS is generated by intrinsic properties of particles and iron content of the dust particles. Secondly, the intensive formation of ROS occurs by the oxidative burst of macrophages and neutrophils activated during phagocytosis and persistent inflammation (Mittal M *et al.* 2014). Thus, the increased level of MDA and GGT suggests that exhaust fumes stimulate free radical generating capacity.

Our result reveals that exposure to petroleum and exhaust fumes could results in oxidative stress in petrol pump workers and in automobile mechanics, because of the presence of lead and other particles as agents of free radicals. This has been depicted by a raised level of oxidative stress parameters (MDA, GGT) in the study group.

Our findings are in agreement with findings of previous studies that observed exposure to petroleum products leads to increase lipid peroxidation (MDA) in rats (Bokolo B *et al.* 2013; Uboh F.E *et al.* 2013, Owagboriaye FO *et al.* 2016 ) and in the human (Odewabi AO *et al.* 2014). Therefore, exposures to petrol fume have a resultant lipid peroxidation effect, and lipid peroxidation is one of the known mechanisms of free radical generation in the biological systems (Uboh F.E *et al.* 2013).

Significantly increased activity of GGT among automobile mechanics observed in North Karnataka. A GGT mediated oxidative stress has been reported. It is capable of inducing oxidation of lipids, protein thiols, alterations of the normal protein phosphorylation patterns and biological effects such as activation of the transcription factor which are in accordance with the results of our study (Dongare NN *et al.* 2010).

Our results are in agreement with other studies which illustrated that MDA was significantly increased with increasing the period of exposure in petroleum station workers. Malondialdehyde concentrations were increased significantly, indicating the amount of cellular damage from lipid peroxidation. Lipid peroxidation initiated by free radicals

generated as a result of diesel intoxication is usually deleterious to cell membranes and is implicated in a number of pathological conditions (Luay AA *et al.* 2014).

Olufunsho Awodele *et al.* noticed similar findings in the petrol tanker drivers. They found a significant increase in their lipid peroxidation level (MDA) compared with the control subjects. It may be due to petrochemical activated metabolites that react with some cellular components such as membrane lipids to produce lipid peroxidation products which may lead to membrane change and tissue damage (Olufunsho Awodele *et al.* 2014).

## 5.7: Cardiovascular changes amongst study and control groups.

### 5.7.1: Results

**Table-5.9: Cardiovascular parameters amongst the study and control group.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F- value | P-value |
|---|---|---|---|---|---|
| S B P (mmHg) | 121.40 ± 8.87 | 125.80 ± 11.58 | 125.14 ± 8.18[c] | 4.230 | 0.016 * |
| D B P (mmHg) | 80.89 ± 5.72 | 83.09 ± 7.15 | 82.63 ± 5.98 | 2.366 | 0.096 NS |
| Pulse Pressure (mmHg) | 40.51 ± 7.124 | 42.71 ± 10.714 | 42.51 ± 6.670 | 1.480 | 0.230 NS |
| MAP (mmHg) | 94.39 ± 6.065 | 97.32 ± 7.299 | 96.80 ± 6.020[b,c] | 4.069 | 0.018 * |
| Heart Rate/Min | 73.66 ± 2.81 | 75.27 ± 6.64 | 74.99 ± 4.79 | 2.080 | 0.127 NS |

Group I–Controls, Group II - Automobile Mechanics (AM), Group III - Petrol Pump Workers (PPW). * depicts 'P'value ≤ 0.05 is significant while 'P' value > 0.05 not significant (NS). Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and II) respectively.

Table 5.9 indicates significantly increased systolic blood pressure (SBP) and mean arterial pressure (MAP) amongst group-II and III as compared to group-I. Significantly increased SBP and MAP were observed in group-II as compared to group-III. Significant increase in SBP was observed in Group III as compared to group-I. Statistically insignificant increase in diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR) was found amongst group-II and III.

### 5.7.2: Percentage change impact on cardiovascular parameters:

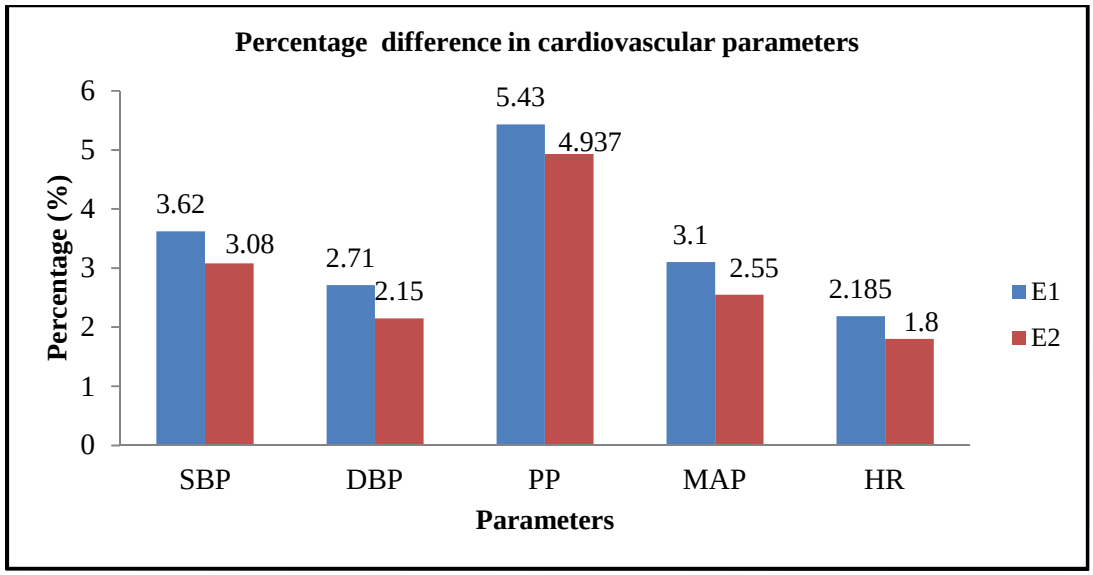



**Figure-5.5:** Percentage change impact on cardiovascular parameters in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure-5.5 depicts there was a less increase in percentage difference of cardiovascular parameters such as SBP, DBP, PP, MAP, and HR in the group- II (AM) compared to group-III (PPW) with respect to control group.

**Table-5.10: Effects of the duration of exposure on cardiovascular parameters in study groups.**

| Groups | Parameters | Group-IIA (n = 38) | Group-IIB (n = 17) | Group-IIB (n = 15) | F- value | P-value |
|---|---|---|---|---|---|---|
| Group-II (AM) (n=70) | S B P (mmHg) | 121.95 ± 7.75 | 125.18 ± 10.54 | 136.27 ± 14.75[b,c] | 41.53 | 0.000* |
| | D B P (mmHg) | 81.79 ± 6.63 | 83.88 ± 5.45 | 85.47 ± 9.49 | 1.589 | 0.212 NS |
| | P P (mmHg) | 48.33 ± 5.774 | 42.86 ± 5.233 | 39.87 ± 6.806 [b,c] | 8.478 | 0.001 * |
| | MAP (mmHg) | 95.18 ± 5.388 | 97.65 ± 6.596 | 102.40 ± 9.791[b,c] | 31.056 | 0.004* |
| | HR /min | 72.68 ± 6.07 | 75.12 ± 5.34 | 82.00 ± 4.54[b,c] | 14.85 | 0.000* |
| Group-III (PPW) (n=70) | **Parameters** | **Group-IIIA (n = 30)** | **Group-IIIB (n = 28)** | **Group-IIIC (n = 12)** | **F- value** | **P-value** |
| | S B P (mmHg) | 119.40 ± 6.81 | 126.36 ± 4.00[a] | 136.67 ± 4.38[b,c] | 44.21 | 0.000* |
| | D B P (mmHg) | 79.53 ± 4.38 | 83.50 ± 5.90 [a] | 88.33 ± 4.96[b,c] | 13.24 | 0.000* |
| | PP (mmHg) | 48.33 ± 5.774 | 42.86 ± 5.233 | 39.87 ± 6.806 [b,c] | 8.47 | 0.001* |
| | MAP (mmHg) | 92.82 ± 4.240 | 97.78± 4.742 [a] | 104.4 ± 3.921 [b,c] | 31.05 | 0.000* |
| | HR /min | 72.53 ± 4.577 | 75.61 ± 4.202 [a] | 79.67 ± 2.06[b,c] | 13.45 | 0.000* |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics.Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB-6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in petrol pump workers.* depicts 'P' value ≤ 0.05 significant, while 'P' value> 0.05 not significant (NS).Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test' was done. Superscript a, b, c expresses the significant difference amongst the (IIA and IIB), (IIA and IIC), (IIB and IIC) and (IIIA and IIIB), (IIIA and IIIC), (IIIB and IIIC) respectively.

Table 5.10 indicates the effect of duration of exposure on cardiovascular parameters amongst group II and III. We observed as the duration of exposure increases there was a significant increase in systolic blood pressure (SBP), pulse pressure (PP), mean arterial pressure (MAP) and heart rate (HR) amongst group-II and III. Diastolic blood pressure (DBP) was significantly increased only in group-III. Significantly increased mean values of

SBP, PP, MAP, and HR were found in group-IIC compared to IIB. Significant increase in SBP, PP, MAP, and HR observed in Group-IIIC compared to IIIB. Significant increase in DBP was noticed in group-IIIC as compared to group-IIIA and IIIB. Insignificantly increased DBP was observed in group-II (AM).

**5.7.3: Discussion:**

Several clinical and epidemiological studies believed that the pathogenesis of heavy metal lead exposure in the development of hypertension is multifactorial. The finding of this study showed that occupational exposure to petroleum and exhaust fumes resulted in a significant increase in SBP, and MAP in petrol pump workers and automobile mechanics. Insignificant increase in diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR) was observed in group-II (AM) and group-II (PPW).

This could be due to inhalation of petroleum and exhaust fumes which enhanced the sensitization of myocardium to catecholamine, impaired vagal tone & increased baroreceptor activity with resultant vasoconstriction and increases arterial blood pressure. Also lead has both direct and indirect effects on the blood vessel and the smooth muscle contractility and it affects blood pressure. Particulate matter (PM) present in exhaust fumes may also elevate blood pressure by inducing autonomic nervous system imbalance and vasoconstriction. It is possible that $PM_{2.5}$ interaction with nociceptive or noradrenergic receptors that can stimulate the sympathetic nervous system (SNS), directly via vasoconstrictive effects of norepinephrine and raising circulating levels of the vasoconstrictor agent Angiotensin-II (Yuanyuan Cai *et al.* 2016).

Gamma glutamyl transferase is also regarded as a cardiovascular risk factor and has been identified as a predictor of cardiovascular mortality. Increased GGT activity found in the present study could be associated with the presence of many cardiovascular risk factors such hypertension, diabetes mellitus, myocardial infarction, metabolic syndrome, and obesity (Yavuz B. B. *et al.* 2008).

Many experimental studies explained the hypertensive effect of lead by various mechanisms. These mechanisms result from the action of lead on the central, peripheral nervous system and on the vessel wall. Heavy metal lead act by promoting sympathetic hyperactivity and by increasing the activity of renin-angiotensin system, by depressing the antioxidant reserves and / or increasing the production of reactive oxygen species (ROS), resulting in oxidative stress (Nunes KZ *et al.* 2015); and by altering the vascular response to vasoactive agents that promote endothelial damage and reduce availability of nitric oxide

(Silveira EA *et al.* 2014); leading to an increase in the vascular tone, and the peripheral vascular resistance.

The present study indicates the change of vascular response to stress. This suggests that lead-induced nephrotoxicity is the probable cause of increased blood pressure in automobile mechanics and petrol pump workers. Fluid & electrolyte retention may be an additional possible mechanism responsible for lead-induced hypertension.

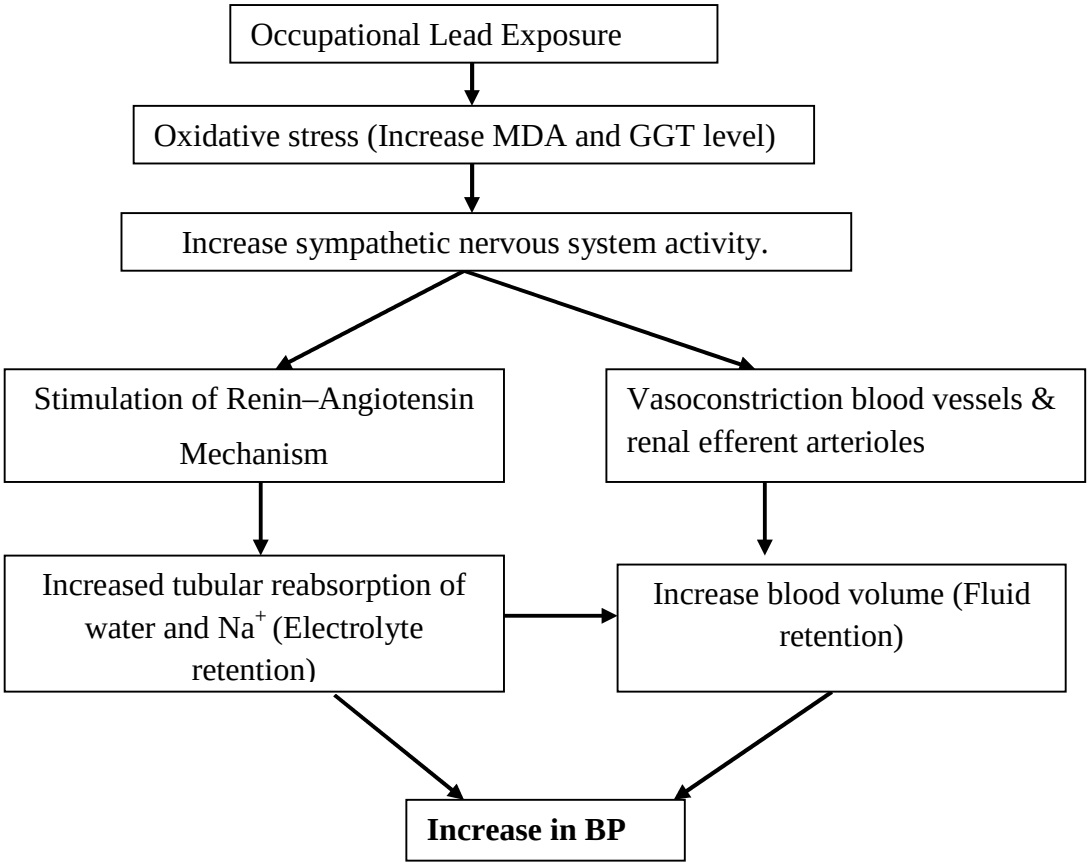



**Figure 5.6:** Possible mechanism for the lead-induced increase in blood pressure.

Our results also are consistent with the result of Dongre NN *et al.* reported slightly increased systolic and diastolic blood pressure in automobile workers as compared to the control group. This indicates that increased blood lead does not alter blood pressure severely, but intense and prolonged exposure to lead might be a cause of hypertension in these workers (Dongre NN *et al.*2011).

A previous animal study reported that inhalation of petroleum hydrocarbons elicited vasoconstriction and impaired vascular tone and increases blood pressure. This finding suggests that petroleum hydrocarbons may have a pressor effect on cardiovascular functions. Our findings correspond with these findings (Azeez OM *et al.* 2012).

Findings of our study coincide with findings of other studies which shows inhalation of petroleum fumes led to a significant increase in systolic, diastolic blood pressure and heart rate in petrol pump workers. This may be due to the ability of petroleum hydrocarbon to enhance the sensitization of myocardium to catecholamine, impaired vagal activity & increased baroreceptor activity with resultant vasoconstriction and increased arterial blood pressure (Patil SV *et al*. 2017; Tiu DN *et al.* 2017*).*

Results of our study correspond with the results made by Zerihun Ataro *et al.* (2018); who reported significantly increased mean systolic ($P<0.0001$) and diastolic blood pressure ($P<0.0001$) in garage workers compared to the control group. This could be due to the occupational exposure to chemicals like benzene, lead and other organic solvent alters blood pressure.

Similarly, higher values of both systolic and diastolic blood pressures were reported in the auto-mechanics. This may be associated with the nephrotoxic effect of lead which indirectly affects blood pressure. The subsequent alterations in tubular function could lead to salt retention and volume overload, eventually resulting in elevated blood pressure (Obi-Ezeani CN *et al.* 2019).

Contrary to these findings, Ajani EO *et al*. (2011); observed no significant differences in the systolic and diastolic blood pressures in automobile mechanics as compared to control the population.

Our observations suggested that the inhalation of petroleum and exhaust fumes increases blood pressure; this is usually because of excessive occupational exposure to heavy metal lead. Lead induces the oxidative stress and it affects the kidney. Kidney compromise and in turn it affects the blood pressure. Duration of exposure has a significant impact on blood pressure and heart rate. This is evidenced by increased SBP, PP, MAP, and HR with increased duration of exposure amongst automobile mechanics and petrol pump workers.

## 5.8: Respiratory changes amongst study and control groups.

### 5.8.1: Results

**Table-5.11: Respiratory parameters amongst study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F-value | P-value |
|---|---|---|---|---|---|
| 40 mmHg test (Sec.) | 34.29 ± 9.88 | 24.64 ± 10.25 | 25.63 ± 10.03[b,c] | 19.48 | 0.000 * |
| PEFR (L/Min) | 523.29 ± 71.24 | 360.43 ± 94.26 | 404.0 ± 93.71[b,c] | 65.64 | 0.000 * |

Group-I–control group, Group-II - Automobile Mechanics (AM), Group-III - Petrol Pump Workers (PPW). * depicts 'P' value≤0.05 is significant while 'P' value> 0.05 -not significant (NS). Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and II), (Group-II and III) respectively. PEFR–Peak expiratory flow rate.

Table-5.11 represents lung function tests in the study and control group. We observed a statistically significant decrease in 40 mmHg endurance time and peak expiratory flow rate (PEFR) amongst group-II and group-III as compared to group-I. Significant decrease in 40 mmHg endurance time and PEFR was observed in group-II as compared to group-III.

### 5.8.2: Percentage change impact on respiratory parameters.

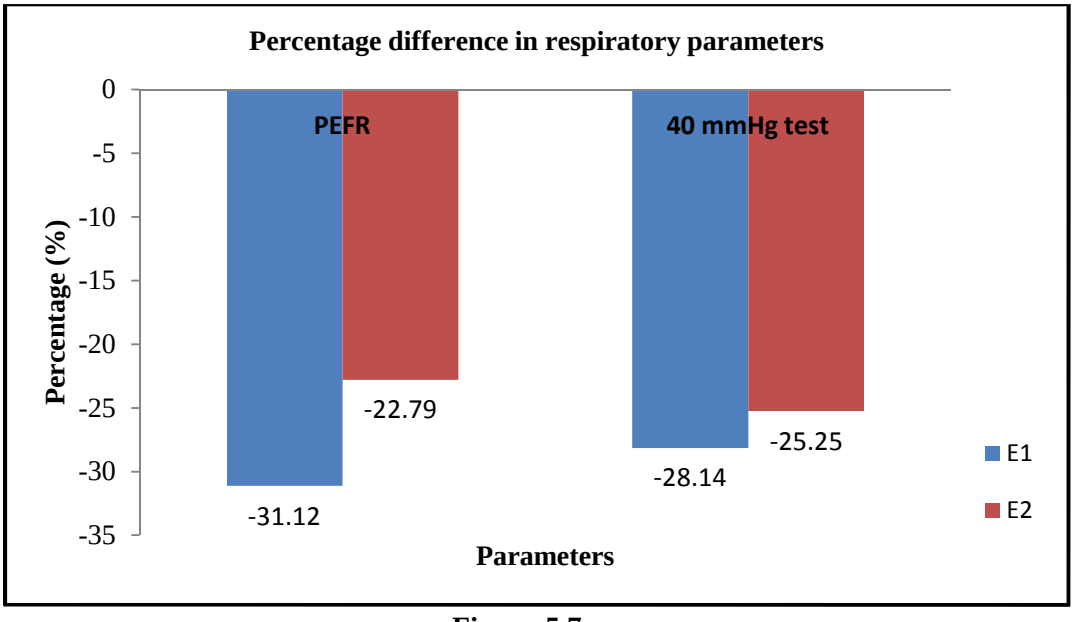



**Figure-5.7**

**Figure-5.7:** Percentage change impact on respiratory parameters in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure-5.7 depicts there was a greater decline in percentage difference of respiratory parameters like PEFR and 40 mmHg endurance tests in group-II (AM) compared to group-III (PPW) with respect to the control group.

**Table –5.12: Effects of duration of exposure on respiratory parameters (Lung Function tests) in study groups.**

| **Groups** | **Parameters** | **Group-IIA (n = 38)** | **Group-IIB (n = 17)** | **Group-IIB (n = 15)** | **F-value** | **P-value** |
|---|---|---|---|---|---|---|
| Group II (Automobile Mechanics) (n=70) | 40 mmHg test (Sec.) | 28.11 ± 8.12 | 21.24 ± 4.51 [a] | 19.73 ± 15.75 [b] | 5.447 | 0.006* |
| | PEFR (L/Min) | 443.42 ± 77.68 | 368.24 ± 73.93 [a] | 344.67 ±107.43 [b] | 9.477 | 0.000* |
| Group III (Petrol Pump Workers) (n=70) | **Parameters** | **Group-IIIA (n = 30)** | **Group-IIIB (n = 28)** | **Group-IIIC (n = 12)** | **F-value** | **P-value** |
| | 40 mmHg test (Sec.) | 30.47 ± 12.03 | 23.93 ± 5.83 [a] | 17.50 ± 4.79 [b] | 9.839 | 0.000* |
| | PEFR (L/Min) | 381.33 ± 91.86 | 360.00 ± 92.06 | 309.17 ± 92.88 | 2.631 | 0.079 NS |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics.Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB -6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in petrol pump workers.* depicts 'P' value $p \leq 0.05$ significant, while p-value> 0.05 not significant (NS).Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c represent the significant difference amongst (IIA and IIB), (IIA and IIC), (IIB and IIC) and (IIIA and IIIB), (IIIA and IIIC), (IIIB and IIIC) respectively.

Table-5.12 describes the effects of duration of exposure on respiratory parameters. 40 mmHg endurance time was significantly reduced with increased duration of exposure in group-II and III. As the duration of exposure increases there was a significant decrease in peak expiratory flow rate (PEFR) in group-II and insignificantly reduced in group-III. We observed a significant decrease in PEFR & 40 mmHg endurance time amongst group-IIB

and IIC compared to group-IIA. Also significant decrease in 40 mmHg endurance time was observed in group-IIIB and IIIC as compared to group-IIIA.

### 5.8.3: Discussion

This significant decrease in PEFR and 40 mmHg endurance time in automobile mechanics and petrol pump workers could be due to,

1. Petroleum and exhaust fumes contain several harmful substances like oxides of nitrogen, sulfur dioxide, carbon monoxide, carbon dioxide, hydrocarbons, unburned carbon particles (soot), benzene, heavy metals, and particulate matters. The particles generated from the exhaust are very small in size of about 0.2 nm. These small particles are easily inhaled and deposit in the lungs. Organic compounds like polycyclic aromatic hydrocarbons and metals adhere to these particles and are carried deeper into the smaller airways of the lungs. These Particulate and gases pollutants may alter the properties and concentration of surfactant. Thus it contributes to the early closure of small airways (Anupama N *et al.* 2012). Hence chronic exposure to them can lead to chronic inflammation of respiratory tract and lung parenchyma. This suggests that a significant decrease in PEFR and 40 mmHg endurance time due to greater involvement of smaller airways.
2. Petroleum hydrocarbons & heavy metal lead exert an irritant effect on the bronchial epithelium; it also affects the cilia and clara cells of the lung and releases proteolytic enzymes from macrophages. A petroleum hydrocarbon impairs the function of type II pneumocytes resulting in a decreased production of surfactant and reduces elastic recoil of the lungs (Azeez *et al.* 2012). Petroleum hydrocarbons and heavy metal also considered as very triggering factors for oxidative stress. Increased oxidative stress leads to the loss of cellular and tissue integrity.
3. Particulate matter ($PM_{10}$) present in automobile exhaust has the greatest effect on human health. PM with a diameter of between 5 and 10 μm is more likely to be deposited in the tracheobronchial tree, whereas PM between 1 and 5 μm can move down to the respiratory bronchioles and alveoli. PM < 1 μm in diameter can penetrate the alveoli and can translocate into cellular tissues and the circulatory system (Eun-A Kim 2017).

Hence, chronic exposure to them can lead to chronic inflammation of the respiratory tract and lung parenchyma. This small airway damage induced by oxidative stress might be the cause for reduced PEFR & 40 mmHg endurance time in automobile mechanics and

petrol pump workers. It was found that petrol pump workers and automobile mechanics are prone to developing small airway disease.

**Possible Mechanism:**

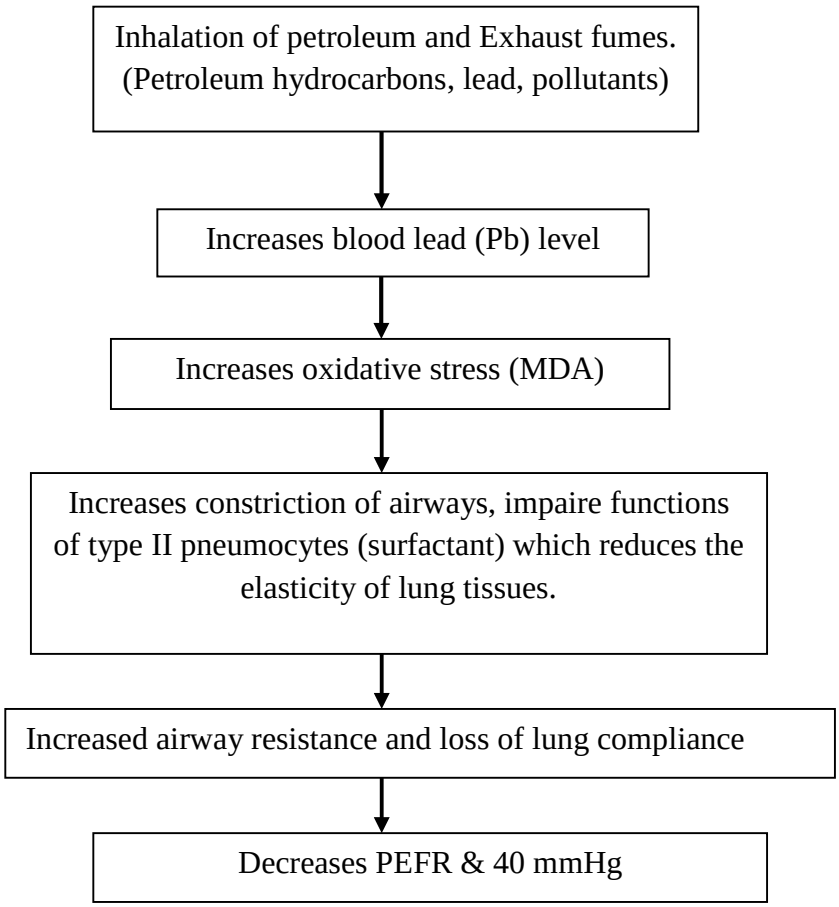



**Figure 5.8:** Possible mechanism for decreased PEFR & 40 mmHg endurance time in occupationally exposed workers.

Decreased PEFR value suggests a restrictive pattern of respiratory diseases. This can be due to continuous exposure of petroleum and exhaust (pollutants) fumes in the working environment. Supporting this statement, a study showed a significant decrease in lung functions can be seen due to their constant inhalation of pollutant emitted by vehicles during filling petrol and diesel at petrol pumps (Sehgal M *et al*. 2011).

Our findings were also supported by the findings of Patil SV *et al.* (2017); reported a significant decrease in breath-holding time and 40 mmHg endurance time in petrol pump workers might be due to petroleum hydrocarbons decreases tolerance to higher $PCO_2$ and low $PO_2$. The mean value of breath-holding time & 40 mmHg endurance time were significantly decreased with increase in the duration of exposure.

Significantly decreased PEFR was observed in the garage workers than the control. Bhardwaj SS *et al.* suggested that peak expiratory flow rate is used to assess the functional status of the airways. They also noticed with increasing duration of exposure to auto exhaust emission; there was a decrease in pulmonary functions (PEFR) in garage workers (Bhardwaj SS *et al.* 2018).

Another study also reported a highly significant decrease in mean PEFR of automobile mechanics than the control subjects. This decreased PEFR in automobile mechanics was due to continuous exposure to dust, fumes, auto exhaust and other pollutants at their workplace (Chandel Rashmi *et al.* 2019).

Contrary to our findings, Anupama N *et al*. observed no significant change in PEFR values, of automobile mechanics and the control population. This indicates automobile mechanics was not showing the obstructive pattern of lung diseases (Anupama N *et al*. 2012).

PEFR and 40 mmHg endurance time was decreased with increased duration of exposure. These findings are in accordance with the findings of earlier studies done by Dube Sushil. *et al.* (2013); Santhalingam B. *et al* (2017); and Kannan GK *et al.* (2018).

Our results indicate that as the duration of exposure increases the accumulation of heavy metal lead within the body also increases. This leads to an increase in oxidative stress (MDA) which decreases PEFR and 40 mmHg endurance time in the study group. This finding suggests that exposure to petrol vapors, fumes, diesel exhaust, and airborne particulate matter leads to impairment in lung functions.

## 5.9: Hematological changes in study and control groups.

### 5.9.1: Results

**Table-5.13: Hematological parameters amongst study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F- value | P- value |
|---|---|---|---|---|---|
| Hb % | 15.429 ± 1.24 | 14.66 ± 1.79 | 15.017 ± 1.73 [c] | 4.024 | 0.019* |
| R.B.Cs Count(millons/$mm^3$) | 5.38 ± 0.89 | 4.85 ±0.81 | 5.06 ± 0.64[b,c] | 8.106 | 0.000* |
| TLC ( /Cu mm) | 7024.28 ± 1241.135 | 9717.14 ± 3589.07 | 9404.28 ± 3844.00[b,c] | 15.600 | 0.000 * |
| Platelet count (Lakh /$mm^3$) | 2.51 ± 0.53 | 2.9670 ± 0.72[a] | 2.6477 ± 0.61[b,c] | 9.586 | 0.000* |
| Poly morphs (%) | 60.46 ± 7.710 | 63.77 ± 8.412 | 60.40 ± 10.553 | 3.239 | 0.041* |
| Lymphocyte (%) | 30.64 ± 8.964 | 35.31 ± 7.310 | 32.70 ± 8.837 [c] | 5.433 | 0.005* |
| Eosinophil (%) | 1.86 ± 0.643 | 2.47 ± 1.954 | 2.37 ± 1.144 [c] | 4.117 | 0.018* |
| Basophil (%) | 0.07 ± 0.310 | 0.26 ± 0.606 | 0.46 ± 0.928 [b] | 5.900 | 0.003* |
| Monocyte (%) | 2.41 ± 1.028 | 2.87 ± 1.227 | 3.29 ± 2.141[b] | 5.583 | 0.004* |
| PCV (%) | 46.16 ± 3.39 | 45.27 ± 5.32 | 43.89 ±4.46 [b] | 4.605 | 0.011* |
| M.C.V. (fL) | 90.98 ± 8.15 | 85. 29± 8.914 | 85. 94± 9.82[b,c] | 8.402 | 0.000* |
| M.C.H. (pg) | 31.01 ± 3.59 | 28.27 ± 3.51 | 29.04 ± 3.36[b,c] | 11.531 | 0.000* |
| M.C.H.C % | 33.99 ± 2.29 | 32.04 ± 2.38 [a] | 33.41 ± 2.00 [c] | 14.090 | 0.000* |

Group I–Control Group, Group II - Automobile mechanics (AM), Group III - Petrol pump workers (PPW). * depicts 'P' value P≤0.05 is significant. Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c indicates the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.13 represents hematological changes in the study and control groups. We observed a statistically significant reduction in Hb%, RBC count, PCV& blood indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in group-II and III as compared to group-I. Statistically significant increase in platelet count, total and differential leucocytes count (polymorphs, lymphocyte, eosinophil, basophil & monocyte) were observed amongst group-II and III as compared to group-I. We found a significant increase in platelet count, TLC, monocytes, lymphocytes, basophil and eosinophil count in group-II as compared to group-III. Significant increase in platelet count, TLC, monocytes, basophils, eosinophils were noticed in group-III compared to group-I. Significant decrease in RBC count, PCV, MCV & MCH were observed in group-III compared to group-I. We noticed a significant decrease in Hb%, RBC count & blood indices like MCV, MCH, MCHC in group-II compared to group-III. The observed difference in all hematological parameters was within the normal range.

**5.9.2: Percentage change impact on haematological parameters.**

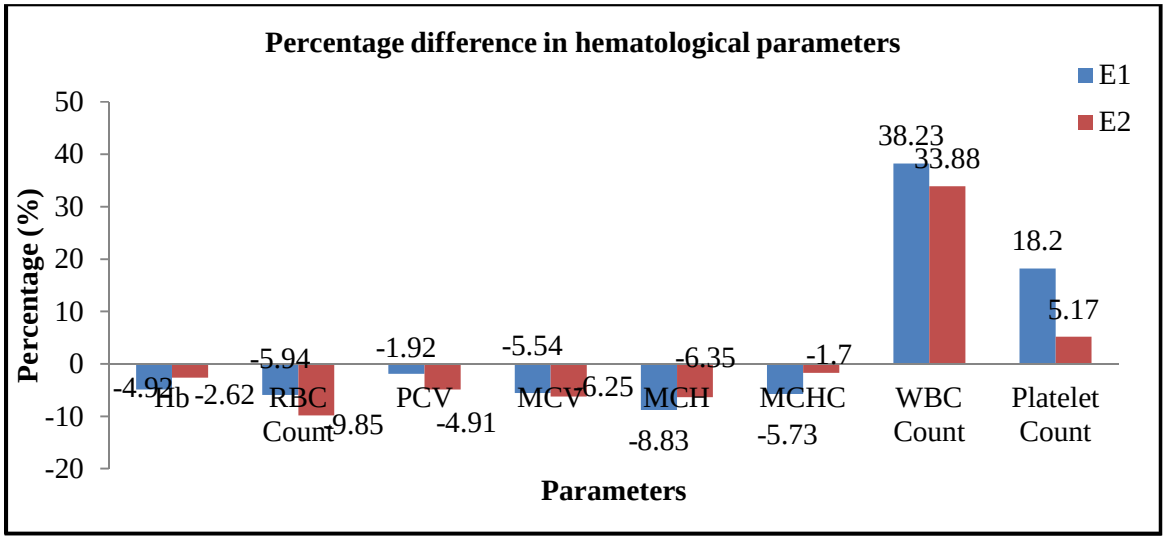



Figure 5.9:- Percentage change impact on hematological parameters in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$ = (Group I- Group III) considering group-I as a control group.

Figure 5.9 shows a decrease in percentage difference of Hb%, RBC count and blood indices (MCV, MCH, and MCHC) among group-II (AM) compared to group-III (PPW) with respect to control group. Total WBC and platelet count were increased to a greater extent in group-II (AM) compared to group-III (PPW) with respect to the control group.

**Table-5.14: Effect of duration of exposure on hematological parameters in group-II workers. (Automobile mechanics).**

| Parameters | Group-IIA (n = 38) | Group-IIB (n = 17) | Group-IIC (n = 15) | F- value | P-value |
|---|---|---|---|---|---|
| Hb % | 15.266 ± 1.57 | 14.724 ± 1.34 | 13.053 ± 1.87 [b, c] | 10.490 | 0.000* |
| R.B.Cs Count | 5.175 ± 0.58 | 4.873 ± 0.56 | 3.993 ± 0.94 [b, c] | 16.919 | 0.000* |
| TLC ( /Cu mm) | 7944.74 ± 1733.72 | 9741.176 ± 3028.41 | 14180.0 ± 3898.02 [b, c] | 29.761 | 0.000 * |
| Platelet count (Lakh/$mm^3$) | 3.059 ± 0.82 | 3.076 ± 0.57 | 2.611 ± 0.54 | 2.388 | 0.100 NS |
| Poly morphs (%) | 63.00 ± 8.16 | 64.76 ± 10.09 | 64.60 ± 7.26 | 0.344 | 0.710 NS |
| Lymphocyte (%) | 31.21 ± 9.69 | 30.35 ± 9.28 | 29.53 ± 6.87 | 0.195 | 0.823 NS |
| Eosinophil (%) | 2.58 ± 2.49 | 2.12 ± 1.17 | 2.60 ± 0.83 | 0.362 | 0.698 NS |
| Basophil (%) | 0.24 ± 0.49 | 0.18 ± 0.53 | 0.40 ± 0.910 | 0.581 | 0.562 NS |
| Monocyte (%) | 2.76 ± 1.28 | 2.76 ± 1.25 | 3.27 ± 1.03 | 0.991 | 0.377 NS |
| PCV (%) | 47.04 ± 2.49 | 46.95 ± 3.62 | 45.46 ± 3.57 | 1.847 | 0.166 NS |
| M.C.V. (fL) | 88.15 ± 8.82 | 84.71 ± 8.13 | 81.71 ± 8.75 [b] | 3.213 | 0.047 NS |
| M.C.H. (pg) | 28.69 ± 3.55 | 28.10 ± 3.85 | 27.38 ± 3.06 | 0.774 | 0.465 NS |
| M.C.H.C % | 32.69 ± 1.34 | 32.07 ± 1.85 | 30.35 ± 3.87 [b] | 5.959 | 0.004* |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics.* depicts 'P' value $\leq 0.05$ significant, while 'P' value> 0.05 not significant (NS). Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c expresses the significant difference amongst groups (IIA and IIB), (IIA and IIC) and (IIB and IIC) respectively.

Table 5.14 indicates the effect of duration of exposure on hematological parameters in group-II workers (AM). With an increase in the duration of exposure, there was a

significant decrease in Hb%, RBC count, MCHC in group-II, but the total WBC count was significantly increased in group-II. Significantly reduced Hb%, RBC count, MCV, MCHC was observed in group-IIC as compared to group-IIA. Significant increase in TLC was observed in group-IIC as compared to group-IIA. Significant decrease in Hb%, RBC count &increase in TLC was found in group-IIC as compared to group-IIB.

**Table-5.15: Effect of duration of exposure on hematological parameters in group-III (Petrol pump workers).**

| Parameters | Group-IIIA (n = 30) | Group-IIIB (n = 28) | Group-IIIC (n = 12) | F- value | P-value |
|---|---|---|---|---|---|
| Hb % | 15.51 ± 1.83 | 14.89 ± 1.79 | 14.08 ± 0.57 [b] | 3.220 | 0.046* |
| R.B.Cs Count millions /cu mm | 5.27 ± 0.59 | 5.08 ± 0.53 | 4.50 ± 0.70[b,c] | 7.429 | 0.001 * |
| TLC ( /Cu mm) | 7633.3 ± 2432.4 | 9210.71 ± 3374.7 | 14283.3 ± 3809.99[b,c] | 19.971 | 0.000* |
| Platelet count (Lakh / Cu mm) | 2.74 ± 0.66 | 2.55 ± 0.59 | 2.65 ± 0.59 | 0.665 | 0.517 NS |
| Poly morphs (%) | 61.50 ± 11.50 | 58.46 ± 9.66 | 62.17 ± 10.21 | 0.797 | 0.455 NS |
| Lymphocyte (%) | 30.93 ± 9.247 | 34.79 ± 8.203 | 32.25 ± 8.915 | 1.411 | 0.251 NS |
| Eosinophil (%) | 2.20 ± 1.06 | 2.61 ± 1.37 | 2.25 ± 0.62 | 0.998 | 0.374 NS |
| Basophil (%) | 0.57 ± 1.07 | 0.21 ± 0.49 | 0.75 ± 1.22 | 1.808 | 0.172 NS |
| Monocyte (%) | 3.07 ± 2.10 | 3.50 ± 2.15 | 3.33 ± 2.35 | 0.294 | 0.746 NS |
| PCV (%) | 43.54 ± 5.23 | 43.88 ± 1.62 | 42.17 ± 4.54 | 0.162 | 0.851 NS |
| M.C.V. (fL) | 86.73 ± 8.22 | 84.78 ± 12.32 | 82.90 ± 6.51 | 0.707 | 0.497 NS |
| M.C.H. (pg) | 28.98 ± 3.33 | 29.12 ± 3.84 | 29.00 ± 2.30 | 0.013 | 0.987 NS |
| M.C.H.C % | 33.49 ± 1.00 | 33.86 ± 2.51 | 32.14 ± 2.17 [c] | 3.370 | 0.040 * |

Group- IIIA-1 to 5 yrs. of exposure, Group- IIIB - 6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in Petrol pump workers.* depicts 'P' value ≤ 0.05 significant, while 'P' value > 0.05 not significant (NS). Data are presented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference amongst groups (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively.

Table 5.15 indicates the effect of duration of exposure on hematological parameters in group-III. We observed as the duration of exposure increases there was a significant decrease in Hb%, RBC count, MCHC in group-III. With an increase in the duration of exposure, there was a significant increase in total leucocytes count in group-III. Significantly decreased Hb%, RBC count, MCHC was found in group-IIIC as compared to group-IIIA. There was a significant increase in TLC was observed in group-IIIC as compared to group-IIIA. There was a significant decrease in RBC count, MCHC and a significant increase in TLC in group-IIIC as compared to group-IIIB.

**5.9.3: Discussion:**

Complete blood count (CBC) has been considered as an easy and quickly available screen for hematotoxicity following occupational exposure.

The present study shows a significant decrease in hematological parameters like Hb, RBC count, PCV & blood indices (MCV, MCH, MCHC) in AM and PPW which could be due to decreased heme concentrations or decreased erythropoietin hormone or decreased iron absorption, or decreased maturation of RBC by petroleum hydrocarbons and heavy metal lead.

Exposure to heavy metals present in petroleum fumes may suppress the bone marrow's activity leading to ineffective hematopoiesis. Lead affects the hematopoietic system and decreases the Hb synthesis, but this occurs only with high levels of exposure. It might be due to decreased haem and globin synthesis or erythrocyte formation and function. In experimental lead-poisoned rats, it is observed that the globin synthesis is inhibited by lead in bone marrow cells at a concentration as low as 1 µmol/L and this may decrease the erythrocyte formation (Dresner DL 1982). Erythrocyte formation is regulated by erythropoietin hormone and the serum level of this hormone is decreased by the lead (Patil AJ. 2004).

Benzene is the major noxious substance present in petroleum fumes, which is capable of covalent interaction with cellular macromolecules, such as DNA, as well as it inhibits the protein and RNA synthesis. This could result in bone marrow depression with

inadequate production of hemoglobin and RBC. Benzene and a heavy metal lead have the ability to induce oxidative stress with a consequent alteration in the DNA structure, which leads to mutagenesis and it alters the normal hematopoietic process. Benzene has the potential to inhibit erythropoietin release from the kidneys (Murtala BA *et al.* 2015).

Oxidative stress-induced by heavy metal lead disrupts the membrane integrity and this could possibly have accounted for the susceptibility of the red cell membrane to oxidative attack giving way to hemolysis. Lead is known to have some toxic effects on membrane structure and functions. It alters the RBC membrane since RBCs have a high affinity for lead and the majority of the lead is found in RBCs. Hence, RBCs are more vulnerable to oxidative damage than any other cells.

Anemia has been commonly associated with the adverse effects of occupational lead exposure. Decreased RBC count, Hb % and blood indices in the present study might be due to nutritional deficiency of iron, folic acid and vitamin $B_{12}$ in the diet, this is evidenced by their economic and educational status in this study.

**Mechanism:**

A number of mechanisms have been proposed to explain the adverse effect of lead on the hematopoietic system. These include lead-induced oxidative stress and interference of lead with enzymes of the haem biosynthetic pathway**.**

Heavy metal lead inhibits several steps involved in the biosynthesis of haem. Lead interferes with heme biosynthesis by altering the activity of three key enzymes d-aminolevulinic acid synthetase (d-ALAS), d-aminolevulinic acid dehydratase (d-ALAD) and ferrochelatase. Lead indirectly stimulates the mitochondrial enzyme ALAS, which catalyzes the condensation of glycine and succinyl Co-A to form - aminolevulinic acid or ALA. Lead also inhibits the zinc-containing cytosolic enzyme ALAD, which catalyzes the condensation of two units of ALA to form porphobilinogen. Finally, the mitochondrial enzyme ferrochelatase catalyzes the insertion of a ferrous ion ($Fe^{2+}$) into protoporphyrin IX to form heme it is also impaired by lead (Patil Arun J *et al.* 2007). Heavy metal lead also depresses serum level of erythropoietin; a hormone that regulates erythrocyte formation.

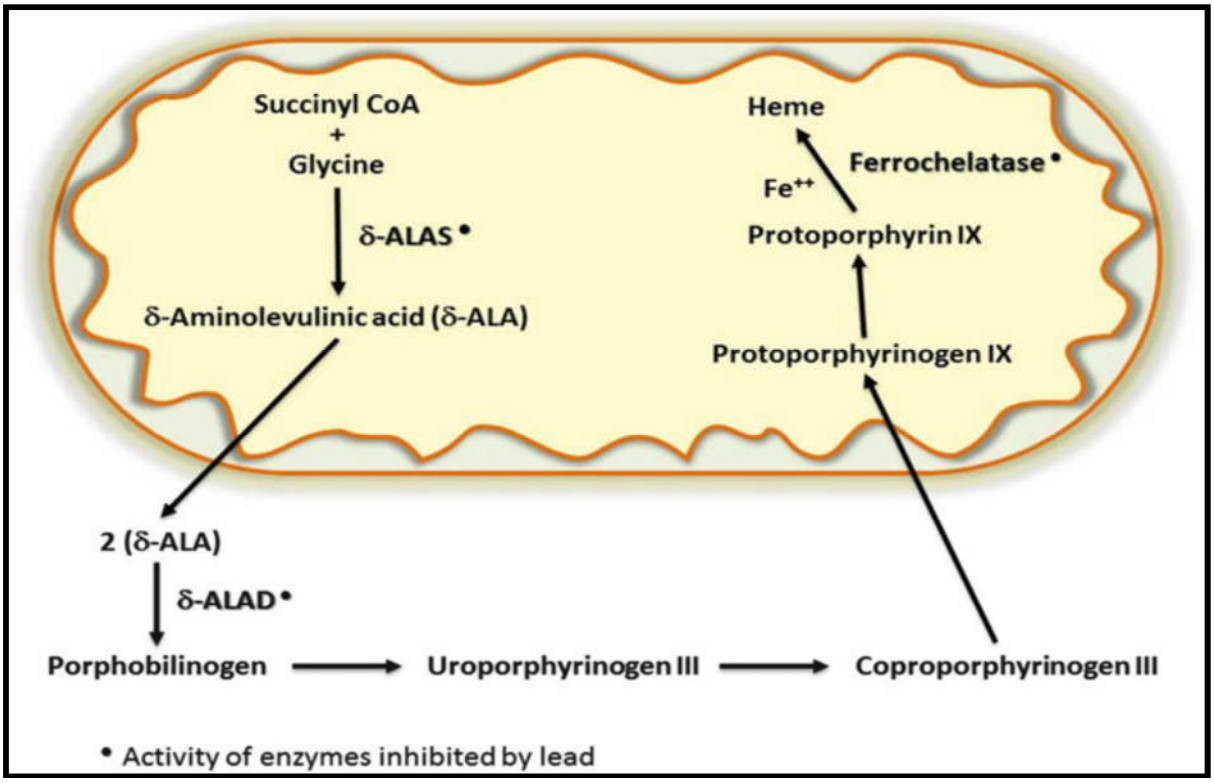



**Figure 5.10:** Effect of lead on the hemopoietic system.

**Source:** Oluwatobi AKEFE.*et al.* Mitigate the effects of antioxidants in Lead Toxicity. Clin Pharmacol Toxicol Jour.2017; 1(1:3):1-9.

Petroleum and exhaust fumes are therefore environmental pollutants that could have a serious consequence on hematological parameters in exposed humans. The hematopoietic system is highly sensitive to most of the air pollutants because these cells recycle continuously. The solvents and air pollutants may lead to defective heme synthesis and reduced life expectancy of red blood cells.

Significantly increased total & differential WBC counts in occupationally exposed AM and PPW could be due to more occupational exposure to dust or fumes of petroleum hydrocarbon & possible heavy metal lead. This also may be due to certain inflammatory and infectious conditions. Significant increase in the neutrophil count found among occupationally exposed groups due to the stressful nature of their work, with the resultant increase in the generation of free radicals with resultant cellular damage. The immune system responds to these damages caused by the production of oxidants during stressful conditions. This is suggestive of a high degree of infection. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages (Ear T. *et al.* 2008). During the periods

of stress, neutrophils from the marginated pool can be recruited to the circulating pool, thereby rapidly increasing the circulating neutrophil count. Eosinophils primarily are associated with parasitic infections and an increase in their number may indicate parasitic infection. Significant increase in platelet count (PLT) could be due to petroleum hydrocarbons may have catalyzed the activity of the bone marrow to produce more platelets (Seriki SA *et al*. 2016).

Significantly reduced RBC count, Hb% observed in car mechanics in car repair shops of Zagazig City. This might be due to exposure to organic solvents especially benzene, has been shown to have a deleterious effect on bone marrow which are in accordance with the results of the present study (El-Saadawy M.S. *et al*. 2011).

In automobile workers, the levels of Hb%, hematocrit (PCV), MCV, MCH, MCHC, RBC count were significantly decreased as compared to control group while total WBC count was increased as compared to the controls. This clearly indicates that the absorption of lead is more in automobile workers and it affects the heme biosynthesis and altered hematological parameters in this study group (Dongare NN *et al.* 2011).

Another study shows a significant increase in total WBC count and platelet count in gasoline vapor exposed rat could be as a result of the body's defense mechanism trying to protect the body from being vulnerable to infections having been poisoned by a foreign body (gasoline). It implies that gasoline has the potential to inhibit erythropoietin release from the kidneys to enhance erythropoiesis. Findings of the present study correspond with these findings (Seriki SA *et al*. 2016).

Similarly, Lavanya M. *et al.* observed a statistically significant reduction in RBC count, Hb%, among petrol pump workers when compared with the control group. It suggests that chronic exposure to petrol fumes has a toxic effect on hematological parameters leading to bone marrow depression. Contrary to our findings Lavanya M. *et al.* observed no significant changes in platelet count, while a significant decrease in WBC count among petrol pump workers and control groups (Lavanya M. *et al.* 2016).

Our findings are consistent with the findings of Zerihun Ataro *et al* (2018); they observed mean RBC count, hemoglobin level, hematocrit (PCV) and MCV values of garage workers were significantly lower than those of the control group. This might be due to decreased heme concentration, iron absorption, or maturation of RBCs by different toxic chemicals. Different chemical exposure times affect the hematopoietic system and result in altered hematological parameters. Significantly higher WBC and platelet counts were observed among garage workers as compared to the control group. This could be due to

certain inflammatory and infectious condition and more exposure to dust or fumes of toxic chemicals.

Our results disagree with the results of Rehab Adam Ahmed Hamad *et al.* 2015; showed significantly higher values of RBC count, hemoglobin level. MCV, MCHC and significantly reduced platelet count in benzene station workers than the control group. This might be due to stimulation of erythropoietin a factor which stimulates erythropoiesis which ultimately leads to the production of more RBCs and hemoglobin in circulating blood.

More reduction of RBC count, Hb%, MCH, and MCHC observed in automobile mechanics than petrol pump workers. This could be due to increased BLL in automobile mechanics than petrol pump workers. These findings disagree with the findings of Anslem OA *et al.* (2014); who observed more reduction in RBC count, Hb, MCH and MCHC in fuel attendants than auto mechanics when compared with the control. These could be due to the effects of benzene and xylenes. Fuel attendants exposed to gasoline fumes beyond 2 years have lower PCV, Hb, MCH, and MCHC than those exposed for 2 years or less. Duration of exposure plays a role in affecting hematological indices. Auto mechanics of over two years had their RBC count, Hb%, MCH, and MCHC significantly lower than auto mechanics of two years and below. This shows an association between duration of exposure and effect on hematological indices.

However, in the present study, all the parameters of total blood count and hemoglobin levels in occupationally exposed petrol pump workers and in automobile mechanics were within the normal range. These parameters also have prognostic value to monitor adverse effects of heavy metal lead in occupationally exposed workers. In the present study, the duration of exposure was also found to be an important predictor for most of the hematological parameters. Declines in hemoglobin, MCH, and MCHC were predicted by the duration of exposure in AM and PPW.

## Biochemical Changes amongst study and control group

## 5.10: Liver Function Tests

### 5.10.1: Results.

**Table-5.16: Liver function tests amongst study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F-value | P- value |
|---|---|---|---|---|---|
| S.G.O.T. (IU/L) | 31.59 ± 10.299 | 33.11 ± 12.855 [a] | 39.86 ± 18.314 [b] | 6.70 | 0.002* |
| S.G.P.T. (IU / L) | 29.84 ± 11.495 | 37.61 ±14.69 | 34.77 ± 25.40 [c] | 3.26 | 0.040* |
| Serum Alkaline Phosphatase (IU/L) | 62.20 ±15.31 | 83.88 ± 24.16 | 90.27 ± 25.33 [b,c] | 31.13 | 0.000* |
| Serum Bilirubin (total) (mg/dl) | 0.55 ± 0.23 | 0.85 ± 0.47 [a] | 0.66 ± 0.28 [c] | 12.98 | 0.000* |
| Serum Bilirubin (Direct) (mg/dl) | 0.32 ± 0.13 | 0.51 ± 0.33 [a] | 0.38 ± 0.18 [c] | 14.04 | 0.000* |
| Serum Bilirubin (Indirect)(mg/dl) | 0.240 ± 0.10 | 0.334 ± 0.19 | 0.354 ± 0.43 [b] | 3.39 | 0.036* |
| Serum Protein (Total) (gm %) | 7.07 ± 0.670 | 6.26 ± 0.965 | 6.55 ± 0.681 [b,c] | 18.72 | 0.000* |
| Serum Protein (Albumin) (gm% ) | 4.10 ± 0.44 | 3.87 ± 0.39 [a] | 3.89 ± 0.38 [b] | 6.87 | 0.001* |
| Serum Protein (Globulin) (gm%) | 2.95 ± 0.568 | 2.77 ± 0.485 | 2.74 ± 0.543 [b] | 3.24 | 0.041* |

Group I–Controls, Group II - Automobile mechanics (AM), Group III - Petrol Pump Workers (PPW). * depicts 'P' value ≤0.05 is significant. Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc t' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.16 represents the liver function tests amongst study and control groups. We observed a statistically significant increase in liver enzyme levels like serum glutamate oxalate transaminase (SGOT) or alanine aminotransferase (ALT), serum glutamate pyruvate transaminase (SGPT) or aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (total, direct and indirect) amongst group-II and group-III as compared to group-I. There is a statistically significant decrease in serum proteins (total, albumin, globulin) observed amongst group-II and III as compared to group-I. We found a significant decrease

in serum SGOT and albumin in group-II as compared to group-I. Statistically significant increase in serum bilirubin (total and direct) was observed in group-II as compared to group-I. We observed a statistically significant increase in serum SGPT, ALP, bilirubin (total, direct) level in group-II as compared to group-III. Significant decrease in serum total protein observed in group-II as compared to group-III.

### 5.10.2: Percentage change impact on Liver functions.

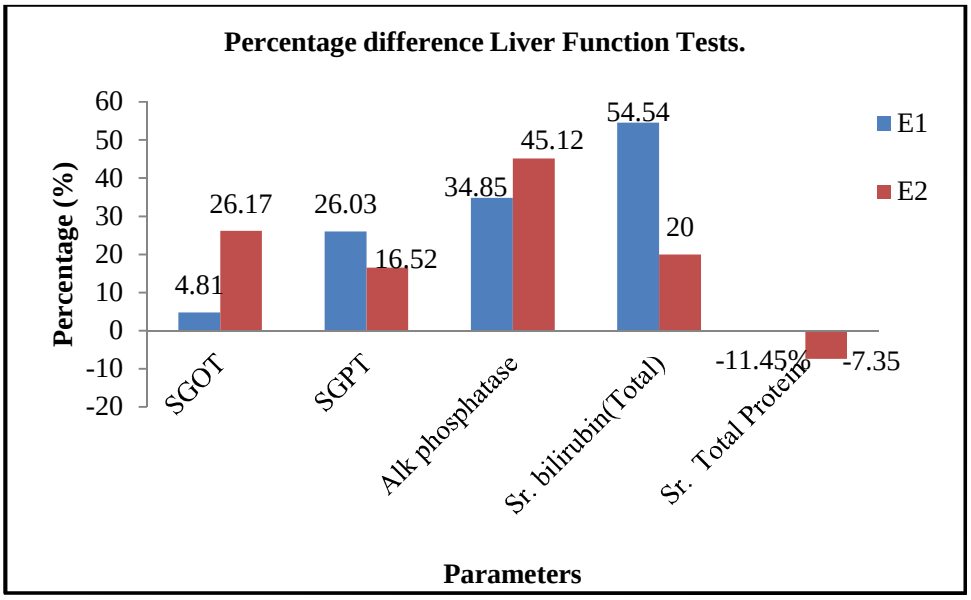



**Figure 5.11:** Percentage change impact on liver functions parameters in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure 5.11 depicts the percentage change impact on liver functions. There was a greater increase in percentage difference of serum SGOT, SGPT, ALP level among group-III (PPW) compared to group-II (AM). Percentage difference of serum total bilirubin was more in Group-II compared to group-III. The more decline in percentage difference of serum total protein was observed ingroup-II (AM) compared to group-III (PPW) with respect to the control group.

**Table- 5.17: Effect of duration of exposure on liver function tests in group-II (Automobile mechanics).**

| Parameters | Group-IIA (n = 38) | Group-IIB (n = 17) | Group-IIC (n = 15) | F-value | P-Value |
|---|---|---|---|---|---|
| S.G.O.T. (IU/L) | 26.00 ± 10.34 | 35.00 ± 6.27 [a] | 49.00 ± 8.65 [b,c] | 34.303 | 0.000* |
| S.G.P.T. (IU/L) | 29.11 ± 10.04 | 38.94 ± 4.63 [a] | 57.67 ± 11.97 [b,c] | 48.55 | 0.000* |
| Serum Alkaline Phosphatase (IU/L) | 67.87 ± 8.70 | 95.06 ± 23.46 [a] | 111.7± 19.12 [b,c] | 47.09 | 0.000* |
| Serum Bilirubin (total) (mg/dl) | 0.23 ± 0.06 | 0.31 ± 0.08 | 0.59 ± 0.09 [b] | 0.770 | 0.047* |
| Serum Bilirubin (Direct) (mg/dl) | 0.57 ± 0.42 | 0.49 ± 0.15 | 0.41 ± 0.15 | 1.337 | 0.270 NS |
| Serum Bilirubin (Indirect) (mg/dl) | 0.32 ± 0.19 | 0.37 ± 0.19 | 0.33 ± 0.15 | 0.427 | 0.654 NS |
| Serum Proteins (Total) (gm %) | 7.11 ± 0.53 | 6.48 ± 0.42 [a] | 5.74 ± 0.89 [b,c] | 27.99 | 0.000* |
| Serum Proteins (Albumin) (gm %) | 4.292 ± 0.36 | 4.024 ± 0.33 [a] | 3.720 ± 0.47 [b] | 12.58 | 0.000* |
| Serum Proteins (Globulin) (gm %) | 2.94 ± 0.49 | 2.60 ± 0.41 [a] | 2.51 ± 0.38 [b] | 6.113 | 0.004 * |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs .exposure in automobile mechanics.* depicts 'P' value ≤ 0.05 significant, while 'P' value > 0.05 -not significant (NS).Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference amongst the group (IIA and IIB), (IIA and IIC) and (IIB and IIC)respectively.

Table 5.17 reveals the effect of duration of exposure on liver function tests in automobile mechanics. We noticed that as the duration of exposure increases there was a significant increase in liver enzymes like serum SGOT, SGPT, ALP and total bilirubin group-II (AM).

Statistically significant increase in serum SGOT, SGPT, ALP, and serum bilirubin (total, direct) and a significant decrease in sr. proteins (total, albumin, and globulin) was observed in group-IIB and IIC compared to group-IIA. There was a significant increase inSGOT, SGPT, ALP in group-IIC compared to group-IIB. Serum total protein concentration was significantly decreased in group-IIC compared to group-IIB.

**Table-5.18: Effect of duration of exposure on liver function tests in petrol pump workers.**

| Parameters | Group-IIIA (n = 30) | Group-IIIB (n = 28) | Group-IIIC (n = 12) | F-value | P-value |
|---|---|---|---|---|---|
| S.G.O.T. (IU/L) | 38.07 ± 19.78 | 40.93 ± 17.70 | 41.82 ± 16.93 | 0.255 | 0.776 NS |
| S.G.P.T. (IU/L) | 35.93 ± 35.01 | 33.79 ± 16.99 | 34.16 ± 10.04 | 0.054 | 0.947 NS |
| Serum Alkaline Phosphatase (IU/L) | 74.87 ± 9.40 | 91.03 ± 13.48[a] | 126.98 ± 35.03[b,c] | 37.212 | 0.000* |
| Serum Bilirubin (total) (mg/dl) | 0.697 ± 0.36 | 0.593 ± 0.16 | 0.750 ± 0.23 | 1.752 | 0.181 NS |
| Serum Bilirubin (Direct) (mg/dl) | 0.40 ± 0.23 | 0.33 ± 0.10 | 0.44 ± 0.15 | 2.073 | 0.134 NS |
| Serum Bilirubin (Indirect) (mg/dl) | 0.39 ± 0.57 | 0.33 ± 0.34 | 0.31 ± 0.12 | 0.258 | 0.773 NS |
| Serum Proteins (Total) (gm %) | 6.797 ± 0.59 | 6.346 ± 0.712 | 6.433 ± 0.6651 | 3.658 | 0.031 * |
| Serum Proteins (Albumin) (gm %) | 3.960 ± 0.32 | 3.85 ± 0.39 | 3.82 ± 0.48 | 1.906 | 0.407 NS |
| Serum Proteins (Globulin) (gm %) | 3.05 ± 0.53 | 2.84 ± 0.65 | 2.95 ± 0.46 | 0.911 | 0.375 NS |

Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB - 6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in Petrol pump workers.* depicts 'P' value ≤ 0.05 significant, while 'P' value > 0.05 -not significant (NS). Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference amongst the group (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively.

Table 5.18 shows the effect of duration of exposure on liver function tests in petrol pump workers. As the duration of exposure increases, there was a significant increase in the

level of serum ALP in group-III (PPW). Significantly decreased serum total protein was observed with increased duration of exposure in group-III (PPW). We also observed a significant increase in serum ALP level in group-IIIC compared to IIIB.

### 5.10.3: Discussion:

**Increased liver enzymes like SGOT, SGPT & ALP:**

Significantly increased activity of liver enzymes like SGOT, SGPT & ALP in occupationally exposed AM and PPW indicates hepatocellular damage, which could be due to accumulation of heavy metal (lead) and petroleum hydrocarbon in liver and exerts its toxic effect that leads to oxidative damage to hepatic cell membranes causing transaminase to liberate into the serum (Dongre NN *et al.* 2010; Oluwatobi AKEFE 2017). ALP is an enzyme in the cells lining the biliary ducts of the liver. If there is an obstruction in the bile duct, ALP levels in plasma increase. ALT is an enzyme present in hepatocytes (liver cells) and it leaks into the blood when liver cells are damaged. ALT rises dramatically in acute liver damage (such as viral hepatitis and paracetamol overdose) and during liver inflammation. AST is similar to ALT in that it is another enzyme associated with liver parenchymal cells. Petroleum hydrocarbons and heavy metal (Pb) may affect transport functions of the hepatocytes or of the biliary tree and can cause elevation of serum ALP activity. These hepatic enzymes are markers of hepatocyte organelle or biliary duct. They can leak into the circulation as a response to hepatocyte injury caused by reactive metabolism in the liver. Hence serum activity of these enzymes is a reflection of the physiological state of the liver and their activity in the blood depends on the severity of the cellular damage. The effect of the elevated BLL on the liver function of occupationally exposed subjects shows that in mechanics, panel beaters and others, elevated BLL was associated with hepatocellular toxicity. These findings suggest that in lead-exposed subjects the metal may elicit hepatotoxicity resulting in injury/damage to the hepatic cells (E. Chukwu Onyeneke *et al.* 2016).

**Possible Mechanism:**

Petroleum hydrocarbons present in petroleum fumes and air pollutant are also known to be hepatotoxic. After inhalation, benzene, heavy metal lead and the other hydrocarbons present in petroleum fumes are readily absorbed from the lungs and get metabolized in the liver by oxidative pathways which contribute to the production of free radicals and metabolites. These free radicals subsequently bound with hepatic macromolecules and ultimately cause lipid peroxidation (MDA). This metabolite creates a

covalent bond with the hepatic macromolecules, thereby initiating lipid peroxidation and damage of hepatic cell membrane, causing the release of liver enzymes in the circulation. (Rahul *et al.* 2017; Olufunsho Awodele *et al.* 2014).

**Increased serum Bilirubin (total, direct and indirect):**

In present study significantly increased serum bilirubin (total, direct and indirect) levels in the petrol pump workers and automobile mechanics compared to controls. This could be due to hemolysis of erythrocytes because of morphological changes that occur in RBC due to heavy metal (Pb) exposure. This suggests the compromised capacity of the liver to transport and metabolize bilirubin in automobile mechanics and petrol pump workers. Several studies reported that exposure to high lead concentration produces morphological changes in red blood cells and destroys the red cells (Dongre NN *et al.* 2010).

**Decreased serum Proteins (total, albumin, globulin):**

The serum protein tests are used to ascertain the liver's biosynthetic capacity. Albumin is a protein made specifically by the liver, is the main constituent of total protein and a useful indicator of hepatic function. Significantly decreased sr. proteins (total, albumin, globulin) in automobile mechanics and petrol pump workers might be due to binding of lead with the high-affinity binding site of albumin & it forms the albumin-lead complex, so total protein, as well as albumin, get decreased. This indicates a decreased rate of protein synthesis.

The results of this study were in accordance with the research work conducted by Gali RM *et al.* (2012); which shows a significant increase in the levels of plasma liver enzyme such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in petrol hawkers when compared with control subjects. There was also a significant decrease in albumin concentrations of petrol hawkers compared to the control group. This could be attributed to the attitude of the hawkers using mouth in aspirating fuel from larger stock containers while dispensing to the motorist or smaller containers which often cause accidental swallowing that could affect the membranes of the liver cells during metabolism.

Similarly results were observed by Rahul *et al.* (2017); shows liver enzymes like serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) significantly higher among the petrol filling attendants compared to the control group, except serum alkaline phosphatase which was although higher in petrol filling

attendants but statistically insignificant between the two groups. This may be due to inhalation of benzene and the other hydrocarbons present in gasoline readily absorbed from the lungs and get metabolized in the liver and damage of hepatic cell membrane.

Significantly raised liver enzymes like AST, ALT, and ALP observed in petrol pump workers than controls. Increased levels in these enzymes activities in the plasma are linked to hepatocellular damage caused by either toxin, toxins in drugs or herbs. These findings correspond with the findings of the present study (Holkar Shrirang *et al.* 2017).

Dongre NN. *et al.* (2010); and E. Chukwu Onyeneke *et al*. (2016); also observed significantly increased serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin in automobile workers as compared with the control group. Serum total protein, albumin, globulin, and A/ G ratio were not significantly altered in the study group as compared to the control group. Similarly, a significant decrease in serum albumin and total protein observed in petrol station workers than in the unexposed group (Masoud Neghab *et al.* 2016).

However, our observations regarding the liver functions in the present study were in contrast to the findings of Akinosun OM *et al.* (2006); which did not reveal any difference in the levels of liver enzymes in both petrol filling attendants and the controls except for significantly lower levels of alkaline phosphatase. Contrary to our findings, Mashael Bin-Mefrij*et al.* (2017); also shows no significant increase in the mean serum concentrations of ALP, ALT, AST, and GGT observed between petrol pump workers and control group.

Our findings of liver function tests indicate that occupational exposure to petroleum and exhaust fumes found to have hazardous effects on liver enzymes, serum bilirubin, and serum proteins. Levels of all the liver enzymes, total bilirubin significantly increased in petrol pump workers and AM but this change occurs within normal limit. This suggests that no hemolysis or liver damage occurred in these workers but they are prone to develop liver damage if further exposure is there. Duration of exposure has a more significant impact on liver enzymes like serum SGOT, SGPT, ALP, and total bilirubin and serum proteins (total, albumin, globulin) in automobile mechanics compared to petrol pump workers.

## 5.11: Renal Function Tests:

### 5.11.1: Results

**Table-5.19: Renal function tests amongst study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F-value | P-value |
|---|---|---|---|---|---|
| Blood Urea (mg/dl) | 23.96 ± 8.647 | 29.29 ±10.158 | 26.87 ± 8.53[c] | 5.96 | 0.003* |
| Serum Cretinine (mg/dl) | 1.26 ± 0.174 | 1.65 ± 0.923[a] | 1.27 ±0.363 [c] | 9.97 | 0.000* |
| **Electrolytes** | | | | | |
| Na+ (mEq/L) | 138.27 ± 3.91 | 145.35 ±10.18 | 143.47 ± 7.66 [b,c] | 15.87 | 0.000* |
| K+ (mEq/L) | 3.44 ± 0.46 | 4.97 ± 1.31 | 4.73 ± 1.06 [b,c] | 46.66 | 0.000* |

Group I–Controls, Group II - Automobile mechanics (AM), Group III - petrol pump workers (PPW). * depicts 'P' value ≤0.05 is significant. Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c express the significant difference amongst (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.19 displays kidney function tests amongst study and control groups. There was a statistically significant increase in the concentration of blood urea, serum creatinine & electrolytes ($Na^+$ and $K^+$) in group-II and III as compared to group-I. We found a significant increase in blood urea, serum creatinine & electrolytes ($Na^+$ and $K^+$) concentration in group-II as compared to group-III.

### 5.11.2: Percentage change impact on Kidney functions.

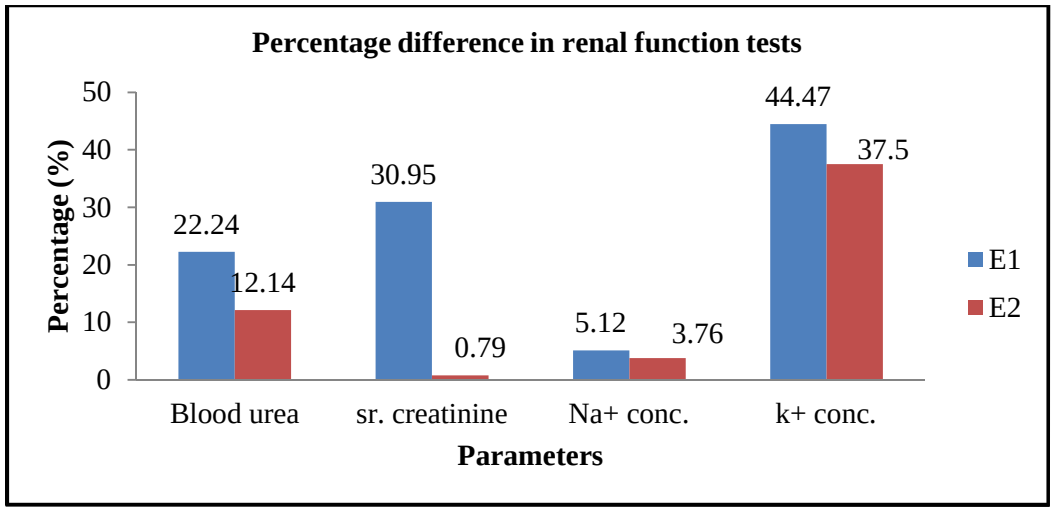



**Figure 5.12:** Percentage change impact on Kidney functions parameters in automobile mechanics (AM) and petrol pump workers (PPW). $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure 5.12 depicts the percentage change impact on Kidney functions. Percentage difference of blood urea, serum creatinine and electrolytes ($Na^+$ and $K^+$) increased to a greater extent in group-II (AM) compared to group-III (PPW) with respect to the control group.

**Table- 5.20: Effect of duration of exposure on renal function tests in group-II (Automobile mechanics).**

| Parameters | Group-IIA (n = 38) | Group-IIB (n = 17) | Group-IIC (n = 15) | F-value | P- Value |
|---|---|---|---|---|---|
| Blood Urea (mg/dl) | 23.63 ± 7.24 | 31.35 ± 7.52 [a] | 41.27 ± 7.73[b,c] | 31.32 | 0.000 * |
| Serum Creatinine (mg/dl) | 0.565 ± 0.09 | 1.025 ± 0.25 [a] | 1.045 ± 0.27 [b] | 11.34 | 0.000* |
| **Electrolytes** | | | | | |
| Na+ (mEq/L) | 139.43 ± 6.486 | 147.35 ± 5.499 [a] | 158.07 ± 9.588[b,c] | 38.53 | 0.000* |
| K+ (mEq/L) | 4.35 ± 1.076 | 5.36 ± 0.907 [a] | 6.10 ± 1.395 [b] | 14.56 | 0.000* |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB-6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics.* depicts 'P' value ≤ 0.05 significant, while 'P' value > 0.05 -not significant (NS). Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc't' test'. Superscript a, b, c expresses the significant difference amongst groups (IIA and IIB), (IIA and IIC) and (IIB and IIC) respectively.

Table 5.20 reveals the effect of duration of exposure on renal function tests in automobile mechanics. As the duration of exposure increases, there was a significant increase in blood urea, serum creatinine and electrolytes ($Na^+$ and $K^+$) concentration. Statistically significant increase in blood urea, serum Creatinine& electrolytes ($Na^+$ and $K^+$) in group-IIB and IIC compared to group-IIA. There was a significant increase in blood urea and the concentration of sodium in group-IIC compared to group-IIB.

**Table-5.21: Effect of duration of exposure on renal function tests in group-III (Petrol pump workers).**

| Parameters | Group-IIIA (n = 30) | Group-IIIB (n = 28) | Group-IIIC (n = 12) | F-value | P-value |
|---|---|---|---|---|---|
| Blood Urea (mg/dl) | 26.17 ± 8.06 | 27.32 ± 9.59 | 27.58 ± 7.57 | 0.189 | 0.837 NS |
| Serum Creatinine (mg/dl) | 1.22 ± 0.43 | 1.21 ± 0.29 | 1.53 ± 0.21[b,c] | 4.062 | 0.022* |
| **Electrolytes** | | | | | |
| Na+ (mEq/L) | 139.50 ± 4.577 | 142.89 ± 6.321 | 154.75 ± 5.675 [b,c] | 32.98 | 0.000* |
| K+ (mEq/L) | 4.21 ± 0.85 | 4.73 ± 0.83 | 6.03 ± 0.94 [b,c] | 19.53 | 0.000* |

Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB - 6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in Petrol pump workers.* depicts 'P' value ≤ 0.05 significant, while 'P' value> 0.05 not significant (NS). Data are represented as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference amongst groups (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively.

Table 5.21 shows the effect of duration of exposure on renal function tests in petrol pump workers. We observed as the duration of exposure increases there was a significant increase in the serum creatinine and electrolytes ($Na^+$ and $K^+$) concentration in group-III (PPW). There was a significant increase in serum creatinine and electrolytes ($Na^+$ and $K^+$) concentration in group-IIIB and IIIC as compared to group-IIIA. We also observed a significant increase in serum Creatinine and electrolytes ($Na^+$ and $K^+$) concentration in group-IIIC compared to IIIB.

### 5.11.3: Discussion:

Urea is a waste product of metabolism that is excreted by the kidneys in urine. It is the most commonly used indicator of renal function. Reduced urea excretion and consequent rise in blood concentration are associated with kidney disease (Adejumo B.I.G *et al.* 2018). Creatinine is produced in muscle by the non-enzymatic conversion of creatine and phosphocreatine. These are synthesized primarily in the liver from the methylation of

guanidine amino acetic acid. It is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Serum creatinine is considered as a more accurate marker of kidney function compared to serum urea.

Significant increase in levels of blood urea & serum creatinine could be due to, slow impairment of glomerular basement membrane caused by deposition & accumulation of lead-protein complex in the kidney (glomerulus and proximal tubules of nephron) that causes alteration in structure & functions of cells of the kidney. Together with bone, the kidney is a primary site of Pb accumulation as Pb is excreted by the kidneys. The major effect of various pollutant like toxic heavy metals lead, PM, and gases on kidney occur by inducing the mitochondrial oxidative stress and inflammation. This results in lipid peroxidation (MDA) and DNA fragmentation. Low-level of lead exposure early in life causes glomerular hypertrophy, which may disrupt glomerular development. Acute exposure to lead causes proximal tubular dysfunction (Fanconi syndrome), and chronic exposure leads to progressive tubulointerstitial nephritis (Baris Afsar *et al.* 2019).

The present findings indicating significantly increased blood urea and higher creatinine in the automobile workers therefore, suggest that these workers may be at risk of renal dysfunction.

Our findings are in agreement with the findings of Nwanjo and Ojiako who reported that petrol attendants had altered renal function values. Olufunsho Awodele *et al.* (2014), Masoud Neghab *et al.* (2015); reported a significant increase in the blood urea and serum creatinine levels of the petroleum tanker driver, petrol station workers compared with the control group. Patil A. J. *et al*. (2007) have also reported a significant increase in blood urea among battery manufacturing workers.

**A possible mechanism for increased blood urea and serum creatinine:**

Lead can cause acute and chronic nephropathies. The proximal renal tubular epithelial cells appear to be the most sensitive tissue in the kidney to lead. Lead is absorbed by the proximal tubular cells of the renal tubules, where it binds to specific lead-binding proteins. These lead-protein complexes are observed as typical intracellular inclusion bodies. However, frequent exposure to lead causes deposition of the lead-protein complex on the kidney (glomerulus and proximal tubules) thereby reducing the glomerular filtration of urea and creatinine leading to their retention in the plasma. An elevation in the serum creatinine concentration usually reflects a reduction in the glomerular filtration rate.

Therefore, the elevation of plasma urea and creatinine may be indicative of disturbed integrity of kidney cells.

**Significantly increased electrolytes ($Na^+$ and $K^+$):**

An electrolyte is of great importance in regulating our nerve and muscle function, our body hydration, blood pH, blood pressure and the rebuilding of damaged tissues. An increased or decreased concentration of body electrolytes causes or can result in biochemical dysfunction which can lead to kidney diseases, heart failure, damaged of tissues in the body, which constitute serious health problem in human being (Emeji Roseline *et al.* 2015).

Significantly increased electrolytes ($Na^+$ and $K^+$) concentration in group-II (AM) and group-III (PPW) could be due to increased serum concentration for urea and creatinine in the exposed group. This suggests impaired excretion of these waste products causes renal impairment. These wastes along with physiological electrolytes are usually eliminated through the urine, and their impaired elimination probably resulted in the higher serum electrolytes ($Na^+$ and $K^+$) concentration in the exposed group. It also may be due to depletion of prostaglandins that may enhance sodium retention. However, the mechanism through which lead exposure raises the plasma concentration of sodium and potassium still remain unclear but it is believed that this could be due to the result of damaged renal tubules by lead. For instance, high blood $Na^+$ concentration was associated with increased blood pressure.

Similarly, blood urea and serum creatinine levels were significantly increased in automobile workers as compared to controls, which indicates slight nephrotoxicity may be due to lead (Dongare NN *et al.* 2010)

Our observations correspond with the findings of Emeji Roseline *et al.* (2015); who reported the significantly increased concentration of electrolyte ($Na^+$ and $K^+$) in the petrol pump attendants than in the control group.

Petroleum pollutants such as fuel fume, kerosene vapors, and petrol are the predisposing factor for impairment of the kidney functions. Also it was observed that, the depletion in the electrolyte concentration among the fuel pump attendants exposed to petroleum pollutants is dependent on the number of years of exposure which means that the more in the number of years of exposure causes more effect on the concentration of the electrolyte which causes diverse body biochemical dysfunction which can result to kidney disease, heart failure, damaged in tissue cells, etc.

Similarly, there was a significant increase in the mean serum concentration of urea and creatinine in automobile mechanics compare to the control subjects. Increase serum urea could be due to slow impairment of glomerular basement membrane caused by the deposition and accumulation of lead in the kidney (Amah UK *et al*. 2014).

Conversely, Amah UK et al. (2014) showed serum electrolytes ($Na^+$, $K^+$) level was not significantly different in both automobile group and control subjects. This could be due to poor sensitivity of electrolytes as renal function markers and the reserve capacity of the kidney to maintain normal function even after the loss of about 50% of the nephron.

Our findings correspond with the findings of Anthony Cemaluk C. *et al.* (2016); who reported a significant increase in the mean serum concentration of urea, creatinine, and electrolytes ($Na^+$ and $K^+$) in the petrol pump workers compared to the control subjects. They suggested renal impairment could be due to enhanced tissue breakdown and release of intracellular nucleotides, perhaps in apparent response to petroleum products vapors intoxication.

Ogunneye AL *et al.* (2014); The kidney function test reveals that petrol attendants show a significant increase in blood urea, creatinine, and electrolytes like serum potassium, chloride, and sodium ion concentrations compared with the control. With an increase in the duration of exposure, there was a significant increase in plasma creatinine, urea, and electrolyte concentration observed in petrol attendants. Long term exposure to petrol fumes could have adverse effects on the kidney.

The present study suggests that duration of exposure have a strong impact on kidney function tests. Blood urea, serum creatinine and electrolytes ($Na^+$ and $K^+$) concentrations increase with an increase in the duration of exposure both in automobile mechanics and petrol pump workers except blood urea in petrol pump workers. The nephrotoxicity observed in this study suggests the presence of some nephrotoxic chemical substances like particulate matter, benzene and heavy metal lead present in petroleum and exhaust fumes. All renal markers were also significantly increased suggesting that automobile mechanics and petrol pump workers are prone to renal damage.

In the present study mean values of all the biochemical parameters in the exposed population (group-II and group-III) were altered. But this change was within the normal range, except values of sr. creatinine, sr. bilirubin (direct), blood lead level and MDA level were slightly exceeding the normal limit in the exposed population (group-II and group-III).

## 5.12: Correlation of blood lead level (BLL) with blood pressure, PEFR, Hb%, RBCs count and MDA in study groups.

### 5.12.1: Results and discussion:

**Table -5.22: Correlations of blood lead level with blood pressure, PEFR, Hb%, RBCs count & MDA in automobile mechanics and petrol pump workers.**

| Parameters | Group-II (Automobile Mechanics) | | Group-III (Petrol pump workers) | |
|---|---|---|---|---|
| | r- value | P-value | r- value | P-value |
| **Blood Pressure** | | | | |
| Systolic BP (mmHg) | 0.408 | 0.000 * | 0.455 | 0.000* |
| Mean Arterial BP (mmHg) | 0.399 | 0.001* | 0.471 | 0.000* |
| PEFR(L/Min) | -4.96 | 0.003 * | -0.218 | 0.070 |
| Hb % | -3.63 | 0.002 * | -0.038 | 0.755 |
| RBC count(Millions/$mm^3$) | -4.38 | 0.000 * | -0.052 | 0.670 |
| Serum MDA(nmol/dl) | 0.585 | 0.001 * | 0.698 | 0.000* |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | | |

Table 5.22 represents the correlation of BLL with blood pressure, PEFR, Hb%, RBCs count & MDA in individual Group-II (AM) and Group-III (PPW).

We observed the significant positive correlation of blood lead level with SBP, MAP and serum MDA in group-II **(**automobile mechanics), while the significant negative correlation of blood lead level with PEFR, Hb%, RBCs count was observed in group-II **(**automobile mechanics). There was a significant positive correlation of blood lead level with SBP, MAP, and serum MDA in group-III **(**petrol pump workers). We did not find a significant correlation of blood lead level with PEFR, Hb%, RBCs count in group-III **(**petrol pump workers).

**Table-5.23: Correlation of blood lead level with blood pressure, PEFR, Hb%, RBCs count and MDA in the combined study group.**

| Parameters | r- value | P-value |
|---|---|---|
| **Blood Pressure** | | |
| Systolic BP (mmHg) | 0.397 | 0.000* |
| Mean Arterial BP (mmHg) | 0.399 | 0.000* |
| PEFR(L/Min) | -0.246 | 0.003* |
| Hb % | -0.257 | 0.002* |
| RBC count (Millions/$mm^3$) | -0.340 | 0.000* |
| Sr. MDA(nmol/ dl) | 0.646 | 0.001* |
| *. Correlation is significant at the 0.05 level (2-tailed). | | |

Table 5.23: represents the correlation of BLL with and blood pressure, PEFR, Hb%, RBCs count & MDA in combined study groups. We observed the significant positive correlation of blood lead level with SBP, MAP and serum MDA, while a significant negative correlation of blood lead level with PEFR, Hb%, RBCs count was observed in combined study groups (group-II and group-III).

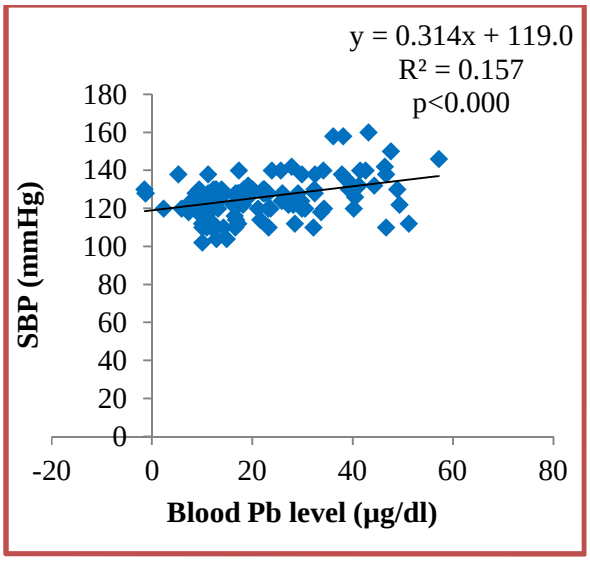



**Figure-5.13**

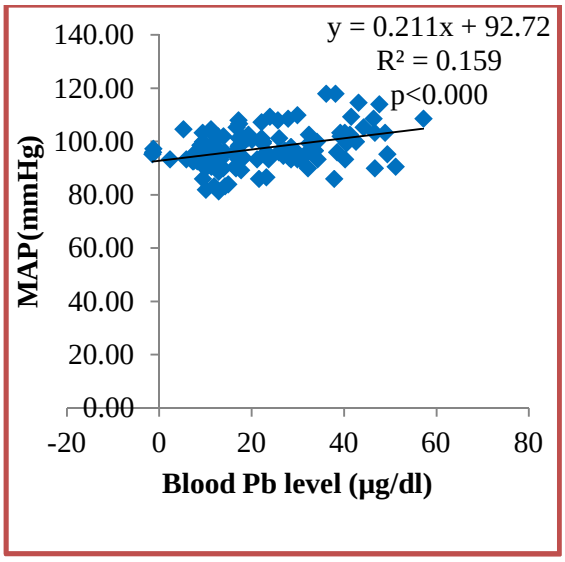



**Figure-5.14**

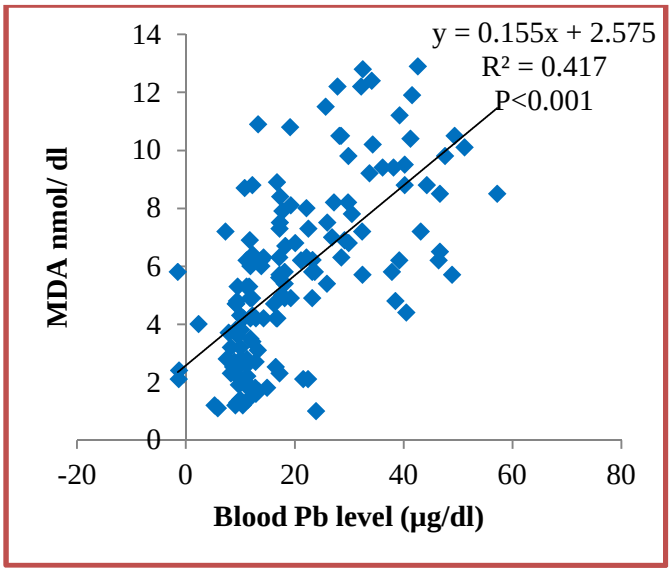



**Figure-5.15**

**Figure-5.13** indicates positive correlation of blood lead level (BLL) with SBP in combined study groups.

**Figure-5.14** shows the positive correlation of blood lead level (BLL) with MAP in combined study groups.

**Figure-5.15** shows the positive correlation of blood lead level (BLL) with MDA in combined study groups.

Our study shows a very strong significant positive correlation between BLL and SBP (r=0.397, p=0.000), MAP (r=0.399, p=0.000) and MDA(r=0.408, p=0.000) in occupationally exposed study groups. This result clearly indicates that the as BLL level increases, the SBP & MAP also increase. The observed correlation between blood lead & blood pressure suggests that lead has an impact on kidney & it induces nephrotoxicity (electrolyte retention mechanism) in AM and PPW.

This result is in conformity with Abdullah A. Alghasham *et al.* (2011) who found a significant positive correlation between blood lead and SBP (r =0.41, P<0.01) and MDA (r =0.51, P<0.05) in hypertensive patients. DA Khan *et al.* (2008) and Zorawar Singh *et al.* (2013); Patil AJ.et al. (2006); also found a significant positive correlation between blood lead level with oxidative stress (MDA) (r=0.71, P<0.01) in occupationally exposed workers.

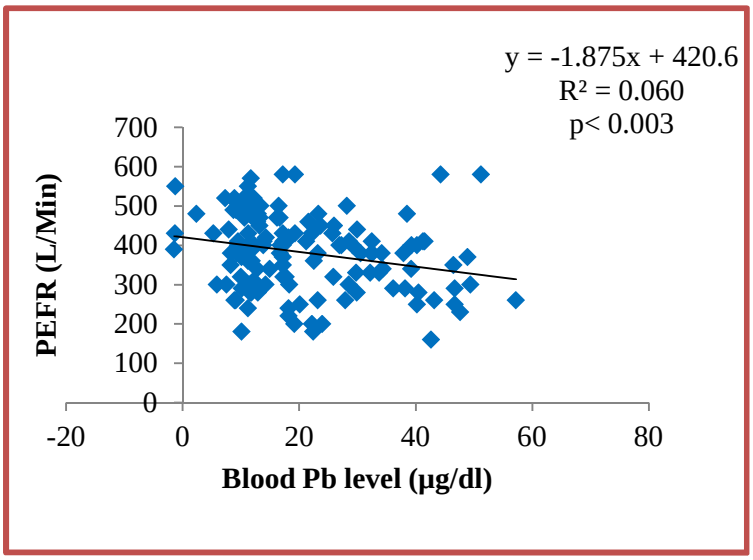



**Figure-5.16** shows the negative correlation of blood lead level (BLL) with PEFR in combined study groups.

A significant negative correlation between BLL and PEFR (r=-0.246, P<0.003) in occupationally exposed study groups. This observed correlation suggests that increased blood lead concentration affect the bronchial epithelium, cilia and Clara cells of the lungs that destroy the lung surfactant and reduce elastic recoil of the lungs. This result is in conformity with results of Stoleski S. et al. (2008); showed a significant negative correlation of BLL with PEFR(r=-0.255, *P*<0.05) in occupationally exposed workers.

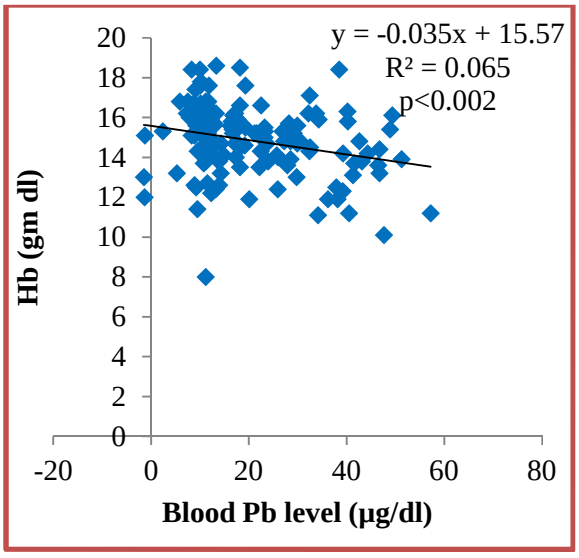



**Figure-5.17**

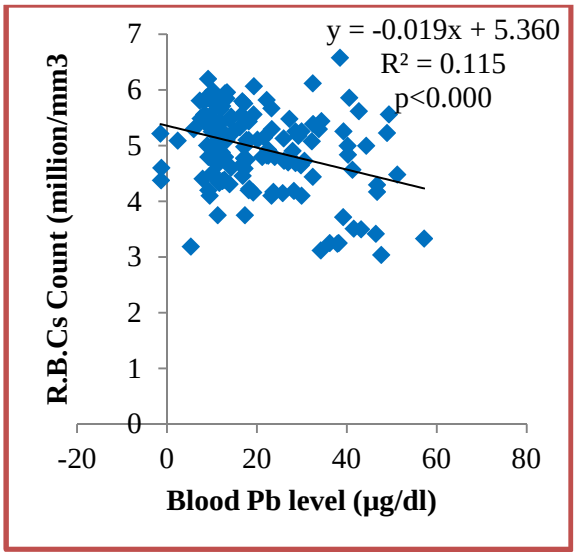



**Figure-5.18**

**Figure-5.17** indicates negative correlation of blood lead level (BLL) with Hb% in combined study groups.

**Figure-5.18** shows the negative correlation of blood lead level (BLL) with RBC count in combined study groups.

A very strong negative correlation was observed between BLL and Hb (r =-0.257, P<0.000) and RBC count (r=-0.340, P<0.001) in occupationally exposed groups. These results indicate that anemia is fairly common at high BLL level in automobile and petrol pump workers. Our observations were supported by Nancy Ibeh *et al*. (2016); shows a strong negative correlation between hemoglobin and BLL (r = -0.53, *P*< 0.01) among petrol pump workers and automobile mechanics of Nnewi, South-East Nigeria.

## 5.13: Cytogenetic alterations (nuclear abnormalities) in exfoliated buccal epithelial cells amongst study and control groups.

### 5.13.1: Results.

**Table-5.24: cytogenetic alterations (nuclear abnormalities) in exfoliated buccal epithelial cells amongst study and control groups.**

| Parameters | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) | F- value | P-value. |
|---|---|---|---|---|---|
| Micronucleated Cell (MNC) | 5.09 ± 3.690 | 8.16 ± 6.140 | 6.34 ± 3.799[c] | 7.616 | 0.001* |
| Binucleated Cell (BNC) | 8.84 ± 3.888 | 11.47 ± 6.812 | 10.80 ± 5.021 [c] | 4.516 | 0.012* |
| Karyorrhexis Cell (KRC) | 12.56 ± 5.112 | 15.34 ± 8.149 | 14.11 ± 4.708 [c] | 3.569 | 0.030* |
| Karyolysis Cell (KLC) | 15.74 ± 6.538 | 21.61 ± 12.028 | 19.63 ± 7.092[b,c] | 7.879 | 0.001* |

Group I–Control Group, Group II - Automobile mechanics (AM), Group III - Petrol pump workers (PPW). * depicts 'P' value ≤0.05 is significant. Data are presented as Mean ± SD; ANOVA followed by Tukey HSD Post Hoc't' test. Superscript b, c express the significant difference between (Group-I and II), (Group-I and III), (Group-II and III) respectively.

Table 5.24: Represents cytogenetic alterations (nuclear abnormalities) in exfoliated buccal epithelial cells in all groups. We found a significant increase in frequencies of micronuclei (MN), binucleate cells (BNC), karyorrhectic cells (KRC), karyolytic cells (KLC) amongst group-II and III as compared to control group. We also found statistically significant increased frequencies of MNC, BNC, KRC, KLC in group-as compared to group-III.

### 5.13.2: Percentage change impact on 'Cytogenetic alterations (nuclear abnormalities)' in exfoliated buccal epithelial cells with respect to control:

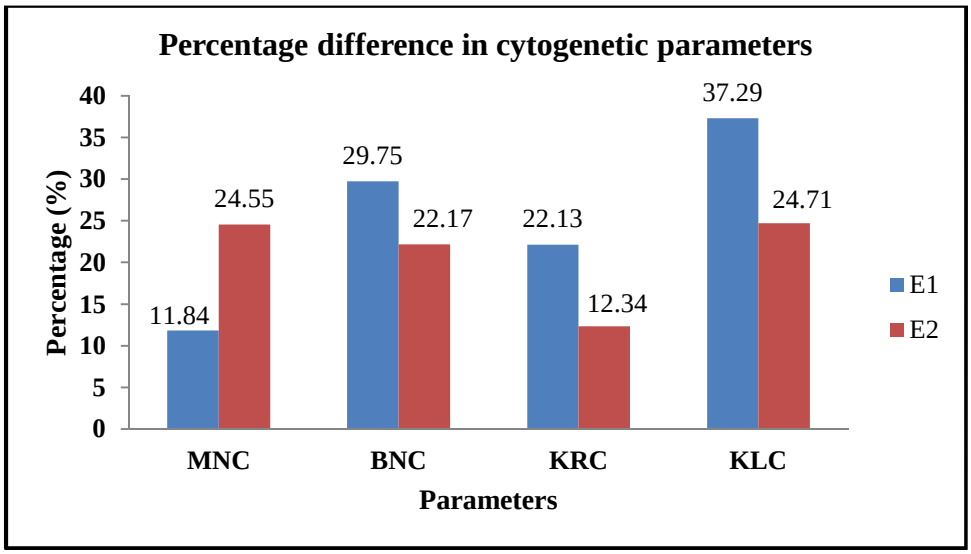



**Figure-5.19:** Percentage change impact on cytogenetic alterations (nuclear abnormalities) in exfoliated buccal epithelial cells of automobile mechanics and petrol pump workers. $E_1$= (Group I- Group II), $E_2$= (Group I- Group III) considering group-I as a control group.

Figure-5.19: depicts the percentage change impact on cytogenetic changes (MN and other nuclear abnormalities) in exfoliated buccal cells. We found a greater increase in percentage difference of MN in group-III (PPW) as compared to group-II (AM). There was a greater increase in percentage difference of BNC, KRC, and KLCingroup-II (AM) as compared to group-II (PPW) with respect to the control group.

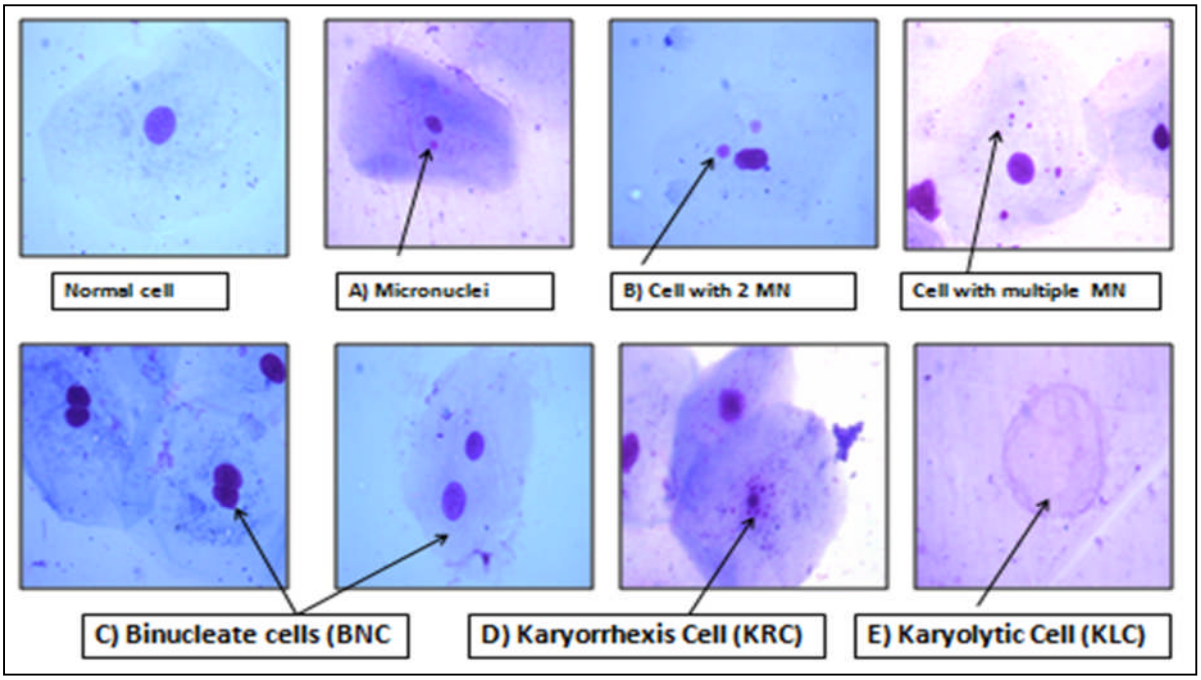



**Figure-5.20:** Micronuclei (MN) & other nuclear abnormalities in exfoliated buccal epithelial cells.

**Table-5.25: Effect of duration of exposure on cytogenetic alterations amongst automobile mechanics and petrol pump workers.**

| Study Groups | Parameters | Group-IIA (n = 38) | Group-IIB (n = 17) | Group-IIC (n = 15) | F-value | P-value |
|---|---|---|---|---|---|---|
| Group-II (Automobile Mechanics) (n=70) | MNC | 5.87 ± 4.23 | 13.18 ± 5.09 [a] | 8.27 ± 8.01 [c] | 10.651 | 0.000* |
| | BNC | 8.79 ± 5.92 | 11.94 ± 3.58 | 17.73 ± 7.73 [b,c] | 12.407 | 0.000* |
| | KRC | 13.13 ± 8.09 | 13.59 ± 4.84 | 22.93 ± 7.01 [b,c] | 10.614 | 0.000* |
| | KLC | 16.71 ± 7.76 | 28.76 ± 15.11 [a] | 25.93 ± 12.07 [b] | 8.725 | 0.000* |
| Group-III (Petrol pump workers) (n=70) | **Parameters** | **Group-IIA (n = 30)** | **Group-IIIB (n = 28)** | **Group-IIIC (n = 12)** | **F- value** | **P-value** |
| | MNC | 3.80 ± 3.27 | 7.57 ± 3.02 [a] | 9.83 ± 2.25 [b] | 20.889 | 0.000* |
| | BNC | 7.77 ± 3.22 | 13.46 ± 5.22 [a] | 12.17 ±4.32 [b] | 13.411 | 0.000* |
| | KRC | 11.63 ± 3.746 | 15.89 ± 4.458 [a] | 16.17 ± 4.877 [b] | 8.999 | 0.000* |
| | KLC | 15.60 ± 4.88 | 21.25 ± 5.51 [a] | 25.92 ± 9.16 [b] | 14.237 | 0.000* |

Group-IIA-1 to 5 yrs. of exposure, Group-IIB- 6 to10 yrs. of exposure, Group-IIC->10 yrs. exposure in automobile mechanics.Group-IIIA-1 to 5 yrs. of exposure, Group-IIIB -6 to10 yrs. of exposure, Group-IIIC->10 yrs. exposure in Petrol pump workers.* depicts 'P' value ≤ 0.05 significant, while 'P' value> 0.05 -not significant (NS).Values are expressed as Mean ± SD; 'ANOVA' followed by Tukey HSD 'Post Hoc 't' test'. Superscript a, b, c expresses the significant difference between group (IIA and IIB), (IIA and IIC), (IIB and IIC) and (IIIA and IIIB), (IIIA and IIIC) and (IIIB and IIIC) respectively. MNC- Micronucleated Cells, BNC-Binucleated Cells, KRC- Karyorrhexis Cells, KLC-Karyolysis Cells.

Table 5.25: Indicates the effects of duration of exposure on cytogenetic alterations in study groups. We observed as the duration of exposure increases, there was a significant

increase in the frequency of MN & other nuclear abnormalities like BNC, KRC, and KLC in group-II and III.

We observed the significant increase in the frequency of BNC, KRC, KLC in Group-IIC as compared to group-IIA. Significant increase in the frequency of MNC, BNC, KRC in Group-IIC as compared to group-IIB. We found a significant increase in the frequency of MNC, BNC, KRC, KLC in group-IIIB and Group-IIIC as compared to group-IIIA.

**5.13.3: Discussion**

Micronuclei (MN) basically arise in mitotic cells due to the dysfunction of the mitotic process and breakages of the chromosome and its detection serves as a biomarker to predict cancer risk (Azubuike NC *et al.* 2019).

Our study clearly indicates that the level of cytogenetic damage (frequencies of MN and other nuclear abnormalities) was significantly higher in group-II (AM) than group-III (PPW) and control group. The frequencies of genotoxic parameters (MNC and BNC) were higher among group-II (AM) and group-III (PPW). Less frequency of MN as compared to BN cells in the exposed groups was observed, this indicates that the workers had very little genetic damage stage. The frequencies of cytotoxic parameters (KRC and KLC) were higher in group-II (AM) and group-III (PPW) as compared to control group, thus depicting the more toxic state of the AM & PPW. The duration of exposure (in years) in petrol pump workers and AM affected the frequencies of nuclear anomalies.

This could be due to the carcinogenic effect of benzene. Epidemiological studies also showed that there is a clear relationship between the increase in MN frequency and exposure to benzene and benzene metabolites (Tompa *et al.* 1994; Turkel B. *et al.* 1994). It also may be due to the consequences of heavy metal lead-induced oxidative stress. Heavy metals lead binding to DNA and proteins causing damage to DNA, thus gene expressions are altered leading to mutations and altered cell cycle and finally dysfunction of the cytoskeleton. Depending on the metal they can give rise to both clastogenic and aneugenic MN (Vipul Jain *et al.* 2017). Significantly increased in frequencies of MN, BN cells reflect chromosomal damage, while a significantly higher rate of nuclear aberration was the indication of cytotoxicity (karyolysis, karyorrhexis).

Micronucleus test in exfoliated epithelial cells seems to be a useful biomarker of occupational exposure to genotoxic chemicals. Other nuclear abnormalities, such as binucleus formation is considered as an indicator of cytotoxicity, while karyorrhexis and karyolysis are considered as indicators of apoptosis. It is also well established that genomic

damage is produced by environmental exposure to genotoxins, medical procedures (eg. radiation and chemicals), micronutrient deficiency (eg.folate), lifestyle factors (eg. alcohol, smoking, drugs, and stress), and genetic factors, such as inherited defects in DNA metabolism and/or repair (Sridhar Reddy *et.al.* 2017)

It was noted that statistically significant increase in frequencies of micronucleated (MN), binucleated (BN), karyorrhexis (KR), karyolysis (KL) cells in >10 years of the exposed group as compared to 1-5 & 6 -10 years of the exposed group. Uniform increasing pattern was observed in both genotoxic (MN, BN) and cytotoxic parameters (KR, KL) respectively. Duration of exposure significantly affected the biomarkers of genotoxicity (MN) in the PPW and AM. It implies the possibility of higher genomic damage in relation to the duration of exposure.

Our findings are also in agreement with those of Singaraja M. *et al.* (2012); who detected a significant increase in frequencies of MN in buccal epithelial cells of petrol bunk workers than controls. This could be due to occupational exposure to genotoxic chemicals (benzene) present in petrol.

Similar results were observed in buccal epithelial cells of petrol pump workers exposed to petroleum fumes. Analysis of buccal cells revealed that MN and NA frequencies in petrol station workers were significantly higher than in control subjects. These findings indicate that the petrol station workers are under the risk of significant cytogenetic damage (Ayla Celik *et al.* 2003).

Findings of our study are in agreement with the findings of recent research work which documented elevated frequencies of MN and other nuclear abnormalities in buccal epithelial cells of automobile spray painters. Lifestyle habits (smoking, alcoholism) increased the MN frequencies of the automobile spray painters. This is due to occupational exposure of automobile spray painters to genotoxins (Azubuike NC *et al.* 2019).

These findings correspond to the current study done by Rajkokila *et al.* (2010), who observed a higher frequency of buccal cells with micronuclei, binucleate, karyorrhexis and karyolytic cells in the study subjects, probably due to the presence of benzene in automobile exhaust and tobacco smoke. A significant difference in micronuclei and nuclear anomalies was observed in workers exposed to petrol for a longer duration (more than 5 years).

Notably, we observed a consistent trend with the increased micronuclei frequencies in previous studies, which were tested in peripheral blood lymphocytes (Pandey A.K *et al.* 2008, Feng Xiong *et al.* 2016). However, our results on cytogenetic damage in petrol station attendants are controversial to the findings of Pitarque M. *et al.* (1996); found no

statistically significant increased frequencies of MN in cultured blood lymphocytes from a group of filling station attendants.

Present study emphasized that petrol station attendants and automobile mechanics are not only exposed to petroleum hydrocarbons present in petrol vapors, but also to the automobile exhaust produced by engines during fuel combustion. These automobile exhausts may also cause cytotoxic and genotoxic effects. Therefore, our study suggests that the increased frequency of micronucleus, BNC, KRC, KLC could also reflect the genetic damage induced by occupational exposure to fuel vapors may be predominantly due to benzene with a substantial effect of co-exposure to toluene and xylene.

## 5.14: Oral Findings amongst study and control groups.

### 5.14.1: Results.

**Table –5.26: Oral health status in the study and control group.**

| Oral Findings | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) |
|---|---|---|---|
| **Mouth related symptoms** | | | |
| Mouth Ulcers | 19 (27.1%) | 39 (55.7%)* | 32 (45.7%) |
| Discolourisation | 15 (21.4%) | 35 (50.0%)* | 25 (35.7%) |
| Halitosis (Bad breath) | 21 (30.0%) | 46 (65.7%)* | 40 (57.1%) |
| Mouth Bleeding | 17 (24.3%) | 45 (64.3%) | 47 (67.1%)* |
| Gingivitis | 15 (21.4%) | 39 (55.7%) | 47 (67.1%)* |
| Periodontitis | 19 (27.1%) | 41 (58.6%) | 45 (64.3%)* |
| Caries | 19 (27.1%) | 39 (55.7%) | 41 (58.6%)* |
| Burtonian line | 1 (1.4%) | 14(20%)* | 11(14.6%) |

Group I–Control Group, Group II - Automobile Mechanics (AM), Group III - Petrol Pump Workers (PPW).* depicts maximum percentage frequency.

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Table-5.22 represents oral health status in all groups. A higher percentage of group-II workers showed mouth ulcers (55.7%), a discolorisation of the tooth (50.0%), halitosis (bad breath) (65.7%). While mouth bleeding (67.1%), gingivitis (67.1%), Periodontitis (64.3%) & caries in the tooth (58.6%) was observed more in group-III. Also, we observed a stippled blue line on the junction of the gums and teeth (burtonian line) in group-II (20%) & group-III (14.6%) workers as compared to controls.

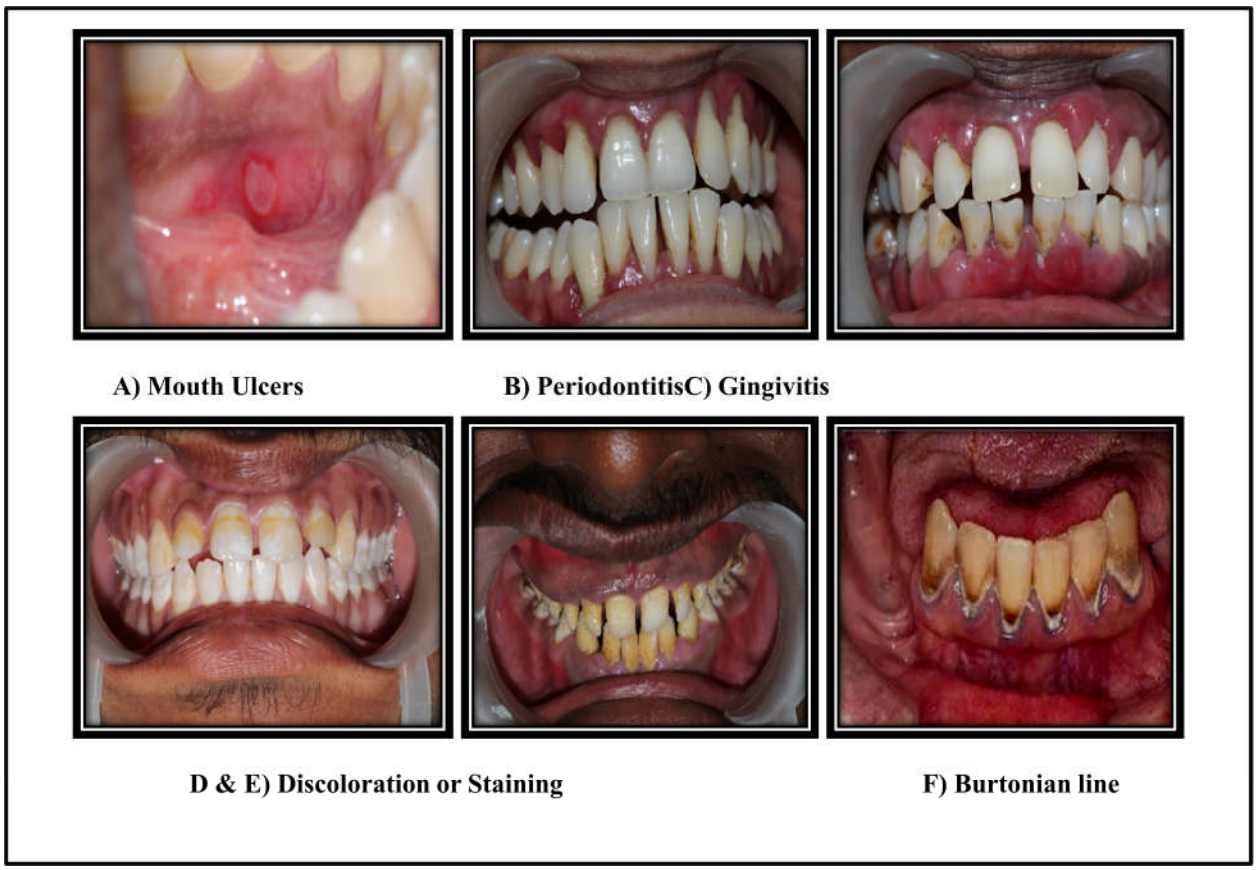


**Figure 5.21: Oral findings in the study and control group.**

**5.14.2: Discussion**.

The biological link between lead exposure and periodontal disease has not been clearly explained, but an increased reactive oxygen species (ROS) has been suggested to be a factor for periodontal disease. Heavy metal lead exposure leads to the generation of ROS and this cause oxidative stress. ROS can also cause damage to proteins and DNA, as well as lipid peroxidation, and increased blood lead level can cause damage to the gingival, periodontal ligament, and alveolar bone tissues (Borany Tort *et al.* 2018).

This oral manifestation occurs may be due to lead-induced oxidative stress. Occupational exposure of the workers in the polluted environment containing lead dust in the air can make easy passage of lead into the body through the mouth. This may cause the mouth and its related tissues very prone to infections, which can cause frequent mouth bleeding. But few studies show the association between dental caries and lead exposure (Moss ME *et al.* 1999; Campbell JR *et al.* 2000; Gemmel A *et al.* 2002). So poor oral health was observed in automobile mechanics and petrol pump workers.

Gingival lead line or Burtonian line was first described by Grisolle in 1836 and later by Henry Burton in 1840. The lead line typically manifests as a purple-blue line within gingival tissue or as a stippled bluish-black line at the junctions of the gums and teeth. Burtonian line caused by a reaction between circulating lead with sulfur ions released by oral bacterial activity, which deposits lead sulfide at the junction of the teeth and gums. It may sometimes appear as a stippled bluish-black line at the junctions of the gums and teeth. The lead line is visible when oral hygiene is poor resulting subepithelial deposition of lead sulfide granules liberated by microorganism from decomposing protein food deposits. Furthermore, other oral changes include sweetish metallic taste in the mouth, halitosis, and dyspepsia (Shetty S R. 2015).

Our findings were supported by Wani Ab Latif *et al.* (2016); who observed symptoms related to mouth and teeth such as dental erosion and discolorization, halitosis (bad breath), ulceration and mouth bleeding indicate that occupational presence of the workers in polluted environment containing lead dust in the air can make easy passage into body through mouth. This may cause the mouth and its related tissues very prone to infections. Even the passage of lead dust to the mouth may cause the epithelial layer of mouth prone to easy damage, which can cause the mouth bleeding frequently. There are no studies, which show the symptoms such as halitosis, ulceration, and mouth bleeding in relation to lead.

## 5.15: Awareness of occupational hazards of lead and their routes of entry into the body.

**Table 5.27: Percentage distribution of awareness of occupational hazards of lead and their routes of entry into the body.**

| Questions | Group-I (n=70) | Group-II (n=70) | Group-III (n=70) |
|---|---|---|---|
| Heard about heavy metal lead | 70 (100%) | 6 (8.6%)* | 14 (20.0%) |
| Heard about the occupational hazards of heavy metal lead. | 67 (95.7%) | 2 (2.9%)* | 9 (12.9%) |
| **Knowledge regarding lead entry in the body.** | | | |
| Inhalation | 70 (100.0%) | 38 (54.3%)* | 53 (75.7%) |
| Ingestion | 65 (92.9%) | 21 (30.0%)* | 27 (38.6%) |
| Dermal Contact | 65 (92.9%) | 22(31.4%) | 13 (18.6%)* |

Group I–Control Group, Group II - Automobile Mechanics (AM), Group III - Petrol Pump Workers (PPW). * depicts lowest percentage frequency

Table-5.27: indicates the awareness of workers regarding the heavy metal lead, occupational hazards of heavy metal lead, & routes of lead entry into the body. We noticed that less number of group-II (AM) and III (PPW) workers aware of the occupational hazards of lead. We also observed less number of group-II workers reported inhalation and ingestion are the routes of entry while less number of group III workers reported their entry through dermal contact of these substances knowledge regarding its entry in the body compared to controls.

Our results are parallel with the results of M. Adejumo *et al.* (2017); who revealed that large proportion of the participants have not heard about lead (89.2 %), lead poisoning (97.3%). This is an indication that automobile mechanics (workers) in the study area had little or no knowledge about lead and lead poisoning. It also shows that automobile mechanics have poor knowledge about the effect of lead and route of entry into the human body. Marahatta SB *et al.* (2018); reported that only 56% had awareness of an occupational hazard and 44.3% of automobile mechanics were using personal protective equipment.

Similar findings were observed by Zerihun Ataro *et al.* (2018); They noticed that all the garage workplaces were dusty and the workers did not follow safety measures. Regarding possible routes of chemical entry into the body, 16 (53.3%) workers mentioned that inhalation was the route of entry, followed by 15 (50%) who reported that ingestion was the route and 13 (43.3%) who claimed that skin was the route of entry. A total of 12 (40%) workers had knowledge about the effects of chemicals on human health. Workers had neither attended training courses nor had health professionals visit their station.

## 5.16: Percentage distributions of practices performed and the use of personal safety measures by the workers.

**Table–5.28: Percentage distribution of practices and use of personal safety measures at workplaces in study groups.**

| Characteristics | Group-II (n=70) | Group - III (n=70) |
|---|---|---|
| **Practices** | | |
| Sucking Fuel with Mouth | 57 (81.4 %) | 3 (4.3 %) |
| Hand wash with fuel | 70 (100.0% ) | 30 (42.9%) |
| **Personal safety measures** | | |
| Wear Nose Mask | 24 (34.3%) | 18 (25.7%) |
| Wear Hand Gloves | 4 (5.7%) | 0 (0%) |
| Wear long Shoes | 27 (38.6%) | 19 (27.1%) |
| **Causes of not using safety equipment** | | |
| Carelessness | 57 (81.4%) | 57 (81.4%) |
| Not provided | 56 (80.0%) | 45 (64.3%) |
| Discomfortable | 55 (78.6%) | 50 (71.4%) |
| Not Necessary | 35 (50.0%) | 28 (40.0%) |
| Group II - Automobile Mechanics (AM), Group III - Petrol Pump Workers (PPW). | | |

Table - 5.28 indicates the percentage distribution of practices and personal safety measures and causes of not using personal safety measures at workplaces in the study group. The higher percentage of group-II workers practiced sucking fuel with the mouth (81.4%) and washing hand with fuel (100%) as compared to group-III workers (4.3 % & 42.3%). We observed that higher percentage of the group-II workers did not wear nose mask (34.3%), hand gloves (5.7%) and long shoes (38.6%) as compared to group-III (25.7%), whenever they were on duty. A higher percentage (80.0%) of group-II workers did not use safety measures (nose mask, hand gloves and shoes) as they were not provided to them. 81.4% were careless about using safety measures and 50.0% felt it was not necessary to use safety measures.

Similar findings were noticed by Patrick Adu *et al.* (2018); who reported similar findings in Ghana such as majority (75.6%) of cases had never received occupational safety training. Whereas 35.1% of automobile mechanics routinely siphoned fuel, 36.4% of automobile sprayers never used nose masks in the discharge of their duties. Amah UK *et al.* (2014); also shows only 3% of the automobile repairers wear nose mask, 87% practices fuel pipetting with the mouth, while 100% wash their hands with fuel. This is an indication that the major sources of exposure are inhalation, ingestion and dermal absorption. In spite of the awareness, similar findings were observed by Thangaraj S *et al.* (2017); who reported about 22.5% (n=27) of the mechanics were observed using hand gloves, 15.26% used eye goggles during welding and 13.68% (n=13) used safety boots at the workplace. Less than half (44%) of them were using personal protective equipment for their safety.

While collecting data we had found some lacunae at the workplaces such as,

- There was no changing room available.
- No washbasin, soap for hand wash and a separate room for dining.
- No toilet facility and general cleanliness surrounding the workplace.

None of the workers attended any training course regarding occupational health hazards & safety. None of the health professionals had visited the petrol pump and automobile workshops for a health checkup.

# SUMMARY AND CONCLUSION

## 6.1: Summary

The purpose of the present study was to know the occupational health status of petrol pump workers (PPW) and automobile mechanics (AM) of western Maharashtra. It was a human cross-sectional study, carried out in western Maharashtra (Sangli, Satara and Kolhapur District). Study participants were divided into three groups, Group-I as a control group, Group-II (automobile mechanics), Group-III (petrol pump workers).

We hypothesized that occupational exposure to petroleum and exhaust fumes with heavy metals lead causes significant alterations in cardiorespiratory, hematological, biochemical and cytogenetic parameters. Occupational exposure to petroleum and exhaust fumes increases oxidative stress biomarkers like malondialdehyde **(**MDA) and γ-glutamyl transferase (GGT) among study and control group.

1. Our findings revealed that petrol pump workers and automobile mechanics had a basic level of education and none of them were illiterate. They are from a low socioeconomic background. The higher percentage of self-reported symptoms observed in petrol pump workers and automobile mechanics.
2. The present occupational health research carried out in Western Maharashtra has clearly documented that there is an increased concentration of blood lead level (BLL) in automobile mechanics& petrol pump workers as compared to the control group. High blood lead level induces oxidative stress in automobile mechanics and petrol pump workers, because of the presence of heavy metal lead and other particles as agents of free radicals. This is evidenced by a raised level of oxidative stress parameters (MDA and GGT level) in the study groups as compared to control. As the duration of exposure increases, there was a significant increase in BLL, MDA and GGT activity in the study group.
3. There was a significant increase in SBP and MAP among group-II and group-III as compared to control. As the duration of exposure increases, there was a significant increase in SBP, PP, MAP, and HR observed in the study group.

4. There was a significant decline in lung functions (PEFR and 40 mmHg endurance time) in study groups as compared to control. Decreased PEFR point out towards adverse effects of petroleum and exhaust fumes on lung functions, mainly on small airways with a restrictive pattern of disease. With an increase in the duration of exposure, there was a significant decrease in PEFR and 40 mmHg endurance time in the study group.
5. There was a significant reduction in Hb%, RBC count, PCV& blood indices (MCV, MCH, MCHC) and significant increase in platelet count, total and differential leucocytes count (polymorphs, lymphocyte, eosinophil, basophil & monocyte) amongst group-II and III as compared to group-I. As the duration of exposure increases, there was a significant decrease in Hb%, RBC count, MCHC and significant increase in total WBC count in study groups.
6. We found significantly increased activity of liver enzymes like SGOT, SGPT, ALP and serum bilirubin (total, direct) and a significant decrease in sr. proteins (total, albumin, and globulin) in group-II and III as compared to the control group. As the duration of exposure increases, there was a significant increase in liver enzymes like serum SGOT, SGPT, ALP and total bilirubin group-II (AM).
7. There was a significant increase in the concentration of blood urea, serum Creatinine & electrolytes ($Na^+$and $K^+$) in group-II and III as compared to control. As the duration of exposure increases, there was a significant increase in blood urea, serum creatinine and electrolytes ($Na^+$and $K^+$) concentration in study groups.
8. There was a significant positive correlation of blood lead level with SBP, MAP and serum MDA, while a significant negative correlation of blood lead level with PEFR, Hb%, RBCs count was observed in combined study groups.
9. From the present study it clear that the automobile mechanics and petrol pump workers have increased risk of cytogenetic damage as tested by MN assay. Higher frequency of other nuclear abnormalities (binucleates, karyolysis, and karyorrhexis) in exfoliated epithelial cells suggested a higher degree of apoptosis, necrosis, and cell death, which is a response towards adverse occupational exposure.
10. Less number of group-II (AM) and III (PPW) workers aware of occupational hazards of lead.
11. The higher percentage of group-II and group-III workers did not use safety measures.

12. Poor oral health was observed in group-II (AM) and III (PPW) workers.

## 6.2:Conclusion:

Our study also provides important information regarding occupational health status, ground picture of awareness level and use of personal safety measures among the petrol pump workers and automobile mechanics of Western Maharashtra.

Heavy metal (Pb) and particulate matter present in petroleum and exhaust fumes induces oxidative stress which affects organs (lungs, liver, and kidney) and systems like a cardiovascular, respiratory, hematopoietic system in automobile mechanics and petrol pump workers

The outcome of this research project has indicated acceptable risk in automobile mechanics than petrol pump workers of Western Maharashtra.

The findings of the present study are an eye-opener, indicating the need for occupational health programs. The Government, occupational health authorities, other relevant statutory bodies, and employers should play active roles in providing the workers with knowledge on occupational health hazards, provision of safety measures and periodic health checkups.

## 6.3: Study Limitations.

- The study is confined only to male workers with a limited sample size.
- We have not estimated lead-induced cell damage via cell signaling pathway.

## 6.4: Future Prospective.

- To find out molecular markers of cell signal transduction.

## 6.5: Recommendations.

In 'India' a large section of the workers are employed in unorganized sectors. The large numbers of workers are being illiterate and are not aware of the occupational hazards associated with their occupation. Similarly, the owners are also unaware about the occupational hazards resulting from the improper workplace. This results in poor implementation of safety measures and enforcement of laws.

To minimize the health-related issues,

1. Use of personal safety measures like nose mask, hand gloves, apron, shoes, goggles by the automobile mechanics and Petrol pump workers.
2. To minimize inhalation rates of toxic fumes improve ventilation and introducing air filtration systems will enhance air quality at the sites.
3. Periodic health check-up camp should be arranged every year.
4. To create better awareness of occupational hazards, workers should receive training courses, health education and awareness program in the workplaces with the supports of policymakers. Awareness program should include information about the health hazards of occupational exposure, the importance of personal hygiene & the use of appropriate personal safety equipment.
5. Workers should be advised to do regular physical exercise and pranayama to strengthen the respiratory system.

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## INFORMED CONSENT FORM

**Study Title:** "Study on occupational health of petrol pump workers and automobile mechanics in western Maharashtra with special reference to cytogenetic alterations".

Study Number: -……

Subject's Full Name: - ……

Date of Birth / Age ……

Address ……

1) I confirm that I have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered.

2) I have read and understood this consent form and information provided to me.

3) I have been explained above the nature of the study.

4) I have been explained about the duration of participation with the number of participants.

5) I have been explained about procedures to be followed and about investigations if any to be performed. I have been explained that I don't have to pay or bear the cost of procedure/investigations.

6) My rights and responsibilities have been explained to me by the investigators.

7) I have been adequately explained the risks and discomforts associated with my participation in the study.

8) I have been explained about the benefits of my participation in the study to myself, community and to the medical profession.

9) If despite following the instructions I am physically harmed because of any substances or any procedures as stipulated in the study plan my treatment will be carried out free of cost at the investigational site and the sponsor will bear all the expenses, If they are not covered by insurance agency or by Government program or any third party. I have had my questions, answered to my satisfaction

10) I have been explained about available alternative treatments.

11) I understand that my participation in the study is voluntary and I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

12) I hereby give permission to the investigators to release information obtained from me as a result of participation in the study to the sponsors, representatives of sponsors, regulatory authorities, Government agencies & ethics committee. I understand that they may inspect my original records.

However, I understand that my identity will not be revealed in any information released to third parties or published.

13) I agree not to restrict the use of any data or results that arise from the study provided such use is only for the scientific purpose (S).

I am exercising my free power of choice, hereby give my consent to be included as a participant in the present study.

I agree to co-operate with the investigator and I will inform him/her immediately. If I suffer unusual symptoms.

I am aware that if I have any questions during the study, I should contact one of the addresses listed. By signing this consent form I attest that this document has been clearly explained to me and understood by me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:_____

Signatory's Name_____ Date_____

Signature of the Investigator_____ Date_____

Study Investigator's Name _____

Signature of the Witness _____ Date_____

Name of the Witness _____

**Contact Numbers:**

1. **Miss Smita V. Patil** (Investigator) – 9503628202 ,8208250236.

   Email –mailmesmita.patil@rediffmail.com

# PROFORMA

**1) Preliminary data:**

Name: ……..

Address: …….

Phone No……

Educational Qualification……..

Name of the petrol pump…….

Age:-……Years Sex: - Male / Female

Height: - ……cm. Weight: - …….kg. BMI …….

Job specification: …….

Year of employment…….

Year of exposure…….....

Working Time - Day / Night

Working Hours/ Day……..

Working Days / Week……

Previous workplace- …….

Diet: - Vegetarian / Non-vegetarian / mixed.

Daily water intake……Liter

**2) <u>History Regarding</u>:-**

Present medical illness:-

Complaints at present:-

**3) <u>Past History</u>:-**

Respiratory / Cardiovascular – Illness in past

Asthma ……

T.B…….

Diabetes……...

Hypertension……

Any other illness: ……

**4) <u>History Regarding Sports</u>:-**

Types of sports played:-……..

Time of practice in a day: -…….. Hours.

Regular exercise other than sports - yes/no

Time duration: - ……..... Hours

Type: - heavy / light

**5) <u>Systemic Examination</u>:-**

It is carried out to rule out any major diseases of,

Cardiovascular system

Respiratory system

Alimentary system

**6) Any frequent Symptoms amongst the following**

Skin irritation –…… Headache…….

Insomnia……. Mental confusion ……

Muscle pain……. Dizziness…….

Weakness…… Nausea……

Convulsions …… Abdominal pain ……

Eye Irritation –…… Chest pain.....

Cough..... Low appetite…….

Nausea…… Vomiting.....

Diarrhoea..... Constipation.....

Conjunctivitis.....

**7) Oral Health** –

Mouth Ulcers …… Mouth Bleeding.....

Gingivitis……... Periodontitis…….

Caries…… Burtonian line……...

## Questionnaire for Awareness of Workers

1) Do you think that exposure to petroleum products & heavy metal lead have effects on human health? …… Yes / No.

2) Are you familiar with the health hazards of petroleum products and the heavy metal lead?

……. Yes / No.

3) Have u attended any training course in health hazard of petroleum product exposure ……..…… Yes / No.

4) If yes, please specify the institution or other sectors which conduct the course …….

5) By which root they enter the body……Inhalation / Ingestion / Contact.

6) Do you think that there is a need to use the mask …….. Yes / No.

7) Do owners provided you the same ……Yes / No.

8) Do you use them……... Yes / No.

9) If no why? .....

10) Do you think that there is a need to use the Gloves……..Yes / No.

11) Are they providing it to you…… Yes / No.

12) Do you use it …….……... Yes / No.

13) If no why? .....

14) Do you think that there is a need to use special shoes…….…...Yes / No.

15) Are they provide to you…….. ……..…Yes / No.

16) Do you use it ……..…...Yes / No.

17) Do you wash your hand before eating ……..Yes / No.

18) Do you wash your hand with soap ……... Yes / No.

19) Does any health professional come to visit your workplace periodically? …….……Yes / No.

20) If yes, please, specify the health professional sector, who conducted the Visit…….

## Inspection at local sites

Changing room…… ……..... Yes / No.

Separate room for Dining…… Yes /No.

Availability of washbasin …….. Yes /No.

Availability of Soap / Hand wash…… Yes /No.

Availability of Toilet facility…… Yes /No.

General cleanliness…….. Yes /No.

# Plagiarism Verification certificate

**BLDE (DEEMED TO BE UNIVERSITY)**

Annexure -I

**PLAGIARISM VERIFICATION CERTIFICATE**

1. **Name of the Student:** Smita Vitthal Patil **Reg No:** 12PHD013
2. **Title of the Thesis:** "Study on occupational health of petrol pump workers and automobile mechanics in western Maharashtra with special reference to cytogenetic alterations".
3. **Department:** Physiology
4. **Name of the Guide & Designation:** Dr. Sumangala Patil, Professor and Head.
5. **Name of the Co Guide & Designation:** Dr. Sampada kanitkar Ex. professor and Head.

**The above thesis was verified for similarity detection. The report is as follows:**

**Software used: Turnitin** **Date: 03/09/2019**

**Similarity Index (%): Five (5%)** **Total word Count: 38540.**

**The report is attached for the review by the Student and Guide.**

**The plagiarism report of the above thesis has been reviewed by the undersigned.**

**The similarity index is below accepted norms.**

**The similarity index is above accepted norms, because of following reasons:**

.....

.....

.....

**The thesis may be considered for submission to the University. The software report is attached.**

**Signature of the Guide**
**Name & Designation**

Prof. and Head
Dept. of Physiology
BLDE(DU) Shri B.M. Patil Medical College, Hospital & R.C.
Vijayapur-586103.

**Signature of Co-Guide**
**Name & Designation**

**Signature of Student**

03.09.2019

**Verified by (Signature)**
**Name & Designation**

B.L.D.E. University's
Shri B.M. Patil Medical College
Bijapur.

# Institutional Ethical Clearance Certificate

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No-104/2014-15 Dec 5, 2014.

**INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE**

*The Ethical Committee of this University met on 1st December 2014 at 11 AM to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate student / Faculty members of this University / college from ethical clearance point of view. After scrutiny the following original / corrected & revised version synopsis of the Thesis / Research project has been accorded Ethical Clearance.*

*Title* " study on Occupational Health of petrol pump workers and Automobile mechanics in western Maharashtra with special reference to cytogenetic alterations."

*Name of PH.D./ P. G. / U. G. Student / Faculty member*. Miss. Smita Vitthal Patil.Department of Physiology.

*Name of Guide* : Dr.Sumangala Patil. Professor Department of Physiology.

**Dr. Sharada Metgud**
Chairperson, I.E.C
BLDE University,
VIJAYAPUR – 586 103

**Dr.G.V.Kulkarni**
Secretary, I.E.C
BLDE University,
VIJAYAPUR – 586 103.

Member Secretary,
Institutional Ethical Committee,
BLDE University, BIJAPUR.

**Note:-Kindly send Quarterly progress report to the Member Secreatary.**

Following documents were placed before Ethical Committee for Scrutinization:

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant documents.

Institutional Ethical Committee * BLDE University, BIJAPUR

Smt. Bangaramma Sajjan Campus, Sholapur Road, Vijayapur – 586103, Karnataka, India.

**University:** Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeuniversity.ac.in, E-mail: office@bldeuniversity.ac.in

**College:** Phone: +918352-262770, Fax: +918352-263019, Website: www.bldeuniversity.ac.in, Emaibmpmc.principal@bldeuniversity.ac.in

# PAPER PRESENTATIONS AND AWARDS

**Paper Presentations:**

**Oral paper presentations at national conferences:**

1) Title: "Effect of occupational exposure on cardio-pulmonary efficiency in petrol pump workers & in automobile mechanics of Western Maharashtra".
   National conference on CSCN-2015, 2nd & 3rd September 2015 at D.Y. Patil Medical College Kolhapur.
2) Title: "Micronuclei (MN) - as a biomarker of genotoxicity in automobile mechanics of western Maharashtra".
   National Conference ASSOPICON- 2016, 15th to 17th September 2016 organized by BLDE University's Shri B. M. Patil Medical College, Hospital and Research Centre Vijaypur.
3) Title: "Effects of occupational lead exposure on the Hematological Parameters and its correlation with blood lead level in petrol pump workers of western Maharashtra (India)."
   National conference on "Cardioprotective Role of Progesterone-A New Perspective" CAPROP-2019 held on 18th January 2019 Organized by Department of Physiology at D.Y. Patil Medical College, Kolhapur, Maharashtra.
   Received **"Second Prize for paper presentation"**

## Oral paper presented at International conferences:

1) Title: "Study of occupational exposure to heavy metal (lead) on the Hematological parameters & its correlation with blood lead level in automobile mechanics of western Maharashtra (India)" 4th International conference on lead and heavy metal toxicity-LEDCON 2018 at North Bengal Medical College and Hospital on 4th& 5th October 2018.

## Poster presentation:

1) **Title:** Effects of occupational lead exposure on the Hematological indices and its correlation with blood lead level in automobile mechanics of western Maharashtra (India). On “Research Day 2018” at BLDE University’s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijaypura.

   Received **“Best poster Award”**

# PUBLICATIONS

1. Patil Smita V, Sumangala Patil, Sampada Kanitkar. Study of Peak Expiratory Flow Rate as the Assessment of Lung Function in Occupationally Exposed Petrol Pump Workers of Western Maharashtra. JKIMSU.April-2016; 5(2):95-100**.**
   **(Indexed in Scopus)**

2. Patil Smita V, Sumangala Patil, Sampada Kanitkar, and Pandurang Gaikwad. Effect of Petroleum Fumes on Cardio -Pulmonary Efficiency in Petrol Pump Workers of Western Maharashtra. RJPBCS. November–December 2017, 8(6) 408-412.
   **(Indexed in Scopus)**

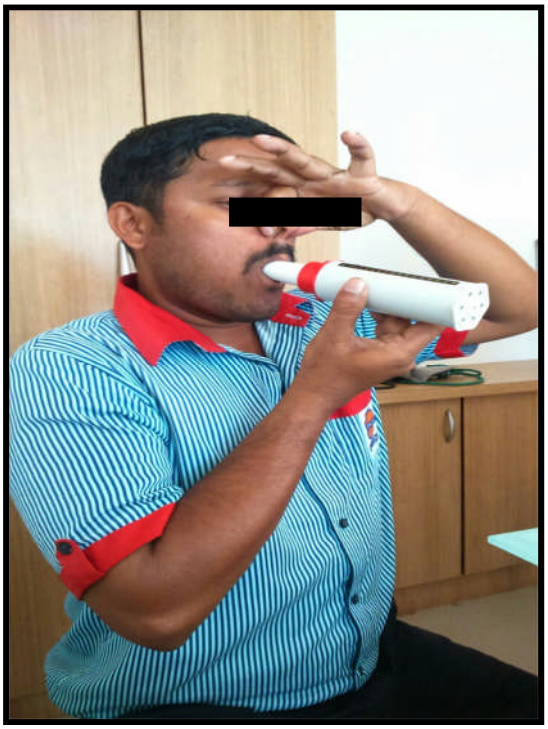
3. Smita V Patil, Sumangala Patil, Sampada Kanitkar, Micronuclei - As A Biomarker Of Genotoxicity In Automobile Mechanics Of Western Maharashtra. 2018 AJPCR. 11( 8): 467-469.
   **(Indexed in Scopus)**

# Photographs

**Photographs taken while collecting data and blood sample from study subject.**

**Photograph taken when doing blood lead estimation on Atomic Absorption Spectrophotometer (AAS).**


**Photograph taken when doing PEFR and MDA estmation.**



ORIGINAL ARTICLE

# Study of Peak Expiratory Flow Rate as the Assessment of Lung Function in Occupationally Exposed Petrol Pump Workers of Western Maharashtra

*Patil Smita V[1*], Sumangala Patil[1], Sampada Kanitkar[2]*

*[1]Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Vijayapur-562135(Karnataka) India, [2]Department of Oral Pathology, Bharati Vidyapeeth University Dental College and Hospital, Sangli-416416 (Maharashtra) India,*

**Abstract**:

*Background*: Fast urbanization trends, rapid industrial growth, globalization, and poor environmental conditions at work places have created a lot of health-related issues. *Aim and Objectives:* The aim of this study is to investigate Peak Expiratory Flow Rate (PEFR) as the assessment of lung function in occupationally exposed petrol pump workers and also check whether PEFR increases or decreases with duration of exposure. *Material and Methods*: The study was conducted on 60 male petrol pump workers between age group of 20-40 years who were working as petrol filling attendants for more than one year from western Maharashtra. 50 normal healthy males with same socioeconomic status were chosen as controls to find out the effect of occupational exposure to petroleum product on PEFR as the assessment of lung function tests. Petrol pump workers were divided into three groups based on their duration of exposure i.e. 1-5 yrs, 6- 10 yrs and more than 11 years. PEFR of petrol pump workers and control was measured by using a Mini Wright peak flow meter which is a portable device for measuring ventilator functions. Comparisons was done using unpaired t-test for 2 groups comparisons and one way ANOVA for multiple groups of exposures. *Results:* The PEFR was significantly lower decrease (p=0.001) around petrol pump workers (389.17) as compared to control (534.2. As year of exposure increased mean value of PEFR was significantly decreased from 452.17, 378.00 and 283.64 respectively in petrol pump workers. *Conclusion:* The results suggested that respiratory functions i.e. PEFR of occupationally exposed petrol pump workers are significantly reduced as compared to controls, also PEFR is significantly reduced with increase in the duration of exposure.

**Keywords:** Occupational Exposure, Petrol Pump Workers, Peak Expiratory Flow Rate, Year of Exposure

## Introduction:

Millions of workers in a variety of occupational settings have the potential to get exposed to hazardous substances. These substances include organic chemicals, intermediates, by-products or end products [1]. Petrol pump workers are exposed to various petroleum products (petrol, diesel).These petroleum products (petrol, diesel) contain various organic compounds such as benzene, toluene, ethylbenzene and xylene (BTEX compound). Petrol pump workers are coming in contact with these BTEX compounds through inhalation, ingestion and dermal contacts. However, the main route of exposure is the respiratory system. The volatile nature of petrol and diesel increases its concentration in air at petrol filling stations, automobile garages and depots. The people working in these areas are continuously coming in contact with these BTEX compounds due to their occupational exposure [2]. Petrol or diesel is mainly used as a fuel for road vehicles e.g. cars, motorbikes, and small van, and also small appliances like lawnmowers, generators, cement mixers, etc.

Peak Expiratory Flow Rate (PEFR) is a sensitive indicator for predicting the magnitude of airway obstruction [3]. Lung reactions to exposure to dust, gases, and fumes at work places have been studied in different populations. The emission level of pollutants that emit particulate matter less than 10 micrometers in size (PM 10) has been found very high in Western Maharashtra. There is a high prevalence of occupational diseases, such as, silicosis, asbestosis, and pneumoconiosis among workers working in different industrial environments in India [4].

Hence, petrol pump workers in western Maharashtra are likely to get exposed to a high level of air pollution along with petrol and diesel vapours, both of these factors can affect the respiratory health of petrol pump workers. Also there is lack of availability of sufficient research on the occupational aspects of petrol pump workers in Western Maharashtra.

**Material and Methods:**

After the approval by institutional ethics committee, a cross sectional study was carried out on 60 healthy petrol pump workers of Western Maharashtra (Sangli, Satara and Kolhapur Dist). They were further divided in to three groups depending on their duration of exposure as Group I (1-5 years), II (5-10 years) and Group III (more than 10 years). 50 control subjects were chosen from paramedical staff of same socioeconomic status from Bharati Vidyapeeth Medical and Dental College and Hospital Sangli. Written informed consent was obtained from subjects. With prior appointment, petrol pump workers were interviewed with the help of pretested questionnaire. Workers were evaluated as per standard proforma, which included questionnaire regarding health status. The workers with, past or present history suggestive of cardiovascular or respiratory illness or any other systemic illness, any family history of asthma or allergic diseases, were excluded from the study. Only nonsmoker workers were enrolled. Subjects performing any type of yoga or pranayama and other physical exercises like resistance training, sports and athletics were excluded from the study. 60 workers were eligible to whom the experimental protocol was explained and written informed consent was obtained from them. PEFR was measured with the help of mini wrights peak flow meter which is a portable device for measuring ventilator functions. The readings were taken in liters per minute. Subjects were advised to wear loose fitting clothing over the chest and abdominal area. Breathing maneuvers was explained in detail and demonstrated for the subjects. Subjects were then made to practice breathing into the mouthpiece of flow meter until they could duplicate the maneuvers successfully on three consecutive attempts.

**Statistical Analysis:**

Results were presented as Mean ± SD. Unpaired t-test and ANOVA test was used to find the significance of study parameters by using SPSS 16.0 version. $P < 0.001$ was considered as statistically significant.

**Results:**

Table 1 indicates that age wise distribution of PEFR in petrol pump workers and in control group. The results showed a significant reduction in the PEFR in petrol pump workers, as compared to the control group as shown in Table 2. Table 3 shows that correlation between years of exposure and PEFR. An observed value of PEFR according to duration of exposure in Table 4 indicates linear decrease in mean values with increasing duration of exposure. Table No 5 shows that between group comparison Using post hock test. Differences between mean values of PEFR in 1-5 years of exposure group with 6- 10 years exposure group and also with 11-15 years exposed group were a found to be statistically significant.

**Table 1 Age Wise Distribution of PEFR in Study and Control Group**

| | Age | N | Mean | SD |
|---|---|---|---|---|
| **Petrol Pump Workers** | 20 - 30 years | 27 | 397.87 | 99.10 |
| | 30 – 40 years | 33 | 374.07 | 92.20 |
| **Control Group** | 20 - 30 years | 33 | 545.75 | 61.39 |
| | 30 – 40 years | 17 | 517.05 | 58.81 |

**Table 2 Showing Mean and SD of PEFR in Petrol Pump Workers and Control Group**

| Parameters | Group | N | Mean ± S.D | t test | P value |
|---|---|---|---|---|---|
| **PEFR (L/min.)** | Petrol pump workers | 60 | 389.17 ± 96.07 | -9.298 | 0.001 |
| | Control group | 50 | 534.29 ±60.89 | | |

**Table 3 Correlation between Years of Exposure and PEFR among Petrol Pump Workers**

| | Mean | SD | N | R value | P value |
|---|---|---|---|---|---|
| **Exposure** | 1.81 | 0.74769 | 60 | - 0.651 | 0.001* |
| **PEFR** | 387.16 | 96.07715 | 60 | | |

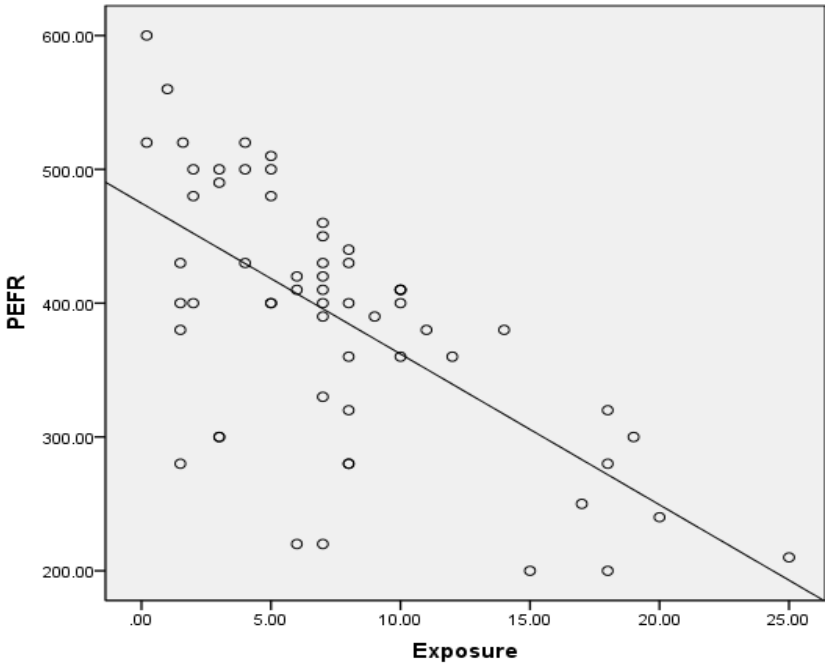
*Pearson correlation test. * Significant*

**Table 4 showing Mean and SD of PEFR in Petrol Pump Workers according to the Duration of Exposure**

| Year of Exposure | N | Mean ± SD | F | P value |
|---|---|---|---|---|
| **1-5 yrs.** | 23 | 452.17±84.151 | 19.515 | 0.001 |
| **6-10 yrs.** | 25 | 378.00±66.771 | | |
| **More than 10 yrs.** | 12 | 283.64±69.609 | | |

**Table 5 showing between Group Comparison using Post Hoc Tests**

| Year of exposure | | Mean Difference | P value |
|---|---|---|---|
| **1-5 yrs.** | 6-10 yrs | 74.174 | 0.001 |
| | More than 10 yrs | 168.538 | 0.001 |
| **6-10 yrs** | More than 10 yrs | 94.364 | 0.001 |




PEFR- Peak Expiratory flow rate (L/min); Exposure – Years of exposure

**Fig.1: Graph showing Correlation Slope and Decrease in PEFR per Year of Exposure**

**Discussion:**

Rapidly increasing number of vehicles in western Maharashtra is responsible for increase in workload at petrol pumps. It has resulted in increase in the workload of petrol pump workers who are constantly exposed to exhaust fumes and fuel vapors throughout their duty hours. In the present study we have tried to assess dynamic lung function PEFR in petrol pump workers. PEFR was compared between petrol pump workers and control group. Our study was different from other studies in the aspects that we compared our workers according to the duration of exposure and we found statistically significant change in PEFR with exposure of 5 years, 10 years and more than 10 years.

**Effect of Petroleum Fumes on the Peak Expiratory Flow Rates of Petrol Pump Workers and Control Group**

From this research finding, there was significant decrease in the mean peak expiratory flow rates of the petrol pump workers as compared to control group (389.17 and 534.29) respectively.

Findings of our study coincide with previous studies of Das *et al* (1991), Tyagi *et al* (2013), Uzma *et al* (2008), Kittad *et al* (2015), Singhal *et al* (2007), Verma *et al* (2001) [5-10]. In these studies, lung function test PEFR was significantly decreased in petrol pump workers and petrol filling workers. Ezejindu *et al* 2014 [11] studied the PEFR of petrol station attendants in Nnewi using a wright peak flow meter, they observed that statistically insignificant decrease in PEFR which could be due to use of small sample size.

**Effect of Petroleum Fumes on the Peak Expiratory Flow Rates of the Petrol Pump Workers Based on Years of Exposure to Petroleum Fumes**

From the result of this research, there was also a decrease in the mean PEFR of the petrol pump workers according to their years of exposure. The mean value of PEFR decreased with increased years of exposure; this change was found to be statistically significant (P-value 0.001). Our results coincide with Akor-Dewu *et al* (2008), Tyagi *et al* (2013) and Sofoola *et al.* (2005) [12,6,13] who carried a research to assess pulmonary function tests amongst adult male petrol station attendants.

Table 4 and 5 represent the lung function versus duration of exposure of petrol pump workers shows that subjects who had worked for a longer duration at the petrol stations had lower mean values of PEFR. This reduction may be not only due to increasing age of subjects but also because of petrol pumps are located on busy roads, hence these workers in addition to petrol and diesel exhausts it also get exposed to air pollution when vehicles coming to fill up petrol/diesel (Table 1).

The occupational solvents like benzene in petrol and diesel fumes get absorbed into the human body either through the respiratory tract or via epidermal contact. This reduction in PEFR in present study may be due to occupational solvents and air pollutant exerts an irritant effect upon the bronchial epithelium, it also affects the cilia and clara cells of the lungs and release proteolytic enzymes from macrophages [14]. These changes destroy the lung substance and reduce elastic recoil of the lungs. These may cause respiratory symptoms and impaired pulmonary functions resulted in reduced PEFR in petrol pump workers as compared to controls [15]. Accumulation of dust laden macrophages leads to varying degree of wall thickening and remodeling in terminal and respiratory bronchioles [16]. This small airway damage might be the cause of reduced pulmonary functions.

**Conclusion:**

From the present study it can be concluded that PEFR of petrol pump workers who are continuously exposed to petroleum fumes is significantly reduced as compared to PEFR of age, weight and height matched control groups. Also it was observed that PEFR of petrol pump worker who had exposed for more than 10 years were more affected than those who had worked for less than 5 years. Also it is observed that there is negative correlation between PEFR and years of exposure.

**Recommendation:**

To minimize the health related issues, periodic health check up along with pulmonary function tests every year should be done every year. Health check-up camps should be arranged frequently. Use of effective personal protection by petrol pumps workers like gloves, apron, long shoes, and appropriate respiratory protective equipments i.e. anti-pollution masks should become mandatory to prevent lung diseases in them.

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[*]***Author for Correspondence****: Dr. Mrs. Smita V. Patil (Gaikwad), Deparment of Physiology, Bharati Vidyapeeth Deemed University Dental College and Hospital, Sangli-416416 (Maharashtra) India*
*Email: mailmesmita.patil@rediffmail.com Cell: 8983020456, 9503628202*

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Effect of Petroleum Fumes on Cardio -Pulmonary Efficiency in Petrol Pump Workers of Western Maharashtra.

**Patil Smita V[1]*, Sumangala Patil[2], Sampada Kanitkar[3], and Pandurang Gaikwad[4].**

[1]Ph.D Scholar, B.L.D.E University, Shri B.M.Patil Medical College Vijaypur, Working at Bharati Vidyapeeth University Dental college & Hospital, Sangli
[2]Ph.D. Guide & Professor in Dept. of Physiology, BLDE University Shri B.M.Patil Medical College, Vijaypur.
[3]H.O.D. & Professor. Dept of Oral Pathology Bharati Vidyapeeth University Dental College & Hospital, Sangli.
[4]M.Sc. Medical Physiology, Assistant Professor. Dept. of Physiology, Prakash Institute of Medical Science and Research Islampur. Dist. Sangli (Maharastra) India

**ABSTRACT**

Although petroleum products are useful chemical compounds which form an integral part of our modern technology, they have been reported that inhalation of petroleum products cause hazardous effect on health. Long term exposure to these chemical has deleterious effect on heart and lungs. This work was undertaken to identify cardio- pulmonary efficiency among the petrol pump workers (PPW)of western Maharashtra. The study was conducted on 60 male petrol pump workers between age group of 20- 40 years who were working as petrol filling attendant for more than one year. 60 normal healthy male with same age & same socioeconomic status were chosen as control to find out the effect of occupational exposure to petroleum fumes on cardio-pulmonary efficiency. Blood pressure & 40 mmHg endurance time was measured clinically with the help of Sphygmomanometer & stop watch. Heart rate & breath holding time was measured with stop watch. There was significant increase in systolic, diastolic blood pressure& Heart Rate (HR) in petrol pump workers as compare to control group. The mean value of systolic and diastolic blood pressure increases with increased years of exposure; this change was found to be statistically significant (P-value 0.001). But the mean value of HR was not increased according to year of exposure. Also there was significant decrease in breath holding time & 40 mmHg endurance time ($p < 0.05$) in petrol pump workers as compare to control. The mean value of breath holding time & 40 mmHg endurance time significantly decreases with increased years of exposure. The cardiopulmonary tests are done & results suggest that cardio-pulmonary efficiency are significantly compromised in petrol pump workers as compared to control subject as they are exposed to traffic vehicle exhaust apart from environmental pollutants.

**Keywords:** Petrol Pump Workers (PPW), Heart Rate (HR), Systolic Blood Pressure (SBP), Diastolic Blood Pressure(DBP), Breath holding Time (BHT), 40mmHg endurance time.

***Corresponding author***

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## INTRODUCTION

India is a rapidly developing country and automobiles running on roads are increasing day by day. Petrol is a generic term for petroleum fuel which is mainly used for internal combustion engines, it mainly containtoxic chemical substances like benzene, toluene, ethylbenzene, xylene (BTEX Compound) and lead. Other compounds found in petrol are manganese, naphthalene, trimethylbenzene and Methyl tert-butyl ether (MTBE).

Petrol pump workers are exposed to petroleum hydrocarbon through ingestion of contaminated food, drinking contaminated water, contact with contaminants (dermal exposure) or inhalation of vapour and air-borne soil. [1]

Urbanization has led to enormous increase in number of automobiles and accordingly in order to increased number of petrol filling stations and workshops for repairing the vehicles. Workers engaged in these places are continuously exposed to petrol and diesel fumes. The particles generated from petrol exhaust are extremely small and are present in the nuclei or accumulation modes, with diameters of 0.02nm and 0.2nm respectively.

In recent years, interest on the adverse effects of petroleum hydrocarbons has grown, and focus has been on the deleterious effects of these products on various systems of the body. Petroleum hydrocarbons not only affect the Systems(respiratory, cardiovascular, immune and nervous) but also it affect organs like heart, liver, lung, kidney, and skin. Petroleum products and its exhaust can cause significant respiratory symptoms like chronic cough, breathlessness and wheezing [2, 3]. Also it has been shown that petroleum products cause tachycardia, dysarrhythmias, dizziness, pulmonary hypertension,[4] pulmonary aspiration, severe respiratory and cardiac failure, impaired regulation of vascular tone and endogenous fibrinolysis. [5,6]

A number of studies are available on toxic effect of petroleum fumes in petrol pump workers, but none of them documented cardio-pulmonary efficiency in petrol pump workers. So, in the present study an attempt has been made to justify toxic effect of petroleum fumes on cardio-pulmonary efficiency in petrol pump workers of western Maharashtra by comparing it with normal subject.

## MATERIALS AND METHODS

After the approval by institutional ethical committee, a cross sectional study was carried out on 60 petrol pump workers of Western Maharashtra (Sangli, Satara & Kolhapur Dist) with age group 20-40 years. They were further divided into three groups depending on their duration of exposure as Group I (1-5 years), II (5-10 years) & Group III (more than 10 years). 60 control subjects were chosen from paramedical staff of same age, gender and socioeconomic status from the same place for comparison. Demographic, occupational and clinical data were collected by using questionnaire and interview. Randomly selected 60 petrol pump workers were eligible to whom the experimental protocol was explained and written informed consent was obtained from them. Non-smokers, non-alcoholic healthy males, who were occupationally exposed to petroleum fumes for more than 6 hr /day with the duration of exposure from 2 to 20 years, were selected for this study. Most of the workers consumed mixed type of diet. Workers with less than 1 year exposure, any history of respiratory, cardiac diseases and smokers tobacco/mawa/Gutkha chewers were excluded from the study

The cardiopulmonary fitness of an individual was found out by performing the following tests :

**Blood pressure (BP)** was measured with the help of Riva Rocci Mercury Sphygmomanometer by auscultatory method, insupine position. Systolic and diastolic BP was expressed as mm/Hg.

**Heart rate** of each subject measured clinicallyfor one min. after 5 min. of rest in supine position.

**Breath holding time**-The workers were asked to take a maximum inspiration after maximum expiration and then hold the breath by plugging the nose as long as possible. The maximum time the subject can hold the breath was noted with the help of stop watch[7](proceedings).

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**40 mmHg endurance test (Flack's Air-Force Manometer Test)-**workers were asked to take deep inspiration and then close the nose. Then immediately blow in rubber tube which was connected to mercury manometer to raise the mercury level up to 40mmHg without blowing the cheeks. The workers were asked to maintain this mercury level up to 40mmHg as long as possible. Time interval between beginning of blow and breaking point was taken as40 mmHg endurance time. Anthropometric measurements which include height & weight were recorded.

All the tests were recorded at noon before lunch. The experimental protocol was explained in detail to the workers & trials were given after the demonstrations. For each volunteers three satisfactory efforts were recorded.

**Statistical analysis -** Data are expressed as mean **±** standard deviation **(SD)** of petrol pump workers and control group. Statistical analyses were done by analysis of variance (ANOVA). Unpaired student *t* – test was used to analyze the level of significance between the petrol pump workers and control groups. $P<0.05$ was considered to be significant.

## RESULTS

Rapidly multiplying number of automobiles vehicles and petrol pumps has increased air pollution. So we had tried to assess cardiopulmonary efficiency in petrol pump workers. These workers are constantly exposed to exhaust fumes and fuel vapours throughout their duty hours which causes decline in cardiopulmonary efficiency.

The observed values of cardio-pulmonary efficiency tests in petrol pump workers & in control groups are given in Table 1. There was significant increase in SBP, DBP, HR in petrol pump workers than in control group. Also there was significant decrease in breath holding time & 40 mmHg endurance time ($p < 0.05$) in petrol pump workers than in control group.

**Table I: Showing mean and SD of cardio-pulmonary efficiency in petrol pump Workers& in control Group.**

| Parameters | Petrol Pump Workers (n=60) Mean ±SD | Control(n=60) Mean ±SD | t- Value | P value |
|---|---|---|---|---|
| Systolic BP (mmHg) | 124.00 ± 8.445 | 120.23 ± 7.439 | -2.162 | 0.033 * |
| Diastolic BP (mmHg) | 83.03 ± 5.758 | 79.70 ± 4.537 | 2.593 | 0.011* |
| Heart Rate /min. | 73.77 ± 3.417 | 70.80 ± 4.657 | -3.979 | 0.000* |
| Breath Holding Time (BHT) (sec.) | 28.78 ± 12.585 | 37.50 ± 11.481 | -3.964 | 0.000* |
| 40mmHg Endurance Test (sec.) | 23.00 ± 10.276 | 34.12 ± 10.451 | -5.875 | 0.000* |

*- statistically significant, NS- Not significant

**Table II: Showing mean and SD of cardio-pulmonary efficiency in petrol pump Workers according to the duration of exposure.**

| Parameters | Year Of Exposure of Petrol Pump Workers | | | | |
|---|---|---|---|---|---|
| | 1-5 yrs (n=20) Mean ±SD | 6-10 yrs (n=12) Mean ±SD | More than 10 (n=8) Mean ±SD | F- Value | P value |
| Systolic BP (mmHg) | 123.74±8.354 | 124.33±9.335 | 128.0±8.485 | 0.249 | 0.780NS |
| Diastolic BP (mmHg) | 82.65±5.964 | 84.00±5.117 | 86.00±5.657 | 5.646 | 0.005* |
| Heart Rate /min. | 71.00 ±3.668 | 70.87 ±4.978 | 68.00 ±0.00 | 0.370 | 0.693NS |
| Breath Holding Time (BHT) (sec.) | 40.00 ± 8.48 | 37.50 ± 7.84 | 26.02±12.54 | 5.511 | 0.005* |
| 40mmHg Endurance Test (sec.) | 23.00 ± 10.27 | 22.23 ± 8.511 | 20.75 ± 7.605 | 12.359 | 0.000* |

*- statistically significant, NS- Not significant

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## DISCUSSION

About 95% of compositions in the petrol vapour are aliphatic and acyclic compounds while less than 2 % are aromatic compounds.[8] Inhalation of petrol fumes is associated with risk of cancer, respiratory and cardiovascular diseases.[9]

This study showed that inhalation of petroleum fumes led to significant increase in systolic , diastolic blood pressure and heart rate(Table no I).The mean value of systolic and diastolic blood pressure increases with increased years of exposure; this change was found to be statistically significant (P-value 0.001). But the mean value of HR was not increased according to year of exposure, this change was not found to be statistically significant (Table no II).

Findings of our study coincide with findings of other study done by Azeez et al. 2015,Azeez et al. 2012, Steffe et al.1996, Chalmers 1991; Levecchio & Fulton 2001; Mills 2005. [10-15]

Also A.Akintonwa et al.2003[16] studied that there was significant increase in both systolic and diastolic blood pressure, about 12.5% of the petrol station attendants had systolic blood pressure range of 141-160mmHg while 28.6% had diastolic blood pressure range from 91-110mmHg. This increase in SBP, DBP & HR are may be due to ability of petroleum hydrocarbon to enhance the sensitization of myocardium to catecholamine, impaired vagal activity & increased Baroreceptor activity with resultant vasoconstriction and increased arterial blood pressure.

Present study showed that inhalation of petroleum hydrocarbon led to significant decrease in breath holding time and 40 mmHg endurance time (Table no I).The mean value of breath holding time and 40 mmHg endurance time decreases with increase in years of exposure,(Table no II) this change was found to be statistically significant (P-value 0.001).

Breath holding time and 40 mmHg endurance is a useful biomarker in determining cardio-pulmonary fitness. These tests are considered as one of the best indicators of pulmonary efficiency. Normal voluntary breath-holding time is 45-55 seconds. During breath-holding, arterial $Po_2$falls and $PCo_2$ rises, resulting in a state of asphyxia. Since both these factors are powerful respiratory stimulants, a point is reached where the respiratory drive becomes so strong that the person cannot hold the breath any longer. In present study there is highly significant decrease in BHT & 40 mmHg endurance time may be due to, petroleum hydrocarbons decreases tolerance to higher $PCo_2$ and low $PO_2$.

Finding of present study suggests that petroleum hydrocarbons may have a pressor effect on cardiovascular functions. The pressor effect could be correlated to the ability of hydrocarbons to cause sensitization of myocardium to catecholamines, impairment of vasovagal event, respiratory depression, hypoxia, and hypercapnia[17,18,19] with consequent sympathetic effect and elevation of arterial blood pressure. This study revealed that inhalation of petroleum hydrocarbons led to increase baroreflex sensitivity impaired vascular tone.

## CONCLUSION

The present study revealed that petrol-pump workers are highly vulnerable for Cardio-pulmonary impairment due to their occupation. It has also shown that Breath holding time and 40 mmHg endurance is a useful biomarker in determining cardio-pulmonary fitness.

Impairment cardio-pulmonary efficiency could be attributed to the lack of health awareness and protective measures during work among petrol pump workers. Hence, considerably improved awareness of the sources of toxins in the community would help the individuals to avoid them.

**Recommendation:** To minimize the health related issues,

- Petrol pump owners should conduct regular health checkup camps in their petrol stations.
- Petrol pump owners should make sure that health regulations are implemented, with employees being provided biennial medical surveillance program.

- There be more stringent hierarchy Petrol pump owners should provide control measures in work place, that include provision of personal protective equipments like gloves, apron, long shoes, appropriate respiratory protective equipments i.e. anti-pollution masks etc.

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Research Article

# MICRONUCLEI - AS A BIOMARKER OF GENOTOXICITY IN AUTOMOBILE MECHANICS OF WESTERN MAHARASHTRA

**SMITA V PATIL[1]*, SUMANGALA PATIL[1], SAMPADA KANITKAR[2]**

**[1]Department of Physiology, BLDE University's, Shri B. M. Patil Medical College, Bijapur, Karnataka, India. [2]Department of Oral Pathology, Bharati Vidyapeeth University, Dental College and Hospital, Sangli, Maharashtra, India. Email: mailmesmita.patil@rediffmail.com**

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**ABSTRACT**

**Objective:** The aim of this study was to assess the potential cytogenetic damage associated with occupational exposure to polycyclic aromatic hydrocarbons (PAHs) among automobile mechanics (AMs) using micronuclei (MNs) and other nuclear abnormalities (NAs) such as binucleate cell (BN), karyorrhexis (KR), and karyolysis (KL) as biomarkers of genotoxicity.

**Methods:** The study was conducted on 60 AMs between age group of 20–40 years who were working in automobile garages for more than 1 year from western Maharashtra, and 60 healthy males with same socioeconomic status were chosen as controls. AMs were divided into three groups based on their duration of exposure, i.e. 1–5 years, 6–10 years, and more than 11 years. The exfoliated buccal cells were obtained and fixed with methanol for 10 min. Then, air-dried and stained it with Giemsa stain. Statistical analysis was done using unpaired t-test for two groups and one-way ANOVA for multiple groups of exposures.

**Results:** The mean values of MN, BN, KR, and KL in AMs (8.20, 13.57, 16.70, and 22.10, respectively) are significantly increased as compared to controls (5.10, 8.82, 12.30, and 16.12, respectively). As the year of exposure increased, the mean values of MN and other NAs were significantly increased in AMs ($p<0.05$).

**Conclusion:** MN and other NAs reflect genetic changes and events associated with carcinogenesis. Therefore, the results of this study indicate that AMs exposed to PAHs are under risk of significant cytogenetic damage. Therefore, it is important to provide and to create better awareness of occupational hazards among these workers to promote occupational safety.

**Keywords:** Micronuclei, Binucleate cell, Karyorrhexis, Karyolysis, Genotoxicity.

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## INTRODUCTION

To live in the 21st century means to live in a toxic world, where daily we get exposed to large number of toxic substances. Millions of workers in a variety of occupational settings have the potential to get exposed to toxic substances. They can be present in the form of gases, vapors, fumes, and particles [1]. Automobile mechanics (AMs), because of their occupation get exposed to toxic substances, which include polycyclic aromatic hydrocarbons (PAHs), which are found in petroleum products. PAHs are a major class of environmentally hazardous organic compounds due to their known or suspected carcinogenicity [2,3] and are known to be toxic.

Micronuclei (MNs) originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division [4]. Biomarkers of genotoxicity (MN) have received a considerable interest as tools for detecting human genotoxic exposure to various chemical carcinogens. Different types of bioassays have been widely used for assessing the genotoxic, mutagenic, and carcinogenic potencies of different substances. Biomarkers have been defined by the National Academy of Sciences (USA) as an alteration in cellular or biochemical components, processes, structures, or functions that are measurable in a biological system or sample. The traditional classification of biomarkers is of three main categories - biomarkers of exposure, effect, and susceptibility - depending on their toxicological significance [5]. Cytogenetic biomarkers are the most frequently used end points in human biomonitoring studies and are used extensively to assess the impact of environmental, occupational, and medical factors on genomic stability [6]. The present study incorporates all three categories of biomarkers to promote occupational safety.

MN assay is one of the most sensitive markers for detecting DNA damage and has been used to investigate genotoxicity of a variety of chemicals. Exposure to gasoline vapors is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans, mainly on the basis of the well-established carcinogenicity of some components such as benzene [7]. Benzene is proven to be carcinogenic in nature. Several studies have shown that benzene can induce various forms of genetic damage including chromosome aberrations, sister chromatid exchanges, MNs formation, and DNA damage [8].

In western Maharashtra, automobile garages are located on the road, and workers at the garages have a higher opportunity for exposure. AMs are engaged in doing routine maintenance and repairing the automotive vehicles. They are commonly exposed to PAH due to sucking of petrol or diesel through tube from the vehicle tank, while repairing the vehicles. They also often wash vehicle parts with petrol without wearing gloves. They are usually negligent regarding use of protective measures. Therefore, the occupational exposure to PAHs and other derivatives may possess genotoxic risk. Occupational exposure to carcinogens is of great public health concern.

Regeneration of cell is dependent on the number and division rate of the proliferating (basal) cells, their genomic stability, and their propensity

for cell death. These events can be studied in the buccal mucosa (BM), which is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study participants. Buccal epithelial cell is an indication of the regenerative capacity of the tissue that is why we collect sample from BM.

In the light of the above discussion, we applied MNs and other nuclear abnormalities (NAs) as a biomarker of genotoxicity in exfoliated buccal cells of AMs of western Maharashtra.

## METHODS

After the approval by institutional ethical committee, a cross-sectional study was carried out on 60 automobile workers of western Maharashtra (Sangli, Satara, and Kolhapur district) with age group of 20–40 years. They were further divided into three groups depending on their duration of exposure as Group I (1–5 years), Group II (5–10 years), and Group III (more than 10 years). Sixty control participants were chosen from paramedical staff of the same age, gender, and socioeconomic status from Bharati Vidyapeeth Medical and Dental College and Hospital Sangli. Workers were evaluated as per standard pro forma, which included a questionnaire regarding health status. Randomly selected 60 automobile workers were eligible to whom the experimental protocol was explained, and written informed consent was obtained from them. Workers with <1 year exposure and tobacco/mawa/gutkha chewers were excluded from the study.

Before buccal cell collection, the participants' mouth was rinsed thoroughly with water to remove any unwanted debris. The exfoliated cells of the BM were obtained by a sterile wooden spatula. For each individual, the slides were prepared in triplicate by smearing the cells onto pre-cleaned slides. The slides were then air-dried and fixed with methanol for 10 min. Then, air-dried again and stained it with Giemsa (Biolab diagnostic Pvt., Ltd.) stain for 15–20 min.

### Scoring method

Slides were evaluated using classification for NAs by Tolbert *et al*. [9]. Frequencies of MNs, binucleation, karyorrhexis (KR), and karyolysis (KL) in exfoliated 1000 cells were scored per slide. Nuclei less than one-third the diameter of the main nucleus were determined as MNs (Fig. 1b). Cells with two nuclei were considered as binucleate cells (BNs) (Fig. 1c). Nuclei fragmented into irregular pieces were scored as KR (Fig. 1d). Nuclear dissolution, ghost-like image of the nucleus remains, was evaluated as KL (Fig. 1e).

Results were presented as mean±standard deviation (SD). Unpaired t-test and ANOVA test were used to find the significance of study parameters using SPSS 16.0 version. $p>0.05$ was considered as statistically significant.

## RESULTS AND DISCUSSION

MNs test has been getting attention as a simple and sensitive short-term assay for the detection of environmental genotoxicity [10]. Analysis of exfoliated buccal cells provides evidence of other NAs such as BNs, KR, and KL [11].

Urbanization has led to enormous increase in number of automobiles and accordingly to increased number of petrol filling stations and workshops for repairing the vehicles. Workers engaged in these places are continuously exposed to petrol and diesel fumes. The particles generated from petrol exhaust are extremely small and are present in the nuclei or accumulation modes, with diameters of 0.02 nm and 0.2 nm, respectively [12].

The occupational solvents such as benzene in petrol and diesel fumes get absorbed into the human body either through the respiratory tract or through epidermal contact [13].

Biomarkers of genotoxicity have received a considerable interest as tools for detecting human genotoxic exposure and its effects, especially in health surveillance programs dealing with chemical carcinogens. Petroleum products are a complex mixture of aliphatic and aromatic hydrocarbons with high volatility. Such types of hydrocarbons exhibit co-mutagenic and co-carcinogenic properties [14]. Benzene is one of such hydrocarbon and is the natural component of petroleum products [15]. Its content in petrol is in range of 1–5%. In India, gasoline contains 3% benzene. The percentage of benzene may be as high as 30% in gasoline in some countries [16].

In the present study, we have tried to assess MN and other NAs as biomarkers of genotoxicity in AMs. In the present study, we found that frequency of MN and other NAs were significantly increased in AMs as compared to control group as shown in Table 1. Furthermore, as the year of exposure increases, frequency of MN and other NAs was significantly increased in AMs as shown in Table 2.

Our findings coincide with the findings of other authors, Khan *et al*. [17] and also our finding are confirm with the findings from the previous studies, which report the increased frequency of MN formation in oral buccal epithelial cells of automobile car mechanics (Sudha Sellappa), petrol pump workers (Paul *et al*.; Metgud *et al*.), and painters (Khan *et al*.) as compared to control [18-21].

BNs are probably indicative of failed cytokinesis following the last nuclear division in the basal cell layer [22]. KR is indicative of more extensive nuclear chromatin aggregation which leads to disintegration of the nucleus [23]. Binucleus formation is considered as indicator of cytotoxicity, while KR and KL are considered as indicators of apoptosis [21]. It has been postulated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, chronic cell injury, and ultimately tumor development [24].

Increased frequency of MN and other NAs in our study reveal that AMs are under the risk of significant cytogenetic damage. MN assay can be regarded as an important biomarker to predict the relative risk of occurrence of cancer [25,26]. MN assay is rapid, easy, cheap,

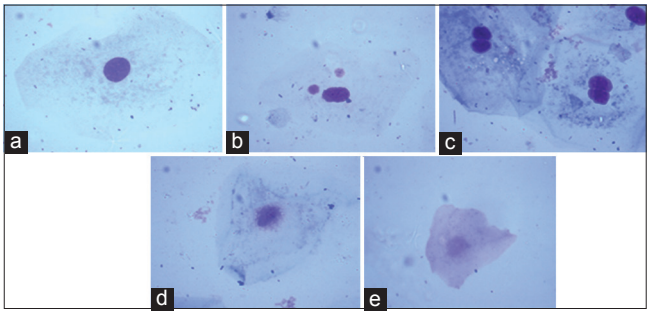


**Fig. 1: (a) Normal cell, (b) micronucleate cell, (c) binucleate cell, (d) karyorrhexis, and (e) karyolysis. Micronuclei and other nuclear abnormalities in exfoliated buccal cells of automobile mechanics and in control group**

**Table 1: Mean±SD of MN and other NAs in automobile workers and control group**

| Parameters | Mean±SD | | t-test | p value |
|---|---|---|---|---|
| | Automobile workers | Control group | | |
| MNC | 8.20±6.134 | 5.10±3.722 | 3.347 | 0.001* |
| BNC | 13.57±7.892 | 8.82±3.855 | 4.189 | 0.00* |
| KRC | 16.70±10.818 | 12.30±5.176 | 2.842 | 0.05* |
| KLC | 22.10±11.685 | 16.12±6.370 | 3.483 | 0.01* |

*: Significant, MNCs: Micronucleated cells, BNCs: Binucleated cells, KRC: Karyorrhectic cell, KLCs: Karyolytic cells, SD: Standard deviation, MNs: Micronuclei, NAs: Nuclear abnormalities

**Table 2: Mean±SD of MN and other NAs in automobile workers according to the duration of exposure**

| Parameters | Group I mean±SD (n=45) | Group II mean±SD (n=10) | Group III Mean±SD (n=5) | F | p |
|---|---|---|---|---|---|
| MNC | 5.80±4.037 | 15.30±4.596[a] | 15.60±8.295[b] | 24.996 | 0.000* |
| BNC | 12.49±7.710 | 13.50±5.642 | 23.40±7.701[b, c] | 4.865 | 0.011* |
| KRC | 15.62±10.654 | 15.70±6.165 | 28.40±14.328[b] | 3.456 | 0.038* |
| KLC | 18.20±7.397 | 29.90±11.170[a] | 41.60±18.243[b] | 18.726 | 0.000* |

*: Significant. Group-I (1–5 years exposure), Group-II (6–10 years exposure), Group-III (more than 10 years exposure). MNCs: Micronucleated cells, BNCs: Binucleated cells, KRCs: Karyorrhectic cell, KLCs: Karyolytic cells. Values are expressed as mean±SD; ANOVA followed by Tukey HSD *post hoc* within group comparison tests was done. Subscript a, b, and c express the significance difference between (I and II) (I and III) (II and III). SD: Standard deviation, MN: Micronuclei, NAs: Nuclear abnormalities

and sensitive tool for the detection of mutagens [27,28]. Micronucleus test in exfoliated epithelial cells seems to be a useful biomarker of occupational exposure to genotoxic chemicals. As demonstrated in this study, other NAs, such as binucleates, KL, and KR, are also useful indices of chemical exposure and toxic response.

These AMs are not aware that they have been exposed to genotoxic agents. Therefore, there is a need to educate those mechanics, about the potential occupational hazards and the importance of using protective measures.

## CONCLUSION

MN and other NAs reflect genetic changes and events associated with carcinogenesis so it is considered as biomarkers of genotoxicity. Therefore, the results of this study indicate that AMs exposed to PAHs are under the risk of significant cytogenetic damage. Therefore, it is important to provide and to create better awareness of occupational hazards among these workers to promote occupational safety.

## AUTHORS' CONTRIBUTION

Mrs. Smita V Patil - conception and design of study and drafting of the manuscript. Dr. Mrs. Sumangala Patil - critical revision of the manuscript for important intellectual content. Dr. Mrs. Sampada Kanitkar - supervision of the study and acquisition of data.

## CONFLICTS OF INTEREST

There are no conflicts of interest for the present study.

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