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### Effect of chronic inhalation of petroleum products on hematological parameters

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#### A B S T R A C T

Petroleum products are used for various reasons by human beings at homes, in manufacturing and petrochemical industries. The individuals most frequently exposed are those working in enterprises concerned with petrol and diesel, like petrol pumps. Chronic exposure to these compounds causes CNS depression and affects the kidney, liver, blood and systems. Hence, the present study was undertaken to assess the extent of damage caused by the chronic inhalation of petrol fumes on the haematopoietic system of human cases. The study was carried out on adult human subjects, 50 fuel attendants as cases and 50 shopkeepers as controls from Shivamogga city. The tests were performed in Department of Physiology, Shivamogga Institute of Medical Sciences, Shivamogga. Hemoglobin estimation was done using the Sahli's Hemoglobinometer and RBC count & TLC were done using improved Neubauer's counting chamber. Statistical analysis was done using unpaired t-test. The mean values of haemoglobin %, RBC count and Total leucocyte count were found to be significantly decreased in the test group when compared with the control group. The results obtained were statistically significant ( $p < 0.05$ ), which concludes that chronic exposure to petroleum fumes has adverse effects on human hematopoietic system, leading to bone marrow depression and resultant pancytopenia.

### Introduction

Petroleum products are used for various reasons by human beings at homes, in manufacturing and petrochemical industries. The daily use of petroleum products both in and outside petroleum industries may have effects on users. Those who work directly in petroleum industries and are occupationally exposed are likely to be more affected than

their counterparts who do not work in these industries.

The individuals most frequently exposed are those working enterprises concerned with petrol and diesel, like the petrol pumps. The workers involved in the job of filling the petrol or diesel in the vehicles are the one

maximally exposed to the petroleum fumes. Sources of petroleum vapours at the petrol pumps include losses from underground tanks, displacement vapour losses from filler pipes during refueling, fuel spillage and evaporative and tailpipe emissions from motor vehicles.

The available toxicokinetic data on petrol/gasoline shows that it is absorbed from all exposure routes, including perinatal. Some petroleum components are absorbed more rapidly than others. For example, aromatic compounds like benzene, toluene, and xylene which have both high blood/air partition coefficients and skin penetration rates are absorbed more rapidly than other petrol components. Acute exposure to petrol/gasoline and benzene, toluene etc. has been associated with skin and sensory irritation, central nervous system depression and effects on the respiratory system, whereas prolonged chronic exposures to these compounds affects these organs as well as kidney, liver and blood systems of these, particularly benzene is considered to be the most hematotoxic (Norbert, 1989).

Benzene, an aromatic hydrocarbon that is a natural component of crude oil and natural gas, is toxic to the blood and blood-forming organs. Chronic hematotoxic effects of benzene exposure, including reduced lymphocyte, neutrophil and platelet counts in peripheral blood, have been detected at occupational exposure below a level that had previously been considered not to cause any health effects. Whether these abnormalities represent bone marrow damage and/or initial events in the development of a true neoplastic disease is not known (Jorunn *et al.*, 2008).

Previous research studies carried out were on composite fumes evaporating from

kerosene, petrol and diesel and such studies were carried out on experimental animals. Hydrocarbons like benzene, metals like lead and volatile nitrates have all been shown to produce harmful effects on the bone marrow, spleen, and lymph nodes (Okoro *et al.*, 2006).

Hence, the present study was undertaken to assess the extent of damage caused by the chronic inhalation of petrol fumes on the haematopoietic system of human cases.

## **Material and Methods**

The study was carried out adult human subjects, from Shivamogga city, aged between 20 to 40 years who gave informed consent to the study. Since most of the petrol pump workers were males, the study involved only male subjects. For the study, 50 fuel attendants (as study group) working in different petrol pumps across the city, and 50 shopkeepers (as control group) from nearby areas were selected, who complied with the inclusion and exclusion criteria.

### **Inclusion criteria common to both the groups**

1. Age group of 20 to 40 years.
2. Subjects who have given written consent.

### **Exclusion criteria common to both the groups**

1. Subjects suffering from significant cardiovascular disorders.
2. Subjects with family history of malignancies.
3. Chronic smokers, smoking at least 20 cigarettes or beedies per day for not less than 10 years.
4. Subjects with chronic Renal or Respiratory diseases.

5. Individuals on corticosteroid therapy, radiotherapy or chemotherapy.

### **Selection of the study group**

50 Presently working fuel attendants in petrol pumps across the city, involved in the profession for not less than 2 years. For this, several petrol pumps across the city were surveyed and the fuel attendants involved in direct handling of fuel, like those involved in filling petrol in vehicles were selected. From these, subjects complying with the inclusion and exclusion criteria were shortlisted and 50 candidates were selected by simple random sampling.

### **Selection of the control group**

Male shopkeepers were selected from the vicinity of the surveyed petrol pumps. 50 subjects matching with the study group with respect to age and the inclusion/exclusion criteria were selected among the shopkeepers.

The study protocol was explained to the subjects included in the study. Informed consent was obtained from each of the participant. A detailed history was taken. The physiological parameters pulse rate and blood pressure were recorded.

- All tests were conducted between 10 am and 1 pm minimum half an hour after a light breakfast.
- Subjects were made to relax and comfortably seated.

Venous blood (2 ml) was taken from a peripheral vein on the arm of each subject and immediately transferred into sterile potassium EDTA anticoagulant vials. The blood samples obtained were analysed on daily basis. The Hemoglobin estimation was done using the Sahli's Hemoglobinometer

and the total red blood cell and white blood cell were count was done using the improved Neubauer's counting chamber.

The parameters recorded are,

1. Haemoglobin- in grams per decilitre (100ml).
2. Red Blood Cell count- in millions per cubic millilitre.
3. Total leucocyte count - in thousands per cubic millilitre.

Analysis of the data was done by using descriptive statistics such as mean and standard deviation and inferential statistics using the unpaired t- test. All values are presented as Mean  $\pm$  Standard Deviation (Mean  $\pm$  SD). Comparison of mean values of parameters between Control and Study group is done by unpaired 't' test. A p-value of less than 0.05 was considered to be statistically significant.

### **Results and Discussion**

The results obtained from the laboratory were tabulated and "means" of the recorded parameters were calculated, which are presented in the Table-01.

Subsequently, the means of respective parameters were compared between the study and control groups, and p-values obtained, which is also presented in the table.

The mean values of haemoglobin % (in grams per decilitre) were  $12.12 \pm 0.07$  in the study group and  $14.46 \pm 0.08$  in the control group. That of RBC Count (in millions per cubic millilitre) were  $4.21 \pm 0.06$  and  $5.06 \pm 0.05$  respectively and the mean values of Total leucocyte count (in thousands per decilitre) were found to be  $4.51 \pm 0.12$  and  $5.62 \pm 0.14$  in the study and control groups respectively.

This shows that mean values of haemoglobin %, RBC count and TLC were significantly decreased in the test group when compared with the control group. The results obtained were statistically significant, p-value being less than 0.05 in case of Red and White cell counts and less than 0.01 in case of haemoglobin values.

The results are graphically depicted in Graph-01. Decrease in haemoglobin content could be due to decrease in red blood cells or impaired biosynthesis of heme in bone marrow (Zayed *et al.*, 1993). Decreased haemoglobin and red blood cell could also be attributed to insufficiency of protein synthesis that mainly induces decrease of essential amino acids and shortage of the energy source of protein synthesis incorporated in haemoglobin production. The decrease in red blood cell count was observed in the exposed population (Gautam and Chowdhury, 1987).

Our findings are in accordance with that of a study, performed in Baghdad city on 292 workers of petrol filling station with five years duration of employment and consequently in which 146 petrol filling workers were found with hematopoietic changes. Significant changes in haemoglobin level were observed as compared with individuals who are not exposed to workplace. Petrol station attendants are workers chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refuelling. The adverse health effects of gasoline exposure may be primarily related to the impairment of the haemopoetic system with bone marrow depression (Ali and Sahb, 2011).

Toxic constituents of crude oil such as Benzene and Lead are reported to be activated in the bone marrow and the cytotoxic effects observed are mediated

through disturbance in DNA function. The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements (Rabble and Wong, 1996).

Another study done in Nigeria on fuel attendants showed similar results, with a global reduction in the mean values of total leucocyte count, red blood cell count, Packed Cell Volume and other red blood cell indices in exposed individuals (Okoro *et al.*, 2006).

White blood cells function primarily in body defence against foreign bodies and this is often achieved through leucocytosis and antibody production. In a study, the white blood cell count decreased significantly in humans of both sexes exposed to petroleum fumes and the decrease was greater in those exposed for more than two years. Benzene is reported to produce haematological changes ranging from pancytopenia to total bone marrow aplasia, affected through its myelotoxic action (d'Azevedo *et al.*, 1996).

Petroleum fumes occupational exposure in petroleum sector has been described to have toxic effects on, immune and nervous systems. Different organs such as the skin, heart, kidneys, and lungs are affected by these toxic effects resulting in various diseases and different forms of carcinogenic, neurotoxic, immunotoxic, genotoxic and mutagenic manifestations (Dede and Kagbo, 2002).

Similarly, the impact of occupational hazards on erythrocyte and haemoglobin levels and menstrual cycle characteristics in women exposed to aromatic hydrocarbons was studied by Georgieya *et al.* (1998) and found that the mean RBC counts and Haemoglobin levels of the subjects exposed to benzene were statistically significantly lower than those of the control group and the

difference was mainly due to the direct influence of aromatic hydrocarbons in the working environment on hematopoiesis.

Potential metabolic mechanisms underlying the haemopoietic toxicity of benzene include bioactivation of phenolic metabolites of benzene by peroxidases in bone marrow and ring opening reactions to generate muconate derivatives. Peroxidase-mediated activation of phenolic metabolites of benzene generates reactive quinones which can be detoxified by NAD (P) Quinone acceptor oxidoreductase (NQO1). The major peroxidase enzyme in bone marrow is myeloperoxidase (MPO) and potential target cells for phenolic metabolites of benzene were characterized in bone marrow stroma on the basis of high MPO: NQO1 ratios. MPO was found to be expressed at the level of myeloid progenitor cells in both murine (lineage negative cells) and human (CD34+ cells) systems. This suggests that progenitor cells may be relevant targets of phenolic metabolites of benzene resulting in aberrant haemopoiesis (Ross, 1996).

Excessive repeated exposure to benzene (>320 mg/m<sup>3</sup>) results in pancytopenia and aplastic anaemia, and is generally associated with a marked decrease in the number of cells in the bone marrow, resulting in severe clinical manifestations including immunosuppression and myelodysplastic syndrome. Lower repeated exposure to benzenes (<96 mg/m<sup>3</sup>) results in cytopenia. Affected people may display a decrease in white blood cells potentially resulting in death due to infection, a decrease in platelet count potentially resulting in death due to haemorrhage, or a decrease in red blood cell count (Fishbeck *et al.*, 1978).

Naza and Ali (2012) designed a study to evaluate the expected toxic effects of long-term exposure to petrol products in 48 gasoline filling workers with an age range

between 27 to 65 years within Sulaimani city area and found significant differences in means of haemoglobin level on most workers.

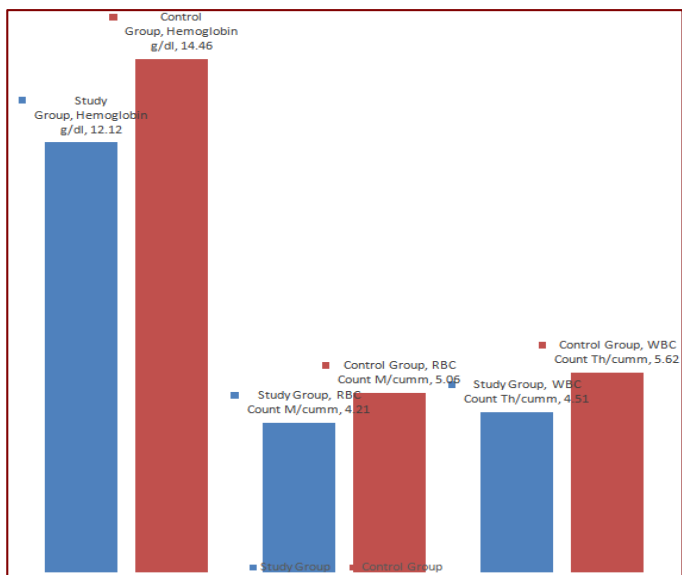
**Table.1** Comparison of haematological parameters between study and control group

Hematological indices	Study Group	Control Group	P - value
Haemoglobin % In g/dl	12.12 ± 0.07	14.46 ± 0.08	< 0.01**
RBC count In millions/cumm	4.21 ± 0.06	5.06 ± 0.05	< 0.05*
TLC In thousands/cumm	4.51 ± 0.12	5.62 ± 0.14	< 0.05*

\*Statistically significant,

\*\*Statistically highly significant.

**Graph.1** Comparison of haematological parameters between study and control group



Hence, the present study concludes that chronic exposure to petroleum fumes has adverse effects on human hematopoietic system, leading to bone marrow depression

and resultant pancytopenia. These findings are also supported by various studies done elsewhere which conclude the adverse effects of the toxic compounds in petrol fumes having adverse effects on various other systems like the nervous, respiratory including the hematopoietic system.

Therefore there is need to periodically evaluate the individuals at risk and prevent further damage either by changing the work type if possible, or provision of protective gear like specialised gowns to protect from transdermal absorption, and gas masks for effective prevention against inhalation the petroleum fumes.

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