# STUDY OF LEUCOCYTE COUNT AND ITS ASSOCIATION WITH BLOOD PRESSURE IN PETROL PUMP WORKERS OF SURAT CITY

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**Abstracts**: **Background & objectives:** Petrol pump workers are exposed to many noxious chemicals like benzene (C6H6) which has carcinogenic and haematotoxic effects. Current study was conducted to find out the effect of benzene and air pollutants exposure on granulocyte & agranulocyte counts, blood pressure and their correlation in petrol pump workers of Surat city. **Methods:** Thirty healthy male petrol pump workers and thirty (30) healthy male non-petrol pump workers aged 20-50 years were selected as study and control groups. Data regarding participant's age, height, weight, pulse rate, blood pressure etc. were recorded. Blood samples were collected in the morning (8-10AM). Data were presented as mean ± SD and compared using unpaired t-test. p value < 0.05 were considered as statistically significant. Pearson's correlation factor (r) was found between granulocyte-agranulocyte counts and BMI, Pulse rate and blood pressure. **Results:** Lymphocyte counts were significantly lower in the study group (%LYM 34±8.6) than control group (38.4±6.7). Monocyte counts were significantly higher in study group (%MON 8.29±3.1) than the control group (6.64±1.6). Granulocyte counts were higher in study group (%GRA 57.17±15.03) than in the control group (54.92±7.29). Mean arterial pressure was significantly higher (92.35±2.2) in study group than in control group (90.22±3.0) and it shows significant positive correlation with leucocyte. **Interpretation & conclusion:** Long term exposure to benzene leads to decreased lymphocyte count which may be due to bone marrow suppression. Monocytes are significantly increased in petrol pump workers which might be due to subclinical inflammation and allergic reactions. Petrol pump workers have raised mean arterial blood pressure which might be due to adverse effect of air pollutants on autonomic nervous system.

**Key Words**: Agranulocyte, Benzene, Granulocyte, Haematotoxicity, Petrol pump workers.

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**Introduction:**

Benzene is a volatile organic compound added in petrol to increase its octane rating and reduce knocking. In India 2-5% benzene (C6H6) is added to petrol that evaporates inside the fuel tank of the vehicle and escape into the atmosphere during refilling. Petrol filling station contains 1 – 25 ppm more benzene than any other places.[1] Exposure mainly occurs through breathing and via epidermal contact. These may cause primary respiratory symptoms, impaired pulmonary and dermatological functions in the exposed populations.[5] Chronic exposure leads to increased risk of developing leukemia, lymphoma, aplastic anemia, pancytopenia and chromosomal aberrations.[2] Benzene is metabolized in liver and bone marrow hence these are the major sites for benzene toxicity. Benzene metabolites covalently binds to cellular macromolecules including (DNA, RNA and proteins) leading to dysfunction of bone marrow. Chronic exposure results in consistent structural and numerical chromosomal aberrations in lymphocytes and bone marrow.[3]

Petrol pump workers are at a higher risk of developing benzene toxicity as they do not use any personal protective equipment. Hence proper biomonitering of petrol pump workers and installation of vapour recovery system are important measure for preventing and protecting them from occupational hazards of higher benzene in their working atmosphere. Several previous studies have shown abnormalities related to PFT, thyroid function and blood parameters. A complete blood count has been recognized as an easy and readily available screening tool for assessing the haematoxicity of benzene.[1] Hence this study has been undertaken to find out the effects of benzene on granulocyte and agranulocyte count in male petrol pump workers of the surat city, and educate and motivate them to use personal protective measures for prevention of benzene toxicity.

Along with benzene exposure, petrol pump workers are also exposed to air pollutants related to vehicular exhausts. Based on the hypothesis that air pollution exposure may induce cardiovascular changes mediated by the autonomic nervous system.[19] Elevated blood pressure is an established risk factor for coronary heart disease and stroke. An increase in 1 mmHg in usual systolic BP is estimated to increase by 2-4% the risk of death due to cardiovascular disease. [25,26] We have tried to assess the effect of air pollution on pulse rate and blood pressure and correlate the changes with leucocyte count in petrol pump workers of Surat city.

Surat had a population of 4.6 million at the 2011 census, making it the second largest city in the state of Gujarat, after Ahmedabad. It is the eighth largest city and ninth largest metropolitan area of India. The magnitude of public health related problems has demanded new studies that can clarify the pathophysiological mechanisms responsible for the adverse health effects attributed to benzene and air pollution in petrol pump workers.

**Material and Methods:**

The study was approved by the institutional ethical committee of Government medical college of Surat. Written informed consent was taken from each participants and petrol pump owners prior to the study.

The present study includes a total of thirty (30) apparently healthy non-smoker males aged 20 – 50 years, working in different petrol pumps of Surat city. They were working at the petrol pump for 8 hours per day, for > 1 year duration. Thirty (30) healthy male non-smokers matching socially and economically with the study group and not exposed to benzene or other air pollutants were selected as control group. Relevant data regarding participant's age, height, weight etc. were collected and a brief physical and general examination was carried out. Body mass index (BMI) were calculated using the formula Weight(kg)/Height(mt)$^2$. Systolic and diastolic blood pressure (SBP and DBP, respectively) were measured in the right arm, using a standard mercury sphygmomanometer after 5 minutes of rest in each subject. Here we excluded subjects with any present and past illness, acute infections (typhoid, malaria, pharyngitis etc.) any systemic disease or allergies (hypertension, diabetes mellitus, rheumatoid arthritis etc.), using medications affecting blood counts. 3 ml of venous blood samples were collected in the morning between 8 – 10 AM, in EDTA vaccutte from median cubital vein under aseptic precautions. All blood samples were delivered to the lab within 6 hours after collection. Samples were analysed by 3 part automated cell counter ABX MICROS 60 at the department of physiology, GMC Surat. Data were presented as mean $\pm$ SD. The mean values of two groups were compared by unpaired t test and p values < 0.05 was considered as statistically significant at 95% CI (confidence interval). Pearson's correlation factor (r) was found between granulocyte-agranulocyte counts and BMI, Pulse rate and blood pressure. All stastical analysis was done by openepi and SPSS version 20 software at the department of PSM, GMC Surat.

**Result:**

**Table 1.shows demographic distribution among study group and control group.**

| Traits | Study group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | |
| Age (year) | 33.48 | 7.6 | 35.58 | 9.8 | >0.05 |
| Height (cm.) | 166.02 | 2.4 | 166.38 | 3.4 | >0.05 |
| Weight (kg.) | 60.12 | 6.5 | 61.35 | 5.4 | >0.05 |
| BMI | 21.87 | 1.9 | 22.15 | 1.5 | >0.05 |

**Data collected were statistically non-significant (p>0.05). Subjects of both the groups are of comparable age, height, weight and BMI.**

**Table 2.shows pulse rate and blood pressure in study and control groups.**

| | Study group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | |
| Pulse rate (beats/min) | 82.06 | 5.6 | 80.86 | 4.8 | >0.05 |
| Systolic blood pressure (SBP) (mm Hg) | 122.67 | 4.4 | 116.8 | 4.1 | <0.05 |
| Diastolic blood pressure (DBP) (mm Hg) | 77.2 | 2.8 | 76.93 | 2.6 | >0.05 |
| Pulse pressure (PP) (mm Hg) | 45.46 | 5.4 | 39.86 | 2.4 | <0.05 |
| Mean arterial pressure (MAP) (mm Hg) | 92.35 | 2.2 | 90.22 | 3.0 | <0.05 |

**Data shows significant increase in SBP, PP and MAP between study and control group. (p<0.05)**

**Table 3.shows % and absolute lymphocyte count in study and control group.**

| | Study group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | |
| % Lymphocyte | 34.08 | 8.6 | 38.44 | 6.7 | <0.05 |
| Absolute lymphocyte count ($x10^3$/cu.mm.) | 2.28 | 0.6 | 2.58 | 0.5 | <0.05 |

**Data shows decrease in lymphocyte count in the study group which is statistically significant (p<0.05).**

**Table 4.shows % and absolute monocyte count in study and control group.**

| | Study group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | |
| % Monocyte | 8.29 | 3.1 | 6.64 | 1.6 | <0.05 |
| Absolute monocyte count ($x10^3$/cu.mm.) | 0.54 | 0.2 | 0.41 | 0.1 | <0.05 |

**Data shows increase in monocyte count in the study group which is statistically significant (p<0.05).**

**Table 5.shows % and absolute granulocyte count in study and control group.**

| | Study group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | |
| % Granulocyte | 57.17 | 15 | 54.92 | 7.2 | P>0.05 |
| Absolute granulocyte count ($x10^3$/cu.mm.) | 4.24 | 1.3 | 4.20 | 0.8 | P>0.05 |

**Data shows increase in granulocyte count in the study group which is statistically not significant (p>0.05).**

**Table 6. Correlation of Exposure, BMI, Pulse, Systolic Blood Pressure (SBP) , Diastolic Blood Pressure (DBP), Pulse Pressure (PP), Mean Arterial Pressure (MAP) with lymphocyte, monocyte, granulocyte count. Table shows significantly positive correlation between SBP and MAP with granulocyte and agranulocyte counts.**

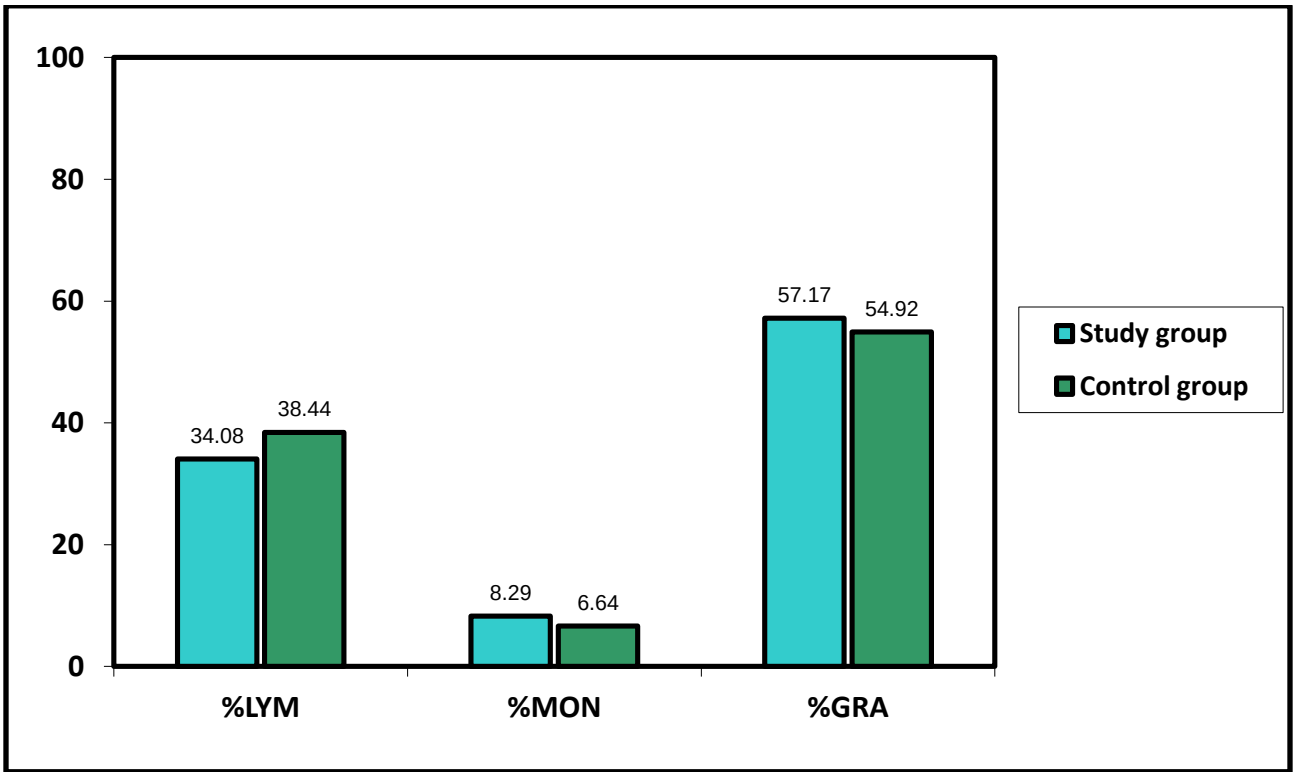
| | | Absolute lymphocyte count | Absolute monocyte count | Absolute granulocyte count |
|---|---|---|---|---|
| Exposure (in yerars) | r | -0.159 | -0.069 | 0.162 |
| BMI | r | 0.341 | 0.149 | 0.348 |
| Pulse | r | 0.388* | 0.234 | 0.333 |
| SBP | r | 0.399* | 0.247 | 0.382* |
| DBP | r | 0.358* | 0.135 | 0.337 |
| PP | r | 0.389* | 0.373* | 0.381* |
| MAP | r | 0.381* | 0.187 | 0.362* |

**r = Pearson's Correlation factor, * = p-Value (<0.05 statistically significant)**

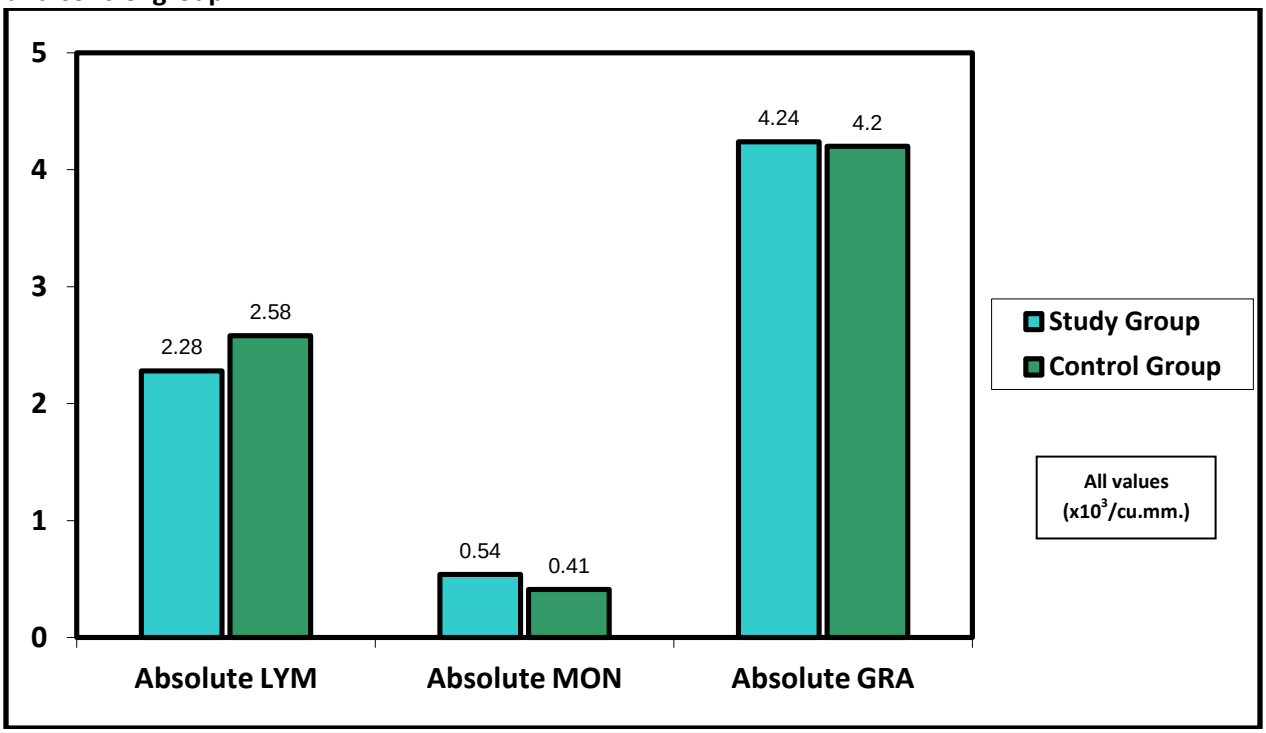
**Table shows-**

1. **Absolute lymphocyte count shows significant positive correlation with pulse, SBP, DBP, PP and MAP.**
2. **Absolute monocyte count shows significant positive correlation with PP.**
3. **Absolute granulocyte count shows significant positive correlation with SBP, PP,MAP.**

**Graph.1. Comparison of % lymphocyte, % monocyte and % granulocyte count in study and control group**



**Graph.2. Comparison of absolute lymphocyte, absolute monocyte and absolute granulocyte count in study and control group**




**Discussion:**

The present study was conducted on petrol-pump workers because they are at high risk of benzene exposure.[1] The physiochemical properties of benzene include low evaporation temperature and vapour pressure which allows its incorporation into the environment directly from the petrol during refilling or from automobiles.[3] Study was important as prolonged exposure to benzene may cause known effect like bone marrow depression, haematological & neurological toxic effects and cancers.[1]

In the present study, granulocyte and agranulocyte counts were measured in the study and control groups. Study shows significant lymphocytopenia along with increase in monocyte and granulocyte count. Statistically significant lymphocytopenia was seen ($p<0.05$) in benzene exposed workers which is suggestive of immune suppression and increased chances of opportunistic infection in them.[2] Benzene causes toxicity to the progenitor cells of WBC and platelets instead of circulating cells.[5] This may also be the reason for lymphocytopenia. While rise in monocyte and granulocyte count are indicative of subclinical inflammation and allergic reactions in workers exposed to benzene and air pollutants.[2]

Various studies have been conducted in the past to find out the effect of benzene on humans. Uzma N et al (2008), Goldwater L (1941) and Rothman N et al (1998) reported lymphocytopenia in benzene exposed workers.[3,6,7] Studies conducted by Wierda & Iron (1982) stated that the benzene and its metabolites (quinone & catechol) are immunotoxic and results in potent B cell suppression as well as block in B cell differentiation and maturation.[8] Ray M et al (2007) had shown rise in eosinophil and monocytes along with fall in T & B lymphocytes in benzene exposed worker.[2] These studies support the result of our study. In China, a cross sectional study was conducted by Bogadi A et al (2000) and Lan Q et al (2004) on benzene exposed shoe making workers showing significant lymphocytopenia.[9,10] Similar results were shown by Jorunn K et al (2008) on shoe manufacturing workers when benzene exposure is >10ppm.[4] Tunsaringkarn T et al (2013) reported exposure to benzene cause bone marrow depression presenting a drop in haemoglobin, haematocrit and eosinophil counts.[11] Singh D et al (2013) had shown significant decrease in eosinophil

count when duration of benzene exposure >15 years.[1] Another study conducted by Pesatori AC et al in 2009 found that the eosinophil count was inversely related to benzene exposure only among smokers, conversely basophil increase with increasing exposure.[12] Schnatter AR et al (2012) have shown that relatively low level of exposure to benzene experienced by petroleum distribution workers was associated with an increased risk of myelodysplastic syndrome (MDS), but not acute myeloid leukemia (AML), suggesting that MDS may be more relevant health risk for lower exposure.[13] These observations were consistent with our results. In contrast, studies conducted by Qu Q et al (2002) and Hameed F et al. (2009) reported decrease in WBC and other cell types in benzene exposed workers.[14,15] Routinely collected surveillance data on benzene exposed workers in USA weren't showing any correlation between lymphocytes and benzene exposure.[16,17,18]

In present study we also found significant increase in SBP and MAP in petrol pump workers, which is significantly correlated with granulocyte and agranulocyte count. The observed increase in systolic blood pressure associated with benzene and other air pollutants which could be related to a change in cardiovascular autonomic control. The cardiovascular system and HR are permanently under the influence of the sympathetic and parasympathetic nervous systems, with a predominance of the latter. Stimuli promoted by outdoor air pollution which may change the balance between the two systems, decreasing parasympathetic influences and increasing the sympathetic tone. This new situation leads to increases in blood pressure and PR. [19,20,21] Air pollutants promote vascular constriction secondary to acute increase in the release of vascular endothelin.[22,23] Wiwanitkit (2007) had shown the prevalence of hypertension was significantly higher in the group with high levels of benzene exposure (100 %) than in the group with lower levels of benzene exposure (49 %). This study suggested that disturbance of the nitric oxide production process may account for benzene-induced hypertension. [24] Brook R et al (2002) had shown brachial artery vasoconstriction after exposure to air pollutants in 25 healthy non-smoker adults.[23] Elevated WBC counts cause a chronic low-grade inflammation that alters endothelial function, affecting nitric oxide and prostacyclin production and consequently, a loss of vasodilator, antithrombotic and antiatherogenic properties of the vascular endothelium. Other postulated mechanisms include increased adherence of the stimulated leukocytes to the vascular endothelium, causing capillary leukocytosis and subsequent increased vascular resistance; a raised WBC count may therefore indicate increased catecholamine levels or enhanced sympathetic nervous system activity, thus causing an increase in blood pressure. [27] Various studies were conducted on petrol pump workers in the past, but our study has shown significant adverse effect of benzene and air pollutants on lymphocyte and monocyte counts and mean arterial blood pressure in the petrol pump workers. Also a positive association has been found between MAP and lymphocyte counts suggesting higher risk of future cardiovascular morbidity, mortality, hematotoxicity and immunosupression in them.

Limitations:

Due to the moderate sample size, the petrol pump workers were not classified based on their years of exposure, to assess the effect of duration of benzene on these studied parameters. Along with WBC, We need to assess various other inflammatory markers including high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-∝). Autonomic function test have not been done to study sympathovagal balance. Further studies with larger sample size and prospective cohort studies are needed to assess the role of air pollutants and chemicals on blood counts and future cardiovascular risk factors in the premises of petrol-pump.

**Conclusion:**

This study demonstrated that prolonged exposure to benzene and air pollutants cause decrease in lymphocyte counts along with increase in monocytes which are significantly correlated with raised mean arterial pressure. These effects are constantly observed in the occupationally exposed petrol pump workers which can be detected by monitoring blood counts at regular intervals. This

study supplies biological plausibility for observational studies on the cardiovascular effects of air pollution and provides additional evidence that public policies must be adopted to reduce air pollution in Surat. In order to prevent these among petrol filling workers, we suggest that medical observation, including pre-employment and periodic medical Checkups, should be performed. Control strategies should be adopted to reduce the benzene concentration in the ambient air and evaporation control. Petrol pump workers must be provided with effective masks to avoid inhalation of noxious substances. However, due to the dissimilarities among studies carried out in different places with specific air pollution characteristics and different populations, further investigations are required on a research topic that is far from concluded.

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