Benzene, a major monocyclic hydrocarbon is widely used as solvent in variety of industrial and commercial processes. It is also generated through processing, combustion and evaporation of gasoline. A large population of humans is exposed to benzene from various occupational and environmental sources. Benzene is an established human and animal carcinogen. Exposure of benzene has been associated with leukaemia so benzene also known as leukemogen (Haseeb, 2007).

Exposure to high levels of benzene in humans and animals has been shown to result in structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells, indicating that benzene is genotoxic (David et al., 2001; Subrahmanyam et al., 1991). *In vitro* cytotoxic effect of benzene on rat bone marrow showed elevated DNA damage by comet assay study (Zhao et al., 2006). Lung function abnormality and gene mutation (*lacI*) in mice were also observed by Ann et al., (1998). According to Snyder et al., (1993) benzene and its metabolites do not function as mutagens but are highly clastogenic, producing chromosomal aberrations, sister chromatid exchanges and micronuclei.
In several studies, increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with increased risk of cancer, especially hematological malignancies. Thus, chromosomal aberrations may be a predictor of future leukaemia risk (Daniel et al., 1999; Min et al., 1999; Yusuke and Shosuke, 1996). Since the 1960s approximately 50 cytogenetic studies in benzene exposed subjects have been conducted and most studies have shown a positive association between benzene exposure and chromosomal aberrations (Ji et al., 2004). In addition Zhang et al., (2005) revealed that benzene exposed individual have risk of aneuploidy of Chromosomes 1, 5, 6, 7, 8, 9, 11, 12 and 21. But the effects of exposure to low levels of benzene are not well understood. Therefore, many studies have been focused on occupationally benzene-exposed workers (Bloemen et al., 2004; Bogadi et al., 2003; Liu et al., 2003; Tompa et al., 1994; Zhu et al., 2001).

Workers exposure to benzene can be higher in certain industries with greater risk, such as the plants for the production of organic chemicals, shoe factories, paving companies and leather manufacturing, printing companies, elevator manufacturing, petrol stations and the petrochemical industry (Cavallo et al., 2006; Glass et al., 2000; Pitarque et al., 2002; Zhang et
al., 1996). Like wise traffic policemen are also exposed by benzene and other different gases exhaled from vehicle engines (Hoxha et al., 2009; Verma et al., 2003).

The human health effect of inhaled benzene depends on the concentration and time of exposure. At 25,000 ppm, benzene is lethal within few minutes (Briefe et al., 1980). Limited inhalation of 4000 ppm can cause giddiness, euphoria and nausea, whereas longer exposure at this level can lead to unconsciousness (Briefe et al., 1980; Haley, 1977). It is generally known that hematopoetic tissues are the most sensitive targets of benzene toxicity because chronic low level exposure correlates with the development of a variety of blood disorders in humans, which range from reduction in the concentration of peripheral blood cells to aplastic anemia, pancytopenia, acute mylogenous leukemia and it variants and lymphoma (Aksoy et al., 1974). In India, 2-5% benzene is blended with petrol as an antiknock agent. Atmosphere of petrol pump contains (1-25 ppm) more benzene concentration than other places because petrol evaporates during refilling, the concentration of benzene in petrol pump station also vary according to topography. The cigarette smoke contain benzene so occupational exposed individuals who are smokers additionally exposed with benzene. If any
individual use to smoke one packet of cigarette per day, it means that he takes approximately 1mg benzene exposure per day (Diane et al., 2006). Passive smoker also exposed with benzene exposure normally indoor benzene concentration is found slightly higher than the normal atmospheric benzene concentration. One study on school children revealed that children were more sensitive to the benzene exposure than the adult (Davidson et al., 2001).

Therefore, in the present study smoker and nonsmoker petrol pump attendants were chosen as chronic low dose exposed individuals. Chromosomal aberrations (CAs), Sister chromatid exchange (SCE), micronuclei (MN) biomarker studies were performed to measure genotoxic effects of benzene on petrol pump attendants as compared to healthy control individuals. Hematological parameters also tested to detect direct cytological changes due to exposure, if any. Biomarkers phenol and trans, trans - muconic acid were also estimated to ensure benzene exposure.

Mitomycin - C is a Streptomyces-derived antitumor antibiotic which is known to disrupt DNA metabolism (Shiba et al., 1959) and to cause cross linking of the complementary strands of DNA (Iyre and Szybalski, 1963). In the course of studying the effects of this drug on human chromosomes, it
was observed that MMC frequently produces chromosomal aberrations which can not observe in normal healthy subjects lymphocyte, so the very low concentration dose of MMC can be use as positive control. As well as the synergistic/co-mutagenic effect with interested toxicant also can be measured. Here in this study low dose of MMC was used as positive control.

In the present study, chromosomal aberrations of Group B₁ (non-smoker petrol pump workers) and Group B₂ (smoker petrol pump workers) did not reveal increased than controls, Group A₁ (non-smoker control individuals) and Group A₂ (smoker controls) respectively. It indicates that structural CAs in lymphocyte culture of petrol pump attendants did not occur due to low occupational benzene exposure, in the present study. But in Mitomycin-C (6ng/ml) treated Group B₂ (smoker petrol pump workers) shows significantly increased chromosomal aberrations than Group A₂ (smoker controls). It indicates that Mitomycin-C treatment acts as synergist with the low dose benzene exposure in smoker petrol pump attendants. Independent study on petrol pump workers by Carere et al., (1995) supports our findings. DNA damage study of shoe workers who were exposed to aromatic hydrocarbon did not observe any significant damage (Pitarque et al., 1999). In other independent study on footwear workers who were
exposed to complex mixtures of solvents were found higher micronuclei frequency and DNA damage by comet assay (Heuser et al., 2005). On the contrary, some authors revealed increased CAs from workers who were exposed to low dose of benzene (Killian and Daniel, 1978; Sarto et al., 1984; Tompa et al., 1994; Zhang et al., 2002; Zhang et al., 2005).

Sister chromatid exchange (SCE) is also one of the important chromosomal end points to measure DNA damage or alteration in repair mechanism. Studies indicate that according to intensity of UV radiation and exposure time SCE frequency alter as well as concentration of chemical exposure also increase SCE frequency (Krishnaja and Sharma, 2003). Even SCE frequency can be increased excessively in some genetic disorder likewise Bloom’s syndrome. Carere et al., (1995) worked on gasoline station workers and observed increased SCE frequency as compared to control. In addition, Ayla and Etem (2005) worked on gasoline station individuals and they reveald that SCE frequency and chromosomal aberrations were elevated in gasoline station attendants but there was no difference among smokers and nonsmokers for SCE frequency.

In present study results of SCE in Group B₁ (non-smoker petrol pump workers) and Group B₂ (smoker petrol pump workers) did not show any
significant difference as compared to results of SCE in Group A₁ (non smoker controls) and Group A₂ (smoker controls) respectively. In addition, comparison between the smoker and non smoker petrol pump attendants’ did not reveal significant change, it indicates that smoking habit of petrol pump attendant did not show any significant difference in SCE frequency. Result of MMC treated study groups revealed many fold increased in SCE frequency than the non treated groups that establishing genotoxic effect of MMC. But MMC treated Group B₁ and Group B₂ were compared with MMC treated Group A₁ and Group A₂ non significant alterations were observed.

On contrary, few studies reported significantly increased SCE frequencies among occupational exposed individuals to 10 ppm benzene exposure (Kasuba et al., 2000; Watanabe et al., 1980; Yardley et al., 1988). Some studies on gasoline pump attendant who were exposed with low dose benzene revealed no significant difference in sister chromatid exchange frequency as compared to respective controls. It has been reported that SCE frequencies differs according to age and sex (Bukvic et al., 1998; Yardley et al., 1988). However Celi and Akbas (2005) and Killian and Daniel (1978) illustrated that benzene exposure at 1–9 ppm for 1–20 years
or 3–50 ppm for 2–12 years failed to produce any significant effect on chromosomal aberrations and SCE in female workers.

Over century ago, **Boveri (1914)** paved the way to mechanistic studies linking chromosomal abnormalities to cancer pathogenesis. Since then, theoretical and empirical evidence has been accumulated, supporting a causal role of these events in the etiology of human cancer. A powerful tool for measurement of chromosomal abnormalities is the cytokinesis-block micronucleus cytome (CBMNcyt) assay.

Micronucleus (MN) frequency in cytokinesis-blocked peripheral blood lymphocytes (PBL) has become one of the best-established biomarkers for studying DNA damage occurring *in vivo* in humans (**Fenech, 2007**). The application of this method in population biomonitoring studies requires a deep understanding of how lifestyle and common host variables may influence MN frequency in cultured lymphocyte. **Michael and Stefano (2011)** described on results from studies reporting on the impact of age, gender, diet and lifestyle factors (e.g. exercise, alcohol, smoking and drugs) on this biomarker. Evidences from these studies show that each of these factors, either in isolation or in combination, can significantly influence MN frequency. Proper control for these factors is required to enable better
measurement of the impact of other conditions, such as environmental exposure to genotoxins or a susceptible genetic background, on MN frequency in PBL.

Studies indicate that chromosomal aberrations and micronuclei frequency in cancer patients increased significantly (El-Zein et al., 2011; Fenech and Crott, 2002; Iarmarcovai et al., 2008). Likewise, MN frequency shown to increased in smokers leading to lung cancer (El-Zein et al., 2008). Yager et al., (1990) found that benzene metabolites likewise hydroquinone, 1,4-benzoquinone, phenol and catechol can induce micromuclei in vitro to cultured lymphocyte.

Most of the MN studies in children were focused on analyses of lymphocytes but in the recent years, more investigators are interested in using exfoliated cells from the oral cavity and other cell types that can be collected non-invasively, which is particularly important in pediatric cohorts as well as occupationally exposed individual (Holland et al., 2011). Micronuclei from exfoliated buccal cells (Cytome assay) of occupational workers who were exposed to polycyclic hydrocarbon mixture, showed notably increased MN frequency than the controls (Karahalil et al., 1999).
In the present study, cytokinesis-block micronucleus cytome (CBMNcyt) assay was performed for all study groups by the method of Fenech and Morley (1985). Results of micronuclei frequencies revealed elevated levels in petrol pump attendant as compared to controls. Group B₁ and B₂ showed statistically significant (p<0.05) increase in micronuclei frequency than Group A₁ and A₂ respectively. Where as, in MMC treated groups, especially Group B₁ and B₂ also showed elevated (p<0.01) levels of micronuclei frequency than Group A₁ and A₂. Due to MMC treatment, MN frequencies were observed 6-7 folds higher which indicate that MMC acts as synergist with benzene exposure.

Study on petrol pump attendants by Carere et al., (1995) did not show any significant excess of MN in bi-nucleated lymphocytes of exposed workers with respect to the age paired control. On the contrary, Celik et al., (2003) showed increased MN frequency from exfoliated buccal cells of petrol pump attendants. Recent study on petrol pump attendants revealed significant increased in micronuclei frequencies as well as bi-nucleated cells, nuclear buds, karyorrhexis and karyolysis cell frequency after exposure to benzene (Gadhia et al., 2010). Studies on shoe factory workers and automobile garage workers showed notably increased MN frequency in
occasionally exposed individuals to aromatic hydrocarbon (Lauwerys et al., 1994; Pitarque et al., 2002; Saborit et al., 2009). Increased micronuclei frequency is known in cancer but some studies also indicate that micronuclei frequency may increased in some progressively degenerative disorders such as Alzheimer’s (Thomas et al., 2007).

Detoxification of inhaled or exposed benzene takes place in liver and enzymes such as cytochrome $p^{450}$ (CYP), oxidase, catalase present in hepatocytes play an important role. The enzyme present in hepatocyte convert benzene into its different metabolites such as phenol, catechol, t, t-muconic acid, 1,4-benzoquinone, S-phenylmercapturic acid and hydroquinone which are further secreted in urine. Thus metabolites of benzene: phenol, t,t-muconic acid and S-phenylmercapturic acid are generally used as biomarkers to measure occupational benzene exposure. Verma et al., (2003) studied urinary phenol level in Indian traffic policemen and found elevated phenol level in urine. It is interesting to note that more than 20mg/dl urinary phenol indicates nearly up to 1 ppm benzene exposure (Diane et al., 2006; Wiwanitkit et al., 2001).

In the present study, urinary total phenolic compounds of petrol pump attendants were measured as a well known biomarker of benzene exposure at
work place. Urinary phenol content in Group B₁ was found significantly higher (p<0.01) than control Group A₁ as well as Group B₂ was found significantly higher (p<0.01) than Group A₂. It means petrol pump attendants were found chronically exposed to occupational benzene exposure.

In the present study an another promising biomarker of benzene, urinary t,t-MA was measured from petrol pump attendants using Lee et al., (2005) method. Urinary trans, trans-muconic acid content in Group B₁ was found significantly higher (p<0.05) than control Group A₁ and Group B₂ showed significantly higher level (p<0.01) than Group A₂. It means petrol pump attendants were found chronically exposed to occupational benzene exposure for sure.

Correlation between urinary phenol and urinary trans, trans-muconic acid in Group A₁ presented in (Chart-VI) do not show any proposal relation. But in Group A₂ urinary phenol concentrations notably found increased with (r = 0.7) urinary trans, trans-muconic acid level (Chart-V). Similarly Group B₁ and Group B₂ also show proposal correlation r = 0.86 (Chart-IV) and r = 0.81 (Chart-III) respectively. These correlations results between phenol and trans, trans - muconic acid indicate that urinary phenol concentration
may increase with benzene exposure and/or could be due to digestion of protein during metabolism. But urinary trans, trans-muconic acid has been reported to increase in the case of only benzene exposure like wise, t,t - MA found increased in Group A2, Group B1, Group B2 but not in Group A1. So, urinary t,t-MA was found promising biomarker for occupational benzene exposure than urinary phenol. Urinary Phenol and t,t-MA concentration in smoker were found significantly higher than non smoker individuals.

Nevertheless, the urinary phenol; levels can be affected by demographic factor, such as diet, smoking and ingestion of medicine. In a study on petrol pump attendant, Gadhia et al., (2010) found that urinary phenol and t,t-MA increased with the benzene exposure and it also correlates with MN frequency. Melikian et al., (1993) found markedly elevated tt MA level in cigarette smokers as benzene exposed individual. Virginia et al., (2000) have indicated that urinary t,t-MA result may be vary due to dietary additive or preservative like Sorbic Acid-preserved foods. Study on DNA damage with respect to low dose benzene also showed increased DNA damage with respect to urinary t,t-MA (Sul et al., 2005).

In the present study, Hematological parameters like Hb, RBC count, WBC count, Platelets count and differential count in petrol pump attendants
did not show significant difference than controls. It indicates that effect of occupational benzene exposure on petrol pump attendants did not have any direct effect on hematological parameters.

A study on benzene exposed workers of the standard biomarker for benzene exposure, urine phenol level and the basic blood marker for allergy, eosinophil count were found no correlation (Wiwanitkit et al., 2005). Similarly study between the urine trans, trans-muconic acid (t,t-MA) level and red blood cell parameters were studied in 30 Thai subjects who were occupationally exposed to benzene (Wiwanitkit et al., 2007). In an other study by Wiwanitkit et al., (2004) found that urinary t,t-MA and platelets did not correlate each other and platelets also did not differ significantly from the controls.