Despite of the available scientific data on asthma etiology and treatment facilities, the incidences of asthma morbidity and mortality are continuously increasing among children and adults which represent a global concern.

One of the major initiatives of research on asthma has been to understand the genetic and environmental triggers for bronchial asthma. Asthma clusters in families and twin studies suggest a strong genetic component. Having a parent with asthma doubles a child's risk of asthma, and having two affected parents increases the risk 4-fold (Gilliland et al., 2001).

There is not a single gene or mechanism associated with asthma but, many genes, gene-gene interactions and gene-environment interactions are associated with asthma. For instance 5q23-31, 12q15-24.1, 19q13 and 21q21 are some of the important marker regions that have been shown to be associated with asthma (Ober et al., 1998) and some antioxidant enzymes related genes GST and NQO1 family have been reported to play important role in inflammatory responses (Cross et al., 1999; Baulig et al., 2003; Ahn et al., 2006; Wan et al., 2006).

It is well known that environmental factors like diet, smoke, chemicals also influence asthma and the chronic inflammation associated with it. Many epidemiological studies on the effect of environmental tobacco smoke on adults have reported an increased risk of asthma, dose-dependent relationship with wheezing, and a greater risk for more severe airflow obstruction (Jindal et al., 1994; Hu et al., 1997; Jindal et al., 1999; Kunzli et al., 2000; Thorn et al., 2001; Jaakkola and Jaakkola, 2006). The intake of antioxidant rich foods have been shown to reduce the severity and improve the lung function (Misso et al., 2005; Riccioni et al., 2007; Prescott and Dunstan, 2007).

Since the 1980s, numerous studies have reported significant relations between parental smoking and development of asthma in children (Cook and Strachan 1997, 1998; Jaakkola 2000; Jaakkola and Jaakkola 2002). Meta-analyses based on these studies have shown a dose-dependent increase in children's rates of asthma related to increasing number of household smokers, with the strongest effect observed in the youngest children (California EPA 1997; Cook and Strachan 1997; Strachan and
Maternal smoking has been reported to be strongly associated than other household members’ smoking, raising concern of the potential role of prenatal tobacco smoke exposure.

In the present study, systemic oxidant-antioxidant levels were measured because blood is an easily available source and an important pool of anti-oxidant defenses in the body (Toth et al., 1984; Van et al., 1985; Agar et al., 1986).

5.1 Oxidative stress and asthma

The oxidative stress leads to a series of biochemical and physiological changes, thus, altering normal body homeostasis (Boveris et al., 1972). It is now well known that the activities of antioxidant enzymes correlate with various physiological or pathological conditions, including oxidative stress (Czene et al., 1997; Wojtaszek, 1997). Oxidative damage adversely affects metabolic pathways and also amplifies the inflammatory process. The oxidant-antioxidant imbalance and oxidative damage can be determined in bronchoalveolar lavage fluid (BAL), plasma, serum, tissue and as well as in exhaled breath condensate (Babusikova et al., 2012).

The generation of ROS and oxidative damage are important factors contributing to the onset as well as persistence of airway inflammation in asthmatic patients (Babusikova et al., 2012). The generation of ROS is a part of inflammatory response and occurs either endogenously or exogenously through external stimuli. The inflammatory response is mediated by infiltration of eosinophils, neutrophils, mast cells and T-lymphocytes into the airway epithelium. These stimulated cells uptake molecular O$_2$ and release oxygen radicals like O$_2^{-}$ and OH$^{-}$ that are the mediators of oxidative stress (Halliwell and Gutteridge, 1990). These ROS through a variety of modifications in DNA trigger many signaling pathways, generate toxicants, alter gene expression and disrupt normal repair mechanism (Poulsen et al., 2000; Cadet et al., 2003; Halliwell and Gutteridge, 2007). Further, the endogenous antioxidant defense mechanism, which is present to combat these oxidative forces, is impaired in asthma. Imbalance of these two leads to potential damage. The damage inflicted by the ROS to various molecules like lipids, DNA is a measure of this oxidative stress.

The declined status of antioxidant enzymes like catalase, antioxidant status of plasma and the elevated level of lipid peroxidation provide great evidence for increased oxidative stress in asthma (Rahman et al., 1996; Wood et al., 2003; Bowler,
2004; Ahmed et al., 2012). The increased level of genomic damage can be measured using MN assay in the buccal mucosa and comet assay in the peripheral blood lymphocytes (Stich and Rosin, 1983; Collins et al., 1997, 2008; Tice et al., 2000; Collins, 2004; Møller, 2006; Holland et al., 2008).

A combined study of all kinds of biomarkers of oxidative damage (proteins, lipids, DNA), together with assessment of antioxidant status and genotype of relevant genes in the same time in asthmatic patients can be more reproductive for the selection of best treatment. In the present study, attempt was made to study both the oxidative stress and DNA damage in asthma patients by measuring the catalase and FRAP activity, lipid peroxidation and DNA damage in buccal mucosa as well as peripheral blood.

5.1.1 Catalase activity

Catalase is a tetrameric, endogenous, ubiquitous, antioxidant enzyme, present in the peroxisomes that decomposes hydrogen peroxide (H₂O₂) to H₂O and O₂, thereby helps the cells in combating oxidative stress. Apart from decomposition of H₂O₂, catalase inactivates several environmental mutagens, prevents chromosomal aberrations caused by hypoxanthine/xanthine oxidase (Kono and Fridovich, 1982).

Studies have reported alteration in CAT activity in diseases accompanied by oxidative stress like, asthma (Novak et al., 1991; Mohan and Das, 1997; DeRaeve et al., 1997; Tekin et al., 2000; Shanmugasundaram et al., 2001; Mak et al., 2004; Ahmad et al., 2012). CAT polymorphism may be associated with increased risk of asthma (Mak et al., 2006; Polonikov et al., 2009) and also with increased oxidative damage in asthmatic subjects (Babusikova et al., 2012).

In the present study, it was observed that CAT activities were greatly reduced in asthmatic patients as compared to control subjects in total as well as according to various correlates (Table 4.4, 4.5). The reason behind this might be the increased and prolonged oxidative stress due to increased ROS production in the asthmatics and oxidation of tyrosine residues, in particular tyrosine 358 of the enzyme that has been linked to loss of catalase activity (Ghosh et al., 2006).

Many previous studies have also reported decline in catalase activity in asthmatic subjects (Novak et al., 1991; Shanmugasundaram et al., 2001; Ghosh et al., 2006; Fabian et al., 2011; Ahmad et al., 2012; Al-Aly, 2012). The lower activity of catalase in the red blood cells of asthmatic patients has also been reported by Rai and
Phadke (2006). The results in the present study are in line with these studies. On the contrary, many workers have reported no alteration in the CAT activity in asthmatic subjects (Smith et al., 1993; DeRaeve et al., 1997; Mohan and Das, 1997; Tekin et al., 2000; Nadeem et al., 2003, 2005), while many others have suggested an increase in CAT activity in asthmatic subjects as a positive feedback mechanism of oxidative stress in asthmatic subjects (Mak et al., 2004; Yang et al., 2011). Pennings et al. (1999) observed that erythrocyte CAT activity increased among asthmatic patients with the treatment of beclomethasone (an inhaled corticosteroid).

Recently, Ahmad et al. (2012) reported decreased catalase activity in asthmatic subjects as compared to control subjects and also among the asthmatic patients with increased severity of disease. They also found a negative correlation of the catalase activity with duration of the disease while a positive correlation with measured FEV1 of the subjects. In the present study also, the CAT activity was found to be significantly negatively correlated with duration of asthma and the correlation with FVC and FEV1 was non-significantly positive (Table 4.12) which clearly indicates that the enzyme activity decreases with disease progression.

In the present study, subjects were compared according to various correlated factors, and age, gender, diet, smoking and alcohol drinking have been considered as confounding factors as recommended in previous biomonitoring studies (Moller et al., 2000). CAT activity was found to be significantly higher among asthmatic vegetarians as compared to asthmatic non-vegetarians but no significant difference was found in case of control subjects in relation to dietary habits. During the present study, CAT activity was found to be lower in females as compared to males in both asthmatics (Table 4.5) and controls (Table 4.6) but the difference was non-significant. Anyasor et al. (2009) observed increase in CAT activity of non-vegetarians after the meal but, no difference was observed among vegetarians. They suggested this could be due to lower oxidative stress among vegetarians as compared to non-vegetarians.

Considering smoking as a confounding factor, subjects were compared according to smoking habits during the present investigation and it was found that smokers exhibited significantly lower CAT activity in comparison to non-smokers among the control subjects (Table 4.6). But, no significant difference was observed in case of asthmatic subjects (Table 4.5). Similar trend was observed for biomass exposed subjects in both the groups. Previously, Yildiz et al. (2005) observed decreased CAT activity in active and passive smokers and also with increased dose of
smoking and Gani et al. (2000) observed declined CAT activity among women who smoked and exposed to biomass smoke; whereas, Goth (1989); Powell et al. (1994); Comhair et al. (2000); Bogdanska et al. (2003) and Zaharaie et al. (2005) found no difference in CAT activity between smokers and non-smokers. On the contrary, Toth et al. (1984) reported increased CAT activity in smokers as a positive feedback defense mechanism against increased oxidative stress. Novak et al. (1991) and Shanmugasundarsn et al. (2001) observed no variation in CAT activity of asthmatic subjects due to smoking.

During the present study, CAT activity was found to decline markedly among control subjects who consumed alcohol as compared to abstainers while among the asthmatics, no significant differences were observed. No marked changes were found even with increased dose of alcohol consumption per month (Table 4.9). Earlier studies on CAT activity among alcohol abusers are conflicting. Tarasova (1998); Zhou and Chen (2001) and Bogdanska et al. (2005) reported that CAT activity was decreased among alcohol abusers. On the other hand, no alteration in CAT activity among alcoholics was observed by Tanner et al. (1986); Bjorneboe et al. (1988); Guemori et al. (1991, 1993) and Bogdanska et al. (2003), while Negru et al. (1995) and Temel et al. (2002) reported increased CAT activity among alcohol consumers.

Subjects were also compared according to their physical activity in the present study but no significant differences were observed in the CAT activity. Previously, Amatuni and Safarian (1986) observed that CAT activity was reduced in bronchial asthma patients after physical exercise while, Galassetti et al. (2006) observed no variation in CAT activity in the subjects who exercised for seven days.

İnal et al. (2001) reported that CAT activity deceased with advancing age (above 40 years). They found a positive correlation between age and CAT activity. Akila et al. (2007) observed decline in CAT activity in the erythrocytes of old aged subjects (60-75 years old) as compared to young control subjects (20-32 years old). On comparison of the CAT activity between different age group in both asthmatic and control subjects during the present study, it was found that CAT activity declined slightly with advancing age in both the groups (Tables 4.5 and 4.6) and there was non-significant negative correlation between age and CAT activity (Table 4.12).
5.1.2 Lipid peroxidation

Another instance of oxidative damage is increased lipid peroxidation level which cause damage to the biological membranes and other lipid containing biomolecules and structures and further leads to declined fluidity of the membranes (Kaplan et al., 2003). The concentration of MDA, measured as TBARS, is a great biomarker of lipid peroxidation, which is a secondary end product of auto-oxidation and enzymatic degradation of polyunsaturated fatty acids (Walsh, 1994; Halliwell, 1994; Gutteridge, 1995; Eken et al., 2005; Babusikova et al., 2012). The TBARS assay is a well-recognized, established method for the estimation of lipid peroxides (Yagi, 1998; Dawn-Linsley et al., 2005).

During the present investigation, the serum MDA was found to be significantly elevated in asthmatic subjects as compared to controls, when compared in total, as well as when compared according to various correlated factors (Table 4.13). The present results are consistent with previous studies that have also reported marked elevation in MDA level in asthmatic subjects in serum and plasma (Shanmugasundaram et al., 2001; Bowler and Crapo, 2002; Sharma et al., 2003; Nadeem et al., 2003, 2005; Narula et al., 2007; Babusikova et al., 2009; Al-Abdulla et al., 2010; Fabian et al., 2011; Ahmad et al., 2012; Al-Aly, 2012). Many earlier studies have reported increased MDA concentration in exhaled breath condensates (EBC) of asthmatic subjects as compared to control individuals (Antczak et al., 1997; Corradi et al., 2003). Ozaras et al. (2001) observed increased MDA level in peripheral blood samples and BAL fluid from asthmatic patients as compared to controls.

In the current investigation, asthmatic females had non-significantly higher MDA level than males indicating possibly higher level of lipid peroxidation among females as compared to males (Table 4.14). Similar finding were observed by İnal et al. (2001). They also found elevated MDA level with advancing age and a positive correlation between MDA and age. During the present investigation also, it was observed that the lipid peroxidation level was higher in the higher age group individuals (>50 years old) as compared to lower age group (<50 years) among the asthmatic subjects (Table 4.14) but, the difference was statistically non-significant. However, a significantly positive correlation was found between MDA level and age of the subjects which correlates well with previous studies (Nuttall et al., 1999; Mutlu- Türkoğlu et al., 2000, 2003; İnal et al., 2001; Sreeramulu et al., 2004; Akila et al., 2007).
In the present study, smoker asthmatic subjects were found to have non-significantly higher serum MDA concentration as compared to non-smoker asthmatics. While among the control subjects, serum MDA concentration was found to be significantly elevated in smokers as compared to non-smokers (Tables 4.14 and 4.15). Similarly, biomass smoke exposed patients and patients who had sedentary lifestyle were also observed to have elevation in serum MDA level than biomass smoke non-exposed patients and light to moderately active patients. But, all these differences were non-significant (Table 4.14). In case of control subjects, significantly higher serum MDA was observed in alcohol consumers as compared to abstainers but no significant differences were found according to sex, age group, dietary habits, daily activity and exposure to biomass smoke (Table 4.15). Alcohol has the ability to induce lipid peroxidation either directly by the acetaldehyde through free-radical formation or through destruction of antioxidant substances (Dianzani, 1985).

Previously, Nielsen et al. (1997) and Garg et al. (2006) observed MDA level was non-significantly higher in smokers than non-smokers and increased with increased dose of cigarettes per day. Plasma MDA was observed to be positively correlated with daily exposure to cigarette smoke. Frie et al. (1991) reported increased level of lipid peroxides after cigarette smoke. Lapenna et al. (1995) observed increased level of fluorescent products of lipid peroxidation in smokers while Morrison et al. (1999) reported increased lipid peroxidation in BAL fluid of the smokers. Gani et al. (2000) observed increased MDA level in women exposed to cigarette and biomass smoke. TBARS level was markedly increased in the EBC of smokers as compared to non-smokers and no difference was observed in the TBARS level even with moderate exercise (Nowak et al., 2001). Galassetti et al. (2006) found that lipid peroxidation level was reduced in the subjects who exercised for seven days. The present study is in accordance with these studies.

Gupta et al. (2005) reported significant elevation in MDA level of smokers and alcoholic subjects as compared to control non-smokers and non-alcoholics. They also observed that alcoholic subjects had significantly elevated MDA as compared to smokers. Vendemiaie et al. (1989) reported increased plasma MDA level after consumption of ethanol in healthy male subjects. Recently, Deshpande et al. (2014) reported that cirrhotic patients had significantly higher MDA levels associated with DNA Damage than compensated cirrhotic patients and control group who were not suffering from liver cirrhosis (disease which occurs due to chronic alcoholism).
Whereas, Block et al. (2002) found no association between alcohol intake and plasma MDA levels in healthy subjects.

During the present study, when control subjects were compared in relation to number of cigarettes smoked in a day, no changes were observed in the lipid peroxidation with increased consumption of cigarettes, while among the asthmatic subjects, the value of serum MDA first increased among moderate smokers who consumed 11-20 cigarettes/day and then strangely decreased again in heavy smokers consuming >20 cigarettes/day (Table 4.17). This might be due to activation of body’s defense mechanism against increased oxidative forces, but at present it is mere speculation and a subject of further investigation. Previously, Block et al. (2002) and Reejmol and Swaminathan (2013) observed that lipid peroxidation level was highly increased with increased dose of cigarettes/day in patients with periodontitis.

During the present study, among the control subjects, no major change was found in the level of lipid peroxidation with increased consumption of alcohol. While among the asthmatic subjects, level of serum MDA was significantly increased in heavy alcoholics as compared to moderate alcoholics (Table 4.18). The synergic effect of alcohol intake and cigarette smoking on the lipid peroxidation level was also explored, but no significant difference was found between the different categories of smokers and alcohol consumers among the asthmatic as well as control subjects (Table 4.16). However, lipid peroxidation level was greatest among those subjects who were consuming both cigarettes and alcohol (group 1). All these results suggested that smokers and alcoholics exhibited greater oxidative stress than non-smokers and abstainers.

Krajčovičová-Kudláčková et al. (1995) reported no significant variation in lipid peroxidation level between vegetarian and non-vegetarian subjects. No significant differences were observed according to dietary habits in the present study either.

In the present investigation, asthmatic subjects who were prescribed short acting beta-2 agonists or corticosteroids for quick relief were observed to have elevated serum MDA level than the subjects who were not given any such prescription. Studies on the effect of inhaled corticosteroids and beta-2 agonists are conflicting. Nielson and Hadjokas (1998) suggested that beta-2 agonists block the steroid actions that decrease eosinophils superoxide anions, thus increasing the inflammatory mediators and ROS generation. Roberts et al. (1999) suggested that
beta-2 agonists improve clinical indices of asthma but does not improve the inflammatory process associated with the disease. Ozaras et al. (2001) reported that after one month of treatment, inhaled corticosteroids significantly decreased the MDA level in plasma of the patients but it was still significantly higher than the control subjects.

During the present study, asthmatic subjects were also compared according to their family history but no significant difference was observed in the level of serum MDA. Previously, Al-Abdulla et al. (2010) have also studied MDA concentration among asthmatic children according to their family history and found no significant variation in MDA level.

In the current study, serum MDA was compared in the asthmatics according to the level of severity and it was found to be lowest in the patients with intermittent asthma and highest in those with severe asthma. The lipid peroxidation was observed to be continuously increasing with increased severity of asthma (Table 4.20). The correlation of serum MDA with FVC and FEV1 values of patients were also significantly negative suggesting that lipid peroxidation increased with the progression of the disease (Table 4.21). Kanazawa et al. (1991) also observed variations related to severity with acute exacerbations. Plasma TBARS has also been shown to correlate negatively with the percent predicted FEV1 in a previous study, indicating that lipid peroxidation is associated with airflow limitation (Schunemann et al., 1997). Al-Abdulla et al. (2010) also observed significant elevation in serum MDA level of asthmatic subjects with increased severity. In a recent study, Ahmad et al. (2012) studied the lipid peroxidation level in asthma in relation with increased severity and they found that plasma TBARS was increased with increasing severity. They also found a negative correlation between the plasma MDA and FEV1 values of the patients. The present study correlates well with these studies and others who have also reported positive association between MDA level and asthma severity (Bowler and Crapo, 2002; Sharma et al., 2003; Narula et al., 2007). Besides this, significant positive correlation between asthma duration and serum MDA (Table 4.21) was also observed, showing increased level of lipid peroxidation with increased duration of disease.
5.1.3 Antioxidant status of plasma

FRAP assay is used to detect the reducing power of non-enzymatic antioxidants in the plasma, mainly uric acid (Liu et al., 1982; Benzie and Strain, 1999) and ascorbic acid (Benzie and Strain, 1999), but it does not detect reduced glutathione level (Vassalle et al., 2004). Thus, FRAP technique have been suggested as the most appropriate technique for determining antioxidant due to its high reproducibility, simplicity, low cost, rapidity of performance and the high correlation with ascorbic acid, uric acid and other antioxidants, (Benzie and Strain, 1999; Vassalle et al., 2004; Thaipong et al., 2006).

During the present investigation, it was observed that asthmatic subjects exhibited significantly lower FRAP value than control individuals implying that antioxidant power which is equipped to fight the oxidative forces is reduced in the asthmatic subjects which caused increased oxidative damage. The FRAP values of asthmatics and control subjects were also compared in relation to various correlates like age, sex, dietary, smoking and drinking habits, exposure to biomass smoke and daily activity. Asthmatics had significantly lower plasma antioxidant status as compared to controls in relation to all the correlates like sex, age, smoking, drinking, dietary habits, exposure to biomass smoke and physical activity (Table 4.22).

Many earlier studies have shown reduced antioxidant capacity in asthmatic subjects (Rahman et al., 1996; De Raeve et al., 1997; Comhair et al., 2000; Kanazawa et al., 2002; Nadeem et al., 2005; Wood and Gibson, 2009; Ahmad et al., 2012). Nadeem et al. (2003) reported no alteration in total antioxidant capacity in asthmatic subjects but later in another study (Nadeem et al., 2005), it was reported that FRAP value was significantly reduced in patients with acute asthma. Total antioxidant capacity was found to decrease in serum of asthmatic children as studied by Liao et al. (2004).

In the present study, FRAP value was observed to be significantly higher in asthmatic as well as control males as compared to females of the respective categories. Previous studies regarding gender-based alteration in antioxidant power of plasma are quite conflicting. Choy et al. (2000) studied the total antioxidant activity using FRAP assay and ascorbic acid concentration using FRASC assay (a modified form of FRAP assay) in the human tears. They found no significant difference in men and women in terms of total antioxidant activity in tears but men had significantly higher ascorbic acid concentration as compared to women. On the contrary, Benzie et
al. (1998) had reported that plasma antioxidant level measured as vitamin E and plasma ascorbate level was higher in females than males. Yamamoto et al. (2002) reported that female rats have comparatively higher antioxidant level. Katalinic et al. (2005) also determined the antioxidant capacity in heart, kidney and liver tissues of rats using FRAP assay and reported higher antioxidant capacity in female than male rats.

When asthmatic subjects were compared according to their age group, in the present investigation, it was found that patients with advancing age (<50 years old) were having a slightly but non-significantly higher value of FRAP as compared to the younger age group (>50 years of age). Control individuals with higher age (<50 years) were having non-significantly higher FRAP value as compared to younger individuals (>50 years of age). These finding clearly showed that the antioxidant power declined with advancing age and it might play an important factor in increased oxidative stress with ageing. Previously, Nuttall et al. (1999) and Mutlu-Türkoglu et al. (2003) observed decreased antioxidant level in old aged subjects compared to young individuals. The age-related increase in oxidative stress has also been reported in several earlier studies (Marzani et al., 2004; Yildirim et al., 2005; Cencioni et al., 2013).

Among the asthmatic subjects, the value of FRAP was non-significantly higher among non-vegetarians as compared to vegetarians while in case of control subjects, the vegetarians had non-significantly lower FRAP value compared to non-vegetarians, during the present study. Smoker asthmatic subjects had lower value of FRAP as compared to nonsmokers but the difference was statistically non-significant (Table 4.23). Whereas, control smokers had significantly reduced antioxidant level compared to control non-smokers (Table 4.24). When the plasma antioxidant value was compared among control subjects in relation to number of cigarettes smoked in a day, plasma antioxidant value was first observed to decline among the control subjects with increased number of cigarettes smoked daily in moderate smokers but then it decreased a bit, but non-significantly among heavy smokers (Table 4.26). Similar trends were observed in case of the asthmatic subjects. The value of FRAP was non-significantly higher among the moderate and heavy smokers as compared to mild smokers (Table 4.26). The present results are in accordance with the previous studies that have also reported decline in antioxidant level with smoking.
There are evidences that the oxidants in cigarette smoke, either \textit{in vitro} or \textit{in vivo}, markedly decrease plasma antioxidants level. This may be a result of marked depletion of plasma protein sulphhydrils due to exposure to cigarette smoke. Depletion of plasma antioxidants further reduces the protection against cigarette smoke-induced plasma membrane peroxidation (Petruzelli \textit{et al.}, 1990; Reznick \textit{et al.}, 1992; Cross \textit{et al.}, 1993; Bridges \textit{et al.}, 1993; O’Neill \textit{et al.}, 1994). Petruzelli \textit{et al.} (1990) demonstrated low levels of antioxidant capacity in smokers, which were negatively correlated with the levels of 3-nitrotyrosine. Rahman \textit{et al.} (1996) found that plasma antioxidant capacity was greatly reduced in smokers, asthmatic subjects and COPD patients in comparison to control non-smokers. Richard (2007) reported reduced antioxidant capacity in young cigarette smokers compared to non-smokers.

During the present study, control subjects who were exposed to biomass fuel smoke were also observed to have significantly lower antioxidant status as compared to non-exposed subjects (Table 4.24) whereas, no significant difference was found among the asthmatic subjects regarding the exposure to biomass smoke (Table 4.23). Earlier, Dietrich \textit{et al.} (2003) reported a strong association between smoking, exposure to biomass smoke and reduction of plasma antioxidants including uric acid. Sezer \textit{et al.} (2006) studied combined effect of biomass and cigarette smoke exposure on rabbits and found that antioxidant level was decreased with exposure to biomass smoke without altering the oxidant level. Mondal \textit{et al.} (2010) observed that total antioxidant status was reduced significantly among the subjects who were exposed to biomass smoke. The present study correlates well with the previous studies.

During the current investigation, non-significant differences in the FRAP value were obtained between abstainers and alcohol consumers among both asthmatic as well as control subjects. Dose–wise comparison of FRAP value was also made among both asthmatic and control subjects. The value of FRAP was lowest in heavy alcoholics and highest among those who consumed <1L/month, while among the asthmatic subjects, FRAP value was significantly lower in heavy alcoholics as compared to mild alcohol consumers. No significant difference was found between moderate and heavy alcoholics (Table 4.27).

A significantly lower FRAP value was observed in those subjects who were given prescription for short acting beta-2 agonist drugs as compared to those who were not given prescription of these medications, during the current study (Table 4.23). The prescription of short acting beta-2 agonists is given in severe cases. The
results in the present investigation clearly indicated that antioxidant level is reduced in severe cases.

When asthmatic subjects were compared for the FRAP value according to their family history, it was observed that patients with a family history of asthma had non-significantly lower antioxidant capacity as compared to the subjects without a family history of asthma (Table 4.23). This suggests that patients with a family history of the disease exhibited comparatively more oxidative stress.

FRAP values were also compared among the asthmatic and control subjects to study the synergic as well as individual effect of smoking and alcohol intake on the antioxidant power (Table 4.25), and it was observed that among the asthmatic subjects, FRAP value was lowest in group 2 comprising subjects who were smokers but abstainers and highest among group 3 i.e. alcohol consumers but non-smokers implying that smoking caused more obliteration to the antioxidant status of asthmatic subjects comparatively. Among control subjects also, lowest FRAP value was observed among group 2 followed by group 1 (alcohol consumers + smokers) and highest among group 3 (alcohol consumers but non-smokers).

The FRAP value was observed to decline with increased duration as lowest among the patients with more than 10 years of asthma and a marked decline in the FRAP value was observed in subjects with moderate and severe asthma (Tables 4.28 and 4.29). The FRAP value was significantly positively correlated with FVC and FEV1 values of asthmatic subjects. A remarkable negative correlation was found between FRAP value and duration of asthma (Table 4.30). This showed decline in the antioxidant capacity with increased progression of disease. The current findings correlate well with previous studies. Comhair et al. (2000) suggested that decreased antioxidant level in plasma and BAL fluid during stable asthma and in exacerbation, provides strong evidence for increased oxidative stress in asthma. Asthmatic patients with severe exacerbation of the disease have decreased serum total antioxidant status (Katsoulis et al., 2003). Wood and Gibson (2009) reported that patients with uncontrolled or severe asthma show comparatively low level of plasma antioxidant level than those with controlled or partly controlled asthma. In a recent study, Ahmad et al. (2012) observed that antioxidant capacity of plasma was decreased in asthmatic patients with increased severity of disease and also in comparison to healthy controls.
5.2 Genetic damage and asthma

5.2.1 Micronucleus assay

The expression of micronuclei in exfoliated buccal mucosa cells is a good source for biomonitoring genetic damage. The suitability of this assay for human biomonitoring studies was first described in exfoliated cells of the buccal mucosa by Stich et al. (1982, 1983). The buccal micronucleus (MN) assay is a simple, cost effective and minimally invasive technique to determine genetic damage in human subjects (Holland et al., 2008). In the present investigation, cytological damage was measured by evaluating the mean frequencies of MN, TMN, BN, BE, KL and KH. Many studies have reported the elevated frequency of MN and BN in head and neck cancer (Khliifi et al., 2013) and its correlation with cancer development (Cao et al., 2011), type-2 diabetes (Martinez-Perez et al., 2007), COPD (Maluf et al., 2007) and many other diseases.

In the current investigation, asthmatics were observed to have comparatively higher frequency of all the nuclear anomalies with around two-fold or more increase in MNC, TMN, BN, BE and KL (Table 4.31). The asthmatic subjects had comparatively higher level of cytological anomalies in relation to all the correlated factors viz. age, sex, smoking, drinking, dietary habits, exposure to biomass smoke and physical activity (Table 4.32).

Previously, Herrströma et al. (1998) observed that asthmatic subjects had comparatively increased frequency of MN in B-lymphocytes. The three markers of atopic disease, asthma, hypersensitivity to pollen and IgE levels were observed to be significantly associated with increased frequency of MN in B-lymphocytes. Rossnerova et al. (2011) reported a slightly higher frequency of MN in asthmatic children from Ostrava in comparison with controls. They also found slightly greater genetic damage in girls in comparison with boys among asthmatic subjects. This difference was significant in the control subgroup.

A significantly higher MN frequency and other nuclear anomalies were encountered in exfoliated buccal cells of the asthmatic subjects. Also, it was found that the mean value of MNC, TMN, KL and KH were non-significantly higher in male asthmatics as compared to females while mean frequency of BN and BE were higher among female asthmatics than males. In case of control group, the frequencies of all the nuclear anomalies were higher among males as compared to females. In a
study on healthy Indian male and female population, the results showed a significantly higher level of basal DNA damage in males as compared to females (Bajpayee et al., 2002). The results in the present study correlate well with this study. In an earlier study, on the contrary, Ganguly (1993) observed higher MN frequency among healthy females compared to healthy male subjects. In yet another study involving 127 donors, MN were increased with age and gender however, gender variation was mostly in people aged < 50 and women having higher MN frequencies (Wojda et al., 2006, 2007).

In the present study, smoker asthmatic subjects had markedly higher frequency of BN, BE and KL compared to control smokers. Among the asthmatic subjects, the mean frequency of MNC, TMN, BN, BE and KH were non-significantly higher among smokers as compared to non-smokers whereas, among the control subjects, mean frequencies of MNC, TMN and BN were significantly higher in the smokers than non-smokers. These results are in accordance with the previous studies that have reported the increase in number of micronucleus due to smoking and various other forms of tobacco (Stich et al., 1982; Sarto et al., 1987; Piyathilake et al., 1995; Ozkul et al., 1997; Konopacka, 2003; Wu et al., 2004; Haveric et al., 2010).

Earlier, Bonassi et al. (2003) reported that MN frequency was not increased in moderate smokers; only heavy smokers (30 cigarettes or more per day) showed a significant increase in genetic damage as measured by the micronucleus assay. Wu et al. (2004) suggested the positive relation between micronuclei frequency and smoking intensity with increased number of MNi in heavy smokers. Hoffmann and Speit (2005) reported increased MN frequency in smokers but the difference was statistically non-significant. Rossnerova et al. (2011) reported that MNi in asthmatic children classified as active smokers were significantly higher in comparison with healthy smoking children. No significant DNA damage in asthmatic children from smoking families was observed as compared to healthy children from nonsmoking families.

In the present study also, the micronuclei frequency in buccal cells was found to be markedly higher in heavy smokers (consuming >20 cigarettes a day) than light to moderate smokers (consuming 1-10 cigarettes a day). Similar results were obtained for BN, KL and KH frequency, but the variations in the mean frequencies were non-significant, implying a positive relation between smoking and genomic damage among the control subjects (Table 4.37). Among the asthmatic subjects, the
frequencies of all the nuclear anomalies were increased with increased number of cigarettes smoked/day and significant increase was observed in MNC, TMN and KL frequency (Table 4.38). Also, a dose response relationship was obtained between the consumption of cigarettes and frequency of nuclear anomalies of both asthmatic and control subjects.

Regarding the alcohol drinking habits, no significant differences were found among the asthmatic subjects (Table 4.33), whereas among the control subjects, significantly higher frequency of MNC, TMN, BN and KH were found in the alcohol consumers as compared to abstainers (Table 4.34). A dose response relationship was obtained between the nuclear anomalies and alcohol consumption in both asthmatic and control subjects which implies increased genomic instability with increased dose of alcohol. Many authors have suggested a possible clastogenic and aneugenic effect of ethanol and also a possible role in chromosome malsegregation (all these cause MN formation) and that alcohol consumption is associated with increased MN frequency (Stich and Rosin, 1983; Crebelli et al., 1989; Castelli et al., 1999; Maffei et al., 2000, 2002; Burim et al., 2004; Iarmarcovai et al., 2007). On the contrary, in a reanalysis of total 30 databases containing 5424 subjects by Bonassi et al. (2011), no association was found between alcohol consumption and increased MN frequency. In a recent investigation, Santovito et al. (2015) found no significant differences in the MNi frequency between alcoholics and non-alcoholics and no association between MNi and duration of alcohol abuse.

Stich and Rosin (1983) observed increase in the MN frequency in the alcohol drinkers who were consuming 2-4 packs of cigarettes a day. Those who were consuming cigarettes alone did not show any significant rise in the MN frequency. Similar findings were observed recently by Jindal et al. (2013). Du et al. (2000) suggested that the synergism between tobacco and alcohol in causing cancer might be due to the enhancement of the effects of tobacco carcinogens by ethanol. Reis et al. (2002) found that frequency of micronuclei in buccal mucosa cells was higher in the group of alcoholic individuals when compared to the control group, although the difference was not statistically significant. Sellappa et al. (2010) found increase in MN frequency due to smoking and alcohol consumption in control as well welder subjects exposed to hexavalent chromium. Welders who were both smoker and alcoholic showed even more MN induction than other groups.
During the present investigation also, it was observed that in case of asthmatic subjects, mean MNC, TMN, BN, BE and KH values were highest among those who were smoker but abstainers and lowest in those who were alcohol consumers but non-smokers but the differences were non-significant (Table 4.35). Among the control subjects (Table 4.36), the MNC, TMN, and BN frequencies were highest in those who were both smokers and alcohol consumers (group 1) and lowest in group 4 (non-smokers + abstainers) showing that the synergic effect of alcohol consumption and smoking was more hazardous than smoking or alcohol drinking alone. The frequency of KH was also highest in group 1 and no KH was observed in group 2 (smokers + abstainers).

When asthmatic subjects were compared with respect to their dietary habits, no significant differences were found between vegetarians and non-vegetarians (Table 4.33). Among the control subjects, mean frequency of all the nuclear anomalies except KL was higher among the non-vegetarians as compared to vegetarians but significant difference was observed in BN and KH frequency (Table 4.34).

Exposure to biomass smoke also affects the frequency of nuclear anomalies in human subjects. During the present investigation, the asthmatic and control subjects were also compared according to their exposure to biomass smoke. Among the asthmatics, no significant difference was found between the exposed and non-exposed group. While among the controls, the mean frequencies of all the anomalies except KH were found to be higher in the exposed group as compared to non-exposed group and significant differences were observed in BE and KL frequency. A previous study by Mondal et al. (2011) also reported increased MN frequency in buccal exfoliated cells of the women exposed to biomass smoke.

No significant differences were observed between light to moderately active subjects and those subjects who had sedentary lifestyle in both asthmatic and control category during the present study. Previous studies have also reported the effects of physical exercise on MN in PBL but the findings are conflicting which range from a reduction in MN in association with moderate exercise or following a triathlon race in fit subjects (Stefanie et al., 2008; Huang et al., 2009), to null effects (Hartmann et al., 1998; Pittaluga et al., 2006) or even an increase in MN in healthy subjects after acute exhaustive exercise (Schiffl et al., 1997; Umegaki et al., 1998).

The comparison of nuclear anomalies among the patients was also done on the basis of prescription of short acting beta-2 agonists (Table 4.33). Mean frequencies of
all the nuclear anomalies were observed to be elevated in the subjects who used to have frequent episodes and were given prescription for short acting beta-2 agonists for quick relief during an episode and a significant difference was observed in mean MNC, TMN, BN, and BE frequencies. This depicts that subjects who have frequent episodes of asthma exhibit more genomic damage comparatively. No significant differences were found in the nuclear anomalies with respect to family history of asthma.

Among the asthmatic subjects (Table 4.33), the incidences of nuclear anomalies were observed to elevate with advancing age. The mean MNC, TMN, and BE were significantly higher in the older age group (50-80 years) as compared to younger age group (<50 years). Among the control subjects as well (Table 4.34), the MNC, TMN, BE, KL and KH frequencies were observed to increase with advancing age but significant difference was found only in mean TMN value. Pearson correlation of age was also significantly positive with MNC and BE frequency. Frequency of BN, KL and KH were also positively correlated with age but the correlation was non-significant depicting increased genomic instability with advancing age. The increase of MN with age might be due to a combination of some factors like, (i) the cumulative effect of acquired mutations in genes involved in DNA repair, chromosome segregation and cell cycle checkpoint and (ii) numerical and structural aberrations in chromosomes caused by exposure to endogenous genotoxins, inadequate nutrition, exposure to environmental or occupational genotoxins, as well as a wide range of uncontrol lifestyle factors (Fenech and Bonassi, 2011).

In some of the previous studies also, an age related increase of spontaneous chromosome instability was showed (Fenech and Morley, 1985b; Ganguly, 1993; Barnett and King, 1995; Bolognesi et al., 1997; Fenech, 1998). Wojda et al. (2007) also observed increased MN frequency in higher age group (>50 years old). Rossnerova et al. (2011) observed increased MN frequency in the lymphocytes of asthmatic subjects with advancing age. On the contrary, Santovito et al. (2015) observed no significant correlations between the age of subjects and the MN frequency in the blood lymphocytes.

During the present investigation comparison of nuclear anomalies was also done among asthmatic subjects with respect to duration of asthma (Table 4.41). Though nuclear anomalies increased slightly with increased duration but, no
significant variations were observed. The Pearson correlation between duration of asthma and various nuclear anomalies were also non-significant (Table 4.44).

When patients were compared according to increased severity of asthma, the mean values of MNC, TMN, BN, BE were observed to increase significantly with increased severity of asthma while, mean KL and KH were observed to increase non-significantly. The Pearson correlation of various cytological observations with the severity of disease in terms of FVC and FEV1 values of patients were also investigated (Table 4.42). The MNC, TMN, BN, BE and KL were significantly negatively correlated with measured FVC values of the patients. Mean BN and KL were also significantly negatively correlated measured FEV1 of the patients (Table 4.43).

5.2.2 Comet assay

The comet assay is a useful and highly reliable biomarker for detecting different types of DNA damage. It is widely used to detect single- and double-strand breaks, protection and repair at the level of individual cells (Collins et al., 1997a, b, 2008; Tice et al., 2000; Collins, 2004; Møller, 2006). The percentage of DNA in tail is related to DNA break frequency (Ross et al., 1995), therefore, increase or decrease in DNA damage will ultimately lead to increase or decrease in head DNA (%), tail DNA (%), tail length, tail moment, olive moment, head area and tail area, so, all these parameters can be used to detect level of DNA damage. However, the comet tail length, tail DNA (%) and olive tail moment are the most commonly used parameters for detecting DNA damage (Collins, 1992, 2004; Ahuja and Saran, 1999; Collins et al., 2008).

During the present study, the DNA damage was observed to be markedly elevated among asthmatic subjects as % head DNA was significantly declined and % tail DNA, tail length (µm), tail area (µm²), tail moment and olive moment were all significantly increased compared to controls (Table 4.45). This depicts the increased genetic damage among the asthmatic subjects which might be due to increased oxidant-antioxidant imbalance. Previous studies have also reported increased DNA damage among asthmatic subjects. Zeyrek et al. (2009) reported increased lymphocyte DNA damage level in children with bronchial asthma and suggested that the elevated DNA damage may be related to increased oxidative stress. DNA damage in children with asthma bronchiale was studied by Hasbal et al. (2010). Before and
after 8 weeks of anti-asthmatic therapy blood samples were taken, DNA strand breaks and Fpg-sensitive sites in peripheral leukocytes were determined by comet assay. DNA strand breaks and Fpg-sensitive sites in the asthma group were found to be increased as compared to control group. Earlier, Fortoul et al. (2003) reported that asthmatic subjects had less damage in their leukocytes than non-asthmatic individuals, but more DNA breaks in their nasal epithelial cells than did their non-asthmatic counterparts.

During the present investigation, no significant difference was encountered in the comet parameters between male and female subjects. However, the genetic damage was slightly higher among males as compared to females. Previous studies have reported increased DNA damage in males in Indian population (Bajpayee et al., 2002) and in European population (Betti et al., 1994, 1995). Lam et al. (2002) reported higher DNA damage in elevator manufacturing male workers in China. Genotoxic differences by sex in nasal epithelium and blood leukocytes in subjects residing in a highly polluted area were demonstrated by Fortoul et al. (2004). Higher DNA damage in nasal cells and leukocytes was found in males compared to females and healthy subjects. The percentage of squamous metaplastic changes in the nasal epithelium was also higher in males compared with females and controls.

Regarding smoking habit as a confounding factor, the present work has revealed that smokers exhibit more damage than non-smoker patients that in turn had higher values than non smoking controls (Figure 4.36). The tail length and tail area were significantly increased in the smokers as compared to non-smokers. % tail DNA, tail moment and olive moment were non-significantly higher in the asthmatic smokers, whereas, a very slight increase was observed in the tail area, tail moment and olive moment among the control smokers (Table 4.48) implying that smoking somehow enhanced the genomic instability more among asthmatic subjects than the controls. The asthmatic subjects were compared according to the number of cigarettes smoked per day (Table 4.52). No significant differences were observed in the comet parameters. But the trend of the comet parameters viz. % tail DNA, tail length, tail moment and olive moment were increasing non-significantly with increased number of cigarettes. In case of control subjects, the tail length, tail moment and olive moment increased non-significantly with increased number of cigarettes/day while, the tail area was found to increase significantly (p<0.05) with increased number of cigarettes smoked/day (Table 4.51).
Many earlier studies reported elevated DNA damage in smokers and heavy smokers compared to non-smokers (Betti et al., 1994, 1995; Rojas et al., 1996; Piperakis et al., 1998, 2000; Dhawan et al., 2001; Lam et al., 2002; Hininger et al., 2004; Fracasso et al., 2006). Various in vitro studies have linked tobacco smoke to DNA damage (Hopkin and Evans, 1979; Hopkin and Evans, 1980; Bond et al., 1989; Au et al., 1991; El-Zein et al., 2010; Song et al., 2010). Lam et al. (2002) found that smoking, male gender, age and even passive smoking increased DNA strand breaks in lymphocytes of elevator factory workers. Hoffmann et al. (2005) did a metaanalysis of 38 studies and observed that DNA damage was significantly higher among smoking human subjects as compared to non-smokers. In contrast, many workers have reported no change in DNA damage level due to smoking (Wojewodzka et al., 1999; Moller et al., 2000; Dusinska et al., 2001; Nia et al., 2001; Maluf et al., 2001; Speit et al., 2003; Garaj-Vrhovac and Kopjar, 2003; Vodicka et al., 2004; Hoffmann and Speit, 2005). Nia et al. (2001) used a multi-biomarker approach to study effect of smoking on the oxidative DNA damage, repair and antioxidant defense mechanism. They however did not find any significant difference between smoker and non-smokers.

During the present investigation, no significant difference was observed in the genetic damage between alcohol consumers and abstainers. Also, no significant changes were found in the DNA damage with increased dose of alcohol (Tables 4.53, 4.54) however, it increased slightly among heavy alcoholics. When the synergic effect of smoking and alcohol intake was studied, the genomic damage was least among the group constituting abstainers and non-smokers while, subjects who were both alcohol consumer and smoker, exhibited highest value of tail length, tail area and tail moment. But the variations were non-significant. Van Zeeland et al. (1999) investigated the level of 8-OHdG in DNA from peripheral leukocytes among 102 healthy adults living in North Italy. No association was observed of DNA damage with alcohol consumption. No significant difference was observed in the DNA damage measured as mean comet tail length between alcohol drinker and non-drinker subjects (Sellappa et al., 2010).

On the contrary, Mutlu-Türkoğlu et al. (2000) observed increase in H₂O₂-induced DNA damage in the lymphocytes in patients with chronic alcoholism. Recently, Deshpande et al. (2014) reported that cirrhotic patients had significantly higher MDA levels associated with DNA Damage than those with compensated
cirrhosis and control group who were not suffering from liver cirrhosis. These results highlighted a significant higher degree of DNA damage in chronic alcoholics associated with oxidative stress as shown from greater average DNA migration in decompensated cirrhotic patients than in the compensated cirrhotic patients with low level of oxidative stress.

As for the confounding factors, no significant variations were observed in the comet parameters with respect to dietary habits, however, a slightly increased DNA damage was found among the non-vegetarian asthmatic subjects than the vegetarian asthmatics (Table 4.47) during the present investigation. Previously, Dhawan et al. (2001) reported increased tail moment in healthy non-vegetarians subjects.

During the current investigation, level of DNA damage measured as various comet parameters, was also compared according to exposure to biomass fuel smoke and the DNA damage was found to elevate non-significantly in the exposed individuals suggesting increase in the genetic damage due to use of biomass fuel in the domestic chulha. In some previous studies on the effect of biomass fuel smoke on female population, Pandey et al. (2005) and Mondal et al. (2010, 2011) reported increased % tail DNA, tail length and olive tail moment. These findings clearly show that exposure to biomass fuel smoke enhances the genomic instability.

Hartmann et al. (1994) reported increased DNA damage due to physical activity but during the present study, but the present study did not show any major variation in the comet parameters due to physical activity. The current investigation showed that the % tail DNA, tail length, tail area, tail moment and olive moment were increased slightly in the subjects who were prescribed the short acting beta-2 agonist drugs for quick relief (Table 4.47). The slightly increased DNA damage in these subjects possibly corresponds to increased severity and frequent episodes due to which they were prescribed such drugs. Levels of strand breaks and Fpg-sensitive sites were found to decrease in the asthmatics after the treatment implying that anti-asthmatic therapy including gluco-corticosteroids not only controls asthma but also decreases mutation risk in children with asthma bronchiale (Hasbal et al., 2010).

When DNA damage in terms of comet assay was compared according to family history of asthma, it was observed to decrease in the subjects who had a family history as compared to those without a family history of asthma, during the present study (Table 4.47).
In the present study, DNA damage in terms of comet assay was compared among control and asthmatic subjects according to their age group. DNA damage was found to increase with advancing age (>50 years) among both the categories but significant variations were found in the asthmatic subjects only. The correlation between DNA damage measured as comet assay and age of the subjects was also positive. The % tail DNA and tail length exhibited significant positive correlation with age indicating the increased genetic damage with advancing age of the subjects. The present findings are in agreement with previous work which reviewed that there is a positive correlation between age and the level of DNA damage as observed by comet assay (Møller, 2006) and various other studies that have reported increased DNA damage with advancing age.

Betti et al. (1994) used regression lines to study the effect of age on genetic damage and they found a significant effect of age on the DNA damage measured as comet assay and Rojas et al. (1996) reported elevated level of DNA damage with advancing age using SCGE assay in the buccal mucosa cells. Increased DNA damage and decreased DNA repair capacity among old aged people has been reported earlier using SCGE assay (Singh et al., 1990; Dhawan et al., 2001). Piperakis et al. (1998) reported increased DNA damage with advancing age in the lymphocytes of healthy subjects. Inter-individual differences in DNA damage assessment carried out for the evaluation of DNA damage in urinary bladder washing cells in the comet assay and the DNA migration was found to increase significantly with age (Gontijo et al., 2001).

During the current study, DNA damage was analyzed with increased duration of asthma. The % head DNA was reduced while the % tail DNA was increased significantly, from group 1 with 0-1 year duration to group 4 with >10 years of duration. Tail length and tail area increased significantly up to group 3 with 5-10 years duration but then, decreased in group 4 (Table 4.55). The correlation of duration of asthma with % head DNA, tail length and tail area was non-significantly negative, while with % tail DNA, tail and olive moment, it was non-significantly positive (Table 4.58). All these findings showed that duration of asthma might affect the level of DNA damage among patients.

Level of DNA damage measured as comet assay was also found to elevate among asthmatic subjects with increased severity of disease (Table 4.56). Severe asthmatics exhibited highest tail area, tail moment and olive moment compared to mild and intermittent asthmatic subjects. The % head DNA showed markedly positive
correlation while, % tail DNA, tail moment and olive moment showed significant negative correlation with FVC and FEV1 values of the patients. All these findings indicate the rise in genomic instability with the increased severity of disease.

5.3 Association of ABO and Rh allele frequency with asthma

The ABO blood group system plays important role in transfusion and transplantation practices (Mohanty, 2007). The oligosaccharides expressed on the surface of epithelial cells determine the ABO blood type. The terminal structure of the glycoconjugates expressed in the O blood group differs from those expressed in the other ABO phenotypes (Oriol, 1995; Schenkel-Brunner, 2000). This difference creates different binding sites on the terminal structure of the oligosaccharide chains (Henry, 2001). These glycoconjugates act as potential receptors for microorganisms. The glycoconjugates expressed in the O blood group may bind allergens and influence the immune response (Karlsson et al., 1992; Henry, 2001; Linden et al., 2008).

During the present investigation, it was found that the phenotype frequency of blood group O was highest among asthmatic subjects, while among the controls, that of blood group B was found to be highest. The allele frequency of O allele was highest in the asthmatics followed by allele B and allele A, whereas in control subjects, there was very less difference observed between the frequencies of allele B and O. Frequency of allele A was higher among control group in comparison to asthmatics. However, the chi square value was observed to be non-significant in the asthmatics, whereas it was significant in the control subjects, which showed that there was lack of association of ABO allele frequency in asthma patients.

Data on association of ABO blood group frequency with asthma has been inconsistent. Bijanzadeh et al. (2009) studied 200 asthmatic and 2000 healthy subjects. They observed no significant difference in the distribution of ABO alleles in both asthmatic and healthy population; however, they also found maximum number of patients with O blood group. Other workers have also reported no association of the ABO blood group frequencies with asthma (Denborough and Downing, 1968). The present work correlates well with these studies.

On the contrary, De la Vega et al. (1976) found significant difference in the distribution of blood group A and O between the asthmatics and controls. Brachtel et al. (1979) observed higher frequency of blood type A and B among 239 German subjects with atopic conditions (allergic rhinitis, asthma, atopic dermatitis, hay fever
and acute urticaria) than the normal subjects. Kauffmann et al. (1996) investigated coal miners with asthma and found that asthma was significantly associated with non-secretor blood group O. They further suggested that subjects with blood type O are more prone to have lower lung function, wheezing and asthma. They also observed lower lung function in blood group A than blood group B. Many other researchers have reported an association between the O blood group and asthma in Italian (Ronchetti et al., 2001) and Taiwanese children (Chen et al., 2005) and in Najaf population (Al-shamma et al., 2008).

**Conclusion**

Bronchial asthma is a complex multifactorial disease in which environmental factors, oxidative stress and genetic factors are responsible for initiating and modulating the progression of the disease. Oxidative stress represents as one of the important factors contributing to the onset and persistence of airway inflammation in asthmatic subjects. It plays a significant role in the pathophysiology of bronchial asthma, therefore, represents a potential target of the therapy in asthmatic patients.

The present findings suggest that the oxidative stress increases in asthma as a consequence of reduced antioxidant level which further causes DNA damage. Increased lipid peroxidation and reduced catalase activity are the contributing factors in the elevation of oxidative damage. Significant differences observed in the comet parameters and nuclear anomalies between the patients and control individuals suggest increased genomic instability due to chronic inflammation in asthmatic subjects and the effect was even more exacerbated with poor lifestyle and dietary habits, smoking, alcohol intake and exposure to biomass smoke. From the present findings, it can be concluded that asthma is associated with increased oxidative stress followed by an accumulation of DNA damage. The oxidant-antioxidant imbalance in favor of the former suggests that amplification of the antioxidant defenses might be recommended as a part of therapy. Moreover, multiple genotype analyses are necessary as well because gene polymorphisms may have a significant effect for asthma development.

However, several questions still remain unexplored, for instance, what is the role of short-acting beta-2 agonists in oxidative stress management; the effect of supplementary diets such as intake of fruits, vitamin A and C and other supplementary foods. Also very few smoker and alcoholic subjects were available in asthma patients
as compared to control category due to which we could not find strong evidence on
the role of smoking and alcoholism in asthma pathogenesis.

For future study the following points are recommended:

1. There is a need to understand the various underlying factors, both genetic as
   well as environmental that contribute to onset and persistence of asthma and
   might affect an individual’s susceptibility to develop asthma.

2. It is highly important to diagnose the patients properly in order to provide
   appropriate therapy accordingly as many times asthma cases become very
   severe due to improper treatment as the patients was not correctly diagnosed.

3. Understanding of the role of various factors like diet, antioxidant intake,
   exercise, yoga in the treatment of asthma is also important as these factors
   affect the frequency of asthma episodes.

4. Proper analysis of various treatments given to the individuals is highly
   mandatory. The study of effects of steroids and other drugs on the individual
   and their correlation to oxidative damage as well as asthma severity is crucial
   for the proper treatment.

5. Effect of environmental and occupational exposure, active and passive
   smoking, traffic pollution, biomass smoke and medical history are needed to
   be analyzed during the therapy.

6. A genetic screening and molecular analysis of the individual suffering from
   asthma might be helpful in understanding the possible triggers for the
   individual and accordingly treatment can be given. Molecular analysis can
   also provide information regarding the precautions that an individual can take
   to prevent future episodes.

   Hence, it is strongly suggested that estimation of all kind of markers of
   oxidative damage (proteins, lipids, DNA), together with estimation of antioxidant
   defense status, production of reactive oxygen species and genotype of relevant genes
   in the same time in asthmatic patients can be helpful for the selection the best
   treatment.