Increasing incidences of respiratory diseases is a major concern worldwide and asthma is one of them. Asthma has become more common in children and adults. Asthma is a pulmonary disorder which is characterized by the generalized reversible obstruction of airflow to a variety of stimuli and to define reversibility as a greater than 12% increase in the patient’s forced expiratory volume in 1 second (FEV1) that occurs either spontaneously or with therapy.

Asthma is a major cause of chronic morbidity and mortality with an estimated 300 million individuals affected worldwide and over 180,000 deaths each year and there is evidence that its prevalence has increased considerably over the past 20 years, especially in children. In 2009, asthma caused 250,000 deaths globally. According to a WHO survey, in 2012, asthma along with other respiratory diseases caused 4 million deaths globally. If the current trends continue, it is estimated that there may be an additional 100 million more asthmatics by 2025. As per an Indian survey, it is estimated that 2 to 4% of the adult population in India is affected by asthma. The prevalence of asthma is considerably higher in rural areas than in urban areas and is slightly higher among males than among females in case of rural areas. Occupational asthma is also prominent in the country with high frequency of asthma in the industrial regions. Alarming increase in the indoor pollution, lack of education and proper medical care, the situation has become more terrible. There are evidences of increased prevalence of childhood asthma with up to threefold increase incidences among children.

Asthma is associated with chronic inflammation of the airways, airways obstruction, oxidant-antioxidant imbalance which further leads to oxidative stress. The inflammatory cells that are recruited to the airways are capable of producing oxidants. Once recruited, these become activated and generate reactive oxidants in the airways, in response to various stimuli. Activated eosinophils, neutrophils, monocytes, and macrophages, and other resident cells such as bronchial epithelial cells, can generate oxidants. The ROS interact with a wide variety of molecules in the biological systems resulting in lipid peroxidation and enzyme dysfunction and enhancement of proinflammatory cell signaling, thereby profoundly altering cellular
function. The reactive oxygen species interfere with the basic structure of lipids, proteins and DNA which causes genetic damage.

The present study was undertaken to assess genetic damage and oxidative stress in asthmatics as very less research has been done in this area. In the present study Catalase activity and lipid peroxidation in serum and total antioxidant capacity of the plasma have been detected. The DNA damage in the exfoliated buccal epithelial cells by the MN assay and oxidative damage to lymphocyte DNA was measured by the comet assay.

Objectives of the present study are as follows:

1. To conduct an epidemiological survey of the asthmatic human subjects and healthy matched controls.
2. To measure the level of oxidative stress by measuring total antioxidant capacity of plasma (FRAP assay), lipid peroxidation level in serum (MDA concentration) and catalase activity of serum.
3. To study the DNA damage using comet assay, in the asthmatic subjects along with healthy matched controls.
4. To make a comparison of the frequency of micronuclei in buccal epithelial cells of the asthmatic subjects with controls.
5. To provide a baseline data based on which further studies may be planned.

The present study was conducted in Human Genetics Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra. The epidemiological data of asthmatic and healthy matched control individuals with their blood samples and buccal smears was collected with the help of registered medical practitioner. The data was recorded on a standard proforma. Before obtaining the samples an informed consent was taken from each subject and a questionnaire was filled up to determine their lifestyle (smoking, alcohol drinking, disease etc.). Before sampling, ethical clearance from IEC, K.U. Kurukshetra was obtained vide letter no. IEC/13/329 dated 27-04-13. The collected samples were brought to the laboratory in ice box. Plasma and serum extraction was done and were processed by below mentioned techniques to analyze catalase activity, MDA concentration and protein concentration in serum and
FRAP value of plasma. For the evaluation of genetic damage Micronucleus assay in the exfoliated buccal cells and Comet assay in blood lymphocytes were used.

The present thesis work has been organized into five chapters. Chapter 1 embodies the general introduction and facts about asthma, the global burden of asthma and prevalence in Asia and India. The causes and triggers behind asthma, diagnosis and the treatment of asthma are also included in this chapter. Chapter 2 comprises the review of literature and the research works done in the past on asthma, oxidative stress, genetic damage and its association with asthma. Chapter 3 entails the detailed laboratory and statistical methodology followed during the present study to analyze the samples. In Chapter 4, the results have been compiled. This chapter includes the figures of MN and comet assay, 60 tables and 45 graphs and a brief description of the results. Chapter 5 embraces the brief discussion of the results and inferences of the present study and its correlation with the previous studies. The salient finding, conclusion and the recommendations for future study are also incorporated in this chapter.

A total of 210 subjects (120 asthmatics and 90 matched controls) with average age of 42.48 years (range 13-80 years) were studied. Among the 90 control subjects, there were 53 males and 37 females. Out of 90, there were 29 smokers and 25 alcohol consumers. 42% of the control individuals were light to moderately active. 29% of the control individuals were exposed to biomass fuel smoke i.e. they were using biomass fuel in the domestic chulhas. Out of 120 asthmatic subjects, 76 were females while 44 were males. 84% of them were non-smokers and only 16% were smokers. Most of asthmatic subjects studied were abstainers (86%) and around 68% were vegetarians. There were only 20% individuals who were light to moderately active, while 80% were not active. Around 30% of the asthmatics were exposed to biomass fuel smoke.

Statistically significant difference was observed among the asthmatics and controls regarding the activity of catalase. In asthmatics, statistically significant difference (p<0.05) was observed in the vegetarians (2.75±0.22) and non-vegetarians (1.94±0.27). Comparison of catalase activity was also made among the asthmatic as well as control subjects according to various correlated factors. Vegetarian asthmatics (0.23±0.02) were found to exhibit higher CAT activity than the non-vegetarians (0.15±0.02, p<0.05). Asthmatic subjects were compared on the basis of their family
history of asthma and prescription of short acting beta-2 agonists but no significant differences were observed in the CAT activity. In case of control subjects, the activity of catalase was found to be greatly reduced in smokers as compared to non-smokers (p<0.05), in alcohol consumers as compared to abstainers (p<0.05) and in biomass smoke exposed individuals as compared to non-exposed individuals (p<0.05). No significant changes were observed regarding CAT activity with increased dose of cigarettes per day in both asthmatic and control subjects. However, the CAT activity slightly decreased with increased dose of cigarettes among control smokers but then again increased in those who were smoking more than 20 cigarettes per day. The CAT activity showed a non-significant positive correlation with FVC and FEV1 of the asthmatic subjects while negative correlation with age and duration. The CAT activity was significantly negatively correlated with the duration of asthma.

The concentration of MDA in serum was found to be significantly higher in asthmatics (0.75±0.08) as compared to controls (0.37±0.02). In all the correlates, serum MDA concentration was higher in asthmatics as compared to controls. The lipid peroxidation level was higher in the higher age group individuals (>50 years old) as compared to lower age group (<50 years) but the difference was statistically non-significant (p>0.05). Similarly smokers, biomass smoke exposed patients and patients who had sedentary life style were observed to have higher serum MDA concentration as compared to non-smokers, biomass smoke non-exposed patients and light to moderately active individuals. But all these differences were non-significant.

The lipid peroxidation level was greatest among those subjects who were consuming both cigarettes and alcohol (group 1), while lowest among those who were non-smokers but alcohol consumers (group 3). In case of asthmatics, the serum MDA was significantly higher (p<0.05) in those patients who were prescribed short acting beta-2 agonists for quick relief (0.85±0.08) in comparison to those who were not prescribed any drug for treatment (0.53±0.10). This was due to increased severity of disease. The lipid peroxidation was also significantly higher (p<0.05) in the patients with duration as well as severity of the disease. A significant negative correlation was observed between the measured FVC and FEV1 of the patients and MDA concentration while a significant positive correlation was there with the age and
duration of the disease showing that as the severity and the duration of the disease increases, the lipid peroxidation also increases.

Plasma FRAP value was significantly lower in the patients (367.39±9.95) as compared to control individuals (477.95±12.11, p<0.01) in total as well as in relation to all the correlates. This might be due to the increased oxidative stress and reduced antioxidant level in the patients. The FRAP value was significantly higher in male asthmatic subjects (393.41±17.05) as compared to females (352.32±11.98, p<0.05). Among the control subjects also, males had significantly elevated FRAP value as compared to females (p<0.01). A significantly lower FRAP value was observed in control smokers (444.69±17.07) than non-smokers (493.77±15.59, p<0.05). FRAP value was lowest in asthmatic subjects who were smokers but abstainers (289.52±19.95) and highest among alcohol consumers but non-smokers (443.43±55.77, p<0.05), implying that smoking caused more harm to the antioxidant status of asthmatic subjects.

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 using these medications. In asthmatics, a significant decrease in plasma FRAP value was observed with the severity of the disease. Pearson correlation was markedly positive with the FVC and FEV1 value of the patients showing more ferric reducing ability of plasma in mild or controlled patients and control individuals while negative correlation with duration and age shows less antioxidant level in the older patients.

Significant differences were observed in the nuclear anomalies between the patients and controls. Frequency of MNC was markedly higher in asthmatics (3.07±0.26) than controls (1.97±0.17). Similarly, frequency of BN, BE, KL were also significantly higher in asthmatics (12.08±0.68, 5.74±0.41, 10.88±0.65 respectively) than control individuals (3.70±0.27, 1.78±0.19, 0.74±0.11). No significant difference was observed in the mean frequency of nuclear anomalies between males and females, in case of the asthmatic subjects. The mean frequency of MNC, TMN, BN, BE and KH were non-significantly higher among asthmatic smokers as compared to non-smokers. Among the asthmatic subjects, a remarkable dose-response relationship was observed between the smoking and cytological observations. 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 implying increased genomic damage due to heavy smoking.

The frequency of MNC and TMN were observed to be significantly increasing with increased dose of alcohol/month in case of both asthmatic and control subjects. Significantly higher BE and KL frequency were observed in the subjects exposed to biomass smoke as compared to non-exposed subjects among controls. A significant higher mean MNC, TMN, BN, and BE frequency were observed in those who were given prescription for short acting beta-2 agonists as compared to those who were not given prescription of these drugs. No significant difference was observed in the nuclear anomalies with the duration of the disease. There was statistically significant difference among the patients with increasing severity of the disease. The severely asthmatic patients showed high incidences of MN (4.31±0.52), BN (16.08±1.50) and BE (8.34±0.81) as compared to mild and moderate asthmatics. Pearson’s correlation coefficient between various nuclear anomalies and duration of the disease was found to be non-significant while it was significantly positively correlated with the age of the patients. The negative correlation of the nuclear anomalies with FVC and FEV1 value of the asthmatic subjects revealed increased genomic instability with disease progression.

The asthmatic and control subjects were observed to have significant differences for all the comet parameters (p<0.01). The value of % head DNA was significantly declined among asthmatic subjects (62.20±1.01) as compared to controls (79.37±0.57, p<0.001). The % tail DNA, tail length (µm), tail area (µm²), tail moment and olive moment were all observed to be significantly increased among the asthmatics in comparison to controls suggesting the increased genetic damage among the asthmatic subjects. The mean tail length was observed to be markedly higher (27.00±4.41) in smoker asthmatic subjects as compared to non-smokers (21.03±0.86, p<0.05) and also in the asthmatic subjects with a family history of asthma in comparison to those who did not have a family history of asthma. No significant changes were found in the comet parameters with increased dose of alcohol, however, the % head DNA was found to be decreasing, while % tail DNA and tail area were observed to be increasing with increased consumption of alcohol, but non-
significantly. However, it was found that smoking caused more harm than alcohol and synergic effect of smoking and alcohol was even more hazardous than smoking alone.

DNA damage was found to increase with advancing age (>50 years) among both asthmatic and control subjects, but significant variations were found in the asthmatic subjects only. The % tail DNA and tail length exhibited significant positive correlation with age which proved that the genetic damage increased with advancing age of the subjects. The mean values of % head DNA, % tail DNA and tail length were found to be increasing with duration of disease. Level of DNA damage measured as comet assay was also found to elevate among asthmatic subjects with increased severity of disease. Severe asthmatics exhibited highest tail area, tail moment and olive moment compared to mild and intermittent asthmatic subjects. The % tail DNA, tail moment and olive moment showed significant negative correlation with FVC and FEV1 values of the patients showing the rise in genomic damage with increased severity of asthma.

The 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. 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.

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 important biomarkers of elevated oxidative stress. 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. In conclusion, it was observed that the oxidative stress was increased in asthmatics due to oxidant-antioxidant imbalance which resulted in increased genetic damage. The present study will be helpful in creating awareness among people about the oxidative stress and genetic damage associated with asthma and also providing a baseline for the future research work in this field.
However, several questions still remain unexplored, for instance, the role of short-acting beta-2 agonists in oxidative stress management and the effect of supplementary diets such as intake of fruits, vitamin A and C and other supplementary foods. Hence for the future studies, it is recommended to understand the role of various factors like diet, antioxidant intake, exercise, yoga in the treatment of asthma and to analyze the effect of environmental and occupational exposure, active and passive smoking, traffic pollution, biomass smoke and medical history on asthma. The study of effects of short acting and long acting beta-2 agonists and other drugs on the individual and their correlation to oxidative damage as well as asthma severity is crucial for the proper treatment. 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.

Hence, the 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 is strongly suggested for providing best treatment and care to the patients.