A growing area of research is the relationship between genetic damage and oxidative stress in diabetic patients. Accumulating evidence indicates that oxidative stress, a condition of excessive reactive oxygen species, may play a role in the aetiology of type 2 diabetes mellitus (by inducing insulin resistance in the peripheral tissues) and type 1 diabetes mellitus (impairing insulin secretion from pancreatic beta-cells). The fast so-called progressive westernization of the society is predisposing increasing numbers of the population to higher rates of oxidative stress and may be the results of the increase in the prevalence of diabetes and other dysmetabolic conditions. The development of a simple screening tool may help to identify individuals at high risk of development of such dysmetabolic states in the society.

Biomarkers are the indicators of molecular and cellular events that may illuminate relationships between environmental hazards and human health effects and disease processes. Of late, the molecular epidemiological approach using these molecular markers has become quite established (Dusinska and Collins, 2008). Genetic damage due to oxidative stress in lymphocytes and buccal mucosa cells can be evaluated by micronucleus assay and single cell gel electrophoresis assay. Other methods to investigate oxidative processes are via measuring catalase activity, malondialdehyde levels and ferric reducing ability of plasma.

2.1 MICRONUCLEUS (MN) ASSAY

The micronucleus assay involves examination of circulating lymphocyte cells or any other type of dividing cells to determine the prevalence of cells with micronuclei. These are extranuclear bodies composed of chromosomes or chromosomal fragments that failed to be incorporated into daughter nuclei at mitosis (Schmid, 1975).

Micronuclei (MNi) are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosomal fragments or intact whole chromosomes that lag behind at the anaphase stage of cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis, and is regarded as promising biomarker to assess the genotoxicity or impaired DNA repair (Jenssen and Ramel, 1980).
The buccal cell micronucleus (MN) assay was first proposed by Stich et al. (1982a) and still continued to gain popularity as a biomarker of genetic damage in numerous applications. They developed a protocol for the micronucleus assay with exfoliated human epithelial cells.

Callisen et al. (1986) developed in-house-assembled image analysis system for determination of the number of lymphocytes with micronuclei among the proliferating population.

Chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division give rise to MNi. Thus, MNi provide a measure of both chromosome breakage and chromosome loss and it is at least as sensitive an indicator of chromosome damage as classical metaphase chromosome analysis (Fenech and Morley, 1986; Muller and Streffer, 1994; Tucker and Preston, 1996; Evans, 1997; Fenech, 1997; Kirsch-Volders, 1997; Miller et al., 1997).

Livingston et al. (1990) suggested that use of smokeless tobacco may cause exposure-dependent genetic damage, including micronuclei (MNi), to cells in the human oral epithelium.

Micronucleus test on exfoliated cells has been used to quantify chromosomal breakage occurring in the human oral cavity, esophagus, cervix, lung, nasal cavity and urinary bladder. Intervention trials on high-risk populations have shown that supplementation with chemopreventive agents can modulate this breakage. This assay can be used as a biological marker for the efficacy of a chemopreventive regime (Rosin, 1992).

Tolbert et al. (1992) demonstrated that other nuclear anomalies are at least as common as micronucleation and therefore, there are chances of misclassification. So, they proposed refinements in micronuclei scoring criteria and the inclusion of other nuclear anomalies in the scoring system.

Moore et al. (1993) described new techniques that employ fluorescence in situ hybridization (FISH) with centromeric and chromosome-specific DNA probes to detect aneuploidy and micronucleus formation in exfoliated human epithelial cells.

According to Beliën et al. (1995), at least 10,000 exfoliated cells should be screened to monitor a significant result in the MN assay. Therefore, they recommended the automation of counting of micronuclei to lessen the time required to complete the screening.

Blount et al. (1997) studied that folic acid deficiency increases spontaneous chromosomal damage by massive incorporation of uracil into human DNA (4 million per cell), which produces chromosomal breakage and micronucleus formation and contributes to the increased risk of cancer.
Casartelli et al. (1997) used different staining techniques namely, Feulgen, Giemsa stain, Hoechst 33258 and Propidium iodide for micronucleus staining. Cells were collected by either light or vigorous scraping of oral mucosa. They concluded that Hoechst 33258 can be recommended for micronucleus staining in squamous epithelial cells of oral mucosa. Differences in scraping may affect the measurement of micronucleus frequency and hence, scraping should therefore be carefully standardized in each laboratory.

Fenech (1999) reported that the micronucleus index in cytokinesis-blocked lymphocytes is significantly negatively correlated with plasma vitamin B12 concentration and significantly positively correlated with plasma homocysteine.

Fenech (2001) studied that folate deficiency caused expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation and DNA hypomethylation. This activity is compromised when vitamin B12 (B12) concentration is low because methionine synthase activity is reduced, lowering the concentration of S-adenosyl methionine (SAM) which in turn may diminish DNA methylation and cause folate to become unavailable for the conversion of dUMP to dTMP.

Fenech (2002) suggested that marginal deficiencies in folate, vitamin B12, niacin and zinc impact significantly on spontaneous chromosome damage rate. Compelling evidence of the important role of micronutrients in maintenance of genome integrity is provided by micronucleus formation in blood and epithelial cells. The need to revise current RDAs for these micronutrients based on minimisation of DNA damage was also highlighted.

Fenech and Crott (2002) studied that in folic acid deficient human lymphocytes, micronuclei (genomic instability), nucleoplasmic bridges (chromosome rearrangement) and nuclear buds (gene amplification) are introduced and it was explained by Breakage–fusion-bridge (BFB) cycles. The CBMN (cytokinesis-block micronucleus) assay may be a useful model for the study of the BFB cycle which may be one of the key mechanisms for the hypermutability phenotype required for the rapid evolution of cancer cells.

Kirch-Volders et al. (2002) termed the micronucleus assay as multi-endpoint test for genotoxic responses to clastogens/aneugens because of the multi directional approach of interpreting the different kind of mechanism leading to the formation of micronuclei and they also made use of fluorescent in situ hybridization with human pan-centromeric DNA probes for
discriminating the negatively labelled micronuclei containing acentric chromosome fragments and positively labelled micronuclei containing one or more chromosomes.

Interest in evaluation of cytogenetic damage in epithelial cells has increased recently as the frequency of micronucleus was extensively used as a biomarker of genotoxic exposure and early biological effect in human biomonitoring studies (Norppa and Falck, 2003; Nersesyan and Llin, 2007).

Norppa and Falck (2003) proposed that MN are segregated randomly between the daughter cells and the frequency of MN in binucleated cells (where both daughter nuclei are present) gives an indirect estimate of chromosome loss (or gain) due to micronucleation, however, the fate of micronuclei remains unclear.

Dhillon et al. (2004) postulated that it seems possible that some MNi may be retained within a cell and may be capable of functioning as an independent extra-nuclear entity capable of chromosomal replication and possibly other cellular functions.

The worldwide success and adoption of the MN technique, for in vitro and in vivo studies of genomic damage have been the contributions of high reliability and low cost of this biomarker. It can also be used for the early detection of carcinogenic effects in the cell exposed to various carcinogenic agents (Bonassi et al., 2005).

Iarmarcovai et al. (2006) suggested that micronuclei (MNi) are formed as a result of aneugenic events containing a single centromere (C1 + MN) and two or more centromeres (Cx + MN) may arise through different pathways. Chromosome migration impairment would lead to increased C1 + MN frequency whereas centrosome amplification would induce Cx + MN with three or more centromeric signals. Additional studies that target cellular defects on the centrosome (microtubule nucleation, organization of the spindle poles, cell cycle progression) are required to better understand the aneuploid cell production.

Bonassi et al. (2007) provided preliminary evidence that MN frequency in peripheral blood lymphocyte is a predictive biomarker of cancer risk within a population of healthy subjects. The wide-spread use of the MN assay provides a valuable opportunity to apply this assay in the planning and validation of cancer surveillance and prevention programs.

The MN assay in exfoliated buccal epithelial cells is a useful and modestly invasive method for monitoring populations exposed to genotoxic agents. Epithelial tissue exfoliated cells are derived from actively dividing basal layer. These cells migrate towards the surface within 5 to 14
days and can exhibit nuclear damage happen at this time. Basal layer also provides the first barrier against potential carcinogens. Thus, it is more likely to suffer damage by these agents before reflecting a systemic condition. The presence of micronuclei (MNi) and other nuclear anomalies within these cells has been shown to be associated with genetic defects in genome maintenance, accelerated ageing, exposure to genotoxic agents, oral cancer risk and neurodegenerative diseases and was also useful in chemo-preventive studies (Holland et al., 2008).

Glaviano et al. (2009) assessed the genetic damage in the human fibroblast cells with the use of micronucleus assay and reported that combined exposure of cadmium and radiations showed a synergistic effect.

Weng and Morimoto (2009) reported differential responses among CD4+ T-cells, CD8+ T-cells, B-cells and NK-cells exposed to different mutagens, using different assay methods, such as micronuclei (MNi), sister chromatid exchanges (SCEs), single cell gel electrophoresis (SCGE), and fluorescence-activated cell sorting (FACS). These methods are mostly used for genetic biomonitoring of human populations exposed to potential mutagens.

In a meta-analysis of 63 human population studies with exfoliated buccal MN assay, Ceppi et al. (2010) reported a statistically strong correlation between the increase in frequency of micronucleated cells in PBLs and buccal cells in genotoxic exposed groups. Thus, supporting the use of buccal MN assay as a biomarker of DNA damage in epithelial cells.

Dhillon et al. (2011) reviewed that genetic polymorphisms in XRCC1 (Arg280His), ERCC2 (Lys751Gln), CYP2E1 (c1/c2) and MTR (A2756G) were consistently associated with the MN formation and genotype may influence MN frequency in human cells.

Kashyap and Reddy (2012) described micronuclei in exfoliated oral buccal cells as internal dosimeters for determining tissue-specific genotoxic damage in different disease conditions, dietary deficiencies, individuals exposed to environmental and occupational carcinogens.

An automated method for scoring in vitro MNi with human TK6 cells using combination of flow cytometric analysis, compound exposure, processing, and sampling in a single 96-well plate has been described by Bryce et al. (2013).

François et al. (2014) compared the results of visual scoring method with laser scanning cytometry technique and observed highly significant correlation between them. The high-throughput nature of laser scanning cytometry can provide distinctive advantage in future DNA damage studies.
Bolognesi et al. (2015) reviewed the inter- and intra-laboratory variability in the results of MN assay and recommended the need for automation of the MNi scoring system.

Khan et al. (2015) examined the exfoliated buccal cells to study the cytotoxic and genotoxic effects of cotton dust and other toxic, allergic and infectious substances exposure in cotton weavers. They reported that long term exposure may indicate an increased risk of oropharyngeal cancer.

2.1.1 MN Assay in T2DM

Martínez-Pérez et al. (2007) showed increase in frequency of micronuclei in Mexicans with type 2 diabetes mellitus and been treated with oral hypoglycemic drugs (sulfonylurea and/or metformin), with no microvascular or macrovascular complications.

Jajarm et al. (2008) evaluated oral mucosa epithelium in type 2 diabetic patients by an exfoliative cytology method and reported that cytoplasmic area and nuclear area is significantly larger in patients with diabetes as compared to controls for both tongue dorsum and buccal mucosa. Cytoplasmic/nuclear ratio was larger in controls. The frequency of nuclear changes like karyorrhexis, multi-lobed nuclei and cytoplasmic localization was high in buccal mucosa of diabetic patients than controls.

Shareef et al. (2008) observed morphologic alterations like nuclear enlargement, karyorrhexis, binucleation in buccal mucosal epithelial cells of type 2 diabetic patients. They reported significantly higher nuclear area and lower cytoplasm to nuclear area ratio in diabetic patients as compared to control group. The cytoplasm area between these two groups was not significantly different. Hence, exfoliative cytology is useful as an additional tool to aid in the diagnosis of diabetes mellitus.

Shaik et al. (2010) studied that type 2 diabetes mellitus patients under long term treatment of pioglitazone and glimepiride in combination, showed increased frequency of micronuclei as compared to controls. The study suggests that the micronuclei assay can be used as a constituent among the panel of biomarkers to assess genotoxicity in type 2 diabetes mellitus patients under long term anti-hyperglycemic drug therapy.

Andreassi et al. (2011) published the association of micronucleus frequency with obesity, diabetes and cardiovascular disease and discussed the future research perspectives in order to elucidate the prognostic power of micronuclei (MNi) as biomarker for the detection and the progression from metabolic syndrome to type 2 diabetes mellitus and cardiovascular diseases. Their
findings supported the hypothesis that cytokinesis-block micronucleus (CBMN) assay may provide a useful tool for screening of the metabolic syndrome and its progression to diabetes and CVD in adults as well in children.

Shettigar et al. (2012) evaluated the induction of micronuclei due to increased glycosylation in type 2 diabetes. The increased glycosylation of haemoglobin seems to induce oxidative damage in the DNA of the diabetic patients, which manifests as an increased micronuclei frequency.

Binici et al. (2013) investigated the frequency of SCE and MNi in T2DM patients compared with healthy controls. T2DM is a condition with genomic instability characterized by an increased level of SCE and MN compared with controls. The SCE frequency was found to be positively correlated with the plasma HbA1c level. Hyperglycemia-induced oxidative stress may be the underlying factor of the increased SCE and MN frequency.

Corbi et al. (2014) evaluated the frequency of micronuclei by cell culture cytokinesis-block MN assay and found elevated frequency of micronuclei in patients affected by type 2 diabetes, dyslipidemia and periodontitis. Hence, the micronuclei assay might be useful as a biomarker for DNA damage in individuals with chronic degenerative diseases.

Toneline et al. (2014) reported that cells from diabetic patients showed an increased frequency of micronuclei and nuclear budding when compared to the control group, indicating a higher DNA damage due to oxidative stress and possibly related vascular complications of diabetes. These nuclear changes have the potential to be used as biomarkers of cell damage in diabetic patients.

2.1.2 MN Assay in T1DM

Zuniga-Gonzalez et al. (2007) demonstrated that either controlled (glycosylated haemoglobin levels, <7%) or uncontrolled diabetic patients (glycosylated haemoglobin levels, >7%) had about 2-fold higher frequency of micronuclei in buccal mucosa samples than healthy subjects. There was also evidence of an increased micronuclei frequency among patients with uncontrolled type 1 diabetes as compared with patients with a good metabolic control. Furthermore, a significant reduction in micronuclei was observed after folate supplementation for 30 days.

Cinkilic et al. (2009) reported that patients with type-1 diabetes mellitus showed a higher frequency of sister chromatid exchange compared with controls, but there was no significant correlation between the duration of diabetes, glycated haemoglobin and sister chromatid exchange.
No significant difference was found in chromosomal aberration or micronuclei frequency in type-1 diabetic patients compared with controls. Hyperglycemia-induced oxidative stress may be the underlying factor of the increased sister chromatid exchange frequency.

Witczak et al. (2014) reported increased frequency of MN in diabetic mothers and their newborns than the corresponding controls in both the groups using the cytokinesis-block micronucleus (CBMN) test.

2.1.3 MN Assay involving both T2DM and T1DM

Batista-González et al. (2006) reported increased micronucleated erythrocytes and micronucleated polychromatic erythrocytes in premature children born to mothers with diabetes mellitus as compared to healthy mothers.

2.2 SINGLE CELL GEL ELECTROPHORESIS (SCGE) / COMET ASSAY

Cook et al. (1976) investigated the nuclear structures based on the lysis of cells with non-ionic detergent and high molarity sodium chloride. Due to this, the membranes of the cell and organelles lysed and left behind the nucleoid, which consisted of nuclear matrix including RNA, protein and negatively supercoiled DNA. The survival of the supercoils implied that DNA could not rotate freely. Thus, a model with the DNA attached at intervals to the matrix was proposed so that it is effectively arranged as a series of loops, rather than as a linear molecule (Cook and Brazell, 1976).

Rydberg and Johanson (1978) made the earliest attempts to quantify DNA strand breaks directly with cells embedded in agarose on microscope slides and lysed under mild alkaline conditions. After neutralization, the cells were stained with acridine orange and the extent of DNA damage was quantified by measuring the ratio of green (indicating double stranded DNA) to red (indicating single stranded DNA) fluorescence using photometer. They observed that following X-ray irradiation the nuclei of lysed cell became increasingly diffused, in a dose dependent manner.

Ostling and Johanson (1984) described a micro-electrophoretic procedure for the direct visualization of DNA damage in individual cells in which mammalian cells suspended in a thin agarose gel on a microscopic slide were lysed by detergents and salts at high concentrations, electrophoresed under neutral conditions, and stained with a fluorescent DNA binding dye. During
electrophoresis, the broken and relaxed DNA fragments migrated further than the nucleus towards the anode.

Roti and Wright (1987) performed the halo assay to study DNA loops in nucleoids from HeLa cells and to investigate the DNA damage and repair mechanisms.

Singh et al. (1988) carried out electrophoresis under highly alkaline conditions (pH>13) which allowed the DNA supercoils to relax and unwind and made possible the detection of alkali labile sites and SSBs in DNA during electrophoresis. This technique was sensitive, flexible, low cost, easy, quick and needed small number of cells per sample.

Each cell, with damage in its DNA, gives the appearance of comet (due to migration of DNA fragments towards anode during the electrophoresis), while the undamaged cell is seen as a halo. Therefore, this assay is called as comet assay (Olive, 1989; Collins, 1992). The rate of migration is inversely related to the size of fragments.

Singh et al. (1990) used microgel electrophoretic assay to study age-dependent changes in distribution of DNA damage and repair among individual cells. They found that basal (pre-irradiation) levels of damage were independent of the age of the donor. An age-dependent increase in DNA damage was observed immediately following irradiation. However, the study indicated an age-related decline in DNA repair competence among a small subpopulation of lymphocytes.

Gedik et al. (1992) reported that SCGE technique has very high sensitivity. As few as 0.1 DNA breaks per $10^9$ of Dalton were detected in this assay.

Collins et al. (1996) suggested that the comet assay (single cell gel electrophoresis) can be used to detect oxidative DNA damage in form of DNA breaks.

Collins et al. (1997) pioneered a modification of the comet assay which enormously increases its range, sensitivity and specificity. The DNA in the gel, following lysis, was digested with a lesion-specific repair endonuclease, which introduced breaks at sites of damage and any lesion for which a repair endonuclease exists can be detected in this way. They also reported that the percentage of DNA in the comet tail was probably the most useful descriptor for comparing the results within or between laboratories.

At the International Workshop on Genotoxicity Test Procedures (IWGTP) held in Washington, DC, March 25-26, 1999, an expert panel identified the minimal standards for obtaining reproducible and reliable comet data deemed suitable for regulatory submission and developed guidelines for the use of the comet assay in genetic toxicology (Tice et al., 2000).
In conjunction with the 4th International Comet Assay Workshop (Ulm, Germany, 22-25 July 2001), an expert panel reviewed existing data and recent developments of the comet assay. They gave the detailed guidelines for conducting in vivo alkaline comet assay ranging from selection of animals, experiment conduction to statistical analysis. They emphasised on uniformity in conducting of the comet assay and reproducibility of the results across different laboratories all over the globe (Hartmann et al., 2003).

Collins (2004) explained the principles, applications and limitations of the comet assay. The intensity of the comet tail relative to the head reflects the number of DNA breaks. The assay has applications in testing novel chemicals for genotoxicity, monitoring environmental contamination with genotoxins, human biomonitoring and molecular epidemiology, and fundamental research in DNA damage and repair.

Olive and Banáth (2006) presented a procedure for the comet assay, a gel electrophoresis–based method that can be used to measure DNA damage in individual eukaryotic cells. It measured DNA single-strand breaks but modifications to the method allowed detection of DNA double-strand breaks, cross-links, base damage and apoptotic nuclei. The limit of sensitivity was approximately 50 strand breaks per diploid mammalian cell.

Lovell and Omori (2008) recommended that the % tail DNA is a suitable end point for analysis and has the advantage of a defined scale from 0 to 100% which is comparable across studies and sample size of 50 cells per slide were probably satisfactory to be scored to get statistically reliable results.

Smith et al. (2008) recommended the design of the rodent comet assay as a standard technique for evaluating DNA damage in vivo, as an extension of the traditional comet assay used for biomonitoring studies. They proposed methylnitrosourea to be a good alternative positive control inducing DNA damage in all tissues examined in the respective study.

The comet assay is advantageous as compared to other DNA damage assays. It is a fast, simple and sensitive assay. It can detect DNA damage at single cell level. It can detect low levels of DNA damage and requires less amount of sample. It is easy to perform and low cost technique. It is a flexible assay as it can evaluate various type of DNA damage. It is readily modifiable for adaption to a variety of experimental requirements (Liao et al., 2009).
Forchhammer et al. (2010) studied the comet assay protocols used across different laboratories and found that image analysis rather than the laboratory procedure was an important source of the inter-laboratory variation.

Zhang et al. (2011) modified the protocol for single cell gel electrophoresis slide preparation in order to cast five or more samples on one glass slide and can be processed. Slide and sample handling was simple, easy to master, and did not require any sophisticated equipments. This protocol retained the sensitivity of detection of the conventional alkaline comet assay while improving the efficiency of sample preparation and processing simultaneously.

The ComNet project was launched in 2011 during the International Comet Assay Workshop meeting in Kusadasi, Turkey, with the aim of establishing the comet assay as a reliable biomonitoring tool for human studies and to define the relevance of DNA damage (as measured with the comet assay) for human health and disease (Collins et al., 2012).

Mozaffarieh et al. (2013) reported that the cryopreservations leads to increases in number of ssDNA breaks in leukocytes, thus, affecting the results of comet assay analysis. They recommended performing the comet assay analysis on freshly prepared leukocytes for obtaining more accurate results.

Collins (2014) proposed that a simple modification i.e. incorporating a digestion of DNA with a lesion-specific endonuclease, makes it possible to measure oxidised bases. The assay can be used to monitor the cellular or in vitro repair of strand breaks or oxidised bases. It also has applications in assessing the antioxidant status of cells.

Gyori et al. (2014) described OpenComet, an open-source software tool providing automated analysis of comet assay images. It uses a novel and robust method for finding comets based on geometric shape attributes and segmenting the comet heads through image intensity profile analysis. Due to automation, OpenComet is more accurate, less prone to human bias, and faster than manual analysis. Live analysis functionality also allows users to analyze images captured directly from a microscope. OpenComet have been validated on both alkaline and neutral comet assay images as well as sample images from existing software packages. OpenComet achieves high accuracy with significantly reduced analysis time.
### 2.2.1 Comet Assay in T2DM

Dinçer et al. (2002) determined glutathione (GSH) level and, formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites, using the comet assay, which indicates oxidised guanine in freshly isolated blood from age-matched type 2 diabetics and controls. They reported that decreased GSH level may be a contributory factor for enhanced oxidative DNA damage in type 2 diabetics; and chronic hyperglycemia derived from poorly-controlled diabetic conditions may induce oxidative DNA damage in these patients.

Pitozzi et al. (2003) used the comet assay (single cell alkaline gel electrophoresis) to evaluate DNA strand breaks and DNA base oxidation, measured as FPG (formamidopyrimidine DNA glycosylase)-sensitive sites, in peripheral blood cells (PBC) from type 2 diabetes patients and healthy controls. Oxidative DNA damage in leukocytes was increased in diabetic compared to normal subjects. There is higher vulnerability to oxidative damage of polymorphonuclear as compared to mononuclear leukocytes in type 2 diabetes.

Blasiak et al. (2004) evaluated the extent of DNA damage, the sensitivity to exogenous mutagens (hydrogen peroxide and doxorubicin) and the effectiveness of DNA repair in peripheral blood lymphocytes of type 2 diabetes mellitus patients and healthy individuals using comet assay. They suggested that type 2 diabetes mellitus may be associated not only with the elevated level of oxidative DNA damage but also with the increased susceptibility to mutagens and the decreased efficacy of DNA repair.

Bagatini et al. (2008) used the comet assay to assess the levels of DNA damage before, immediately after and 48 hours after the hemodialysis session in patients with type 2 diabetes mellitus and healthy individuals and increased levels of DNA damage was observed in hemodialysis-dependent T2DM than healthy individuals. Damage levels increased immediately after the hemodialysis session and 48 hours after hemodialysis evidenced damage removal, which may be suggestive of DNA repair.

Oxidative DNA damage as measured by the comet assay in leukocytes obtained from diabetic patients was reported to be significantly higher compared to healthy subjects (Lodovici et al., 2008).

Arif et al. (2010) quantified the level of DNA damage in lymphocytes of T2DM patients using comet assay. A significant increase in mean comet tail DNA, indicating DNA damage, was observed in diabetic patients compared with controls.
Ibarra-Costilla et al. (2010) assessed whether general DNA damage levels evaluated by comet assay (length of tail comet, tail extent moment and olive tail moment) differ in Mexican patients with T2DM. The results indicated a lack of association between diabetes and general elevated levels of DNA damaged.

Thakkar and Jain (2010) evaluated the apoptotic DNA levels in blood leukocytes of patients with type 2 diabetes, hypothyroid, hyperthyroid, and patients suffering from both diabetes mellitus and hypothyroid, with the help of single-cell gel electrophoresis. Type 2 diabetic patients possessed greater cell damage than hypothyroid or hyperthyroid patients and further increase in cell damage was reported in patients with diabetes mellitus and hypothyroid.

Sheth et al. (2011) studied the effect of Indian Gentian (Enicostemma littorale Blume), an herb, as a genoprotective agent in T2DM patients with the help of comet assay and sister chromatid exchanges. Gentian was reported to significantly reduce DNA damage and attenuate sister chromatid exchanges in T2DM patients.

El-Wassef et al. (2012) concluded that T2DM patients have more oxidative DNA damage than normal controls and poor glycemic control may aggravate this damage. They observed that dyslipidemia is not a contributing factor for DNA damage in diabetes.

Palazzo et al. (2012) evaluated the levels of DNA damage using the comet assay in hemodialysis patients with type 2 diabetes mellitus. The same blood samples were also evaluated using the cytochalasin B micronucleus assay and results of the two assays were compared. The frequencies of micronuclei and nuclear buds were higher in patients than in controls. There was a correlation between the frequency of micronuclei and DNA damage with the results of the comet assay.

Tatsch et al. (2012) used comet assay to evaluate DNA strand breakage and its association with oxidative, inflammatory, and endothelial biomarkers in type 2 diabetes patients. NOx (nitrate/nitrite), interleukin-6 (IL-6), urinary albumin, fasting glucose, and glycated hemoglobin (HbA1c) levels were evaluated. The study showed higher DNA damage in type 2 diabetes patient with poor glycemic control than control subjects. Association between DNA damage and NOX, IL-6, urinary albumin, fasting glucose and HbA1c were reported. The comet analysis showed that the control group had the most compact DNA and maintained the circular form of a normal nucleus, with no evidence of comet formation. In contrast, cells from type 2 diabetes patients exhibited a distorted appearance, indicating substantial DNA damage.
2.2.2 Comet Assay in T1DM

Collins et al. (1998b) measured the levels of DNA damage in lymphocytes in patients with insulin-dependent diabetes in form of DNA strand breaks, oxidized pyrimidines (endonuclease III-sensitive sites) and altered purines (sites sensitive to formamidopyrimidine glycosylase), with the help of comet assay and reported that the mean values of strand breaks, oxidized pyrimidines and formamidopyrimidine glycosylase-sensitive sites were significantly higher in diabetics when compared to normal individuals. A strong correlation was seen between formamidopyrimidine glycosylase-sensitive sites and serum glucose concentrations.

Hannon-Fletcher et al. (2000) used the alkaline comet assay to measure DNA damage (single-stranded DNA breaks and alkali-labile sites) in freshly isolated whole blood, lymphocytes, monocytes, and neutrophils and statistically insignificant elevated levels of DNA damage (expressed as % comet tail DNA) was observed in the lymphocyte, monocyte and whole blood fractions from insulin dependent diabetes mellitus subjects as compared to controls. But elevated basal levels of DNA damage in the neutrophil fraction in insulin dependent diabetes mellitus subjects compared to controls was found to be significant.

Dinçer et al. (2003) evaluated strand breakage and formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites by the comet assay in DNA from leukocytes of the T1DM patients. Nitrite level, superoxide dismutase activity (SOD) and glutathione peroxidise (G-Px) activity of the leukocytes were also measured by spectrophotometric kits. Strand breakage and Fpg-sensitive sites were found to be increased, SOD and G-Px activities of the leukocytes were found to be decreased in both men and women of patient group as compared to their respective controls. Hence, impaired antioxidant defense in leukocytes of patients with T1DM may be one of the responsible mechanisms for increased DNA damage in T1DM patients.

Varvarovská et al. (2004) demonstrated increased oxidative stress (lower superoxide dismutase and glutathione) in children with T1DM. Although, their oxidative DNA damage (measured as DNA strand breaks with comet assay) was not substantially altered but DNA repair capacity was significantly increased in children with T1DM compared with normals.

Kushwaha et al. (2011) evaluated the extent of oxidative stress-induced DNA damage in different organs (lung, liver, heart, aorta, kidney, pancreas and peripheral blood lymphocytes) with the progression of diabetes. They were analyzed for both alkaline and modified comet assay with endonuclease-III (Endo III) and formamidopyrimidine-DNA glycosylase (FPG). The extent of
DNA damage was found to increase with the progression of diabetes as revealed by the parameter of olive tail moment.

### 2.2.3 Comet Assay in both T2DM and T1DM

Anderson *et al.* (1998) found that IDDM patients had lower level of DNA damage in the comet assay than in the control and a slightly lower level in the NIDDM patients, which indicated that these cells are handling more oxidative damage on a regular basis. The confounding factors (smoking, drinking and vitamin intakes, etc.) appeared to have no effect.

Öztok *et al.* (1999) detected higher oxidative damage to DNA in diabetes mellitus (NIDDM and IDDM) as compared to control subjects with the help of comet assay. DNA damage in the comet assay was at higher level in NIDDM patients and slightly lower level in IDDM patients which may indicate that these cells are handling more oxidative damage on a regular basis. Also, a synergistic effect of smoking on DNA damage (with high levels of tailed nuclei) was observed in smoking diabetic patients in comparison with smoking non-diabetic controls.

Sardaş *et al.* (2001) reported increased oxidative DNA damage in the comet assay in both NIDDM and IDDM than controls. Greater extend of DNA migration was found in NIDDM patients by comparison with IDDM patients which might indicate that IDDM patients are handling more oxidative damage on a regular basis. Smoker individuals had higher frequencies of cells with migration by comparison with the non-smokers in both groups.

Botto *et al.* (2002) found that oxidative DNA damage as observed in the comet assay was higher in individuals with type 2 diabetes mellitus as compared to those with type-1 diabetes.

### 2.3 OXIDATIVE STRESS

#### 2.3.1 T2DM studies

Baynes *et al.* (1991) stated that glycoxidation products like Nc-(carboxymethyl)lysine, Nc-(carboxymethyl)hydroxylysine, and the fluorescent cross-link pentosidine accumulate in tissue collagen with age and at an accelerated rate in diabetes. The glycation-derived free radicals can cause protein fragmentation and oxidation of nucleic acids and lipids leading to further increased free radical production and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress.
Leinonen et al. (1997) published new biomarker evidence of oxidative DNA damage in patients with non-insulin dependent diabetes mellitus and stated that high glycosylated hemoglobin was associated with a high level of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG). The increased excretion of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) indicated an increased systemic level of oxidative DNA damage in non-insulin dependent diabetes mellitus patients.

Hinokio et al. (1999) investigated the possible contribution of oxidative DNA damage to the pathogenesis of diabetic complications and measured the content of 8-hydroxy-2'-deoxyguanosine in the urine and the blood mononuclear cells of type II diabetic patients by HPLC-electrochemical detection method. The content of 8-hydroxy-2'-deoxyguanosine in the urine and the mononuclear cells of the type II diabetic patients were much higher than that of the control subjects. Thus, augmented oxidative DNA damage in diabetes is speculated to contribute to the pathogenesis of diabetic complications.

Rehman et al. (1999) used gas chromatography-mass spectrometry to measure the oxidative DNA damage in diabetic subjects and controls. Levels of multiple DNA base oxidation products, but not DNA base deamination or chlorination products, were found to be elevated in white blood cell DNA from patients with type II diabetes as compared with age-matched controls. This showed that the products generated by oxidative DNA damage are significantly elevated in T2DM and the pattern of modification was the same as one expected from the attack of the hydroxyl radical (OH') upon DNA.

Shin et al. (2001) assayed the serum 8-hydroxyguanine (8-OHG) concentration using high-pressure liquid chromatography. In type 2 diabetic patients, higher level of serum 8-hydroxyguanine levels was reported as compared to control subjects. Hence, serum 8-OHG is a novel convenient method for evaluating oxidative DNA damage.

Tanaka et al. (2002) reported that glucose toxicity leads to oxidative stress in pancreatic β-cells and glutathione peroxidise protects from oxidative stress.

2.3.2 T1DM studies

Ha et al. (1994) published DNA damage in the kidneys of diabetic rats exhibiting microalbuminuria. 8-hydroxydeoxyguanosine (8-OxodG) and urinary albumin excretion was increased in the kidneys of diabetic rats as compared to control rats, and insulin treatment reduced
both 8-hydroxydeoxyguanosine (8-OxodG) formation and urinary albumin excretion in the kidney. Therefore, oxidative damage is closely related in the process of diabetic nephropathy.

Orie et al. (1999) concluded that reactive oxygen species may play a role in the metabolic syndrome of non-insulin dependent diabetes mellitus (NIDDM) but not in essential hypertension (EH) and evaluated increased levels of reactive oxygen species (ROS) (both resting and stimulated levels of intracellular reactive oxygen species) in the pathogenesis of non-insulin dependent diabetes mellitus (NIDDM) than essential hypertension or controls were measured in lymphocytes with the dye, dihydrorhodamine-123 in the presence or absence of superoxide dismutase (superoxide scavenger), sodium azide (singlet oxygen/hydrogen peroxide scavenger), genistein (tyrosine kinase inhibitor), or bisindolylmaleimide (protein kinase C inhibitor).

Park et al. (2001) studied the effects of insulin and antioxidant on plasma 8-Hydroxyguanine and tissue 8-Hydroxydeoxyguanosine in streptozotocin-induced diabetic rats. They reported elevated level of plasma and tissue 8-Hydroxydeoxyguanosine in diabetic rat than control rats. They suggested that plasma 8-OHG could be a useful biomarker of oxidative DNA damage in diabetic subjects.

Hata et al. (2006) determined the concentrations of acrolein-lysine adducts, 8-hydroxy-2′-deoxyguanosine (8-OHdG) using competitive enzyme-linked immunosorbent assay, and nitric oxide metabolites were measured using the colorimetric, non-enzymatic assay in young type 1 diabetic patients. Increased urinary concentration of 8-OHdG in diabetic group indicates increased oxidative stress may be present at early stages of type 1 diabetes.

Van Dyke et al. (2010) studied the occurrence of oxidative or nitrosative stresses triggers type I diabetes. They investigated 30 children and found that urate is decreased by 25% in all these diabetic children each over the age of 3 years. Urate is a major portion of blood-antioxidant load and this decrease in urate indicates ongoing oxidative or nitrosoactive stress and hence, detectable in humans.

2.3.3 T2DM and T1DM combined studies

Krapfenbauer et al. (1998) published glycoxidation, protein and DNA oxidation in patients with diabetes mellitus and reported significantly increased glycoxidation and DNA oxidation in patients with type I and type II diabetes, but protein oxidation was not different from controls by
measuring urinary o-tyrosine, 8-hydroxy-2'-deoxyguanosine and pentosidine with the help of high pressure liquid chromatography (HPLC).

Lee and Chung (1999) found that the flux of glucose through the polyol pathway is the major cause of hyperglycemic oxidative stress in this tissue.

Formation of advanced glycation end products (AGEs) due to elevated nonenzymatic glycation of proteins, lipids and nucleic acids is accompanied by oxidative, radical-generating reactions and thus represents a major source for oxygen free radicals under hyperglycemic conditions. Once formed, AGEs can influence cellular function by binding to several binding sites including the receptor for AGEs (RAGE). Binding of AGEs (and other ligands) to RAGE results in generation of intracellular oxidative stress and subsequent activation of the redox-sensitive transcription factor NF-kappaB in vitro and in vivo (Mohamed et al., 1999).

Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is closely associated with aging and a number of diseases including cancer, cardiovascular diseases, diabetes and diabetic complications. Regular physical exercise is an important tool in the prevention and treatment of diseases including diabetes. Although acute exhaustive exercise increases oxidative stress, exercise training has been shown to up regulate antioxidant protection (Atalay and Laaksonen, 2002).

Glucose autoxidation, protein glycation and formation of advanced glycation endproducts, and the polyol pathway are known to be involved in the genesis of the oxidative stress, which has been reported both in experimental diabetes in animals and in type 1 and type 2 diabetic patients. Reciprocally, oxidative stress is involved in the origin of type 1 diabetes, especially via the apoptosis of pancreatic beta-cells, as well as insulin resistance in type 2 diabetes. Glucose control plays an important role in the prooxidant/antioxidant balance. Macromolecules such as molecules of extracellular matrix, lipoproteins and deoxyribonucleic acid are also damaged by free radicals in diabetes mellitus. A supplementation with antioxidants has been proposed as a complementary treatment, and some antidiabetic agents may by themselves have antioxidant properties independently of their role on glucose control. (Bonnefont-Rousselot et al., 2000).

Free radicals are formed disproportionatley in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development
of insulin resistance. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, non-enzymatic glycosylated proteins, and hyperglycemia occur in diabetes (Maritim et al., 2003).

Excess glucose metabolites traveling along glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, sorbitol formation, and oxidative phosphorylation pathways might cause beta-cell damage. However, all these pathways have in common the formation of reactive oxygen species that, in excess and over time, cause chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis. Chronic hyperglycemia is the proximate cause of retinopathy, kidney failure, neuropathies, and macrovascular disease in diabetes. The beta-cell steadily undergoes deterioration, secretes less and less insulin, and becomes a participant in a downward spiral of loss of function. (Robertson, 2004).

Van Campenhout et al. (2006) investigated the impact of diabetes mellitus on the relationships between iron-, inflammatory- and oxidative stress status. Glycaemic control, serum iron, proteins involved in iron homeostasis, global antioxidant capacity and levels of antioxidants and peroxidation products were measured in type 1, type 2 diabetic patients and control subjects. Circulating proteins, body iron stores, inflammation, oxidative stress and their interrelationships were found to be abnormal in patients with diabetes and differed between type 1 and type 2 diabetes.

Lenzen (2008a) stated that beta-cell is vulnerable to oxidative stress as antioxidative defence mechanisms of pancreatic beta-cells are weak and can be overwhelmed by redox imbalance arising from overproduction of reactive oxygen and reactive nitrogen species. The redox imbalance leads to lipid peroxidation, oxidation of proteins, DNA damage and interference of reactive species with signal transduction pathways, which contribute significantly to beta-cell dysfunction and death in type 1 and type 2 diabetes mellitus.

Moussa (2008) concluded that diabetic patients undergo an important oxidative stress that is low in non-insulin dependent diabetes mellitus (NIDDM) compared to insulin dependent diabetes mellitus (IDDM), suggesting metabolic differences between the two types of diabetes.
2.4 CATALASE (CAT) ACTIVITY

Catalase is a 244 kDa tetrameric protein, comprising four identical subunits of 59.7 kDa. Each subunit is composed of 527 amino acid residues, one haem group, namely iron (III) protoporphyrin IX, and a tightly bound molecule of NADPH. The enzyme catalase is an endogenous antioxidant present in all aerobic cells helping to facilitate the removal of hydrogen peroxide. The enzyme consists of 4 subunits of the same size, each of which contains a heme active site to accelerate the decomposition of $H_2O_2$ to water and oxygen. Catalase activity varies greatly between tissues with highest activities in the liver, kidney and erythrocyte, and lowest activity present in connective tissues. In eukaryotic cells, the enzyme is concentrated in sub-cellular peroxisome organelles. (Kodydkova et al., 2014).

Haining and Legan (1972) described a modification of the polarographic assay for catalase that is based upon automatic titration of a buffered $H_2O_2$-catalase reaction mixture with a more concentrated $H_2O_2$ solution such that there is no significant change in the volume of the reaction mixture. The improved assay was found to work satisfactorily for measuring catalase activity in rat tissue homogenates.

Sinha (1972) described colorimetric assay for catalase activity using $K_2Cr_2O_7$/acetic acid reagent.

Del Río et al. (1977) increased the sensitivity of catalase determination at least 20 times higher than that of previous methods by means of the Clark oxygen electrode. The assay was based on measurement of the initial rate at which oxygen was released by catalase in an oxygen-free buffer. Displacement of oxygen was brought about by flushing with nitrogen, and the substrate used was hydrogen peroxide at a 33.5 mm final concentration. The method was rapid and can be used with crude catalase preparations.

The polarographic method of catalytic currents applied to a wave of oxygen permits the simultaneous assay of superoxide dismutase and catalase in biological materials with high speed and reproducibility and minimal manipulation of tissues as proposed by Rigo and Rotilio (1977).

Johansson and Borg (1988) exploited the peroxidatic function of catalase for the determination of enzyme activity. The method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde produced was measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as a chromogen.
Pigeolet *et al.* (1990) estimated the inactivation of antioxidant enzymes (glutathione peroxidise, superoxide dismutase and catalase) by at least one of the oxidative reactive molecules (hydrogen peroxide, cumene hydroperoxide, t-butyl hydroperoxide and hydroxyl and superoxide radicals). They concluded that in the case of high oxidative stresses such an inhibition could take place, leading to an irreversibly autocatalytical process in which the production rate of the oxidants will continuously increase, leading to cell death.

Automated assays for catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase were presented by Wheeler *et al.* (1990).

Göth (1991) developed a rapid, cost-efficient, spectro-photometric assay for serum catalase activity which was a combination of optimized enzymatic conditions and the spectrophotometric assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate. 17.7% higher catalase activity was reported in males than in females. The serum catalase was found to increase with age between 14–60 years.

Leff *et al.* (1992) found that serum from individuals with Acquired Immunodeficiency Syndrome (AIDS) had significantly more catalase activity than serum from healthy control subjects. Serum catalase activity increased progressively with advancing human immunodeficiency virus (HIV) infection (i.e., AIDS > symptomatic infection > asymptomatic infection > controls).

Yasmineh *et al.* (1992) developed a simple, kinetic method for the determination of catalase activity in which the enzyme catalyzes the peroxidation of ethanol by hydrogen peroxide to acetaldehyde and water, and the acetaldehyde so formed is rapidly oxidized to acetic acid and NADPH by the addition of an excess of NADP+ and aldehyde dehydrogenase. The rate of NADPH production was monitored at 340 nm in a COBAS centrifugal analyzer.

Michiels *et al.* (1994) reviewed the importance of selenium-glutathione peroxidase, catalase, and Cu/Zn-superoxide dismutase for cell survival against oxidative stress.

A new method of hydrogen peroxide determination for the measurement of catalase activity and rates of hydrogen peroxide removal by erythrocytes from human subjects, rats and mice was described by Masuoka *et al.* (1996). Hydrogen peroxide was determined by converting it to the indamine dye with a water-soluble iron porphyrin and measuring the absorbance at 590 nm.

Bolzán *et al.* (1997) analyzed the influence of sex, age, and cigarette smoking on superoxide dismutase (SOD), catalase (CAT), and seleno-dependent glutathione peroxidase (GSH-Px) activities in human blood. Females showed higher SOD and CAT activities but lower GSH-Px
activity than males. SOD activity was found to increase with advancing age while opposite trend was observed for GSH-Px activity. No effect of age on CAT activity was observed. CAT activity in females was found to be lower in smokers than in non-smokers. They suggested that for clinical purposes it might be necessary to consider the sex and age of the subjects involved in the study.

İnal et al. (2001) measured erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels. There were positive correlations between CAT, GPx and MDA levels and age. While negative a correlation between SOD activities and age was reported.

Wu et al. (2003) described a method for the fluorometric determination of the activity of the enzyme catalase, based on the finding that H$_2$O$_2$ in the europium (III)–tetracycline–hydrogen peroxide system is consumed by catalase.

Li and Schellhorn (2007) examined the applicability of a kinetic microassay to quantify catalase from two different sources. The micorassay allows simultaneous evaluation of many samples (up to 96) in a short time (<5 min) and is thus well-suited to applications, such as high-throughput screening, where many parallel assays are required.

Shivakumar et al. (2011) proposed an assay for the determination of catalase activity based on the enzymatic consumption of hydrogen peroxide using iso-nicotinicacidhydrazide (INH) and pyrocatechol (PC). Quantification of catalase activity has been carried out in mycelia mats and culture media.

Bakalaa et al. (2012) found that glycation damages targeting catalase with aging may severely affect its activity, suggesting a link between glycation stress and the age-related decline in antioxidant defense in the mitochondria.

Iwase et al. (2013) assessed the applicability of the assay for measuring the catalase activity of various samples using laboratory strains of Escherichia coli, catalase-deficient isogenic mutants, clinically isolated Shiga toxin-producing E. coli, and human cells.

Wei and Ge (2013) reported that catalase activity is inhibited by graphene oxide by using molecular spectroscopic techniques such as UV–visible absorption, fluorescence, circular dichorism (CD) and fourier transform infrared (FT-IR) spectroscopy, and conducting in vitro enzymatic activity assay. Graphene oxide induced the decrease of α-helical content and the increase of β-sheet structure in catalase, resulting in the loosening and unfolding of the protein skeleton.
2.5 MALONDIALDEHYDE (MDA) LEVEL

MDA (C7H16O4) is a dialdehyde, naturally occurring product of lipid peroxidation of polyunsaturated fatty acids. It is a convenient and sensitive method for quantitative estimation of lipid peroxide concentration in different types of samples including drugs, food products and biological tissues from humans and animals. The most common method of measuring MDA is based on the reaction with thiobarbituric acid (TBA). The reaction results in formation of a pink coloured MDA-TBA adduct which can be measured spectrophotometrically at 532nm. Hence, MDA level can be used as indicators of effects of oxidative stress on lipids and can be utilized as an invaluable tool for the clinical management of different diseases (Romero et al., 1998).

Placer et al. (1966) described a new thiobarbituric acid method for the estimation of the malonyl dialdehyde produced as a result of lipid peroxidation in which the trimethine colored substance is estimated in alkaline solution. Alkaline pyridine-butanol mixture dissolves both proteins and lipids that are precipitated or opalescent during the reaction, yielding a more accurate MDA estimation as well as simplifying the procedure.

Yagi (1976) proposed a simple fluorometric assay for lipoperoxide in blood plasma using a small amount of blood, less than 0.05 ml of blood.

Uchiyama and Mihara (1978) described a thiobarbituric acid (TBA) test procedure with reasonable reproducibility applicable to the assay of lipoperoxides in various animal tissue homogenates. They concluded that the deproteinization of homogenate prior to coloration is not needed, but double wavelength measurement is necessary to avoid interference and the reaction should be performed with phosphoric acid at a definite pH near 2.0.

Ohkawa et al. (1979) developed a standard procedure for the assay of lipid peroxide level in animal tissues by their reaction with thiobarbituric acid. According to the protocol, ten percent (w/v) tissue homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.5), and aqueous solution of thiobarbituric acid. After heating at 95°C for 60 min, the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532nm.

Yagi (1987) described a method to determine the lipid peroxide level in human serum or plasma and its profile of change in several human diseases. Intervention of lipid peroxides in the pathogenesis of certain diseases was also mentioned.

According to Esterbauer and Cheeseman (1990), 4-Hydroxynonenal (HNE) is produced as a major product of the peroxidative decomposition of polyunsaturated fatty acids (PUFA) and
possesses cytotoxic, hepatotoxic, mutagenic, and genotoxic properties. Increased levels of HNE are found in plasma and various organs under conditions of oxidative stress. In addition to HNE, lipid peroxidation generates many other aldehydes that may also be of toxicological significance. Malondialdehyde is in many instances the most abundant individual aldehyde resulting from lipid peroxidation, and its determination by thiobarbituric acid (TBA) is one of the most common assays in lipid peroxidation studies. In vitro MDA can alter proteins, DNA, RNA, and many other biomolecules.

Gutteridge and Halliwell (1990) reviewed that the more specific the assay used, the less peroxide is found in healthy human tissues and body fluids. Lipid peroxidation can arise as a consequence of tissue injury in many disease states and may sometimes contribute significantly to worsening the tissue injury.

At low pH and elevated temperature, MDA readily participates in nucleophilic addition reaction with 2-thiobarbituric acid (TBA), generating a red, fluorescent 1:2 MDA: TBA adduct. The availability of facile and sensitive methods to quantify MDA have led to the routine use of MDA determination and, particularly, the “TBA test” to detect and quantify lipid peroxidation in a wide array of sample types (Janero, 1990).

The 2-thiobarbituric acid (TBA) test has been widely used for the measurement of the oxidative state of biological and food materials (Hoyland and Taylor, 1991).

Draper et al. (1993) compared the conventional spectrophotometric procedure and different high performance liquid chromatographic (HPLC) procedures for the determination of malondialdehyde (MDA) as the thiobarbituric acid (TBA) derivative in liver, fish meal, serum, and urine. They proposed the use of modified HPLC procedure for the determination of MDA as the TBA-MDA complex.

İnal et al. (2001) reported peroxidative injury is raised in the aging process as evaluated by plasma MDA levels in healthy subjects.

Dawn-Linsley et al. (2005) presented the modifications of the standard assay for thiobarbituric acid-reactive substances (TBARs) for analyses of both cell cultures and brain tissue homogenates. They described the usefulness of TBARs assay to assess the overall impact of oxidative stress-inducing and neuroprotective agents.
Pothiwong et al. (2007) had recognized the usage TBARS assay for determination of lipid peroxidation and oxidative injury in biological samples including brain homogenates obtained from bovine, canine, hen, rat, and swine.

Domínguez-Rebolledo et al. (2010) compared the thiobarbituric acid reactive species (TBARS) method and the BODIPY 581/591 C (11) (B581) and BODIPY 665/676 C (11) (B665) fluorescent probes to measure induced peroxidative damage. They concluded that both B581 and B665 might be used for lipid peroxidation analysis. The TBARS method offered comparatively limited sensitivity, and further research is needed in order to determine the source of that limitation.

Oxidative stress has been identified as a likely mechanism of nanoparticle toxicity, and cell-based in vitro systems for evaluation of nanoparticle-induced oxidative stress are widely considered to be an important component of biocompatibility screens. Potter et al. (2011) measured the products of lipid peroxidation, lipid hydroperoxides, and aldehydes, such as MDA via thiobarbituric acid reactive substances (TBARS) assay in human hepatocarcinoma cells.

2.6 FERRIC REDUCING ABILITY OF PLASMA (FRAP) ASSAY

Benzie and Strain (1996) presented a novel method for assessing antioxidant power i.e. ferric reducing ability of plasma (FRAP) assay. It is a simple and automated test which measures ferric to ferrous ion reduction at low pH and thereby a colored ferrous-tripryidyltriazine complex is formed. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects.

Choy et al. (2000) evaluated a novel method (FRASC) for total ferric reducing (antioxidant) activity and ascorbic acid concentration applied to human tears and found it suitable for measuring total antioxidant activity.

FRAP assay works with oxidation of metal ions. FRAP analyse the ability to reduce the ferric ion. The FRAP assay measures the formed ferrous ions by increased absorbance. The assay determines the antioxidant activity in the micromolar range needing minutes to hours (Schlesier et al., 2002).
Katalinic et al. (2005) measured antioxidant capacity of heart, kidney, liver and brain tissues of male and female rats. Antioxidant capacity of heart, kidney and liver tissues was found to be higher in female than male rats indicating stronger defense against oxidative damage in females for all observed tissues.

Katalinic et al. (2007) studied the antioxidant potential of the serum and the level of lipid oxidation products in the sera of apparently healthy adult males. They gave the mean values of FRAP, thiobarbituric reactive species (TBARS) and ferrous oxidation in xylenol orange version 2 (FOX2) levels. The mean±SD sera FRAP level of the 166 apparently healthy adult males was 1047±131 mmol/L (779–1410 range). The mean±SD level of sera TBARS was 1.2±0.3 mmol/L of the sera (0.5–2.2 range). The mean±SD level of lipid hydroperoxides in the fresh sera, determined as FOX2, was 3.9±1.5 mmol/L of the sera (1.9–6.9 range).

Gupta et al. (2009) developed the microplate method for the estimation of total antioxidant status in serum sample. It allows more number of samples to be analyzed per day. The reagent volume required is one fourth than that for the original procedure thereby cutting cost.

Sochor et al. (2010) discussed the behaviour, kinetics, time courses and limitations of the fully automated spectrometric method for FRAP assay. They found that the automatic analyzer allowed us to analyse simultaneously larger set of samples, to decrease the measurement time, to eliminate the errors and to provide data of higher quality in comparison to manual analysis.

Hayes et al. (2011) found that previously reported value of molar extinction coefficient i.e. 19,800 was an underestimate. They determined that the molar extinction coefficient was 21,140. The value of the molar extinction coefficient was also shown to depend on the type of assay and was found to be 22,230 under iron assay conditions.

Jansen et al. (2013) determined the effect of temperatures for storage and handling of serum samples on oxidation and antioxidant status of serum. They concluded that handling of serum samples at +4 and +20°C during short-time periods did not affect the quality and performance of the oxidation (reactive oxygen metabolites) and antioxidant assays (ferric ability of plasma, total antioxidant status and biological antioxidant potential) during day-to-day analyses.
2.7 Catalase (CAT) activity, Malondialdehyde (MDA) level and ferric reducing ability of plasma (FRAP) Assay in T2DM

The study of Gopaul et al. (1995) reported plasma levels of a specific non-enzymatic peroxidation product of arachidonic acid, esterified 8-epi-PGF2 alpha, from healthy- and non-insulin dependent diabetes mellitus individuals as an index of oxidative stress in vivo. Furthermore, it was studied that non-insulin dependent diabetes mellitus is connected with increased plasma lipid peroxidation.

Nourooz-Zadeh et al. (1995) measured plasma lipid hydroperoxides (ROOHs) in non-insulin-dependent diabetes mellitus patients and controls by the ferrous oxidation with xylenol orange assay coupled with the selective ROOH reductant triphenylphosphine. ROOHs concentration was found to be higher in non-insulin dependent diabetes mellitus patients. No difference between concentrations of plasma malondialdehyde measured as thiobarbituric acid-reactive material (TBARM) in NIDDM or control subjects was found.

Ceriello et al. (1997) found that TRAP, vitamin A, SH groups, and uric acid were significantly reduced, whereas the level of vitamin E was significantly increased in NIDDM patients. Oxidative stress may condition coagulation activation in diabetics. They suggested that it is the total antioxidant capacity rather than any single plasma antioxidant that is the most relevant parameter.

In the survey of Ceriello et al. (1998) in type 2 diabetes, free radical production has been reported to be increase in diabetic patients and to be implicated in the development of diabetic complications. After the meal, plasma malondialdehyde and vitamin C increased, while protein SH groups, uric acid, vitamin E, and total plasma radical-trapping parameter decreased more considerably in the diabetic subjects than in control subjects. This result demonstrates that in the absorptive phase, free radicals are produced in diabetic patients. Hyperglycemia may play a significant role in the generation of postprandial oxidative stress in diabetic patients.

Kedziora-Kornatowska et al. (1998) examined lipid peroxidation and activities of key antioxidant enzymes in non-insulin dependent diabetes (NIDDM) without nephropathy and in those with diabetic nephropathy. MDA content was significantly elevated in erythrocytes of NIDDM patients without nephropathy vs the control group and even higher in erythrocytes of NIDDM patients with diabetic nephropathy. SOD and CAT activities were lower in erythrocytes of NIDDM patients without nephropathy than in the control group and lowest in erythrocytes of NIDDM
patients with nephropathy. Hence, the intensity of oxidative stress appeared to be greater in NIDDM patients with nephropathy than in NIDDM patients without nephropathy.

Sözmen et al. (1999) reported higher catalase activities of either hypertensive patients with type 2 DM or type 2 DM patients without complication than controls. A positive correlation between the catalase activities and fasting glucose levels and HbA1c concentrations in hypertensive patients with type 2 DM was found.

Sözmen et al. (2001) revealed that enhanced catalase/superoxide dismutase and catalase/paraoxonase ratios are correlated with HbA1c levels as observed in type 2 diabetic patients. Thus, these ratios may be used as markers of poor glycemic control and as risk factors in the development of diabetic complications.

Özmen et al. (2002) analyzed the activities of the antioxidant enzymes such as Cu,Zn Superoxide Dismutase (Cu,Zn-SOD) and catalase in the cataractous lenses of the type 2 diabetic group and cataractous lenses of the senile group. They indicated that the antioxidant capacity in the diabetic cataractous lenses were decreased and suggested role of antioxidant enzymes in the genesis of diabetic cataracts.

Kimoto et al. (2003) suggested that gliclazide has radical scavenging activity and hence, reduces oxidative stress of β-cell which is induced by H2O2.

Memisoğullari et al. (2003) investigated erythrocyte glutathione levels, lipid peroxidation, superoxide dismutase, catalase, and glutathione peroxidase and some extracellular antioxidant protein levels of patients with type II diabetes mellitus and healthy controls. Higher levels of erythrocyte lipid peroxidation, serum ceruloplasmin and glucose levels, HbA1C levels, and erythrocyte catalase activity and decreased levels of serum albumin and transferrin levels, erythrocyte glutathione levels, and glutathione peroxidase activity were reported.

Sailaja et al. (2003) evaluated the extent of lipid peroxidation (LPO) and antioxidant defense system i.e., levels of glutathione (GSH), glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and catalase (CAT) in reticulocytes and erythrocytes of type 2 diabetic males and age-matched controls. Type 2 diabetics showed increased lipid peroxidation and decreased levels of GSH, GR, GPx, G6PDH, and GST both in reticulocytes and erythrocytes compared to controls, indicating the presence of oxidative stress and defective antioxidant systems in these patients. CAT activity was found to be enhanced in both the reticulocytes and erythrocytes of diabetics.
Góth et al. (2008) reported blood catalase activity in the type 2 diabetic subjects was decreased compared with that in the non diabetic control subjects. Also, blood catalase decreased with age in type 2 diabetic subjects when compared with the control group.

Lodovici et al. (2008) found that the FRAP level was significantly (P < 0.05) lower in diabetic subjects with poor glycaemic control than healthy subjects, while patients with good glycaemic control had FRAP values similar to controls. In patients with poor glycemic control FRAP values were reported to be positively correlated with glycaemic levels and HbA1c. They suggested that addition of antioxidant supplements to the current pharmacological treatment could have potentially beneficial effects in diabetic patients with poor glycaemic control.

Arif et al. (2010) assessed serum levels of MDA and protein carbonyl, and serum activity of superoxide dismutase and the protein thiol group in T2DM patients. Diabetic patients had significantly higher levels of MDA and protein carbonyl in parallel with significant decreases in levels of SOD and the protein thiol group compared with controls. They suggested that the status of oxidant-antioxidant imbalance may be one of the mechanisms leading to the DNA damage detected in the lymphocytes of type 2 diabetic patients.

Prasad and Sinha (2010) examined free radical activity in type 2 diabetic patients with hypertension compared to those without hypertension and reported reduced serum levels of superoxide dismutase and nitric oxide with a concomitant increase in serum malondialdehyde levels is consistent with an increase in free radical activity in hypertensive type 2 diabetics.

Al-Rawi (2011) assessed the salivary content of lipid peroxidation and antioxidants in patients with type 2 diabetes. Salivary MDA levels, a product of lipid peroxidation, were significantly increased among diabetics together with uric acid and might be of great importance in evaluating the disease activity and severity.

Bhutia et al. (2011) found increase in malondialdehyde level in type 2 diabetic patients. Factors like male sex, addiction to tobacco (smoking and smokeless inclusive), longer duration of diabetes (≥5 years), and presence of complications (both microvascular and macrovascular) contributed to significantly increased malondialdehyde level.

Chakraborty et al. (2011) evaluated the effect of metformin on oxidative stress, nitrosative stress and inflammatory parameters in diabetic subjects and explored that metformin treatment restores the antioxidant status, enzymatic activity and inflammatory parameters in type 2 diabetic
patients. Metformin therapy improves the status of oxidative and nitrosative stress altered in type 2 diabetes. Metformin administration also improves Na\textsuperscript{+}K\textsuperscript{+}ATPase activity in erythrocyte membrane.

Huang et al. (2011) studied that hyperglycemia induces oxidative stress in type 2 diabetic patients by activation of NADPH oxidase in monocytes. Increased NADPH oxidase activity was investigated by plasma membrane distribution of p47phox with the help of confocal immunofluorescence microscopy. Glucose unregulated p22phox expression. Higher oxidative stress state is indicated by decreased superoxide dismutase activity and elevated malondialdehyde, reactive oxygen species and protein carbonylation level. Significant positive bivariate correlation was found between glucose and MDA level as well as p22phox expression.

Kouchak et al. (2011) studied the effect of omega-3 fatty acids on serum paraoxonase activity, vitamins A, E, and C in type 2 diabetic patients. Spectrophotometry was used to measure serum paraoxonase activity and vitamin C levels and high performance liquid chromatography was used to measure vitamin A and vitamin E levels. It was found that supplementation of omega-3 fatty acids increased paraoxonase activity in diabetic patients but omega-3 intake caused no significant change in serum vitamin A, C, and E.

Savu et al. (2012) found that the antioxidant activities, lipid peroxide level, copper concentration and caeruloplasmin activity were significantly increased in the plasma of patients with diabetes compared with control subjects, despite an absence of chronic complications.

2.8 Catalase (CAT) activity, Malondialdehyde (MDA) level and ferric reducing ability of plasma (FRAP) Assay in T1DM

Urano et al. (1991) reported reconstituted liposomes prepared from aged diabetic erythrocyte lipids were highly susceptible to superoxide-induced oxidative stress as significantly higher amounts of unsaturated fatty acids, arachidonic acid and docosahexaenoic acid were observed in the erythrocyte membranes of diabetics. Vitamin E was found to be highly effective in suppressing the peroxidative lysis of liposomes composed of diabetic erythrocyte lipids.

Dominguez et al. (1998) evaluated lipoperoxidation in blood samples of 54 diabetic children, adolescents and young adults along with 60 age- and sex- matched control subjects and found progressively higher (P<0.0001) plasma MDA levels in diabetic children and adolescents than in control subjects.
Lubec et al. (1998) studied that kidney malondialdehyde concentrations, reflecting lipid peroxidation; pentosidine, and nepsiion-caboxymethyllysine concentrations, reflecting glycoxidation, were significantly elevated in diabetic mouse strains db/db and kk, and the diabetic BB rat to these parameters in the streptozotocin-treated rat. Aromatic hydroxylation was significantly elevated in the streptozotocin-induced diabetic state exclusively. This suggested that it is streptozotocin that generates hydroxyl radical and not the diabetic state in animal model.

Aragno et al. (1999) observed that hyperglycemic rats had higher thiobarbituric acid reactive substances formation and fluorescent chromolipids levels than controls. Liver, kidney and brain homogenates from dehydroepiandrosterone-treated animals showed a significant decrease of both thiobarbituric acid reactive substances and fluorescent chromolipids formation. The effect of dehydroepiandrosterone on the cellular antioxidant defenses was also investigated. In kidney and liver homogenates, dehydroepiandrosterone treatment restored the cytosolic level of reduced glutathione, as well as the enzymatic activities of superoxide-dismutase, glutathione-peroxidase, and catalase. In the brain, increase of catalase activity was reverted with dehydroepiandrosterone treatment. Dehydroepiandrosterone treatment was found to reduce oxidative stress products in the tissues of streptozotocin-treated rats.

Feillet-Coudray et al. (1999) evaluated the oxidative stress related parameters in streptozotocin-induced diabetes in rats and found that induced liver thiobarbituric acid reactive substances increased after 4 weeks of diabetes, in spite of increased liver vitamin E content. Plasma antioxidant capacity tended to increase after 4 weeks of diabetes and was correlated with plasma vitamin E levels.

In the study of Marra et al. (2002), it was examined whether type 1 diabetic patients with short duration of disease and without complications have an altered oxidative status and whether there are differences between men and women. The findings indicated that reduced antioxidant activity and increased oxidative stress occur early after the diagnosis of type 1 diabetes, especially in women, and this might explain the increased susceptibility of diabetic women to cardiovascular complications.

Martin-Gallan et al. (2003) ascertained the potential role of oxidative stress in the onset of disease-related pathophysiological complications in young type 1 diabetes patients. It was found that erythrocyte glutathione peroxidase activity, glutathione content, and plasma β-carotene to be significantly lower in diabetic patients compared with control subjects. Antioxidant enzyme i.e.
superoxide dismutase activity along with lipid peroxidation indices measured in plasma included malondialdehyde, lipid hydroperoxides, and lipoperoxides, were significantly elevated in diabetic patients regardless of the presence of complications.

In the survey made by Varvarovska et al. (2003), the results in diabetic children with type 1 diabetes showed extensively decreased glutathione peroxidase and plasma antioxidant capacity and increased malondialdehyde when compared with children that were healthy. Also almost the same findings were found in their siblings but not to the same degree.

Ramakrishna and Jailkhani (2007) found that hyperglycemia induces the overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation. A significance difference in the mean plasma concentration of total antioxidant status was observed in IDDM patients. They suggested that diabetes in an altered metabolic state of oxidation-reduction and that it is convenient to give therapeutic interventions with antioxidants.

Goodarzi et al. (2010) measured glycated serum protein (GSP) and MDA by colorimetric assay. Urinary 8-OHdG measurement was carried out using ELISA. Urinary 8-OHdG, HbA1c, plasma MDA, and GSP levels were progressively higher in diabetics than in control subjects. Significant correlation between urinary 8-OHdG and HbA1c was observed in diabetic group. Also, fasting blood sugar showed significant correlation with GSP and MDA.

2.9 Catalase (CAT) activity, Malondialdehyde (MDA) level and ferric reducing ability of plasma (FRAP) Assay in both T2DM and T1DM

Walter et al. (1991) found that diabetes can alter copper, zinc, magnesium, and lipid peroxidation status. Perturbations in mineral metabolism are more pronounced in diabetic populations with specific complications.

Ghiselli et al. (1992) published salicylate hydroxylation as an early marker of in vivo oxidative stress in diabetic patients and proposed measurement of 2, 5-dihydroxybenzoic acid and 2, 3-dihydroxybenzoate, following oral administration of salicylate in its acetylated form (aspirin) for assessment of oxidative stress. They reported significantly higher 2,3-dihydroxybenzoate levels in diabetic patients than in controls and plasma levels of thiobarbituric acid-reactive material (TBARM) values were not significantly different between groups.
Anderson et al. (1998) examined antioxidant capacity in both T1DM and T2DM patients with respect to controls. No differences were detected in control and diabetic patient groups in terms of creatinine levels and antioxidant capacity.

Davi et al. (1999) reported that diabetes mellitus is associated with increased formation of F2-isoprostanes, as a correlate of impaired glycemic control and enhanced lipid peroxidation. Formation of the F2-isoprostane 8-isoprostaglandin (PG)F2α, a bioactive product of arachidonic acid peroxidation, contributes to platelet activation.


Glucose intake stimulates reactive oxygen species generation and p47phox of as oxidase; increases oxidative load; plasma TBARS concentration and causes a fall in α-tocopherol (Mohanty, 2000).

Góth et al. (2001) found no significant difference between the mean blood catalase activities of the patients with type 1 diabetes and the patients with type 2 diabetes in the group lacking familial catalase deficiency.

Seghrouchni et al. (2002) evaluated plasmatic concentrations of thiobarbituric acid reactive substances (TBARS), fatty acids, total antioxidant status (TAS), alpha-tocopherol, and erythrocyte reduced glutathione (GSH) were measured as well as enzymatic activities of superoxide dismutase (SOD), and glutathione peroxidase/reductase in insulin dependent diabetes mellitus (IDDM), non insulin dependent diabetes mellitus (NIDDM) and insulin-treated type 2 diabetes mellitus (ITDM2). Diabetic patients had significant increase of SOD activity, TBARS concentration (concomitant with low levels of unsaturated fatty acids) and significant decrease of GSH and alpha-tocopherol. NIDDM have significantly lower levels of GSH and higher levels of TBARS compared to IDDM. ITDM2 values are intermediate between IDDM and NIDDM but are far from reaching those of IDDM.

Cojocaru et al. (2004) revealed an increase in lipid peroxidation and decrease in plasma CAT activity in patients with diabetes mellitus and ischemic stroke as compared to patients with diabetes mellitus without ischemic stroke with the use of spectrophotometric method. The mean value of plasma CAT activity was lower for females than for males. In diabetic patients, the decrease in plasma CAT activity is the consequence of oxidative modifications.
2.10 OTHER STUDIES

2.10.1 T2DM

Molnar et al. (2004) studied that ACE gene insertion/deletion (I/D) polymorphism is a well-known risk factor of hypertension, cardiovascular diseases and progression of diabetic nephropathy. They reported that D allele may contribute (via increased glycation and oxidative stress) to the target organ damage in type 2 diabetes.

Shorter telomeres are found to be associated with type 2 diabetes in Asian Indians (Adaikalakoteswari et al., 2005).

Asfandiyarova et al. (2006) investigated the risk for stroke in patients with T2DM and it was found that people with diabetes are more likely to get the disease such as stroke.

Mutations in mitochondrial DNA and decreases in mitochondrial DNA copy number have been connected to the pathogenesis of type 2 diabetes (Rolo and Palmeira, 2006).

Sheth et al. (2006) reported that there was a significant increase in sister chromatid exchange frequency in type 2 diabetic patients compared with healthy controls. This could be an early marker of DNA damage and a predisposing factor for some diabetes related complications.

Boehm et al. (2008) studied in women of the Ludwigshafen Risk and Cardiovascular Health, a high level of stable chromosomal aberrations in peripheral lymphocytes is associated with type 2 diabetes than healthy subjects and directly correlated with the risk of early diabetes related death.

Bastos et al. (2011) investigated the prevalence of oral mucosa alterations in patients with type 2 diabetes and identified possible risks associated with type 2 diabetes. Patients with diabetes mellitus showed high percentage of potentially malignant disorders.

Hallikerimath et al. (2011), Sonawane et al. (2011) and Suvarna et al. (2012) performed cytomorphometric analysis of buccal mucosa of type 2 diabetes patients and reported increase in mean nuclear area in study group in comparison to control group whereas mean cytoplasm area did not show any statistically significant difference. The mean cytoplasmic to nuclear ratio was significantly lower in the study group (p<0.001). They suggested that type 2 diabetes mellitus can produce morphologic and functional alterations in oral epithelial cells which can be used in the diagnosis of the disease.
2.10.2 T1DM

Jeanclos et al. (1998) reported that telomere length in WBCs from patients with type 1 diabetes, an autoimmune disease in which subsets of immune cells are involved in the destruction of pancreatic β-cells, is reduced compared with age-matched nondiabetic controls.

Astley et al. (1999) studied lymphocyte DNA from the diabetes group appeared to show less oxidative change with hydrogen peroxide than that of control subjects (with the help of SCGE), which suggests greater resistance in the diabetes group than in control subjects at baseline.

Hsieh et al. (2005), studied the possibility of 8-hydroxy-2’-deoxyguanosine (8-OHdG) serving as a sensitive biomarker of oxidative DNA damage and oxidative stress. Reactive oxygen species (ROS) have been reported to be a cause of diabetes induced by chemicals such as streptozotocin (STZ) in experimental animals. The study demonstrated that oxidative mtDNA damage might occur in multiple tissues of STZ-induced diabetics rats and that intervention with rice bran oil treatment may reverse the increase in the frequency of 8-OHdG.

Hathout et al. (2006) concluded that cumulative exposure to ozone and sulfate in ambient air may predispose to the development of type 1 diabetes in children. Early infant formula feeding and passive smoking in the household may precipitate or accelerate the onset of type 1 diabetes.

Francescato et al. (2014) found that the oxidative stress was not exacerbated due to a single bout of prolonged moderate intensity aerobic exercise. They also reported that oxidative defence increased at the end of exercise in both type 1 diabetic patients and controls, suggesting beneficial effects of prolonged aerobic fatiguing exercise.

2.10.3 T2DM and T1DM

Hiramatsu and Arimori (1988) observed elevated rate of $O_2^-$ production in the diabetic hypertriglyceridermic mononuclear cells. $O_2^-$ may be involved in the pathogenesis of atherosclerosis in diabetic hypertriglyceridermic patients when atherogenic factors specific to diabetes are concomitantly present.

Lenzen et al. (1996) studied the gene expression of superoxide dismutase, catalase, and glutathione peroxidase in pancreatic islets using a sensitive northern blot hybridization technique and for comparison in various other mouse tissues (liver, kidney, brain, lung, skeletal muscle, heart muscle, adrenal gland, and pituitary gland). Low levels of antioxidant enzyme gene expression may
provide an explanation for the extraordinary sensitivity of pancreatic beta-cells towards cytotoxic damage by diabetogenic compounds and during the development of human and animal diabetes.

Suzuki et al. (1999) studied that oxidative mtDNA damage is speculated to contribute to the pathogenesis of diabetic complications through a defect in mitochondrial oxidative phosphorylation or other mechanisms. 8-hydroxy-2’-deoxyguanosine (8-OHdG) and delta mtDNA4977 are useful markers to evaluate oxidative mitochondrial DNA damage in the diabetic patients. Delta mtDNA4977 and the 8-OHdG content in muscle DNA increased in proportion to the severity of diabetic nephropathy and retinopathy.

Decoction of kernels of Eugenia jambolana and extracts of Tinospora cordifolia are used as a household remedy for diabetes as their formulations significantly decreased glucose in the moderate and severe diabetes (Grover et al., 2000).

Dietary antioxidants are useful radioprotectors and play an important role in preventing many human diseases such as cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes (Fang et al., 2002).

Merkel et al. (2003) determined with the help of comet assay that glucose and insulin are important regulators of xeroderma pigmentosum complementation group D (XPD), and prolonged exposure to toxic levels of glucose reduces the insulin-dependent regulation of DNA repair.

Soto et al. (2003) suggested the protective effect of silymarin on pancreatic damage induced by alloxan may be due to an increase in the activity of antioxidant enzymes that, in addition to the glutathione system, constitute the more important defense mechanisms against damage by free radicals.

Demirbag et al. (2006) measured levels of DNA damage, total antioxidant capacity (TAC), total peroxide and oxidative stress index (OSI) and found significantly increased levels of TAC and DNA damage in subjects with metabolic syndrome (MetS) than controls. TAC levels were significantly decreased in MetS than in control. They proposed that increase in DNA damage might occur because of the increase in the imbalance between the production of oxidants and antioxidant defences in subjects with MetS.

Viktorínová et al. (2009) concluded that patients with diabetes mellitus had altered metabolism of Cu, Zn, and Mg which may be related to increased values of glycated hemoglobin. Impaired metabolism of these elements may contribute to the progression of diabetes mellitus and diabetic complications.
Ross (2011) stated that somatic gene conversion could be a significant causative factor in each of the seven diseases (bipolar disorder, cardiovascular disease, crohn's disease, hypertension, rheumatoid arthritis, type-1 diabetes and type-2 diabetes).

2.11 DIABETES AND CANCER

Gullo et al. (1994) proposed that diabetes is not a risk factor for pancreatic cancer. In patients with pancreatic cancer, diabetes is frequently of recent onset and presumably caused by the tumor.

Diabetes mellitus was evaluated as risk factor for pancreatic cancer (Everhart and Wright, 1995; Calle et al., 1998 and Fisher, 2001). They reported elevated frequency of pancreatic cancer among individuals with long-standing diabetes.

Steenland et al. (1995) found that risk of all cancers increased for males diabetics but women diabetics had no excess risk for all cancers. Males had elevated risk of colorectal and prostate cancers. In women, site-specific elevated risks were observed for breast, lung, and colorectal cancer.

Dandona et al. (1996) assayed 8-OHdG by high-pressure liquid chromatography and reactive oxygen species (ROS) by chemiluminescence. Insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus patients showed greater oxidative damage to DNA, with increased generation of reactive oxygen species (ROS) and 8-OHdG than controls and postulated that such changes might contribute to accelerated aging and atherogenesis in diabetes and to the microangiopathic complications of the disease.

Tucker and Preston (1996) stated that structural chromosome aberrations, micronuclei, aneuploidy, and sister chromatid exchanges are the major cytogenetic endpoints used most frequently in hazard identification assays as the first step in the genetic and cancer risk assessment process.

La Vecchia et al. (1997) investigated that history of diabetes mellitus could explain about 8% of cases of liver cancer in this population.

Weiderpass et al. (1997) studied that metabolic and endocrine alterations in patients with diabetes mellitus could contribute to an increased incidence of hormone-related cancers and patients with diabetes mellitus have an increased incidence of endometrial and breast cancers as compared with the general population.
Hu et al. (1999) stated that T2DM is associated with an increased risk of colorectal cancer in women.

Giovannucci (2001) reviewed that high levels of insulin (non-insulin dependent diabetes mellitus and hypertriglyceridemia) and IGF-1 (acromegaly) are related to increased risk of colon cancer, and increased circulating concentrations of insulin and IGF-1 are related to a higher risk of colonic neoplasia. Dietary patterns that stimulate insulin resistance or secretion, including high consumption of sucrose, various sources of starch, a high glycemic index and high saturated fatty acid intake, are associated with a higher risk of colon cancer.

Schneider et al. (2001) observed that one oral diabetes medication, metformin, inhibits pancreatic cancer development in animal (hamster) studies and explained the association between pancreatic cancer and obesity, which is usually associated with peripheral insulin resistance.

Bonelli et al. (2003) investigated the role of cigarette smoking and diabetes mellitus as important risk factors for exocrine pancreatic cancer.

Pancreatic cancer may be a cause of new-onset diabetes especially within 3 years before their cancer diagnosis was suggested by Chari et al. (2005).

Suba and Ujpal (2006) conducted a study with tumour cells and found that increases in glucose levels intensifies DNA synthesis and increases free radical release, cytokines and growth factors, which may destroy DNA as well as the enzymes that take part in the repair process. It was found that insulin resistance promotes tumor progression.

Wang et al. (2006) conducted a population-based case-control study of pancreatic cancer in the San Francisco Bay Area between 1995 and 1999 and reported that individuals with pancreatic cancer were more likely to report a history of diabetes than were controls. Risk for pancreatic cancer varies with duration of diabetes. The recent-onset diabetes, but not diabetes of ≥10-years duration, was associated with increased risk for pancreatic cancer. They also found out that use of oral diabetes medication or insulin for ≥5 years was not associated with pancreatic cancer, but insulin use of <5 years was associated with a 6.8-fold risk for pancreatic cancer.

Vigneri et al. (2009) studied that there is considerable evidence of an increased cancer (including pancreas, liver, breast, colorectal, urinary tract, and female reproductive organs) risk in diabetic patients, being more evident for primary liver cancer and pancreatic cancer. Hyperinsulinemia, obesity, hyperglycemia, and increased oxidative stress may contribute to increased cancer risk in diabetes.
At molecular level, a number of studies are going on to identify the different loci associated with diabetes (Noble and Erlich, 2012; Bakay et al., 2013; Sun et al., 2014). Till date, around 70 susceptibility genes have been reported to be involved in the pathogenesis of T2DM at genome-wide level (Sun et al., 2014). In a recent review, approximately 60 susceptibility loci have been identified as being associated with T1DM (Bakay et al., 2013) with the help of high-throughput technologies.