Abstract
The highly reactive electrophile, Methylglyoxal (MG), a breakdown product of carbohydrate is a major environmental mutagen and has potential genotoxic effects. It is ubiquitous in beverages and foods, such as coffee, toast and soy sauce, as well as in cigarette smoke. Its mutagenicity has also been reported in *Escherichia coli*, wherein it causes mutations at G:C base pairs, as well as in *Saccharomyces cerevisiae*. Previous studies have suggested that MG reacts with free amino groups of proteins and DNA under physiological conditions and forms advanced glycation end products resulting in the generation of hydroxyl (\(\cdot\)OH) and superoxide (\(O_2^-\)) ions which play an important role in the pathophysiology of ageing and diabetic complications. Moreover, in glycation reaction the free carbonyl groups of the sugar react with the free amino residues of the macromolecules in a series of chemical processes known as Maillard reaction. Initiation of glycation occurs by the formation of acid-labile Schiff base adducts which undergoes Amadori or Heyn’s rearrangements into more stable products. Glycated DNA has attracted much attention in the past few years and is considered to be a pathogenic factor for diabetes mellitus. During the disorder in the metabolism of reducing sugars, the sugar molecules can initiate glycation of DNA *in vivo*. Previous investigations by several scientists using biochemical and molecular biological methods have shown that DNA structure and function are affected by the addition of sugars and carbonyls (MG), resulting in deleterious modifications and other mutations.

Diabetes mellitus refers to group of common metabolic disorders that share the phenotype of hyperglycemia. Several types of diabetes mellitus exist and are caused by a complex interaction of genetics and environmental factors and viral infection. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs) and protein kinase C. In diabetes mellitus, low insulin levels prevent cells from absorbing glucose; as a result glucose builds up in the blood. Absolute insulin deficiency caused by autoimmune-mediated destruction of pancreatic \(\beta\)-cells characterizes type 1 diabetes. The main cause of the beta cell loss is a T-cell mediated immune attack. There is a strong association of type 1 diabetes with individuals who possess particular HLA haplotypes. Viruses, such as enteroviruses, coxackie virus and
rubella, have been proposed as culprits but have not been definitively shown to induce type I diabetes. None of these theories has evolved into a clear cause-and-effect initiator of diabetes. By whatever initiating mechanism, the autoimmune destruction of β-cells leads to a progressive decline in the body's insulin secretory capacity.

Like type I diabetes, type II diabetes also involves both genetic susceptibility and environmental factors. It is caused by a combination of insulin resistance and relative insulin deficiency with increased hepatic glucose production. Insulin resistance is generally thought to precede insulin deficiency. Before type II diabetes develops, the pancreatic β-cells increase their production of insulin to compensate for increased insulin resistance. For unclear reasons, β-cell secretory capacity gradually declines in some individuals, leading to the development of type II diabetes. The cause of β-cell failure in type II diabetes is unknown.

In the present study, commercially available human DNA was modified by MG and lysine in the presence of Cu²⁺. DNA modifications were analyzed by various spectroscopic and analytical techniques like, UV, Fluorescence, CD, thermal denaturation studies, HPLC, NMR, LC-MS and ESI-MS. Moreover, agarose gel electrophoresis, nuclease S1 digestibility and comet assays were also performed to assess the modification of the MG-Lys-Cu²⁺ system. Furthermore, antibodies were induced in rabbits against native and modified DNA. The induced antibodies were characterized with respect to antigen binding specificities by direct binding and inhibition ELISA. Antigen binding specificities were further confirmed by band shift assay. These antibodies have been used as an immunochemical probe to detect reactive carbonyl species (RCS) induced DNA damage in diabetes patients. In order to assess the possible role of MG-Lys-Cu²⁺ modified DNA in the etiology of type I diabetes mellitus, sera from patients were assessed for their binding to native and MG-Lys-Cu²⁺ modified human DNA. Furthermore, binding of serum antibodies for type II diabetes patients with native and MG-Lys-Cu²⁺ modified human DNA was also evaluated.

The UV & CD spectroscopical analysis, agarose gel electrophoresis, nuclease S1 digestibility assay and thermal denaturation studies, suggest structural perturbation in DNA as a result of modification. This might be due to the generation of single-stranded regions, destabilization of hydrogen bonds and modification of nitrogenous
bases which result in the destruction of chromophoric groups through attack on the sugar-phosphate back bone. The fluorescence spectroscopy study suggests formation of advanced glycation end-products. This is further proved by anti-glycation study and UV- spectroscopy. The genotoxicity of MG-Lys-Cu\(^{2+}\) formed AGEs was confirmed by using the comet-assay as an endpoint for DNA damage. This is evident from the DNA breakage and subsequent formation of the comet tail. Moreover, the adduct (CEdG) formed due to the glycation reaction of MG-Lys-Cu\(^{2+}\) with human DNA was detected by HPLC, supported by NMR and further confirmed by LC-MS.

The reaction of MG and lysine generates free radicals which were confirmed by quantitation of hydroxyl and superoxide radicals. The generation of these free radicals was further proved by quenching studies. Therefore, results presented here indicate that the glycation reaction of MG with lysine in the presence of Cu\(^{2+}\) may lead to oxidative damage of DNA through a mechanism that involves hydroxyl radicals.

The MG-Lys-Cu\(^{2+}\) modified human DNA proved to be a potent antigen, eliciting high titre immunogen specific antibodies in rabbits. The antigenic specificity of anti-MG-Lys-Cu\(^{2+}\) modified human DNA IgG was ascertained by competitive binding assay. A maximum of 88.5% inhibition in the antibody activity at inhibitor (immunogen) concentration of 20 \(\mu\)g/ml and just 2.8 \(\mu\)g/ml of the inhibitor concentration caused 50% inhibition, clearly indicating very high specificity and affinity of the induced antibodies towards the immunogen, i.e. the MG-Lys-Cu\(^{2+}\) modified human DNA. Affinity purified immune IgG showed higher specificity as compared to serum. Moreover, visual detection of interaction between immune IgG and the immunogen was done by band shift assay. The result shows high affinity of the induced antibodies for the immunogen. The induced antibodies though highly specific for MG-Lys-Cu\(^{2+}\) modified human DNA also exhibited polyspecificity, recognizing various nucleic acid conformers and nitrogenous bases in competitive inhibition assay. The results suggest that MG modification of DNA in presence of lysine and Cu\(^{2+}\) caused structural perturbations generating new epitopes thus transforming it into a potential immunogen. The modified DNA may be one of the factors for the induction of circulating anti-DNA antibodies in diabetes.
The clinical study focuses on the possible involvement of MG-Lys-Cu\(^{2+}\)-modified and the unmodified human DNA in diabetes mellitus (both type I & II) was probed. Out of 40 sera in type I diabetes, 67.5% showed preferentially high binding to MG-Lys-Cu\(^{2+}\) modified human DNA as compared to its native analogue. These results indicate substantial recognition of MG-Lys-Cu\(^{2+}\) modified human DNA by the auto-antibodies in diabetic (type-I) patients. The affinity purified IgG from diabetic patients showed appreciably high binding towards MG-Lys-Cu\(^{2+}\) modified human DNA, reiterating the results obtained with serum antibodies. The strong binding of auto-antibodies from diabetes type I patients to MG-Lys-Cu\(^{2+}\) modified human DNA is evidence towards the involvement of modified bases and single strand regions in disease pathogenesis. The spontaneous production of auto-antibodies in type I diabetes might be a result of the generation of the antigenic epitopes on the DNA molecules that are recognized as ‘non self’ by the body’s immune system. It could, therefore, be one of the factors of the immune response in diabetes. In type II diabetes, out of 45 sera only 35.5% showed low to moderate binding to MG-Lys-Cu\(^{2+}\) modified human DNA as compared to its native analogue. In type II diabetes, the recognition of auto-antibodies against MG-Lys-Cu\(^{2+}\) modified human DNA is quite low as compared to type I diabetes. The results show ample evidence of the involvement of MG-Lys-Cu\(^{2+}\) modified human DNA in type I diabetes while the same is less established for the type II.

In view of all the above studies it could be concluded that MG-Lys-Cu\(^{2+}\) modification of human DNA resulted in the formation of single strand breaks and base modification causing perturbation in the structure of DNA. Moreover, modified human DNA was highly immunogenic in experimental animals. The induced antibodies, though highly specific for the immunogen, also exhibited polyspecific binding. The antibodies showed significant binding with various nucleic acid conformers and nitrogenous bases. Higher recognition of MG-Lys-Cu\(^{2+}\) modified human DNA by diabetes type I autoantibodies is a clear indication of MG-Lys-Cu\(^{2+}\) induced DNA damage in these patients. It could, therefore, be one of the factors for the autoimmune response leading to the induction of circulating anti-DNA autoantibodies in diabetes mellitus, type I. Furthermore, antibodies from type II diabetes patients exhibited low to moderate binding with MG-Lys-Cu\(^{2+}\) modified human DNA.