Reactive oxygen species (ROS) are a ubiquitous part of human life having both beneficial and harmful effects. These are produced in cells under physiological conditions during the oxygen metabolism and other redox processes that are of vital importance for the biological function. However, excess of these radicals react with cellular lipids, proteins and nucleic acids leading to local injury and eventual organ dysfunction. Oxidative stress has a probable role in the pathogenesis of a variety of human diseases.

Among ROS, hydroxyl radical is the most reactive oxidant. It is widely produced in many biological processes through Haber-Weiss, Fenton reactions or by ionizing radiations. It reacts to most biomolecules at diffusion controlled rates causing oxidative damage. DNA is a vulnerable and important target for oxidant induced damage causing destruction of bases and deoxy-ribose sugars or single or double strand breaks. The oxidative damage to DNA is an important factor in mutagenesis and carcinogenesis. Hydroxyl initiated DNA damage is a threat to the integrity of genes, especially tumor suppressor genes. ROS have been implicated in various autoimmune diseases. Abnormal production of ROS may damage surrounding tissues and lead to diseases such as SLE.

Systemic lupus erythematosus is a multisystem chronic, autoimmune disease encompassing a spectrum of diseases defined by clinical criteria. Both cell mediated and humoral autoreactivity precipitates SLE. One of the characteristics of SLE is a long term high affinity immune response to self antigens. SLE is associated with the presence of autoantibodies against tissue and cellular components such as DNA, RNA, cytoplasmic elements found in serum, phospholipids, ribonucleoproteins and histones. However, the main autoantibodies found in SLE are antinuclear autoantibodies that react with nuclear antigens such as dsDNA, ssDNA and histones. The titre of anti-DNA autoantibodies in SLE patients correlates with the disease activity. DNA modified with ROS, is thought to be involved in the development of autoantibodies in SLE.

In the present study plasmid Bluescript DH5α”KS” DNA and native calf thymus DNA fragments of approximately 400 bp referred as native DNA were isolated and modified with hydroxyl radical, generated by the UV irradiation of hydrogen peroxide at 254 nm. The generation of ·OH radical was confirmed using mannitol, a quencher of ·OH radical. The hydroxyl radical induced modifications referred as ROS modifications in 400
bp DNA were analysed by UV spectroscopic and thermal denaturation studies. The modified DNA sample showed a marked hypochromicity at 260 nm as compared to native DNA. The data reflects the presence of single stranded regions in the modified DNA. The Tm of ROS-DNA was found to be 78°C, whereas, native DNA showed a Tm of 88°C. A net decrease of 10°C in the Tm value of ROS-DNA indicates a partial destruction of its secondary structure. The single strand breaks, induced in DNA as a result of ROS modification were detected by nuclease S1 sensitivity assay and hydroxyapatite column chromatography. The ROS-DNA was employed in competitive assay to delineate the epitope recognition of induced antibodies against ROS- plasmid DNA.

The modifications in plasmid DNA were analysed by UV and fluorescence spectroscopic techniques. The modified plasmid DNA showed hypochromicity as compared to native plasmid DNA, thereby reflecting the presence of single stranded regions in the modified plasmid DNA. The single strand breaks induced in the plasmid DNA upon ROS modification were further ascertained by agarose gel electrophoresis and nuclease S1 sensitivity assay.

The antigenicity of ROS-modified plasmid DNA was probed by inducing antibodies in rabbits. The repertoire of specificities of induced antibodies were evaluated by direct binding and competition ELISA. The induced antibodies exhibited polyspecificity, a property commonly associated with SLE anti-DNA autoantibodies. Anti-ROS plasmid DNA antibodies showed preferential recognition of ROS-modified conformers of DNA and nitrogenous bases in a competitive inhibition assay.

A group of SLE sera with high anti-DNA antibody titre were investigated for their specificity towards ROS-modified and native plasmid DNA by ELISA. Direct binding ELISA results showed preferential binding of SLE autoantibodies to ROS-plasmid DNA in comparison to native plasmid DNA. Inhibition ELISA reiterated the direct binding results. Gel retardation assay further substantiated the binding of native and modified plasmid DNA with anti-DNA antibodies. The retarded mobility of ROS-plasmid DNA complexed with SLE anti-DNA antibodies confirmed that the autoantibodies in SLE recognize the modified DNA more as compared to the native plasmid DNA.
Similarly, cancer sera were screened for the presence of antibodies reactive with native and ROS-plasmid DNA. The study consisted of 29 sera from patients with various types of malignancies. Direct binding ELISA showed greater recognition for ROS-plasmid DNA as compared to the native form. Four sera from breast cancer showed higher recognition of ROS-plasmid DNA than native DNA. Four sera from cancer of head and neck also showed higher reactivity with ROS-plasmid DNA. A large group comprised of cancer of respiratory system, which included six sera of oral cavity and three sera of lung cancer. All the oral cancers showed greater reactivity with ROS-plasmid DNA as compared to the native plasmid DNA. Whereas one out of three lung cancer sera showed greater binding with native plasmid DNA as compared to the modified form. Patients suffering from cancer of prostrate and gall bladder also showed higher reactivity towards ROS-plasmid DNA. Serum from vulva, Hodgkin’s lymphoma and CML also showed higher reactivity with ROS-plasmid DNA. These studies point out to the fact that free radicals may contribute widely to cancer development in humans.

In conclusion, the hydroxyl radical caused extensive damage to plasmid DNA altering its immunogenicity. The antibodies against modified plasmid DNA are polyspecific in nature, resembling the antigen binding characteristics of SLE anti-DNA autoantibodies. The possibility of hydroxyl radical modified DNA in autoimmunity and carcinogenesis have been indicated.