4-Aminobiphenyl (4-ABP) and several other related arylamines have been shown to be causally involved in the induction of human urinary bladder cancers. ABP induces a wide spectrum of tumors in a number of experimental animals. Arylamines, including ABP, were earlier used in chemical industries and are present in cigarette smoke and in other sources like hair dye, combustion of fossil fuels, rubber, coal, textile and printing industries. Hemoglobin-ABP adducts have been detected in the blood of smokers as well as non-smokers, although at much higher levels among smokers, who show an increased risk of bladder cancer. Thus, ABP and related aryl and heterocyclic amines represent an important class of environmental mutagens. The genotoxic and carcinogenic effects are exhibited when ABP is metabolically converted to a reactive electrophile, the aryl nitrenium ions, which subsequently binds to DNA. The various metabolic activation pathways that generate reactive electrophiles from arylamines or amides have been extensively studied in experimental animals. The activation of arylamines in general involves N-oxidation by hepatic enzymes followed by conjugation with acetate, sulfate, or glucuronate. In bladder carcinogenesis, the N-glucuronides formed by hepatic metabolism are postulated to be transported to the urinary bladder where they are hydrolyzed to the N-hydroxy derivatives. Under the acidic conditions of urine, the latter are thought to generate the aryl nitrenium ions that interact with critical cellular nucleophiles to initiate neoplasia. Alternatively, the procarcinogens or their proximate metabolites, N-hydroxy metabolites, namely N-hydroxy-4-acetylaminobiphenyl (N-OH-AABP) and N-hydroxy-4-aminobiphenyl (N-OH-ABP) could be activated by the enzyme systems that are present in the urothelium. The N-hydroxyarylamines can then be converted to aryl nitrenium ions through the putative intermediate. These electrophilic nitrenium ions interact with DNA to form covalent DNA adducts, thereby exerting their genotoxic effects. In fact, ABP–DNA adducts have been detected in the exfoliated urothelial cells of cigarette smokers and their levels correlate with the levels of 4-aminobiphenyl–haemoglobin adducts in the same subjects. Furthermore, several DNA adducts are formed upon exposure of human bladder cells to N-OH-ABP, N-OH-AABP and N-Acetoxy-4-acetylaminobiphenyl (N-OAc-AABP). However, the major (80%) DNA adduct formed has been identified as N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP).
Bladder cancer is one of the several types of malignant growths of urinary bladder. The most common type of bladder cancer begins in the cells lining inner side of the bladder and is called transitional cell carcinoma (sometimes urothelial cell carcinoma). Tobacco smoking is the main known contributor to urinary bladder cancer; in most populations, smoking is associated with over half of bladder cancer cases in men and one-third of cases among women. There is a linear relationship between smoking and risk, and quitting smoking reduces the risk.

How a normal urothelial cell transforms to a malignant cell and then metastasizes is a complex process that involves the interaction of many different genes, proteins, and other molecules. Several areas of molecular research have contributed to our knowledge about the initiation and progression of bladder cancer. Loss of tumor suppressor gene function or induction of oncogenes can lead to unregulated cell growth and proliferation. Abnormal expression of growth factors, adhesion molecules, and angiogenic factors are important in the progression of bladder cancer. Due to the genomic instability of cancer cells, it has been difficult to identify those genetic, chromosomal, and transcriptional changes found in bladder cancer that are fundamental to the malignant process vs those that represent secondary or epigenetic aberrations. In general, identifying the former would be more useful in developing detection and preventive strategies, while the latter may be more valuable for prognostic purposes. Awareness of both is likely needed to develop effective therapeutic approaches.

In the present study, commercially available human DNA was modified by $N$-OH-AABP. DNA modifications were analyzed by various spectroscopic and analytical techniques like, ultraviolet, fluorescence and, circular dichroic spectroscopy, thermal denaturation studies, HPLC, NMR and LC-MS. Agarose gel electrophoresis, nuclease S1 digestibility and comet assays were also performed to assess the DNA modification by $N$-OH-AABP. 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 competitive inhibition ELISA. Antigen binding specificities were further confirmed by band shift assay. The antibodies were used as an immunochemical probe to detect the lesions produced by $N$-OH-AABP or related aryl amine in the DNA obtained from
the lymphocytes of bladder cancer patients. Sera from bladder cancer patients, with and without the history of smoking, were assessed for their binding to native and N-OH-AABP modified human DNA to evaluate the epitope recognition by cancer autoantibodies.

The UV and CD spectroscopical analysis, agarose gel electrophoresis, nuclease S1 digestibility assay and thermal denaturation studies suggest structural perturbation in the 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 exposure of chromophoric groups through attack on the sugar-phosphate back-bone. The genotoxicity of N-OH-AABP was confirmed by comet-assay as an endpoint for DNA damage, which was evident from the DNA breakage and subsequent formation of the comet tail. The adduct (dG-C8-4-ABP), formed as a result of the reaction of N-OH-AABP with human DNA, was detected by HPLC. It was further confirmed by $^1$HNMR and LC-MS.

The N-OH-AABP modified human DNA proved to be a potent antigen, eliciting high titre immunogen specific antibodies in rabbits. The antigenic specificity of anti-N-OH-AABP modified human DNA IgG was ascertained by competitive binding assay. A maximum of 89.3% inhibition was observed in the antibody activity at inhibitor (immunogen) concentration of 20 μg/ml, with just 3 μg/ml of the inhibitor sufficient to cause 50% inhibition; clearly indicating very high specificity and affinity of the induced antibodies towards the immunogen, i.e. N-OH-AABP 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, which showed high affinity of the induced antibodies for the immunogen. The results suggest that N-OH-AABP modification of DNA has caused structural perturbations in the helix, generating new epitopes and thus transforming it into a potential immunogen. The N-OH-AABP modified DNA may be one of the factors for the induction of circulating anti-DNA antibodies in bladder cancer.
Possible involvement of $N$-OH-AABP modified human DNA in the bladder cancer was studied by evaluating the binding of $N$-OH-AABP-DNA with the serum antibodies from bladder cancer patients. For this we took 40 patients with smoking history and equal number of patients who were non-smokers. Out of 40 sera in the smoker group of bladder cancer, 72.5% showed preferentially high binding towards $N$-OH-AABP modified human DNA as compared to its native analogue. These results indicate substantial recognition of $N$-OH-AABP modified human DNA by the auto-antibodies in bladder cancer patients with a habit of smoking. The affinity purified IgG from bladder cancer patients showed appreciably high binding towards $N$-OH-AABP modified human DNA, reiterating the results obtained with serum antibodies. The strong binding of auto-antibodies from bladder cancer patients, in the smoker group, to $N$-OH-AABP modified human DNA is an evidence towards the possible involvement of modified bases and single strand regions in disease pathogenesis. The spontaneous production of auto-antibodies in bladder cancer patients belonging to smoker group 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 for the immune response in bladder cancer. In non-smoker group of bladder cancer, out of 40 sera only 40% showed low to moderate binding to $N$-OH-AABP modified human DNA as compared to its native analogue. Thus, in the non-smoker group of bladder cancer, the recognition of $N$-OH-AABP modified human DNA by autoantibodies in bladder cancer patients was quite low as compared to the smoker group. Role of $N$-OH-AABP in bladder cancer was further supported by detection of $N$-OH-AABP induced lesions in the DNA isolated from lymphocytes of bladder cancer patients, using experimentally induced anti-$N$-OH-AABP-human DNA-IgG as a probe. DNA obtained from bladder cancer patients, in the smoker group, recognized anti-$N$-OH-AABP-human DNA-IgG, appreciably and inhibited its activity in the range of 60.5% to 77.6% whereas the lymphocyte DNA isolated from the non-smoker group, caused an inhibition in the range of 47.4% to 57.4% in the activity of experimentally induced antibodies against $N$-OH-AABP modified DNA. The results show ample involvement of $N$-OH-AABP modified human DNA in development and exacerbation of antibodies in cancer patients.

In view of the above studies it could be concluded that $N$-OH-AABP modification of human DNA resulted in the formation of single strand breaks and
base modifications causing perturbation in the structure of DNA. The modified human DNA was highly immunogenic in experimental animals, and induced high titre immunogen specific antibodies, which showed significant binding with various nucleic acid conformers and nitrogenous bases. Higher recognition of N-OH-AABP modified human DNA by autoantibodies of bladder cancer patients, in the smoker group, is a clear indication of N-OH-AABP 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 bladder cancer patients with a habit of smoking. Antibodies from non-smoker group of bladder cancer patients exhibited low to moderate binding with N-OH-AABP modified human DNA. Cancer patients are known to have strand breaks and other lesions, that could be the result of exposure to aryl amines, thus presenting epitopes that are recognized to some extent, by experimentally induced antibodies against N-OH-AABP damaged DNA.