In the present study, p16 gene has been screened to study its role in the development of cervical cancer. All the three exons of the p16 gene were screened for mutation by performing SSCP and direct nucleotide sequencing. Methylation status of the p16 promoter region was detected through methylation specific PCR. MSPCR was performed after sodium bisulfite treatment of the genomic DNA. Expression profile of the p16INK4a protein has been find out by the use of immunohistchemistry technique.

Only one silent mutation in exon-2 of the p16INK4a gene was detected. The single silent mutation is for amino acid Glycine. None of the other exons (exon 1 & 3) exhibited any mutation in p16 gene. To best of our knowledge, the mutation we have found has not been reported in any other study. Although mutational inactivation of p16 gene is common event in other tumor types such as lung, esophageal, and pancreatic cancer, it is a rare event in cervical carcinoma. Accordingly, we can suggest that point mutations are not frequent events in human cervical cancer.

105 samples of primary cervical carcinoma tissue and 35 normal cervical tissues were analyzed with MSPCR. 20.9% samples were found hypermethylated while no methylation was detected in normal cervical tissue. Loss of functional p16 might result in unregulated Cdk4/6 activity, leading to persistent pRb phosphorylation and uncontrolled cellular proliferation. Presumably, p16 gene alteration in HPV–positive cervical carcinomas may confer an additional growth advantage to the affected cells or may play an additional role in the progression of these tumors.

Cancer of the cervix is probably the most preventable major form of cancer. The use of a biomarker that can predict the rate of progression or regression of cervical cancer could represent an attractive means for targeting screening or chemoprevention. In view of the fact that cervical cancer is the most prevalent cancer in India and it is curable if detected early. We have selected the p16 gene for molecular analysis in Indian cervical cancer cases, as this gene can be used as a potential biomarker.

The present study thus encompasses the following major findings:

- High-risk HPV DNA, detected by PCR with type-specific primers, was found in 83.8% cervical cancer cases comprising 80.0% HPV 16 and 3.8% HPV 18.
- Only 1 (0.9%) p16 gene mutation, in the form of silent mutation in exon-2, was detected out of 105 cervical cancer patients. None of the other exons (exon-1& 2) exhibited any mutation in p16 gene.
- The single silent mutation occurred due to transition of C→T at nucleotide number 604, codon number 111 of exon-2 of the p16 gene. This led to codon change GGC→ GGT with no change in amino acid.
- Since, only 1 (0.9%) p16 gene mutation was detected out of 105 cervical cancer patients, no statistical analysis could be employed to correlate between expression and mutation of the p16 gene.
- The frequency of hypermethylation of p16 gene in cervical carcinoma cases was 20.9%, while no case of hypermethylation was detected in normal controls.
- Aberrant p16 promoter methylation is associated with loss of p16INK4a expression. 90.9% hypermethylated cases shows abnormal expression with 54.5% loss of gene expression and 36.4% reduced expression of p16INK4a protein.
- Strong correlation was observed between the over expression of p16INK4a protein and HPV 16/18 infection. 81.8% HPV 16/18 cases shows over expression of p16INK4a protein. In contrast to this only 29.4% cases shows over expression that are negative for HR-HPV.
- There is no correlation between the presence of high-risk HPV type-16/18 and the methylation status of the p16 gene in the cervical carcinoma.
- No trend in terms of p16 gene expression with various clinicopathological variables was observed. The difference between varying p16 gene expression and various clinicopathological features was not to be statistically significant.