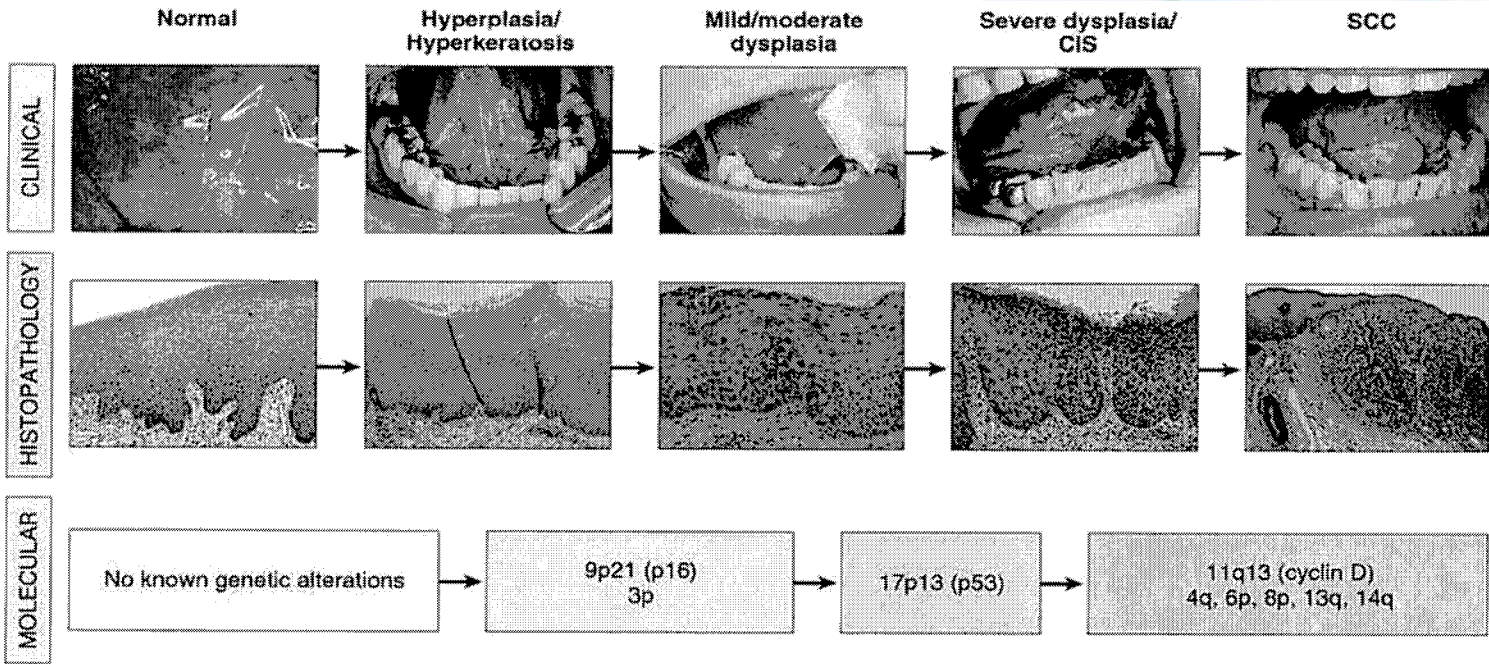
Oral Cancer

ORAL Squamous Cell Carcinoma (OSCC) is the sixth most common cancer and accounts for approximately 5% of all malignant tumors worldwide. In India and South East Asia OSCC is the most common malignancy amounting upto 50% of all malignant tumors. Oral cancer is a predominant cancer in Indian males. It is well known that there is a strong association between gene, cancer and environment. Tobacco use has been suggested as an important risk factor for oral cancers. With the available evidence of high tobacco use among school students there has been recommendations of anti-tobacco health programmes at the school level. Although most of the OSCC is attributed to smoking and alcohol consumption, a significant proportion of oral cancers have been demonstrated to contain anogenital HPV infection. The high risk HPV type 16 tends to be the most predominant type detected in oral cancer. Interestingly, HPV-infected patients were found to have better overall survival than those with HPV-negative tumors and showed well-differentiated tumors. However, the mechanism(s) by which HPV infection improves the overall survival/ prognosis in oral cancer is not yet clearly understood.

Work in our laboratories is divided along two independent lines of enquiry; one is on HPV and the other on initiation of tobacco use among school students. The major aspect that is being delt is to study the role of HPV in oral carcinogenesis and its transcriptional regulation. In addition, clinico-epidemiological studies are being performed for preventing initiation and for cessation of tobacco use among school students and the adults.



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Differential Expression Profile of Nuclear Factor-Kappa B (NF-κB) in Oral Cancer Tissue

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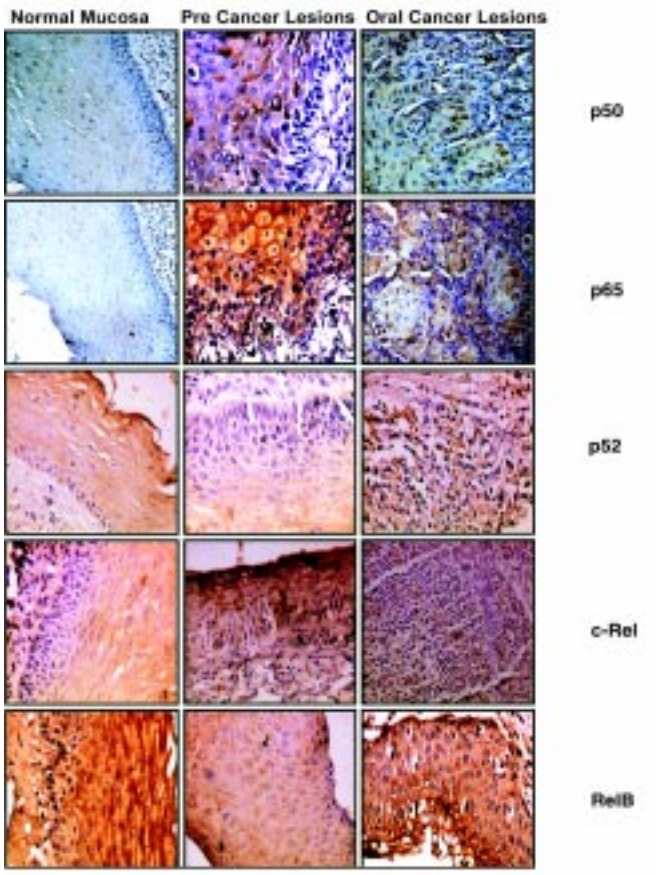
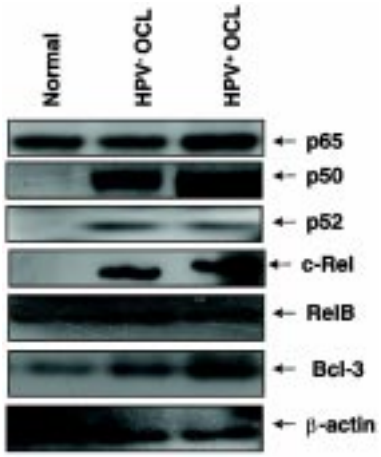
Co-Investigators : **Alok C. Bharti**
Suresh Hedau

Student : **Alok Mishra**

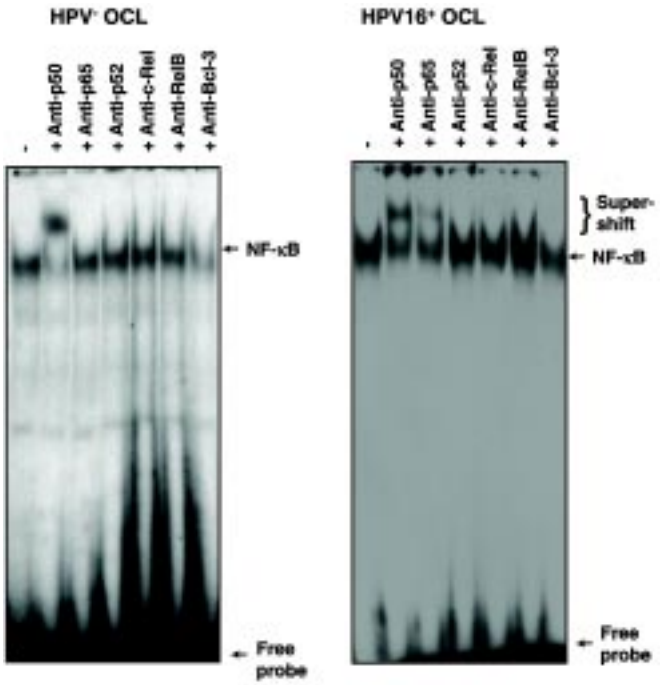
RECENTLY, a significant proportion of oral cancers have been demonstrated to contain anogenital HPV infection in a small but significant percentage of cases. The high risk HPV type 16 tends to be the most predominant type detected in oral cancer and was found mostly in well-differentiated malignant lesions. However, its role in oral cancer and



Immunohistochemical analysis of normal, precancerous (PCL) and cancerous (OCL) oral tissue for expression of different NF-κB members. Increased expression of p65, p50, p52, RelB, and c-Rel in oral cancer lesions. (Original magnification 200×)



Upregulation of p65, p50, and Bcl-3 expression in HPV16-infected oral cancer lesions. protein extracts each from normal, HPV negative (HPV^-) oral cancer lesions (OCL) and HPV16 positive oral cancer lesion (HPV^+ OCL) biopsies was separated on a SDS-PAGE, electrotransferred and probed for p65, p50, p52, RelB, Bcl-3 or c-Rel expression



High risk HPV type 16 infection results in alteration of DNA binding pattern of NF-κB members in oral cancer lesions. Nuclear extracts (10 µg) prepared from HPV⁻ OCL and HPV⁺ OCL were incubated with specific antibodies (2 µg each) either against p50, p65, p52, c-Rel, RelB or Bcl-3 and assayed for NF-κB binding activity by EMSA to study the supershift

association with differentiated cancers remained an unexplored area. High risk HPV type 16 has also been shown to modulate host transcription by altering the activity and expression of host transcription in cervical cancer by modulating the activity and expression of transcription factors such as NF-κB. However, not much is known about its role in oral cancer. NF-κB family of transcription factors plays an important role in the regulation of immune response, inflammation, embryo and cell lineage development, cell apoptosis, cell cycle progression, and oncogenesis.

In view of the above, comprehensive studies of biopsies from 100 oral cancer and pre-cancer lesions obtained from LN Hospital are being done where we are performing detection and typing of HPV in oral pre-cancer and cancer along with analysis of expression of all members of transcription factor Nuclear Factor-κB (NF-κB). The analysis of oral biopsies showed constitutive activation of NF-κB in oral cancer lesions. An up-regulated expression of p50, p65, c-Rel and p52 protein members was also observed during progression of oral cancer. A correlation is being made with the oral cancer cell lines that possess HPV infection. The preliminary in *vitro* studies on cell lines have shown that NF-κB is constitutively activated in HSC-2 (Human squamous carcinoma) cell line. The primary dimerising complex in this case was, however, formed of p50 and p65 members.

Role of Transcription Factor Activator Protein-1 (AP-1) in Oral Carcinogenesis

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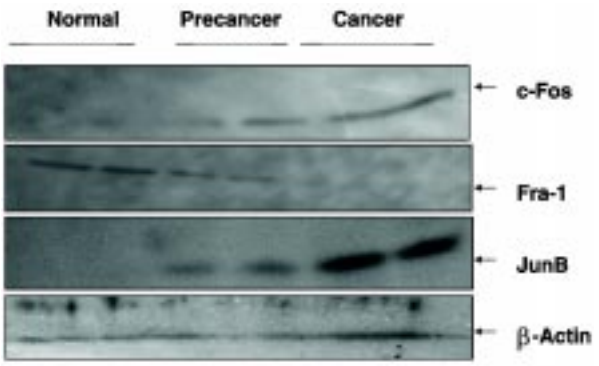
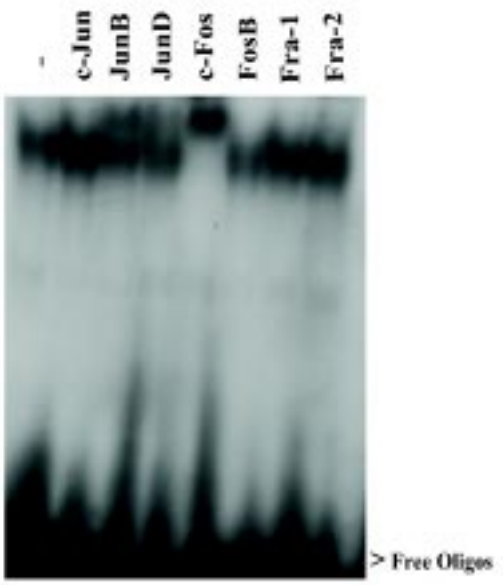
AP-1 transcription factor participates in the control of cellular responses that regulate cell proliferation, differentiation, immune response, cell death and the response to genotoxic agents or stress. To study the role of AP-1 in oral cancer, we studied the activation and expression pattern of AP-1 family of proteins in different stages of oral cancer.

AP-1 binding activity in the oral cancer tissue is highly upregulated as compared to that in normal tissues. But the activation of AP-1 in the precancerous lesions was found to be moderate. The supershift analysis of AP-1 family member proteins has shown that the complex formation occurred among the Jun-B, c-Jun and c-Fos members in oral cancer tissues. The expression of c-Fos and Jun-B protein members were observed to be overexpressed while the expression of Fra-1 member was down regulated. A correlation between activation, composition and expression of these transcription factors is being made with the HPV infection.

Further *in vitro* studies would be performed to analyze the activation and expression profile of AP-1 and NF-κB members after the treatment of herbal anti-oxidants such as curcumin. In oral pre-cancer, curcumin has been specifically shown to have significant curative effect. The molecular mechanisms underlying its anticancer properties in oral cancer are also being investigated.



Expression profile of AP-1 family members in different grades of oral tissue biopsies



Constitutive activation of AP-1 and its Composition in oral cancer tissue specimens

Suppression of Constitutive Activation of Host Transcription Factors, NF-κB and AP-1, in Oral Cancer by Curcumin

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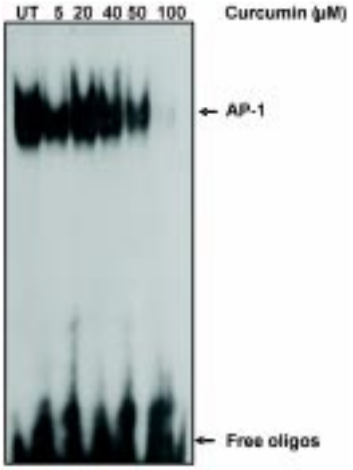
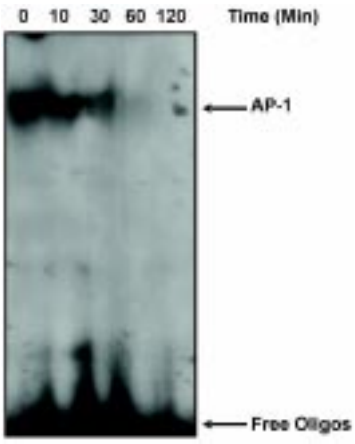


Dose kinetics of curcumin on AP-1 binding activity in HPV-16 positive oral cancer cell line, 93VU147T



Time kinetics of 100 μm curcumin on AP-1 binding activity in HPV-16 positive oral cancer cell line, 93VU147T

IT is a known fact the anti-oxidative drugs interfere with the redox status of the cells. Host transcription factors are the primary sensors and are regulated by cellular redox status. In oral pre-cancer, curcumin has been specifically shown to have significant curative effect. Since we observed a constitutive activity of NF-κB and AP-1 in oral carcinogenesis, we are investigating its therapeutic potential in oral cancer cells *in vitro* which will also have implication *in vivo*.

In vitro studies are being performed to analyze the effect of curcumin on the activation and expression profile of AP-1 and NF-κB members. The molecular mechanisms underlying its anticancer properties in oral cancer are being investigated.

Assessment of Prevalence of Tobacco Use and Impact of Health Education Intervention among School Students

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USE of tobacco currently accounts for 3 million deaths each year worldwide and nearly a third of these deaths occur in India alone. It is well established that chewing of betel quid with tobacco causes oral cancer and is largely responsible for high incidence of oral cancer in several South-Asian countries. Recent trends indicate an early age of initiation of tobacco use and rise in its prevalence among children and adolescents all over the world. Data are presented from 75 sites in 43 countries and the Gaza Strip/West Bank region. Current use of any tobacco product ranges from 62.8% to 3.3%, with high rates of oral tobacco use in certain regions. Current cigarette smoking ranges from 39.6% to less than 1%, with nearly 25% of students who smoke, having smoked their first cigarette before the age of 10 years. Studies from different parts of the world reported current smoking prevalence in school students ranged from 5.3% to 28.8%. In different North Eastern states of India the frequency of current smoking among school children ranged from 19.7 to 34.5%. Even though tobacco use by small children is thought to be culturally not acceptable in Indian society, over 65% users reported initiation at 10 years of age or earlier in different northeastern states. It is in terms of smoking and smokeless tobacco.

Tobacco use has been suggested as an important risk factor for oral cancers. With the available evidence of high tobacco use among school students there has been recommendations of school health programmes for anti-tobacco use. We conducted an extensive literature review and synthesis of published research addressing interventions to reduce tobacco use. It is observed that there are many intervention studies worldwide. However, in India an intervention study on 4,776 school students in 30 schools in Delhi to reduce tobacco habits, used posters, booklets, classroom activities, debates as tools. The study indicated that intervention students were less likely than controls to have been offered, received, experimented with, or have intentions to use tobacco. This is basically intervention programme in urban-based schools.

There is utmost need to have many such school health and intervention programme for preventing initiation and for cessation of tobacco use among school students both in rural and urban areas. Among school students who use tobacco no study examined the presence of risk of different signs of precancerous lesions. We proposed to include in the present study

School students being examined by ICPO's clinicians and scientists for assessment of oral health

School students being educated about the tobacco and subjected to questionnaire by ICPO team

the aspect of oral examination along with magnitude of tobacco use and health intervention. We have chosen NOIDA UP that represents both rural and urban-based schools for this study. The present study will be conducted in three phases for the duration of three years. The overall and specific objective in different phases is as given below: It is aim to determine the magnitude of problems in the first phase. In second phase, we are planning to develop health education module for school students indicating the ill effects of tobacco use and implement health education intervention and oral examination. While in third phase of the study, we will be evaluating the impact of intervention by filling post intervention questionnaires.

In the first phase the frequency of tobacco use and Knowledge, Attitude and practice (KAP) survey among school students of 13–17 years age. In the second phase a health education module will be developed for school students which will indicate the ill effects of tobacco use and health education intervention. In the third and final phase, the impact of intervention of health education in schools will be evaluated by assessment of frequency of tobacco use and KAP in the same schools after three years duration along with a control group of schools in which no intervention is given. It is planned to do this study in a sample of 6,000 students from different schools in and around Noida. This study is planned to be conducted in approximately 3,000 boys and 3,000 girls from both government and public schools. At first stage, we select schools by using simple random sampling (SRS) and in the second stage a random sample of classes will be selected. From each selected class we include all students of every alternative section.

During the year under report a self-administered questionnaire of 34 items was prepared such as demographic characteristics, tobacco use in any form, whether regularly, currently using tobacco in any form, knowledge about use among friends, like to and family

Tobacco use among school students- Frequency of use, Knowledge and Attitude

S. No.	Study Variable	Number (n=299)	Percentage
1.	Ever smoked	27	9.0
	Cigarette smoking	23	7.7
	Bidi smoking	4	1.3
	Regular smoking (Once or More/day)	10	3.3
2.	Ever chewed	18	6.0
	Gutka	4	1.3
	Pan Masala	14	4.7
	Regular Chewing (Once or More/day)	7	2.3
3.	Current smokers	10	3.3
	Current chewers	14	4.7
4.	Ever used tobacco in any form (Smoking or Chewing)	35	11.7
5.	Parents smoke	83	27.8
	Close friends or sibling(s) smoke	83	27.8
6.	Source of buying tobacco (Pocket money)	13	48.1
	Purchase tobacco for Parents and others	71	23.7
7.	View and like actors smoking on screen	162	54.2
8.	Family discussed harmful effects	234	78.3
	Self Knowledge of harmful effects	173	57.8
	School taught about harmful effects	143	47.8

members ever discuss the harmful effects of smoking or chewing tobacco, taught in any of your classes about the dangers of smoking or chewing of tobacco, will use tobacco when you are an adult, smoking/chewing can cause any health problem. The questionnaires were pre tested for its validity. The questionnaire was translated into Hindi language to meet the requirements of Government Hindi Medium schools. Pilot testing of Hindi questionnaire was done and modified accordingly before being formally administered. Director of the Institute formally informed the Principals of schools of NOIDA through a letter mentioning the aims and Objectives of study and permission to conduct the study was taken from them assuring that the information and the identities will remain strictly confidential. The data analysis by using EP Info 6 and Microsoft Excel is under progress. Till now, two schools covering 299 students were included in this study. The frequency of ever smoking was 9.0% and that of chewing was 6.0%. The Current smokers and chewers were 3.3% and 4.7% respectively. Out of 299 students the numbers of girls included in the sample were 55. The frequency of current or ever smoking and chewing among girls was 3.6% and 1.8% respectively. The Details of frequency of use, Knowledge and Attitude towards tobacco use are shown in Table. The study is under progress.

Survey of Tobacco and Alcohol Use in Adult Population

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Investigators : **Shashi Sharma**
Veena Singh
Ashok Seghal

Smoking enhances chances of cancer development

A PILOT survey has been conducted in an adult population of Chiranjiv Vihar (Ghaziabad). Two questionnaires were designed to study the prevalence of smoking, tobacco chewing and alcohol use, and the addiction level amongst the smokers, chewers and alcoholics. In association, the precancerous lesions of oral cavity in the smokers, tobacco chewer and alcohol users will also be detected. One questionnaire was for screening for any habit of smoking, tobacco and non-tobacco chewing and alcoholism. Second questionnaire was designed to take detailed history of those persons who have any one habit.

Total 317 people of 18 years plus, were screened. 46 (14.5%) persons were found to be smokers or chewers or alcohol drinkers. Total smokers were 29 (9.1%) out of which two female were found to be hukka smokers. 22 were cigarette smokers, 5 bidi smokers and 3 hukka smokers. Ex smokers were 9 only. Total numbers of tobacco chewers were 17 (5.4%) out of which two were females and only 1 was ex-chewer. Non-tobacco chewer was only one. Total number of alcohol drinker was 9 (2.8%), no female was found as alcohol taker. Amongst them 4 were ex-alcohol users. Out of 29 smokers 17 (58.6%) and out of 17 tobacco chewers 10 (58.8%) were found to have addiction for tobacco. Further out of 9 alcohol takers 3 (33.3%) were found to have addiction.