Antioxidant enzymes & thiol levels may predict oral cancer risk in healthy tobacco users. MMP-2 and MMP-9 may be useful to detect metastatic phenotype and recurrence of oral cancer.
SUMMARY:
Oral cancer has emerged as “new epidemic” in India as a result of chronic usage of tobacco in different forms and styles. Majority of these patients are diagnosed at later stage of the disease when the disease is locally or regionally spread showing metastatic potentials. This results into low survival and high recurrence rate. Tobacco use in any form is known to generate free radicals resulting into alterations in antioxidant enzymes like, GST, GR, SOD, CAT and GPx as well as oxidative stress related markers such as LPx and thiol. The metastatic potentials of the tumours can be analyzed with matrix degrading enzymes, mainly MMP-2 and MMP-9. Therefore, first aspect of tobacco carcinogenesis in oral cancer patients and their correlation with healthy tobacco users were studied using antioxidant and oxidative stress markers. While another aspect of metastatic potentials of oral cancer was studied by analysis of activation of MMP-2 and MMP-9. Summary of both these aspects are mentioned as follows;

Section-1: Study of antioxidant enzymes and oxidative stress related markers in oral cancer.

Antioxidant enzymes and oxidative stress related markers were analyzed from RBC, plasma, and malignant as well as adjacent normal tissue samples of oral cancer patients by spectrophotometric methods. Expression status of GSTM1 gene was studied by PCR method. These markers were compared between healthy tobacco non-habitués, healthy tobacco habitués and oral cancer patients before and after anticancer therapy. Their usefulness in monitoring oral cancer development in healthy tobacco habitués as well as monitoring treatment response in the patients using these marker panel were assessed. The study included pretreatment blood samples of 140 oral cancer patients and their follow-up blood samples (n=144), classified as CR (n=126) and NR (n=18) according to treatment response. The study also enrolled healthy
subjects as controls including WHT (n=25) and NHT (n=25). Detailed clinical as well as tobacco related informations of the subjects were collected. The observations are summarized below:

- WHT showed significantly low SOD and thiol levels as compared to NHT. WHT revealed significantly higher GPx activity as compared to NHT.
- RBC GST, GR, SOD and CAT activities were significantly higher in PT as compared to WHT. GPx and thiol levels were significantly lower in PT as compared to WHT. Plasma GST values were significantly higher and plasma GR levels were significantly lower in PT as compared to WHT.
- Tobacco use in any form as well as duration, frequency and lifetime tobacco exposure were higher in PT as compared to WHT. RBC SOD activities were significantly associated with lifetime tobacco exposure. Plasma GST activities were significantly higher in smokers as compared to chewers. LPx was significantly higher in chewers than smokers as well as those with both the habits together. Thiol levels were significantly lower in long-term lifetime tobacco exposure than short-term exposure in PT.
- Multivariate analysis revealed that RBC and plasma GST activities were associated with gender, being higher in male than female. RBC SOD activities were significantly higher in patients with advanced stage disease as compared to early stage disease. Plasma GST activities were decreased as stage of the disease advanced. RBC GST activities were positively correlated with RBC GR activities. RBC CAT activities were lower in moderately differentiated tumours as compared to well-differentiated tumours. LPx levels were higher in moderately differentiated tumours as compared to well-differentiated tumours. Plasma GST activities were increased with increase in tumour differentiation.
• WHT with elevated levels of RBC GR, SOD, CAT, lower level of plasma thiol and higher lifetime tobacco exposure showed higher risk of oral cancer development.
• 63% of the PT had GSTM1 null genotype.
• GST, GR and SOD activities were significantly elevated in malignant tissues as compared to adjacent normal tissues. CAT and thiol levels were significantly lower in malignant tissues as compared to adjacent normal tissues. SOD activities in malignant tissues were positively correlated with CAT and GPx activity. Tissue antioxidant enzymes and thiol levels were not associated with other clinico-pathological parameters.
• RBC GST and CAT activities were significantly decreased, while GPx and thiol levels were significantly increased in CR as compared to their pretreatment levels.
• RBC GST, GR and SOD activities were comparable between NR and PT. Mean levels of GPX, CAT, plasma GST, plasma GR and thiol were decreased in NR as compared to PT. Mean plasma LPx levels were increased in NR as compared to PT.

Section-2: Study of MMP-2 and MMP-9 activation as markers of invasion and metastasis in oral cancer.

Activation of MMP-2 and MMP-9 were studied from malignant (n=32) and adjacent normal tissues (n=38) of oral SCCs patients (n=39) using gelatin zymography technique. Activation of MMP-2 and MMP-9 from oral SCCs tissues were analysed to study metastatic potentials of these tumours. Circulating total MMP-2 and MMP-9 were analysed by ELISA method from PT (n=12) and follow-up (CR, n=30 and NR, n=8) plasma samples.
Latent, active and total forms as well as activation ratio of MMP-2 and MMP-9 were significantly elevated in malignant tissues as compared to adjacent normal tissues.

Active MMP-2 levels were higher as compared to active MMP-9 in malignant tissues.

Activation ratio of MMP-2 in malignant tissues was significantly elevated in SCCs with lymph node metastasis as compared to without lymph node metastasis.

No significant differences were found in MMP-2 and MMP-9 activities with age, gender, site, stages, tumour differentiation and nuclear grade of the disease.

There were no significant differences in plasma levels of total MMP-2 and MMP-9 between controls and cancer patients.

Plasma total MMP-9 levels were significantly decreased in CR as compared to pretreatment levels. Plasma MMP-9 levels were significantly lower in CR as compared to NR. Plasma MMP-9 levels were increased in disease recurrence or progression.

There were no significant differences in plasma total MMP-2 and MMP-9 levels between NR and PT.

Plasma levels of total MMP-9 and total MMP-2 could be useful as treatment monitors in oral SCC patients.

Local database and literature search on MMP-2 and MMP-9 have shown that this is the first report from India correlating tissue and plasma levels of MMP-2 and MMP-9 in oral cancer metastasis and treatment monitoring.

**CONCLUSION:**

Tobacco metabolism results into free radical generation, which ultimately results into alterations in antioxidant enzyme levels and oxidative stress related changes in lipid peroxidation and thiol. WHT had significant alterations
in SOD, CAT and GPx activities and thiol levels as compared to NHT. Oral cancer patients showed significant alterations in antioxidant enzyme levels including GST and GR resulting into oxidative stress. All of the oral cancer patients had tobacco habits. 63% of the patients showed GSTM1 null genotype showing susceptibility towards metabolic deficiency of tobacco carcinogens. Tissue antioxidant levels were also associated with oxidative stress in oral cancer patients. Thus development of oral cancer in WHT may be monitored by such genetic and phenotypic alterations. This was observed in WHT with elevated level of RBC GR, SOD, CAT, lower level of plasma thiol and higher lifetime tobacco exposure showed higher risk of oral cancer development. Therefore, the study could be useful for prediction as well as screening of oral cancer among tobacco users. Further, RBC and plasma GST levels may be useful for treatment monitoring of the oral cancer patients. Pretreatment levels of antioxidant enzymes may predict treatment response and may be useful for aggressive treatments, which are based on free radical generation.

Measuring activation of MMP-2 and MMP-9 in malignant oral cancer tissues can be routinely used for identification of metastatic potentials of oral cancer. Activation of MMP-2 and MMP-9 were found to be significantly elevated in malignant tissues as compared to adjacent normal tissues. Activation of MMP-2 predicted lymph-node metastasis in oral cancer patients. While, total MMP-2 and MMP-9 in plasma can be useful for treatment monitoring of the oral cancer patients. Plasma total MMP-9 significantly associated with prediction of the recurrence of the disease as well as response of the therapy.

CONCLUDING REMARKS:

Measures to understand multi-step tobacco carcinogenesis:
Considering tobacco as a major etiological factor, it is necessary to include different mode, frequency and duration of tobacco exposure in different
tobacco users. **Figure-42** depicts further work to understand role of tobacco in causation of oral cancer.

**Figure-42: A model to evaluate multi-step tobacco carcinogenesis**

![Model Diagram]

(AHH: Aryl hydrocarbon hydroxylase; cytP450: cytochrome P450; GST: Glutathione-S-transferase, OPC: Oral Precancerous conditions, NHT: Control without habit of tobacco, WHT: Control with habit of tobacco, GSH: reduce glutathione.)

The tobacco metabolites are activated by phase-I enzymes mainly, Aryl hydrocarbon hydroxylase (AHH) and cytochrome P450 (cyt-P450). The activated tobacco metabolites, if not detoxified by phase-II enzymes (GSTs), damages cellular macromolecules like DNA, proteins and lipids. Analysis of genotypes for enzymes involved in Phase-I and Phase-II activation and detoxification of tobacco metabolites as well as enzymes involved in DNA
repair will be useful in monitoring tobacco induced damages. Inclusion of non-enzymatic antioxidants, biomarkers of tissue damages in different tobacco users like patients with oral precancerous conditions, healthy tobacco abstinence as well as oral cancer patients without tobacco habits may further provide insight into tobacco carcinogenesis.

**Inhibition of MMPs as anticancer (anti-metastasis) therapy:**
Pharmacological inhibition of MMP activities could markedly inhibit the invasiveness of primary and metastatic tumours and therefore, therapeutic benefits to patient with cancer. Several of these approaches as mentioned in figure-43 are important to pharmacological intervention and may be useful for future anti-metastasis therapy. Presently, some of these approaches are studied but lack of effective methods has limited the clinical utility of this approach.

**Figure-43: Schematic representation of the potential therapeutic interventions to inhibit MMPs**

- **Signal transduction**
  - MMP gene
  - Transcription
- **Inhibitors, Gene knockout, Transfection**
  - MMP mRNA
  - Translation
- **MMP anti-sense RNA ribozyme, Tetracycline**
  - Pro-MMP
  - Secretion
- **Blocking of activation by inhibition of activators**
  - MMP
  - Activation
- **TIMPs, Synthetic MMP inhibitors**
  - ECM
  - Degradation
The field of MMPs has grown rapidly and may be said to have grown out of adolescence and reached at certain maturity. It is of interest to consider several of the anti-metastatic approaches for new directions and further development of the field.