Epilogue

Biomarkers might be useful for epidemiological studies on tobacco hazards as well as early diagnosis and management of oral cancer.
Tobacco exposure is one of the leading causes of mortality worldwide accounting for around 4 million deaths per year. If the tobacco usage continues in the similar fashion, by the year 2030, it will kill more than 10 million people annually (Gupta PC, 2001). Globally, over a quarter million new cases of oral cancer were diagnosed in the year 2000, with about 30% of them from India. World’s highest incidence of oral cancer is reported from India and is mainly attributed to high prevalence of tobacco chewing. Tobacco, used in different forms, generates free radicals, which results into several biochemical alterations in human body. Therefore, the documentation of harmful effects of tobacco habits and understanding the basic biology of oral cancer are meaningful ways to control the dreadful disease. The results of the current study might be helpful in planning preventive strategies for oral cancer and better management of oral cancer patients.

Summary:

1. **NO$_2$+NO$_3$ levels in different tobacco products and OR analysis in the subjects**
   - NO$_2$+NO$_3$ levels of the tobacco products, widely used in Gujarat, ranged from 0.13 to 3.39 mg/gm.
   - The OR for OPC and oral cancer were significantly higher in tobacco habitués than non-tobacco habitués (p=0.0001 and p=0.0001, respectively). The OR for oral cancer were significantly increased with duration and frequency of tobacco consumption.
   - Risk of developing OPC and oral cancer was higher in chewers and smokers who had longer duration as well as higher frequency of tobacco usage per day than the non-habituates.

2. **Urinary biomarkers of tobacco exposure in the subjects**
   - Urinary nicotine and cotinine peaks were not observed in children, who neither consumed tobacco nor were exposed to environmental tobacco. The NHT showed either absence or faint presence of
nicotine and cotinine peaks, while WHT revealed prominent peaks of nicotine and cotinine in urine samples.

- Urinary nicotine and cotinine levels were higher in NHT than children. Patients with oral cancer, patients with OPC and WHT showed significantly elevated urinary nicotine (p=0.001, p=0.05 and p=0.001, respectively) and cotinine levels (p=0.009, p=0.043 and p=0.03, respectively) as compared to NHT.

- Urinary nicotine and cotinine levels were significantly higher in tobacco non-abstinence group of oral cancer patients than the abstinence group (p=0.05 and p=0.05, respectively).

- Urinary thioether levels were higher in WHT and patients with OPC as compared to NHT. The oral cancer patients with and without tobacco habits showed significantly elevated urinary thioether levels as compared to NHT (p=0.001 and p=0.05, respectively) and WHT (p=0.008 and p=0.007, respectively).

- Urinary NO$_2$+NO$_3$ levels were higher in WHT than NHT. Patients with OPC and oral cancer patients showed significantly elevated urinary NO$_2$+NO$_3$ levels as compared to NHT (p=0.013 and p=0.031, respectively).

- Urinary nicotine, cotinine and NO$_2$+NO$_3$ levels were higher in controls and patients with tobacco smoking and chewing habits as compared to the controls without these habits.

- Urinary thioether and NO$_2$+NO$_3$ levels were elevated in controls having higher levels of tobacco exposure than their counterpart.

- Risk of oral cancer was associated with higher levels of urinary cotinine and NO$_2$+NO$_3$.

- ROC analysis showed that urinary nicotine, cotinine and NO$_2$+NO$_3$ levels had good efficacy to discriminate between controls and patients.

- Pearson’s correlation analysis revealed that the alterations in urinary thioether were positively associated with the alteration in nicotine
levels. The changes in urinary NO$_2$+NO$_3$ levels were positively associated with cotinine levels.

3. NO$_2$+NO$_3$, prostaglandin E2, antip53 antibodies, MDA, GST, GR, SOD, catalase and thiol in healthy individuals, patients with OPC and oral cancer patients.

- The patients with OPC and oral cancer patients showed significantly elevated plasma NO$_2$+NO$_3$ levels as compared to NHT (p=0.0001 and p=0.0001, respectively) and WHT (p=0.0001 and p=0.0001, respectively).

- Plasma prostaglandin E2 levels were significantly higher in patients with OPC and oral cancer patients as compared to NHT (p=0.0001 and p=0.02, respectively) and WHT (p=0.0001 and p=0.0001, respectively).

- Circulating antip53 antibodies were detected in 25.8% of oral cancer patients, 13.3% of patients with OPC and in none of the controls.

- Mean erythrocyte SOD activity was higher in WHT and patients with OPC than NHT. Oral cancer patients showed significantly lower erythrocyte SOD activity as compared to NHT (p=0.041), WHT (p=0.0001) and patients with OPC (p=0.008).

- Mean erythrocyte catalase activity was significantly higher in WHT and patients with OPC than NHT (p=0.036 and p=0.0001, respectively). Oral cancer patients showed significantly lower erythrocyte catalase activity than patients with OPC (p=0.0001).

- Plasma GST activity was higher in WHT, patients with OPC and oral cancer patients than NHT. Patients with OPC and oral cancer patients also showed higher plasma GST activity than WHT.

- Patients with OPC and oral cancer patients showed significantly lower plasma GR activity than NHT (p=0.031 and p=0.0001, respectively) and WHT (p=0.001 and p=0.0001).

- Mean erythrocyte GST activity was higher in WHT than NHT. The patients with OPC and oral cancer patients showed significantly
elevated erythrocyte GST activity than NHT (p=0.0001 and p=0.009, respectively) and WHT (p=0.0001 and p=0.0001, respectively).

- Patients with OPC and oral cancer patients showed significantly higher erythrocyte GR activities than NHT (p=0.003 and p=0.0001, respectively) and WHT (p=0.0001 and p=0.0001, respectively).

- Oral cancer patients revealed significantly lower plasma thiol levels as compared to NHT (p=0.0001), WHT (p=0.0001) and patients with OPC (p=0.0001).

- Mean erythrocyte GST, SOD and catalase activities were higher in healthy smokers and chewers as compared to NHT.

- Mean plasma MDA as well as erythrocyte SOD and catalase levels were higher in tobacco non-abstinence group of oral cancer patients than the abstinence group.

- A significant increase in OR for oral cancer was observed with increasing quartiles of plasma GST, NO$_2$+NO$_3$ and prostaglandin E2 as well as erythrocyte GST and GR (p=0.022, p=0.0001, p=0.0001, p=0.003 and p=0.0001, respectively). A significant decrease in OR for oral cancer was observed with increasing quartiles and lower levels of plasma GR and thiol as well as erythrocyte SOD (p=0.0001, p=0.0001 and p=0.05, respectively).

- Multivariate analysis revealed that plasma GST activity was associated with types of tobacco habits. Erythrocyte SOD activity and plasma GST activities in oral cancer patients were significantly higher in advanced disease than early disease (p=0.049 respectively). Plasma MDA levels were significantly associated with duration of tobacco consumption (p=0.022).

- Plasma GST and antip53 antibodies as well as erythrocyte SOD levels were higher in advanced disease than early disease.

- Erythrocyte catalase and plasma thiol levels were lower in poorly differentiated tumors as compared to well differentiated tumors.
ROC curve analysis showed that plasma NO$_2$+NO$_3$, prostaglandin E2, GST, GR and thiol as well as erythrocyte GST, GR, SOD and catalase levels revealed good efficacy to discriminate between controls and patients.

Pearson's correlation analysis revealed that the alterations in plasma NO$_2$+NO$_3$ levels were positively associated with urinary cotinine, thioether and NO$_2$+NO$_3$ levels. The alterations in plasma NO$_2$+NO$_3$ levels were positively associated with the changes in plasma GST as well as erythrocyte GST and GR activities. The alterations in plasma NO$_2$+NO$_3$ levels were negatively associated with that in erythrocyte SOD as well as plasma GR and thiol levels. The changes in erythrocyte SOD and catalase levels were positively associated with the alterations in nicotine levels.

Plasma MDA levels were increased, while thiol levels were decreased in non responders as compared to untreated oral cancer patients.

Plasma GST, MDA and prostaglandin E2 levels were lower in non-responders than untreated oral cancer patients.

The alterations in the levels of antip53 antibodies and prostaglandin E2 correlated well with the disease status in oral cancer patients during follow-up.

**Conclusions**

- NO$_2$+NO$_3$ levels in tobacco products were higher which may result in the formation of higher amount of TSNA.
- Risk of oral cancer was higher in tobacco habitués, which was positively associated with duration and frequency of tobacco.
- The modified HPLC method used in the present study for estimation of urinary nicotine and cotinine can be more suitable for tobacco exposure studies on large population.
- Urinary NO$_2$+NO$_3$ and thioether levels can be used as additional markers for tobacco exposure.
The elevation of plasma NO$_2$+NO$_3$, prostaglandin E2 and antip53 antibody levels might be helpful for early diagnosis of oral cancer.

The alterations in erythrocyte SOD and catalase may be useful to identify high risk individuals for OPC and oral cancer.

The alterations in GST and GR levels in healthy individuals, patients with OPC and oral cancer patients might reflect response to carcinogen exposure.

Alterations in erythrocyte SOD, catalase as well as plasma antip53 antibody levels might be useful for prognostication of the patients.

Alterations in plasma prostaglandin E2 and antip53 antibodies might be useful for treatment monitoring of oral cancer patients.

The present study had several approaches; focused on oral cancer prevention, risk determination and management of the disease. It is well said that, "Prevention is better than Cure". In the strong support of this notion, the present study provided interesting clues on etiologic aspects of oral cancer and harmful effects of tobacco. It can be helpful in planning and execution of preventive strategies for oral cancer, the major killer in India. In addition, the biomarkers studied from urine and blood samples would also be helpful in early diagnosis and management of the disease.