SCOPE FOR FURTHER STUDY

It is well known that radiotherapy (RT) induces several side effects such as stomatitis, dysphasia, xerostomia, nausea, trisms, excessive salivation, weight loss and hampers wellbeing and Quality of life of oral cavity cancer patients. These ill-effects are due to immunosuppressive effect of radiotherapy. Above study implies effectiveness of adjunct Ayurvedic treatment in clinical assessment of oral cavity cancer patients treated with radiotherapy. As a further step of this study it is interesting to observe immunological parameters relating the immune status in these patients during and after radiotherapy.

For this pilot study, we enrolled 4 new patients of oral cancer (OC) to investigate if the improvement in the radiation induced symptoms and quality of life in these patients could be related to improvement in the immune status of the patients. The inclusion and exclusion criteria were similar to the clinical study. In addition, two patients included in the study were tobacco chewers showing mild and severe leukoplakia each, without frank malignancy. The leukoplakia patients had tobacco habit for more than 30 years. While OC patients included in the study also chewed tobacco for 12 to 50 years. The Five OC patients received full course of RT along with Ayurvedic medicines. They were observed clinically and assessed for immunological parameters at three time points namely before RT (a), immediately after RT (b) and 1 month after completion of RT (c). Patients included in the study had undergone surgery, and belonged to all stages and grades of the disease.

The following immune parameters were assessed at three time points:
1. Percentage of T and B cells and T cell subsets from peripheral blood mononuclear cells (PBMC) by flow cytometry using antibodies provided by BD Biosciences, San Diego, CA
2. T and B cell proliferation induced by Phytohaemagglutinin (PHA) and Poke Weed mitogen (PWM) respectively, using $^3$HTdR incorporation assay from Sigma (PHA) and Gibco-BRA (PWM)
3. IgA levels in saliva by ELISA using commercial kits provided by Usen Life sciences Inc., China in order to assess local immune response
4. Pro-inflammatory (IL-1, IL-6, IL-8, TNF-α) and anti-inflammatory cytokines (IFN-γ and IL-10) in saliva and sera by ELISA using commercial kits provided by BD Biosciences

**Conclusion of Pilot Study:**
Since the OAM combination used is reported in Ayurvedic literature to boost up immunity, and to reduce inflammation we ventured to study a small group of OC patients treated similarly for their immune status at the same time points. The immune parameters assessed were T cell subsets and B cell counts in PBMCs, and their mitogen induced proliferative responses, salivary IgA levels and serum and salivary levels of cytokines IL-1, IL-6, IL-8, IL-10, TNF-α and IFN-γ. We also studied the same parameters in one patient each of mild and severe leukoplakia.

Although the data reported here is on a very small sample size of 4 cancer patients and 2 patients with leukoplakia, we have reported some interesting observations.

1. We have sequentially studied immune parameters before, immediately after and one month after completion of RT which would indicate basal level, compared with immediate post RT and one month after recovery of RT-induced inflammation.
2. We have correlated levels of pro-inflammatory cytokines IL-1 and IL-6 with post RT inflammatory response.
3. We have correlated reduced IL-10 responses with increased IFN-γ levels, which is indicative of polarization of immune response towards Th1 type.
4. We have shown the significance of saliva as important non-invasive source of bio fluid for analysis of immune parameters especially in OC.
5. We did not find correlation between impairment in immune parameters in leukoplakia parallel with increase in severity of the condition. We feel that improvement of clinical parameters along with immune responses in OC patients could be due to adjunct therapy with oral Ayurvedic medicines given throughout the observation period.

It is however essential to extend this study on a larger number of OC patients and include control group of patients treated with RT alone without additional Ayurvedic medicines, to arrive at definitive conclusion.