DISCUSSION

On a world scale, tobacco consumption is decreasing in the Western countries, while it is on the increase in developing countries like India. About 50 percent of the total tobacco used in India is in smokeless form - zarda, gutkha and pan masala. Tobacco chewing is most prevalent in India, which accounts for 85% of all the oropharyngeal cancers in India. Recent reports point towards increase in usage of smokeless tobacco, especially among urban and rural youth in India. A growing number of internists, pediatricians, pharmacologists, forensic pathologists, psychologists and dentists find that saliva provides an easily available, non-invasive diagnostic medium for a rapidly underlying range of diseases and clinical situations. As used in present study, the fluid most commonly employed for several diagnostic and laboratory purposes is the expectorated whole saliva - a mix comprised largely of secretions from major salivary glands, gingival fluid and serum.

The present salivary IgA concentrations obtained in study do not represent absolute values, but only comparative levels within the context of this investigation. The limitation in estimating salivary IgA concentrations is by loss of immunoglobulins during the process of centrifugation, concentration and by adhesiveness of IgA to surfaces. Moreover, the standard and, therefore, antiserum is serum IgA (7S), while estimation of salivary IgA is predominantly of the (11S) variety. Since the rate of diffusion in agar is dependent upon molecular size, therefore, the larger salivary IgA diffuses slower than serum IgA and the salivary value may be relatively low.
In the present study, a total of 253 adult males between 16 and 35 years of age, either cigarette smokers or users of smokeless tobacco were studied, who were classified on the basis of frequency and duration of habitual tobacco usage. A group of subjects comprising of both smokers and users of smokeless tobacco was studied to evaluate the additive effect of both of these habitual tobacco usages over quantitative salivary IgA estimations. To have a homogenous group, this study recruited only young urban males who started their habitual tobacco usage in teens/adolescent age with commercially available pouches only and continuing to use it till date as tobacco habit. In the present study, an attempt has been made to evaluate the local immune status through saliva of wives, whose husbands smoked in their presence over a period of years with varied frequency, to determine the effects of passive smoking on quantitative S-IgA estimation. The ex-smokers and ex-chewers were included in the present study to have an objective estimate of immune recovery following cessation of tobacco usage over a period of few years.

Immune reactivity and host defence is dependent on multiple variables, of which the most important are nutritional status, socio-economic background and dietary habits. With a view to alleviate any such variables affecting the immune reactivity status, only healthy adult males of good socio-economic status, with minimum education beyond Matriculation, and had never tasted alcohol were recruited for the present study. They comprised of white-collared urban youth of Ahmedabad, and their selection was stringent so as to minimise as much variables.
as possible which affect IgA, including consumption of coffee, alcohol, narcotic drugs, etc.66.

Gingival recession at site of placement of tobacco preparation in chewer subgroups was observed in more than 50% of teeth examined for recession. Gingival recession has been reported in smokeless tobacco users18,50,31,40,68, but not all7 clinical surveys. In this study, the first ever classification of the recession sites at placement of tobacco preparation was carried out. The conclusion arrived at this study was that, as the extent of chewing habit increases, the grades of recession also increase linearly. Thus, it can be hypothesized that recession is directly related to duration of tobacco chewing as also opined in earlier studies54,59. Dose-response studies need to be undertaken in future to assess this effect. It has been advocated that in the absence of plaque induced periodontal diseases, recession results from mechanical and/or chemical injury caused to gingiva underlying a thin alveolar housing or frank alveolar dehiscence60. Alveolar dehiscences associated with labial eruption in the dental arch are common among lower anterior and premolar teeth, a preferred site for placement of smokeless tobacco26. It seems the pH and moisture content of tobacco preparation may also influence periodontal breakdown and recession in tobacco chewers.

The effect of tobacco smoking and chewing on the periodontium has been the subject of many studies5,22,26,29,66. The clinical appreciation of differences in the periodontal status and the tissue response to treatment of smokers, chewers and non-smokers has stimulated extensive research activity60,113. Further interest has been sparked off by the elucidation of immunological interactions in periodontal
diseases\textsuperscript{78,103}. Descriptive cross-sectional studies indicate that smokers and chewer experience more periodontal breakdown\textsuperscript{22}. Exposing periodontal tissues to tobacco smoke and smokeless tobacco effects periodontal breakdown by two potential pathways: (1) directly as a source of gingival irritation, (2) indirectly a systemic mechanism may alter the vascular tissue and haemodynamics\textsuperscript{10}.

The debris in the present study increased in respect of smokers and chewers compared to control, which is in conformity with earlier studies by Solomon (1968)\textsuperscript{78} and Gupta (1964)\textsuperscript{78}. The debris, more or less, remain the same even if the frequency and duration of the habit increase, which is suggestive of poor oral hygiene status among smokers and chewers. Calculus in the present study was increased in smokers and chewers as compared to control; however, there was a progressive increase in calculus in chewers compared to smokers with increase in duration and frequency of the habit. A plethora of studies have reported increased calculus deposition due to habitual tobacco usage, irrespective of oral hygiene practices\textsuperscript{22,78}. Increased calculus in users of smokeless tobacco, compared to smokers, is of great concern as far as maintenance of oral health, especially among youth, is concerned.

Periodontal literature is quiet on the deposition of stains on the external surface of teeth, especially in users of smokeless tobacco. In the present study, increase in stains among smokers and chewers was observed as compared to control. A progressive increase in stains among chewers was found when compared to smokers, as the duration and frequency of the habit increased, indicating a direct relationship between smokeless tobacco usage, severity and-
stains. On an average, staining was more common and more severe in chewers compared to average smokers. The intensity of staining is dependent upon the content and composition of catachu and tobacco leaves in commercially available tobacco pouches and the type of oral hygiene practice. These stains, when deposited in thick layer near the cervical edge of teeth may act as initiating factor favouring plaque accumulation and increasing gingival inflammation\textsuperscript{26}.

At present there are no significant studies carried out to correlate the degree of attrition with frequency and duration of smoking or chewing. Attrition, i.e. functional wear of teeth, in the present study was more common in chewers, which increased progressively as the duration and frequency of the habit increased. Gupta (1964)\textsuperscript{78} reported similar findings. The increase in attrition index may be due to the increased load of mastication forces on the teeth due to constant chewing of betel leaf packets and arecanut, leading to para-functional habit. Surprisingly, it was also observed that, inspite of increased wear of tooth surface, there was no complaints of increased sensitivity, probably because of the deposition of secondary dentin, which again requires further study\textsuperscript{100}.

Studies on plaque organism have not identified any specific bacteria associated with smoking or smokeless tobacco usage, but have pointed to the establishment of more virulent plaque microflora which translates to increased gingival inflammation and pockets in habitual tobacco users\textsuperscript{18,22,80}. Certain investigators have reported increased gingival inflammation, bone loss, pocket depth and periodontal disease among tobacco users, while some others have reported similar gingival inflammation, bone loss, pocket depth and periodontal
disease among non-habitors. An indication of the capacity of smoking to depress gingival inflammation came to light incidentally from a study by Hedin and co-workers in 1981. They observed high levels of cAMP (Cyclic Adenosine Monophosphate) (low levels are associated with gingival inflammation) together with lowered gingival crevicular flow in heavy smokers, (high levels are associated with gingival inflammation), suggesting a suppression of gingival inflammation. In the present study also the change in gingival index is non-significant for light and moderate smokers which supports the observation made by Hedin and co-workers. Gingival inflammation in the present study was pronounced among chewers compared to smokers and the controls. Also, gingival inflammation and periodontal index increased progressively in chewers with increase in chewing intensity, which is suggestive of increased periodontal pathosis in chewers facilitating the loss of tooth supporting bone. In fact, recent studies show that smokeless tobacco extracts are potent stimulators of monocyte secreted PGE2 and IL-IB, inflammatory mediators that have been suggested as major factors in periodontal tissue destruction. As gingival inflammation increases, salivary IgA values increase proportionately, but in the present study, immune-suppressive effects of tobacco metabolites and combustion products seem to overcome this effect, thereby reducing salivary IgA values as habitual tobacco usage and gingival inflammation increase. From the results of scores of indices in smokers and chewers, it is apparent that while the relationship between habitual tobacco usage and periodontal disease needs further study, habitual tobacco
usage is detrimental to periodontal health as it worsens the oral hygiene status of an individual\textsuperscript{22,46,47}.

The results of the present study in relation to quantitative estimation of salivary immunoglobulin A in various subgroups of smokers and chewers, except passive smokers, show a statistically significant decrease in S-IgA level compared to controls, as also reported by Bennet and Reade\textsuperscript{60}. Also, in general, chewers demonstrate more pronounced decreased S-IgA compared to smokers, and there is progressive decrease in S-IgA in smokers and chewers with increase in duration and frequency of smoking or chewing, suggesting a dose-dependent mechanism in decreasing the immune reactivity status\textsuperscript{60}. Maximum decrease in quantitative salivary IgA of combined smoker and chewer supports this hypothesis. It seems, the decrease in S-IgA demonstrated in this study in respect of smokers and chewers can occur either by an influence on the salivary gland cells responsible for the completion of S-IgA molecule, or on cells of the immunologic system involved in the production of IgA molecules\textsuperscript{74}. Experimental studies using animals on tobacco smoking/chewing have suggested that the immuno-suppressive effect is on two cell immuno-surveillance unit consisting of macrophage and lymphocytes, and occurs by the lethal effect on macrophages and depression of lymphocyte functions\textsuperscript{45,76}. Thus, the habitual tobacco usage, in addition to worsening of oral hygiene status of a person, increases periodontal disease, also progressively impairs the defence mechanisms in form of decreased salivary IgA levels (antiseptic coat over oropharyngeal mucous surfaces).
promoting the establishment of both neoplastic and infectious diseases of oral cavity, the lungs as well as other tissues\textsuperscript{22,60}.

It is a generally accepted fact that chronic tobacco usage is associated with the production of a variety of acute and chronic, local or systemic diseases, although the mechanisms have not been well defined\textsuperscript{46,47}. It has been shown that inhalation of tobacco smoke/application of tobacco or arecanut extract over the mucosal surface lead to changes in immunologic responsiveness in experimental animals with moderate exposure, enhancing reactivity and prolonged exposure, ultimately depressing the reactivity\textsuperscript{104}. It is also shown that in human beings, who have smoked cigarettes for long periods of time, a generalized immunologic impairment occurs\textsuperscript{30}, together with interference in the function of macrophages from lung alveoli, phagocytosis function of polymorphonuclear leukocytes\textsuperscript{13} from the oral cavity and natural killer cell (NK cell) function\textsuperscript{19}. There is a depression of local immunologic reactivity or 'surveillance' too with altered distribution of T-helper inducer cell (\(T_4^+\)) and more T-suppressor cytotoxic (\(T_s^+\)) cells\textsuperscript{45,76}. Although not clearly directly linked with tobacco usage, oral cancer - most commonly carcinoma of the oral mucosa - is known to occur more frequently in tobacco habiters\textsuperscript{17}. No doubt, oral cancer constitutes more than 50% of total cancers detected in India, of which more than 50% are attributed to habitual tobacco chewing which differs from race to race and different parts of the country\textsuperscript{48}. The results of the present study also point to the poor oral hygiene status, increase in periodontal disease and more depression of local host defence posture in chewers compared to smokers in general, which may facilitate ingress of tumour antigens through oral
mucosa and periodontum. Due to the habit of retaining tobacco and arecanut preparation in the close-proximity to oral mucosal surfaces for prolonged duration of time, more rapid absorption of nicotine, tobacco specific nitrous amines and polyaromatic hydrocarbons seem to take place in chewers along with antigenic determinants leading to decrease in local immunologic reactivity status as confirmed in the present study\textsuperscript{100}.

In Indian context, diagnostic facilities for immunoglobulin estimations are scarce and, if available, inaccessible to common man. Hence the regression equations derived in the present study for various smoker and chewer subgroups with very high predictivity percentage will be useful in arriving at S-IgA levels of chronic tobacco habiters, when frequency and duration of habitual tobacco usage is known. This is an important aid to motivate tobacco habiters to discontinue the habit and to prevent the risk of contracting infectious or neoplastic diseases\textsuperscript{75}.

It is important to note, for clinical reasons, the work on mice by Thomas, et al.\textsuperscript{105}, which was supported by the data from a study on human beings carried out by Finklea, et al.\textsuperscript{20}, indicated that a restoration of immune function occurs following cessation of tobacco smoking/chewing. This suggests that the ex-tobacco habitor should be restored to a nearer normal state of health with reduced risk of contracting tobacco smoking/chewing related diseases, as the time from cessation of tobacco usage increases\textsuperscript{114}. Accordingly, in the present study, also, there was a gain of 36.7\% for mean abstinence of 4.5 years in ex-smokers and a gain of 25\% for mean abstinence of 4 years in ex-chewers, possibly establishing the concept of "Immune Recovery" as related to quantitative salivary IgA levels for the
first time in literature on quitting tobacco habit. Still, many questions remain unanswered as to whether the immune recovery is consistent or episodic in nature?; what is the interplay of host and other extraneous factors in assessing immune recovery on tobacco cessation? Nevertheless, it is apparent that there is a statistically significant gain in depleted salivary IgA level on tobacco cessation in chronic tobacco habiters.

With a view to monitor the Immune recovery experimentally, this study also envisaged a study on pre-selected tobacco habiters who had high motivation to quit tobacco, but could not do so due to nicotine dependence. These subjects were recruited in a clinical trial for 1 year, post tobacco-quitting made possible by time tested tobacco deterrent pills containing Lobeline sulfate 2 mg as active ingredient.

The theory on which the use of lobeline, the most popular of the smoking deterrent drugs, is based is a plausible one\textsuperscript{107}. Pharmacologically, lobeline has a weak nicotine-like action and substitutes, as it were, for nicotine during the first (pharmacological) phase of tobacco dishabitation, after which lobeline itself is discontinued without withdrawal syndrome\textsuperscript{23,68}. There appears to be no reason to expect the danger of addiction to lobeline from this therapeutic regime, since this drug, unlike nicotine, does not produce, as a rule, pleasurable sensations. In a number of controlled clinical studies, lobeline was found to be more effective than placebos and is being prescribed in many anti-smoking Clinics in the Western countries\textsuperscript{114}. The statistically significant gain in salivary IgA levels, at end of one year clinical trial for "Immune recovery", successfully demonstrates the use of lobeline sulfate as an effective tobacco deterrent drug. It is seen that the
experimental immune recovery achieved in the present study is directly correlated to the duration of time elapsed post tobacco cessation, i.e. more immune recovery is possible if more time/years passes after tobacco cessation, suggesting a somewhat slower recovery of salivary IgA in chronic tobacco habiters who have been smoking/chewing for more than 10 years or so.

The oral mucous lesions - white, folded and striated surfaces that are slightly elevated and diffusely demarcated from surrounding mucosa, i.e. leukoplakia, have been estimated to occur in 16% to 63% of smokeless tobacco users, and 6% to 38% of smokers. The significance of leukoplakia in smokers/chewers and its relationship to oral cancer is a raging debate between the epidemiologists and research workers who have rather confused the cause and effect relationship of leukoplakia with oral cancer, particularly, if the habit is discontinued, or if leukoplakia lesion persists. As reported by P. Robertson, leukoplakia lesions seem to occur rapidly with smokeless tobacco usage, but may restore normalcy quickly with cessation, and are not strongly related to duration. Leukoplakia lesions in smokers are dependent on duration of smoking, and takes time to resolve on tobacco cessation. The present investigation also envisaged a study on leukoplakia severity reversal along with immune recovery in selected leukoplakia patients, to substantiate the remission of leukoplakia lesions on tobacco cessation over a period of 1 year among smokers and chewers. In the present study, the prevalence and severity of leukoplakia lesions in chewers demonstrated a direct dose-response relationship, which is best predicted by the frequency of usage of smokeless tobacco, rather than by the age of the patient.
and duration of usage of smokeless tobacco\textsuperscript{40,100}. It may be for this reason that the decrease in leukoplakic severity was almost 100% in tobacco chewers compared to 73% in smokers on tobacco cessation for 1 year. The prevalence and severity of leukoplakic lesion in smokers in the present study seem to be dependent on duration of smoking, rather than frequency of smoking, and that may be the reason for the somewhat lesser reversal of leukoplakia severity in smokers compared to chewers on tobacco cessation for 1 year. The present data is too small and uncontrolled for leukoplakic lesions in smokers and chewers to possibly hypothesize any direct or indirect relationship of gain in salivary Immunoglobulin A in the reversal of leukoplakia severity. Nevertheless, it is quite encouraging to know that immune recovery and reversal of leukoplakia severity go hand in hand on tobacco cessation over a period of time. The significance of leukoplakia in tobacco chewers and smokers, and its relationship to malignant transformation are not clear, particularly if the habit is continued, or if leukoplakic lesion persists\textsuperscript{40,478,100}.

The present study demonstrated that habitual tobacco usage in form of smoking and chewing progressively worsens the oral hygiene status of an individual\textsuperscript{22}, increases gingival inflammation and periodontal disease\textsuperscript{79,103} as well as there is depression of local immunologic reactivity of oral mucous membrane in form of depletion in levels of salivary immunoglobulin A\textsuperscript{80}. All these changes may facilitate the passage of toxins, nicotine metabolites and tumour antigens to oral tissues, leading to establishment of both infections and neoplastic diseases within oral cavity. Interpretation of the changes in Immune function associated with
tobacco usage, however, may be constrained by the limitations of current knowledge concerning the Immune system, and carcinogenesis\textsuperscript{2,47}. A number of mechanisms act to reduce local defense, but their relative contribution considering the varied host and extraneous factors remains to be elucidated. The conceptual demonstration of "Immune Recovery" on tobacco cessation needs further study in a larger sample base with findings and controlling of the confounding variables of host origin.